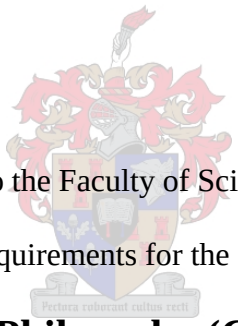
STIR BAR SORPTIVE EXTRACTION FOR THE ANALYSIS OF BEVERAGES AND FOODSTUFFS

A.G.J. Tredoux



Dissertation submitted to the Faculty of Science in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy (Chemistry)

at the

University of Stellenbosch

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Stellenbosch

January 2008

Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: _____

Date: _____

Summary

The main goal of this study was the development of new technologies based on modern analytical techniques for analysis of volatiles in wines. Due to the exponential growth of the wine industry and consumer demands for an enjoyable, safe-to-consume, and high quality product, the need for arose for methodologies aiding the understanding of wine better arose. Chemical analysis is a valuable way of studying the composition of wine in depth. Very sophisticated instrumentation is available nowadays but almost always the sample needs to be cleaned up or concentrated before such analysis. This study investigates the use of stir bar sorptive extraction (SBSE) as such a technique. It is shown that SBSE combined with gas chromatography-mass spectrometry (GC-MS) is extremely suited for a wide number of analyses and during the course of the study the technique was applied for troublesome analytical challenges in various beverages and foodstuffs. The study focuses on the development of a screening technique for volatiles in wine using SBSE and the application of the data to various chemometrical techniques for classification purposes. A second part of the study shows the applicability of SBSE for extraction of pesticides, contaminants and preservatives from wine, water, lemon flavoured beverages and yoghurt. The method is also elaborated upon by development of faster analysis methods for wine and beer and the investigation of using SBSE for headspace sampling of wine. In all the applications, SBSE technology was shown to be sensitive, repeatable, robust and very simple to use.

Opsomming

Die hoofdoel van hierdie studie was die ontwikkeling van nuwe tegnologie, gebaseer op moderne tegnieke vir die analise van vlugtige komponente in wyn. Die eksponesiële groei in die wynindustrie en verbruikers se behoefte aan genotvolle, hoë kwaliteit produkte het die soeke gestimuleer na metodes om die samestelling van wyn beter te verstaan. Chemiese analise is ‘n waardevolle manier om wynsamestelling in diepte te bestudeer. Alhoewel baie gesofistikeerde instrumentasie hedendaags beskikbaar is, is die behoefte aan monstervoorbereiding en -konsentrasie steeds van groot belang. Hierdie studie ondersoek die gebruik van roerstaaf sorptiewe ekstraksie (stir bar sorptive extraction (SBSE)) as so ‘n tegniek. Daar word aangetoon dat SBSE in kombinasie met gaschromatografie massaspektrometrie (GC-MS) besonder geskik is vir die analise van ‘n wye aantal analises en deur die loop van die studie is getoon dat die tegniek toegepas op verskeie problematiese analitiese uitdagings in die voedselindustrie. Die hooffokus van die studie is die ontwikkeling algemene profiel analise van vlugtige komponente in wyn met gebruik van SBSE en die gebruik van hierdie data, met die hulp van chemometriese metodes vir klassifikasie van wyne, ‘ Tweede deel van die studie handel oor die gebruik van SBSE vir ekstraksie van pesbestryders, kontaminante en preserveermiddels vanuit wyn, water, suurlemoendrankies en yoghurt. Die algemene profile analise is ook verder uitgebrei deur dit inniger te maak en toe te pas op wyn en bier. Die gebruik van die roerstaaf vir ekstraksie van die gasfase bo ‘n monster is ook aangetoon. Al die toepassings het getoon dat SBSE ‘n robuste, senstiewe, herhaalbare metode is en besonder maklik is om te gebruik.

Acknowledgements

I wish to express my sincere gratitude to the following people or entities for diverse contributions throughout this study:

Prof. Pat Sandra for giving me the opportunity to conduct this study in an exciting field of research and his expert guidance, inspiration and understanding throughout.

Prof Andrew Crouch for his willingness to become involved in an advanced period of this study and his never ending encouragement, understanding and expert manner of dealing with diverse obstacles encountered during the course of this study.

Andre de Villiers for friendship and scientific stimulation but moreover for his immense contribution to the preparation and (valued) critical editing of the final manuscript.

Prof. Henk Lauer, being involved in the onset of this study for sharing his vast knowledge of separation science, motivation and his excellent way of dealing with frustrating administrative issues.

Frederic Lynen, who took over from Prof Lauer, for his friendship, encouragement, guidance and a unique way of dealing with administrative 'rompslomp'.

Theo Heideman for the kind manner in which he shared his exceptional practical knowledge and enlightening discussions.

Dr Pavel Majek for his competent and enthusiastic assistance with the chemometrical part of this study.

All my co-workers at the Chemistry Department: Astrid Buica, Berhane Weldegergis, and Adriana Stopforth.

Prof Ben Burger (Chemistry Department) for his friendly manner of providing expert advice and support when required.

Prof Helgard Raubeneimer (Head: Chemistry Department) for all his encouragement.

My colleagues at the Institute for Wine Biotechnology (IWBT), including Dr Helene Nieuwoudt, Karolien Roux, Hugh Humat, Claudia Brodard and Rencia van Wyk for their continuous encouragement.

Prof Florian Bauer and the rest of the IWBT management team for their support, understanding and encouragement.

People at the RIC (Research Institute for Chromatogarphy, Kortrijk, Belgium) and the University of Ghent: Eric Baltussen, Bart Tienpont, Frank David and Tom Sandra for accommodating me in their laboratories and making my visits there both productive and very pleasant.

The NRF (National Research Foundation, South Africa), Harry Crossley Foundation and the University of Stellenbosch for funding.

The IOPMS for funding of my research periods in Belgium at the RIC and University of Ghent.

Lastly, and probably most importantly, all my family and friends supporting me throughout this period in innumerable diverse ways.

Table of Contents

List of Publications

Due to collaboration with several co-workers, not all of these publications are included in this thesis. Publications included are marked with an asterisk.

1*. *The Determination of Benzoic Acid in Lemon Flavored Beverages by Stir Bar Sorptive Extraction-CGC-MS*, A.G.J. Tredoux, H.H. Lauer, Th. Heideman, P. Sandra, J. High Resolut. Chromatogr. 23 (2000) 644.

2*. *Stir Bar Sorptive Extraction Applied to the Determination of Dicarboximide Fungicides in Wine*, P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.

3. *A rapid method of diagnosing pulmonary tuberculosis using stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry*, A. Stopforth, A. Tredoux, A. Crouch, P. van Helden, P. Sandra, J. Chromatogr. A 1071 (2005) 135.

4. *Application of a Headspace Sorptive Extraction Method for the Analysis of Volatile Components in South African Wines*, B.T. Weldegergis, A.G.J. Tredoux, A.M. Crouch, J. Agric. Food Chem. 55 (2007) 8696.

5*. *Stir bar sorptive extraction combined with GC-MS analysis and chemometric methods for the classification of South African wines according to the volatile composition*, A. Tredoux, A. de Villiers, P. Májek, F. Lynen, A. Crouch, P. Sandra. Submitted to J. Agric. Food Chem. November 2007.

6. *Chemical characterisation of South African young wines using FTMIR spectroscopy, gas chromatography and multivariate data analysis*, L. Louw, K. Roux, A. Tredoux, O. Tomic, T. Naes, H. Nieuwoudt, P. van Rensburg. Submitted to J. Agric. Food Chem. January 2008.

Abbreviations

AED	Atomic emission detector
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionisation
ASE	Accelerated solvent extraction
BSTFA	*N*,*O*-bis-(trimethylsilyl)trifluoroacetamide
CA	Cluster analysis
CAR	Carboxen™
CE	Capillary electrphoresis
CGC	Capillary gas chromatography
CI	Chemical ionisation
CW	Carbowax
DA	Discriminant analysis
DAD	Diode array detector
DC	Direct current
DCM	Dichloromethane
d_f	film thickness
DMS	Dimethyl sulfide
DSE	Dynamic solvent extraction
DVB	Divinylbenzene
ECD	Electron capture detector
EI	Electron impact
EPA	Environmental protection agency
EPC	Electronic pneumatic control
ES	External standard
FDA	Food and drug organasation
FFAP	Free fatty acid phase
FID	Flame ionisation detector
PFPD	Pulsed Flame photometric detector
ft	film thickness
GC	Gas chromatography
GLC	Gas-liquid chromatography
GPE	Gum phase extraction
GSC	Gas-solid chromatography
HPLC	High performance liquid chromatography
HS-SPME	Headspace SPME
HSSE	Headspace sorptive extraction
ID	Inner diameter
IR	Infra red
IS	Internal standard
ITD	Ion trap detection (mass spectrometry)
$K_{O/W}$	Octanol water partition coefficient
LD	Liquid desorption
LDA	Linear discriminant analysis
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantitation
LVI	Large volume injection
MS	Mass spectrometry
MSD	Mass selective detector
MW	Molecular weight

NIST	National Institute of Standards
NPD	Nitrogen phosphorus detector
OAV	Odour activity value
OD	Outer diameter
OTT	Open tubular trap
PA	Polyacrylate
PC	Principle component
PCA	Principle component analysis
PCB	Polychlorinated biphenyl
PDMS	Polidimethylsiloxane
PFBHA	*O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PTV	Programmed temperature vapourisation
RF	Radio frequency
RI	Retention index
RSD	Relative standard deviation
RTL	Retention time locking
SBSE	Stir bar sorptive extraction
SDVB	Styrene divinyl benzene
SFC	Supercritical fluid chromatography
SIM	Selected ion monitoring
SLDA	Stepwise linear discriminant analysis
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
TD	Thermal desorption
TDS	Thermal desorption system
TMS	Trimethylsilyl
TOF	Time of flight

1

General

Introduction

General introduction and scope

Wine is probably the beverage surrounded by the most romantic perceptions, associated as it is with the notions of well-being, contentment and sophistication. Wine has been produced since biblical times, and probably before, and throughout this period has always been of significant cultural importance, being used in diverse societies as part of religious rituals and celebrations. In recent times, this grape-derived beverage has not lost any of these attributes, and still plays a significant cultural role world-wide, being regularly consumed for social enjoyment, relaxation as well as the potential health benefits attributed to its moderate consumption.

In our modern era, partially driven by the enormous economic impact of the wine industry both internationally and locally, the possibility of unravelling the 'secrets' of wine composition have begun to be extensively explored. Such investigation is spurred by the desire to gain a better understanding of wine chemical composition in relation to its sensory properties, with the overall aim to consistently improve the quality of the product. To achieve this end, chemical analysis provides an essential tool. The characteristics of wine are determined by the chemical composition, which is in turn determined by numerous factors influencing the vine, grapes, wine, etc. The chemical composition of wine undergoes significant changes throughout the lifetime of a wine. The relationship between chemical composition and sensory properties is still only partially understood. Arguably these developments may endanger the mystical notion of wine-making. This is however an inevitable consequence of increasing demands from consumers for consistently high quality, unadulterated, authentic and health-beneficial products. This in turn has led to chemical analysis being applied throughout the wine industry for quality control purposes, contaminant analysis, identification of compounds promoting good health, wine classification and detection of adulteration.

Wine volatiles in particular play a significant sensory role and the analysis of known compounds responsible for specific desirable flavour nuances is essential to gain a better understanding of wine flavour, thereby aiding winemakers to consistently produce sought after products. Volatile analysis is also used as an indispensable tool for discovering (sometimes novel) compounds responsible for sensory defects, or as a means of quality

control, ensuring a product free of defects and of consistent quality reaches the consumer. Furthermore, analytical screening methods in combination with statistical techniques can be used for authentication of wines according to cultivar or geographical origin.

Since more than 1000 different volatile compounds have been found in wine, the analysis of the volatile fraction of wine presents an extremely daunting challenge. Not surprisingly, various analytical tools need to be utilized to accomplish the mammoth task of comprehensive wine volatile analysis (something not achieved to date). Another challenging factor in wine analysis is that the concentrations of the important aroma compounds range from high (mg/L or even %) to ultra-trace levels (sub-ng/L). Thus, it is now known that compounds contributing significantly to the important sensory properties of wine are often those occurring in low, rather than high quantities. Although a variety of state-of-the-art chromatographic techniques, offering high sensitivity, selectivity and robustness, are nowadays available for volatile analysis, only very rarely can a wine sample be introduced directly. This is mainly due to the complexity of the wine matrix, the high sensitivity needed and the requirement to remove interfering compounds prior to analysis.

For this reason, numerous sample pretreatment techniques have been developed for wine volatile analysis (these will be elaborated upon further in this thesis). The foremost aim of all these methods is to reduce the effect of the sample matrix by removing compounds of no interest, to obtain a sample suitable for introduction into analytical equipment, and to concentrate the sample to allow low detection limits. From a review of the various sample pretreatment techniques used for this purpose, it is clear that no ideal method exists for analysis of even most wine volatiles. Although a large number of methods are available for diverse classes of compounds, the continuous drive to reduce analysis costs, increase speed and ease of use, and the desire to identify novel impact odorants, are generally not congruent. Clearly, there exists a considerable need for the development of innovative methods for volatile analysis that would meet some of these criteria.

Within this context, the principle aim of this study was to apply such a novel sample preparation technique, the recently developed stir bar sorptive extraction (SBSE) to wine volatile analysis and to exploit the potential benefits offered by this technology. Therefore, in the first part of the thesis, SBSE was used in combination with gas chromatography-mass

spectrometry (GC-MS) as a powerful analytical tool for the in-depth characterization of the volatile composition of South African wines.

The data obtained in this manner were investigated using chemometric techniques, to determine the suitability for differentiation purposes. Specifically the classification of South African wine according to grape cultivar based on volatile composition was investigated.

Concurrently, the second part of this thesis deals with various other analytical challenges in the beverage and foodstuffs industry. The suitability of SBSE technology for addressing these problems was investigated. Applications developed during this phase include the use of SBSE for analysis of contaminants and pesticides in wine. Moreover, suitable methods based on SBSE are developed for the determination of contaminants in drinking water and preservatives in beverages and foodstuffs. The potential utility of SBSE for volatile screening analysis of wine and beer samples is also explored. Finally, a screening technique using headspace sorptive extraction (HSSE), where stir bars are used in the sample headspace, is reported.

The chapter layout of the thesis is organized according to published work. For this reason some unavoidable repetition will be encountered. This was not rectified in order to retain clarity of the individual chapters.

2

Analytical Techniques and Instrumentation

2.1 Introduction

It is generally agreed by separation scientists that chromatography, and certainly the term, was invented by Russian botanist Mikhial Tswett in 1906 when he succeeded in separating chloroplast pigments on a calcium carbonate stationary phase using petroleum ether as mobile phase [3]. The development of chromatography is extensively discussed in several dedicated publications [1, 2] and the following is not meant to be a comprehensive discussion on the topic, but rather a brief overview of the most important milestones in the development of chromatography. In 1931, Kuhn *et al.* introduced liquid-solid chromatography [4]. Tiselius developed electrophoresis in 1940 [5], and frontal analysis in liquid chromatography a year later [6] for which he was awarded the Nobel Prize in 1948. In 1941 Martin and Synge presented the first model describing column efficiency and developed liquid-liquid (partitioning) chromatography [7], an achievement they received the Nobel Prize for in 1952. Gas-solid chromatography was introduced in 1951 by Phillips [8] and in the same year James and Martin introduced gas-liquid chromatography [9]. In 1957 Golay reported the development of open tubular columns [10, 11], which has led to the modern version, capillary gas chromatography (CGC).

Chromatography can generally be described as the distribution of analytes in a two phase system. These phases can be solid-liquid or liquid-liquid as in modern high performance liquid chromatography (HPLC) and certain modes of capillary electrophoresis (CE), or gas-solid and gas-liquid, as in gas chromatography (GC). If the liquid phase is a supercritical fluid, the technique is termed supercritical fluid chromatography (SFC) [12, 13].

2.2 Gas chromatography

Gas chromatography (GC) is a separation tool in which separation is achieved by interactions between solutes in the gas phase and either a solid adsorbent (adsorption), or a liquid phase (partitioning). Thus GC separations can be subdivided into gas liquid chromatography (GLC), which is most widely used nowadays, and gas solid chromatography (GSC). Since packed columns (either packed with an adsorbent or with particles coated with polymeric liquid on a solid support) were developed before open

tubular capillary columns, these columns are still used, either because re-validation of existing methods on capillary columns will be too costly or for special applications such as gas analysis [12]. Golay illustrated in the late nineteen fifties the vast increase in resolution that can be obtained by using an open tube with a small inside diameter coated with a stationary phase. This improvement is mainly caused by the fact that a capillary column has much less resistance to mass transfer due to a significantly shorter diffusion distance. Furthermore, the low pressure drop across the column makes it possible to increase the column length significantly while still using realistic carrier gas pressures [10, 11, 13]. For these reasons GLC employing wall coated open tubular columns is the preferred technique nowadays. In addition, these columns offer high resolution and robustness, as well as the availability of numerous highly specific liquid polymers. Modern capillary GC (CGC) is characterized by high sensitivity, efficiency and versatility. It is therefore the method of choice for the analysis of relatively volatile and thermally stable organic molecules. Molecules not directly amenable for GC are either derivatised, or are analysed by liquid-based separation methods such as HPLC or CE. In this study, GLC with fused silica open tubular columns were used throughout, and therefore the following discussion will only deal with capillary columns [12].

2.3 Instrumental aspects

Any chromatographic instrument consists of a sample introduction device, column, detector and data collecting system; in addition modern GC versions include accurate electronically regulated pneumatic and temperature control, providing extremely reproducible chromatographic results. A typical chromatographic system will be discussed in terms of capillary gas chromatography (CGC) below.

2.3.1 The column

A wide range of capillary columns are available nowadays, differing in length, inner diameter, film thickness and type of stationary phase. The type of column will be determined by the analytes of interest. In general, a longer column will give better separation but leads to longer run times. Also, a thicker film of stationary phase (d_f) leads to

an increase in retention. Reducing the inner diameter of the column will also improve separation but the capacity is reduced: the amount of analyte the column can handle without overloading the column is less. The most common dimensions used in CGC are ~30 m L × 0.25 mm I.D., 0.25 µm d_f. Shorter and narrower thin film columns (e.g. 10 m L × 0.1 mm I.D., 0.1 µm d_f) give faster separations, but are not as easy to work with and require high pressures. For very complex samples such as found in the petroleum- or food and flavour industries, columns of up to 60 – 100 m in length are not uncommon.

Considering stationary phase selection, a phase with a polarity similar to the analytes of interest is generally selected. In GC, two separation mechanisms may be exploited. The first is separation according to boiling point of the solutes and is most relevant when using apolar stationary phases. Separation based on selective partitioning (interactions with the stationary phase) is most prevalent when using polar columns. A variety of stationary phases ranging from apolar to polar are available for utilizing the optimal combination of these two mechanisms to achieve the desired separation. The most widely used stationary phases are polydimethylsiloxane (PDMS) and polyethelene glycol (wax) phases (**Figure 1**). Numerous specialty phases have also been developed for specific applications, such as the free fatty acid phase (FFAP) which is a wax phase modified with nitroterephthalic acid, resulting in good peak shapes for underivatised polar acidic compounds. Other specialty phases include those incorporating cyclodextrins for chiral separation and siloxane phases stabilized for use at high temperatures for elution of high-boiling analytes [12, 13, 15].

Figure 1. Chemical structures of two of the widely used stationary phases in CGC: apolar polydimethylsiloxane (PDMS, left) and polar polyethylene glycol (PEG or wax, right).

Since the GC separation mechanism relies on the partitioning of solutes between the gas phase and stationary phase, temperature control plays a crucial role. For this reason a GC column is housed in a thermostatted oven with the ability to rapidly heat or cool. The column may be operated at a constant temperature (isothermally) or, more commonly, using a temperature program. When performing low temperature isothermal GC the highly retained compounds elute as very broad peaks, whilst for high temperature isothermal GC the early, closely eluting compounds may not be resolved. This phenomenon is often referred to as the general problem in chromatography. By using a temperature program, it is possible to obtain narrow peak widths and therefore good separation for both early eluting and highly retained compounds [12, 14, 15].

2.3.2 The carrier gas and pneumatic control

In order to sweep the analytes through the column, a carrier gas at a certain pressure is applied to the inlet of the column. Typically hydrogen, helium, or to a lesser extent nitrogen are used for this purpose. All these gases provide comparable efficiency in GC, but in terms of speed H_2 is superior, He somewhat slower while N_2 has its optimum velocity at the lowest carrier gas flow rate. Furthermore, N_2 has the lowest optimum flow rate range of the three and is therefore rarely used [12, 15].

To obtain reproducible retention times, it is critical that the carrier gas pressure is regulated with high accuracy. For this, a high-precision pneumatic system is used. In the past a constant pressure was applied, but since the introduction of electronic pneumatic control (EPC) it is possible to vary the pressure in order to keep the flow constant as the oven temperature increases. The high accuracy of present EPCs also allows for the possibility of retention time locking (RTL), in which the retention times of compounds are locked to a reference compound, thereby increasing the confidence of identification based on retention time [15, 16].

2.3.3 The injector

Since GC is a gas phase technique, compounds need to be vaporised before entering the column. For this, a heated injector is most commonly used. The classical vaporizing, split/splitless injector, in which the sample is introduced in a hot injector and almost instantaneously vaporised, is the oldest and still the most widely used injector. The injector can be operated in the split mode, indicating that, depending on the amount of split flow, only a certain fraction of the total sample is introduced into the column. This technique is commonly used in CGC to avoid overloading of low-volume capillary columns, in cases where sensitivity is not problematic. In the splitless mode on the other hand, in order to obtain higher sensitivity the split valve is closed shortly before injection and only opened after the sample has been transferred to the column to flush the injector. Splitless injection has the benefit of higher sensitivity but can be troublesome when used in combination with capillary columns because of the low volume of the column. Typically, when 1 μL sample is injected and completely transferred to the column, a large solvent peak and excessively broad peaks will be observed due to overloading. In order to overcome this, it is necessary to make use of the solvent effect, where the initial column temperature is low enough (typically 20^{o}C below its boiling point) for the solvent to recondense at the beginning of the column. When the oven temperature is subsequently raised, the evaporation of the solvent film will cause the analytes to be focused into sharp bands. A piece of 1-5 m uncoated fused silica, referred to as a retention gap, is typically used to connect the column to the injector to enhance refocusing [12, 13, 17].

In this study, a programmed temperature vaporizing injector (PTV) (**Figure 2**) was used. The PTV is essentially a split/splitless injector with a much lower injector volume and the possibility to introduce the sample at low temperature. This is followed by rapid heating of the injector to transfer the sample to the column. This accurately controlled heating and cooling feature is responsible for most of the unique features of the PTV. Heating is performed either by direct or indirect resistive heating while for cooling compressed air, carbon dioxide or liquid nitrogen is employed (the degree of cooling is determined by the coolant used) [18, 19]. A PTV offers the possibility of large volume injection (LVI), allowing a relatively large amount of sample to be injected at low temperature (close to the boiling point of the solvent but not the analytes). With the split vent open initially, the

sample solvent is vented from the injector before closing the split vent and heating the injector to introduce the sample to the column. Since the sample is concentrated before injection, this leads to an increase in sensitivity and is beneficial in trace analysis of semi-volatile compounds such as pesticides. Analogously, a PTV can be used to cryogenically trap analytes originating from a thermal desorption (TD) process (discussed below) or headspace sampling prior to injection [18, 20, 21].

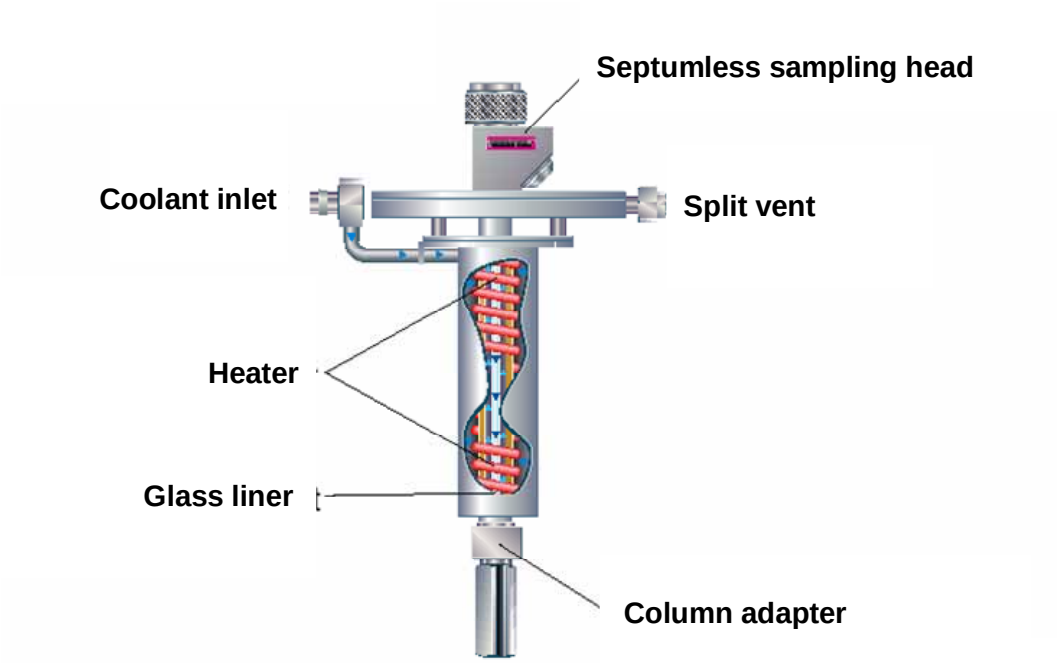

Figure 2. A programmed temperature vaporization (PTV) injector (CIS-4) [29].

2.3.4 Detection

The purpose of a chromatographic detector is to generate an increase or reduction of an electric current based on the chemical and/or physical properties of the analytes as they elute from the column. This change in current is amplified and recorded by the data system as a peak, of which the size (area or height) is indicative of the analyte concentration in, or mass flow of analytes into the detector at a given time. Several detectors have been developed for use in GC, varying significantly in terms of detection limits, linear range and specificity. The flame ionization detector (FID) is the most widely used. The FID is regarded as a universal detector as it gives a response for almost all organic compounds by

ionizing the analytes in a hydrogen flame and producing a response based on the conductivity of the ionized gas mass in the flame. A number of specific detectors have also been developed, for example an electron capture detector (ECD), specific for electronegative species (e.g. halogenated compounds), the nitrogen phosphorus detector (NPD) for nitrogen and phosphorus containing molecules and the flame photometric detector (FPD) for sulphur or phosphorus containing compounds. The mass spectrometer (MS), when coupled to GC, can be used as a selective or universal detector. Due to its versatility, robustness and sensitivity, MS is nowadays one of the most common and valuable detectors available. As this was the detector exclusively used in this study, the MS is discussed in more detail [12, 13].

The first mass spectrometric experiment was performed by Thomson in 1913 [22] and the technique was further developed by Aston, focussing on the analysis of elemental isotopes between 1922 and 1942 [23, 24]. Coupling of GC with MS was first demonstrated by Holmes and Morrell in 1957 [25]. Coupling a mass spectrometer to GC as detector not only offers good sensitivity but provides structural information (in the form of a mass spectrum). By comparing the mass spectra obtained during analysis of an unknown sample with those found in mass spectral databases, the identity of the compound can be tentatively determined. Even in cases where poor mass spectral matching is obtained, the information in a mass spectrum may be used as a guideline as to the type of compounds under investigation and by studying fragmentation patterns the identity may be elucidated [26].

A mass spectrometer essentially consists out of four parts: a sample inlet system, an ion source in which ionization and fragmentation of molecules takes place, a mass analyzer for separation of the ions according to their mass to charge ratio (m/z) and an ion detector, commonly an electronmultiplier.

In GC-MS, the sample is introduced into the MS by positioning the outlet of the GC column, after being transferred to the MS via a heated transfer line, in the ion source as close as possible to the path of an electron beam. The column outlet is sealed with a nut and ferrule to ensure that the only flow into the system is that of the effluent from the column. The ion source consists of a filament providing high energy electrons for ionization, and various lenses for guiding the ions into the analyzer. The electron beam in the source is created by a heated filament and ionization can occur either directly by using electron

impact ionization (EI) or indirectly, by chemical ionization (CI). In CI, a reaction gas such as methane, ammonia or isobutane is ionized by electrons from the filament and the resulting ions ionize sample analytes by charge transfer processes. The result is a softer ionization technique with less fragmentation and a higher possibility of obtaining molecular ions indicative of a compound's molecular weight. In EI, the electron energy used for ionisation is set to 70 eV, substantially above the ionisation potential of most organic molecules, and therefore sufficient to cause both ionisation and fragmentation. Since no two organic molecules will fragment in exactly the same way under the same electron energy, the mass spectrum may be considered a fingerprint of the molecule. For this reason all commercial mass spectral libraries consist of mass spectra generated at 70 eV to facilitate comparison between instruments [12, 13, 15].

Several mass analysers have been developed, the most common being the quadrupole **(Figure 3)**, but others such as the ion trap, time of flight or magnetic sector instruments have been used with equal success. A quadrupole consists of four parallel rods around the flight path of the ions. By applying a radio frequency (rf) on two of the opposing rods and a direct current (dc) voltage on the other two, a magnetic field is created between the rods. This field alters the resonance of all ions in such a way that only one ion of a specific mass to charge ratio (m/z) will have a stable resonance and pass through the quadropole while all other ions will collide with the quadrupole and be lost. Therefore only an ion of a specific m/z passes through the quadrupole at a specific time to be detected by the ion detector. By altering the voltages on the rods it is possible to continuously select different ions to pass through the quadrupole. If the voltages are changed in such a way that ions of sequentially increasing m/z ratios are allowed though the quadrupole, the instrument is being operated in the scan mode. Typically 2 – 5 mass spectra are recorded per second, depending on the chosen mass range. In selected ion monitoring (SIM) mode only the selected ions, characteristic of specific fragments, are monitored. Since more time is spent measuring each of the ions, an increase in sensitivity is obtained in SIM compared to scan mode. This sensitivity increase can be as much as 1000 fold.

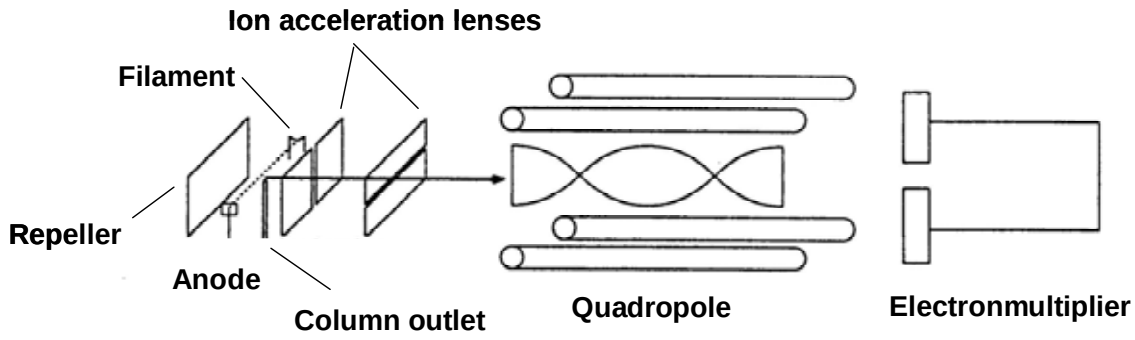

Figure 3. Basic components of a quadrupole mass spectrometer [27].

The electron multiplier registers the ions that passed through the quadrupole and consists of many conversion dynodes, each of which release numerous electrons when hit by an ion or electron. In this manner a cascade effect is produced to deliver a gain in signal in the order of 10^6 [13, 15].

2.3.5 Thermal desorption systems

Thermal desorption (TD) systems are used to thermally desorb analytes present in solid samples and for the desorption of analytes adsorbed on, or sorped into, a trapping phase following sampling of liquid or gaseous matrices. A modern thermal desorption system (TDS) used in combination with a PTV, as depicted in **Figure 4**, consists of a sealed tube holder that can be programmably heated or cooled as in the case of the PTV. A solid sample or a sorptive or adsorptive sampling device is placed in a glass sample tube. Upon heating of this tube, volatiles and semi-volatiles are released and transferred by gas flow via a fused silica transfer line to the PTV for cryo-trapping. The pneumatics of a TDS resembles those of a PTV injector, offering split, splitless or solvent venting modes. During thermal desorption the PTV is typically operated in solvent vent mode while being cooled to trap desorbed analytes, allowing a relatively high flow (typically 50 mL/min) of carrier gas through the tube for desorption, while the TDS remains in the splitless mode. After desorption the PTV will be switched to splitless mode for injection, while the TDS will be in solvent vent mode to flush out impurities remaining after desorption and prevent them

from entering the column. The carrier gas pressure is the same as the column head pressure as it is an open system [28, 29].

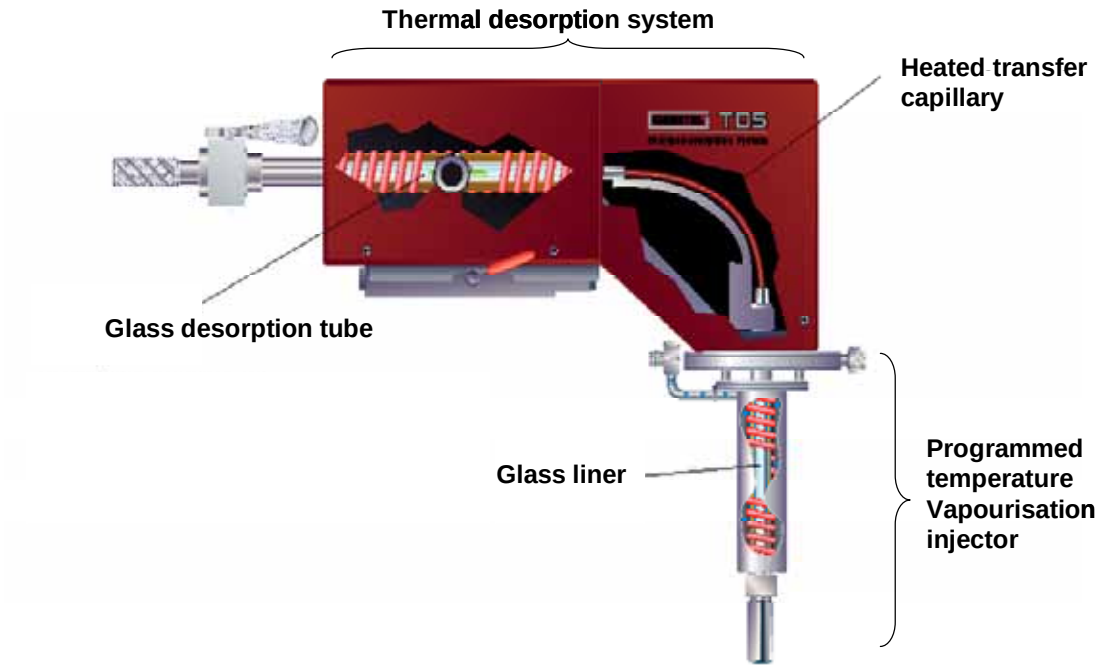

Figure 4. The TDS-2 thermal desorption system used in this study. For typical operating principles, refer to text [29]

2.3.6 Qualitative and quantitative analysis

The purpose of chromatographic analysis is two-fold. Firstly it is used for identification of compounds present in a sample (qualitative analysis), and secondly to determine the amount of each compound (quantitative analysis). In order to determine the identity of an unknown compound in a chromatographic analysis several methods, or ideally a combination of more than one, can be applied. The most common practice is to compare the retention time of the unknown peak with that of a pure standard. This is, however, not always the most reliable method since small changes in instrumental parameters can cause shifts in retention time. A variation to this approach is to analyse the sample spiked with a pure standard in order to confirm that the peak in question increases in size. Another possibility is to make use of relative retention times. The most well known method for doing this is making use of the Kovats retention index (RI) system [30]. This system follows from the observation that for a homologous series of hydrocarbons, when the

logarithms of the retention times of an isothermal analysis are plotted against the carbon number, a straight line is obtained. Hydrocarbons are used as reference compounds, with the RI for each defined as the carbon number times 100 (i.e. the RI for hexane is 600). By adding a series of hydrocarbons to a sample, it is therefore possible to mathematically calculate the theoretical 'carbon number' of the relevant unknown peak by using the hydrocarbons eluting before and after the peak. RIs are dependent on the stationary phase and temperature. Comparison of experimental RIs with library values (either compiled in-house or available commercially) for the same conditions can be used to identify compounds. Retention indices alone are normally not sufficient to unambiguously identify a compound, for this reason RI values under at least two different sets of conditions are required. Other means of determining the identities of unknowns make use of spectroscopic techniques such as infrared (IR), atomic emission detection (AED) or mass spectrometry (MS). Spectroscopic data in combination with retention data are the most reliable, and most common, means of determining the identity of an unknown peak [12, 15].

Quantitative analysis is based upon the fact that the area under or height of a chromatographic peak is proportional to the quantity of the compound injected (within the dynamic range of the detector). In order to determine the quantity (concentration) of a compound several techniques can be used. The simplest of these is to analyse a sample containing known quantities of the analytes of interest in an identical matrix. These data are used to construct a calibration graph of peak area or height versus concentration. Following analysis of the samples containing unknown amounts of the compounds of interest, the previously constructed calibration graph is used to relate peak areas to concentration. This technique is known as external standard (ES) quantitation, and if used properly produces very accurate results [12]. However, the method requires a very reproducible, preferably automated, injection system.

An alternative quantification technique is the internal standard (IS) method. Here a compound not present in the samples is added at a specific concentration before analysis. The area or height of the IS peak relative to those of the analytes of interest are used for quantitation. As a result, the IS method effectively corrects for small variations in peak area/height due to injection and minor operational discrepancies. When the data required are of a relative nature, the area of the peak of interest divided by that of the internal

standard is used. However, more often absolute concentrations are required and for this a calibration graph is constructed by analysis of standards solutions containing a fixed amount of internal standard and several concentration levels of the compound(s) of interest. The area of the standard divided by that of the internal standard is then plotted against the concentration level of the standard divided by the internal standard concentration. This process is graphically illustrated in **Figure 5** where 3 concentration levels of compound **a** are analysed (left) and the calibration graph is constructed by plotting the area ratios versus the concentration ratios (right). From this graph the response ratio is determined as the slope of the straight line.

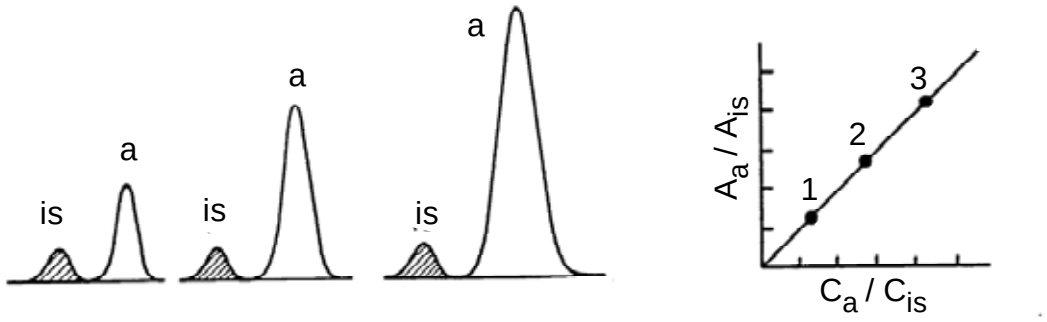

Figure 5. The process of construction a calibration graph for use with IS quantitation.

The careful selection of an IS is of utmost importance since the accuracy of this technique relies on the assumption that the behavior of the IS and the compound(s) being quantified are similar. Therefore it is important for the IS to have the following characteristics compared to the analytes of interest: it should be chemically and physically as similar as possible (therefore deuterated standards are the best), be pure and chemically inert, have a similar retention time and concentration level and be well-resolved from all other chromatographic peaks. Often, in complex samples, it is necessary to use more than one internal standard representative of the different classes of compounds present [12, 13, 15, 27].

A lesser used but equally accurate technique, especially when facing difficulty finding a suitable IS, is the standard addition method. In this method known amounts of the compound to be determined are added to the sample. Following analysis of each sample, a calibration graph of peak area or height versus concentration is constructed and the

unknown concentration is determined from the intercept on the concentration axis. This is illustrated graphically in **Figure 6**, with the compound of interest having a concentration of ~2.5 [12].

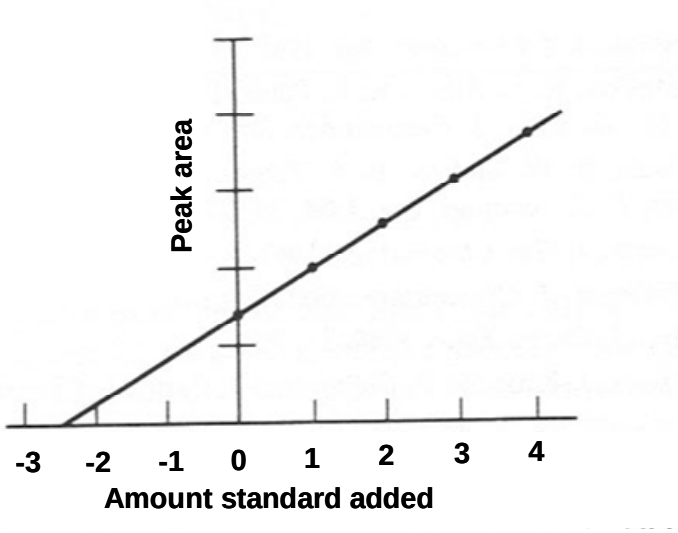

Figure 6. Example of a standard addition graph.

2.4 Sample preparation techniques

2.4.1 Introduction

Although analytical instrumentation has become increasingly sensitive and robust, sample preparation is most often required before analysis, mainly for two reasons. Firstly, sample preparation serves as a means of concentrating the sample before analysis and thereby increasing the detection limits of a specific method. Secondly, sample preparation is used as a clean-up step when the sample matrix is not suitable for direct introduction into a chromatographic system, or for removal of interfering compounds to reduce the complexity of the sample. For numerous samples, pretreatment is performed for both of these reasons [20]. This section is not meant to be an exhaustive discussion on the topic of sample preparation, but rather to discuss the most frequently used sample preparation techniques, with the focus on sorptive techniques used in this thesis.

2.4.2 Liquid-liquid extraction

Liquid-liquid extraction (LLE) is one of the oldest, and still most frequently used sample preparation techniques. As the name implies, LLE is suitable for the extraction of analytes from liquid samples into a second immiscible liquid. Compounds are extracted based on their relative affinities for the two phases as governed by their respective distribution coefficients (K_D) between the phases. The sample matrix is most commonly an aqueous medium, and the extraction liquid a less polar organic solvent if performed prior to GC analysis. LLE extraction can be performed manually or in a semi-automated fashion. The main disadvantages of LLE are a lack of sensitivity and the large consumption of harmful solvents, the latter becoming increasingly a concern nowadays. Several strategies have been devised to address both these problems. The development of micro liquid-liquid extraction (μLLE) drastically reduced the amount of solvent used while simultaneously leading to an increase in sensitivity due to a more favorable phase ratio [20]. Other variants include microwave assisted solvent extraction (MASE) [31] where microwaves are used to enhance extraction efficiency and ultrasonic extraction where ultrasound is used for the same reason. Accelerated solvent extraction (ASE) [32] makes use of temperature and pressure to increase extraction efficiency and reduce extraction time. Recently single drop extraction (SDE) [33], making use of a single drop of solvent, has been described.

2.4.3 Solid phase extraction

Developed as an alternative to LLE, solid phase extraction (SPE) is a very popular sample preparation technique because of its versatility [34]. The mechanism is similar to liquid chromatography, the only difference being that SPE is performed at low pressure. Typically, a suitable sorbent material is packed into a cartridge (or purchased pre-packed) and conditioned before loading the sample. Once the sample is loaded interfering compounds can be rinsed from the cartridge before the analytes of interest are eluted with a strong solvent to remove them from the stationary phase. Flow through the cartridge can be obtained by using a vacuum manifold or by applying positive pressure, for example with a syringe. The versatility of this technique is a result of different separation mechanisms it offers such as adsorption, partitioning, affinity or ion exchange; allowing the user to select

the most suited stationary phase for a particular application. The most common stationary phase for organic analyses are apolar phases such a C18 and polymeric styrene-divinyl benzene (SDVB). SPE offers several attractive benefits such as high sensitivity (high concentration factor), low solvent consumption, high selectivity and the option of automation [34, 35].

2.4.4 Purge and trap

Purge and trap is a less common technique used for extraction of volatiles from liquid samples. This technique is worth mentioning since it is capable of providing high sensitivity and highly purified samples. An inert gas is bubbled through the liquid sample and volatiles are released into the gas phase prior to being trapped on an adsorbent trap (typically Tenax or activated charcoal) at low temperature. After sampling is completed the trapped volatiles are desorped, either thermally in a thermal desorption system, or by using a suitable solvent for elution. Purge and trap systems can be automated and can also be mounted on a GC instrument. The sample obtained is normally very clean since all the non-volatiles have been removed, thus eliminating their interference during chromatographic analysis [15, 21, 27].

2.4.5 Sorptive extraction techniques

2.4.5.1 Introduction

It has been realized for some time that sorptive extraction provides an excellent alternative to adsorptive trapping for sample enrichment, while at the same time offering some unique features. In sorptive extraction the analytes are not adsorbed on the surface of a stationary phase but rather retained in the bulk of a polymeric stationary phase. Sorptive extraction is comparable to LLE since the polymers employed are below their glass transition points at room temperature, thus acting as liquid phases. The most common sorption material is polydimethylsiloxane (PDMS). Since sorption is a weaker process than adsorption, surface-catalysed reactions that easily occur due to the strong bonding on adsorbent material is minimized, and polar compounds are more easily removed from the phase as a result of this

weak bonding. PDMS has also been proven to be much more chemically inert than many commonly used adsorbents and is stable at high temperatures. Moreover, PDMS degradation products can easily be discerned when an MS detector is used, eliminating the possibility of identification of artifacts not originating from a sample [28, 36].

Over the years several sample preparation techniques based on sorption have been developed, including open tubular traps (OTT's), gum phase extraction (GPE) with PDMS particles packed in a glass tube, solid phase microextration (SPME) and stir bar sorptive extraction (SBSE).

2.4.5.2 Open tubular traps and gum phase extraction

An OTT consists of a length of tube, typically between 0.5 and 2 m, coated on the inner walls with a thick layer (up to 12 μm) of PDMS. First reported by Grob et al [37] and further developed by Burger et al [38], the application of OTT's to both headspace and liquid sampling has been demonstrated. Using an OTT, the sample is normally sucked or pumped through the trap until breakthrough of the first analyte of interest occurs. The trapped analytes are consecutively eluted using a liquid such as dichloromethane, or thermally desorbed, for GC or GC-MS analysis. The main disadvantage of this technique is that in order to avoid premature breakthrough, low sampling flow rates have to be used (e.g. less than 1 mL/min for a typical 2 m trap), resulting in long sampling times. In an attempt to solve this problem, Ortner and Rohwer developed a sampling system in which several OTTs are used in parallel, increasing the flow rate to 15 mL/min [39]. However, OTTs have never gained widespread acceptance.

A similar approach is gum phase extraction (GPE), first reported by Baltussen *et al.* in the late 90s [40]. In GPE a glass tube is packed with granulated PDMS instead of previously used Tenax or activated charcoal. The tube is then used for dynamic sampling of gaseous or liquid samples by pumping the sample through the tube at a specific flow rate for a predetermined time. After sampling the analytes can be thermally desorbed in a TDS system, or by means of liquid desorption with a suitable solvent. The principle advantage the traps offers is higher sampling flow rates, up to 2.5 L/min for gases and 100 mL/min for liquids, therefore greatly increasing the speed of sampling. Since the amount of stationary

phase employed is also considerably increased compared to OTTs (or SPME), the traps display a substantial increase in sample capacity and hence sensitivity.

2.4.5.3 Solid phase micro-extraction (SPME)

SPME, pioneered in the early 90's by Pawlizyn *et al.* [41], is originally a sorptive technique, since the stationary phase used in the development thereof was PDMS. Nowadays however, SPME is available with a selection of phases, including both sorbents and adsorbents or mixtures of the two. An SPME device consists of a fused silica micro-fiber (1 cm long) coated with the phase. The fiber is fixed to the stainless steel plunger of a syringe, allowing the fiber to be drawn into the syringe needle when the plunger is retracted. Depending on the nature of the analytes, headspace or immersion SPME is possible. During sampling the syringe (with the fiber retracted) is used to pierce the septum of a vial containing the sample, after which the fiber is exposed to either the headspace or the liquid phase. When sampling occurs in the liquid the extraction is governed by a partitioning of the analyte based on the PDMS/liquid partition coefficients. When sampling from the headspace, extraction is dependent on the distribution of the analytes between the liquid and gaseous headspace (volatility) and also by the respective PDMS/gas partition coefficients. Following sampling, the fiber is retracted into the needle, inserted in a hot GC injector and exposed again while the analytes are desorbed from the fiber coating and introduced in the chromatographic system. A typical SPME sampling procedure is graphically illustrated in **Figure 7**. Several sampling parameters are influential in SPME and these have been extensively studied, the most important being sampling temperature, time, ionic strength and agitation during sampling [42, 43]. Recently, devices that allow liquid desorption of SPME fibers prior to HPLC analysis have been developed [42].

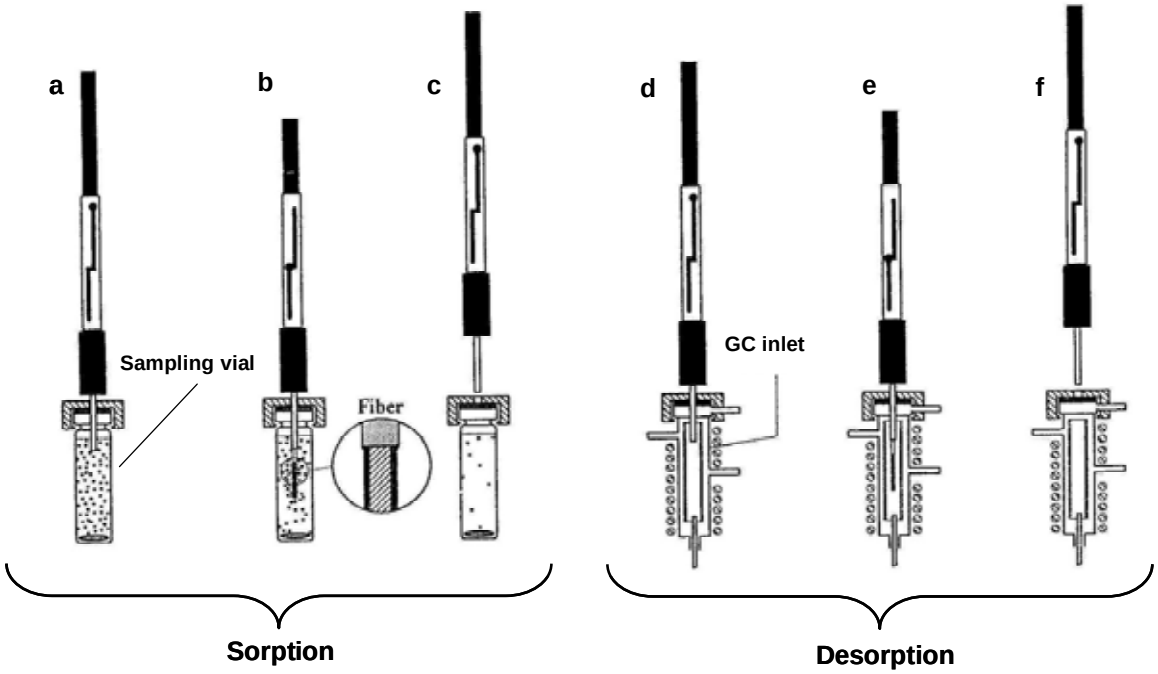

Figure 7. The SPME sorption and desorption process [27]

Some variations on the idea of SPME using PDMS have also been reported. In SPDE (solid phase dynamic extraction) the PDMS layer is coated on the inside of a syringe needle and by continuously filling and emptying the syringe with sample, dynamic sampling is achieved [44]. Burger *et al.* developed a sample enrichment probe (SEP) using a glass rod coated on the one end with a thick layer of PDMS. The advantage of this design is that it can be desorbed as with SPME in a standard GC-injector, but the thick layer of PDMS ensures higher sensitivity and cryotrapping is not required [45].

2.4.5.4 Stir bar sorptive extraction (SBSE)

In SBSE a magnetic sir bar is encapsulated in a glass sleeve and coated with PDMS. This technique was developed by Baltussen *et al.* in 1999 [46]. SBSE is a technique for the sorptive extraction of aqueous samples offering the sensitivity of GPE combined with the simplicity of SPME. The stir bar is introduced in the aqueous sample and sorptive extraction occurs whilst stirring. After stirring for a certain time, the stir bar is removed from the sample, dried with a lint free cloth and introduced into a glass desorption tube. Thereafter the analytes are thermally desorbed and transferred to a GC-MS instrument. A variant of SBSE is head space sorptive extraction (HSSE), where sampling takes place in

the headspace above a sample, similar to headspace SPME [47, 48].

SBSE can either be performed dynamically where sampling is terminated before establishment of equilibrium, or statically where extraction under equilibrium conditions is performed. The former is faster but more susceptible to small variations and may therefore be less repeatable, while the latter takes longer but is potentially more sensitive.

Varying amounts of PDMS can be used depending on the length and thickness of the coating applied. Typically 55 μL PDMS is used for a 10 mm stir bar, but up to 219 μL for a 4 mm stir bar can be used. This increased amount of stationary phase explains the high gain in sensitivity when compared to SPME where the maximum amount of PDMS is ~0.5 μL (100 μm thickness, illustrated in **Figure 8**). In theory, SBSE is described similarly to SPME [49]. By assuming that the partitioning coefficients between PDMS and water ($K_{PDMS/W}$) are approximately proportional to the octanol-water partition coefficients ($K_{O/W}$) the following equation can be used:

$$K_{O/W} \approx K_{PDMS/W} = \frac{C_{SBSE}}{C_W} = \frac{m_{SBSE}}{m_W} \times \frac{V_W}{V_{SBSE}} \qquad (1)$$

Here, C_{SBSE} and C_W are the analyte concentrations in the PDMS and the water phases, m_{SBSE} and m_W the mass of analyte in the PDMS and the water phases, respectively, V_{SBSE} and V_W the volumes of the PDMS and water phases, respectively. Replacing V_W/V_{SBSE} with the phase ratio, β, equation (1) can be re-written as:

$$\frac{K_{O/W}}{\beta} = \frac{m_{SBSE}}{m_W} = \frac{m_{SBSE}}{m_0 - m_{SBSE}} \qquad (2)$$

where m_0 is the total mass of analyte originally present in the water sample. Equation (2) may then be re-arranged in such a way as to determine the extraction efficiency or recovery from the water sample as follows:

$$\frac{m_{SBSE}}{m_0} = \frac{\left(\frac{K_{O/W}}{\beta}\right)}{1+\left(\frac{K_{O/W}}{\beta}\right)} \qquad (3)$$

From equation (3) it is evident that the only variables governing the recovery of an analyte from the sample are the partition coefficient (K) and the phase ratio (β), implicating.that should $K_{O/W}/\beta = 1$ the recovery will be 50%. At low $K_{O/W}/\beta$ the recovery is approximately

proportional to $K_{O/W}/\beta$, while at $K_{O/W}/\beta$ values higher than 5 extraction is virtually quantitative as illustrated in **Figure 8**.

As mentioned above, the maximum volume of stationary phase coated onto an SPME fiber is *ca*. 0.5 μL (for 100 μm film thickness). This implies that for a sample volume of 10 mL the phase ratio equals 2×10^4 and therefore quantitative extraction is only obtained for compounds with a $K_{O/W}$ higher than 10^5. Only a very limited number of analytes exhibit such high $K_{O/W}$ values and, moreover, it was shown that apolar solutes strongly adsorb onto Teflon coated stir bars and glass vials commonly used when performing SPME [28, 36, 46, 50].

In practice, solely as a result of the increase in phase ratio, Figure 8 clearly illustrates that SBSE benefits more polar solutes (having a $K_{O/W}$ of between 10 and 500). In fact most organic compounds commonly analysed by GC fall within this range of polarities. In a recent review, David and Sandra present an in-depth overview of the application of SBSE for environmental, food and flavour as well as biomedical analyses [51].

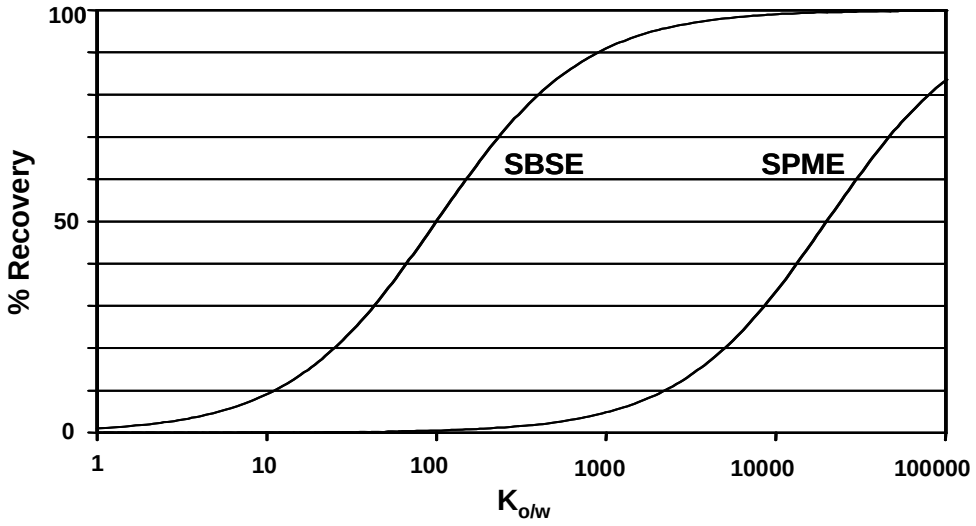

Figure 8. Theoretical recovery obtained by SBSE and SPME as a function of analyte octanol-water distribution coefficient (Ko/w) [28].

Despite the superior performance of SBSE compared to SPME in most instances, the fact that PDMS currently the only commercially available phase for SBSE inevitably leads to low recoveries of highly polar molecules. Since a wider selection of stationary phases are

available for SPME (notably Carbowax, being more polar), SPME remains a viable alternative for polar analytes. Also to be kept in mind is that the high sensitivity SBSE offers may not always be an advantage when extracting compounds present in higher concentrations as this often results in column overload. Furthermore, SBSE requires thermal desorption instrumentation, and as yet cannot be fully automated (contrary to SPME). In an attempt to overcome the discrimination of PDMS against polar and very volatile compounds, Bicchi *et al.* developed dual phase stir bars, incorporating PDMS as well as various forms of activated charcoal. This was shown to provide superior performance for polar analytes, however these stir bars are not yet available commercially [52].

While thermal desorption is most commonly used in combination with SBSE, liquid desorption has also been demonstrated to be effective for thermally labile, non-volatiles compounds prior to HPLC or CE analysis [53, 54]. It may however be argued that the availability of SPE and it's broad versatility (and option of automation) is a more sensible approach for such compounds.

Keeping the above in mind, the applicability of SBSE for analysis of diverse chemical compounds in a range of beverages and foodstuffs is demonstrated in This thesis.

2.5 References

[1] L.S Ettre, Anal. Chem., 43 (1971) 20A.

[2] V. Heines, Chem. Technol., 1 (1971) 280

[3] M.S. Tswett, Ber. Dtsch. Bot. Ges. 24 (1906) 316.

[4] R. Kuhn, A Wunterstein, E. Lederer, Hoppe-Seyler's Physiol. Chem. 197 (1931) 141.

[5] A. Tiselius, Ark. Kemi. Mineral. Geol, 14B (1940) 22.

[6] A. Tiselius, Ark. Kemi. Mineral. Geol, 15B (1940) 6.

[7] A.J.P. Martin, R.L.M. Synge, Biochem. J. 25 (1941) 1358.

[8] C.S.G. Phillips, J. Griffiths, D.H. Jones, Analyst (1952) 897.

[9] A.T. James, A.J.P. Martin, Biochem. J. 50 (1952) 679.

[10] M.J.E. Golay, Anal. Chem. 29 (1957) 928.

[11] M.J.E. Golay, Nature 180 (1957) 435

[12] C.F. Poole, S.K. Poole, Chromatography Today, Elsevier, Amterdam, 1991.

[13] R.L. Grob, Modern Practice of Gas Chromatography, Wiley-interscience, New York, 1995.

[14] W. Jennings, E.Mittlefehld, P. Stremple, Analtical Gas Chromatography 2nd edition, Academic Press, New York, 1997

[15] P. Sandra, H. Lauer, Introduction to Separation Science, University of Stellenbosch, 2003.

[16] L.M. Blumberg, M.S. Klee, Anal. Chem. 70 (1998) 3828.

[17] L.S. Ettre in 'Sample Introduction in Capillary Gas Chromatography Volume 1', P.Sandra (Ed), Dr. Alfred Huetig Verlag, New York, 1985.

[18] W. Voght, K. Jacob in 'Sample Introduction in Capillary Gas Chromatography Volume 1', P.Sandra (Ed), Dr. Alfred Huetig Verlag, New York, 1985.

[19] G.J. Mol , Trace Analysis with Gas Chromatography Using On-Line Enrichment and Large Volume Injection, PhD Thesis, Technische Universiteit Eindhoven, 1995

[20] A.J. Handley (Ed.), Extraction Methods in Organic Analysis, Sheffield Academic Press, London, 1999, pp 1- 53.

[21] R. Soniassy, P. Sandra, C. Schlett, Water Analysis, Hewlett-Packard, Publication 23

5962-6216E (1994), 12.

[22] J.J. Thomson, Rays of Positive Energy, Longmans, Green, London, 1913.

[23] F.W. Aston, Isotopes, Edward Arnold, London,1922.

[24] F.W. Aston, Mass Spectra and Isotopes, Edward Arnold, London, 1942.

[25] J.C. Holmes, FA Morrell, Appl. Spectros. 11 (1957) 86

[26] F.W. McLafferty, The Interpretation of Mass Spectra, University Science Books, Sausalito, USA (1993).

[27] A. de Villiers, Separation Science Course, University of Stellenbosch, 2007.

[28] E. Baltussen, New Concepts in Sorption Based Sample Preparation for Chromatography, PhD Thesis, Technische Universiteit Eindhoven, 2000.

[29] Courtesy of Gerstel GmBH, Germany, 2007.

[30] E. Kovats, Helv. Chim. Acta (1958) 41 1915.

[31] A.J. Handley (Ed.), Extraction Methods in Organic Analysis, Sheffield Academic Press, London, 1999, pp 166-194.

[32] J. Gan, S.K. Papiernik, W.C. Koskinen, S.R. Yates, Environ. Sci. Technol. 33 (1999) 3249.

[33] M. Michulec, W. Wardencki, Chromatographia, 64 (2006) 191.

[34] E.M. Thurman, M.S. Mills, Solid Phase Extraction: Principles and Practice, John Wiley and Sons, 1998

[35] A.J. Handley (Ed.), Extraction Methods in Organic Analysis, Sheffield Academic Press, London, 1999, pp 54-72.

[36] E. Baltussen, C. Cramers, P. Sandra, Anal. Bioanal. Chem. V373 (2002) 3.

[37] K. Grob, A. Habich, J. Chromatogr. A 321 (1985) 45.

[38] B.V. Burger, Z. Munro, J. Chromatogr. A 402 (1986) 95.

[39] E.K. Ortner, E.R. Rohwer, J. High Resolut. Chromatogr. 19 (1996) 339.

[40] E. Baltussen, H.-G. Janssen, P. Sandra, C.A. Cramers, J. High Resolut. Chromatogr. 20 (1997) 385.

[41] J. Pawliszyn, C.L. Arthur, Anal. Chem. 62 (1990) 2145.

[42] S.A. Wercinski, Solid Phase Microextraction: A Practical Guide. Marcel Dekker Inc.: New York, 1999; pp 177-202.

[43] J. Pawliszyn, Solid Phase Microextraction: Theory and Practice. Wiley VHC, Inc.: New York, 1997; pp 97-140.

[44] C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, J. Chromatogr. A 1024 (2004) 217.

[45] B.V. Burger, B. Marx, M. le Roux, W.J.G. Burger, J. Chromatogr. A 1121 (2006) 259.

[46] E. Baltussen, P. Sandra, F. David, C.Cramers, J. Microcolumn Sep. 11 (1999) 737.

[47] B. Tienpont , F. David, C. Bicchi, P. Sandra, J. Microcolumn Sep. 12 (2001) 577.

[48] B.T. Weldegergis, A.G.J. Tredoux, A.M. Crouch, J. Agric. Food Chem. 55 (2007) 8696.

[49] C. Arthur, L.K. Pratt, S. Motlagh,, J. Pawliszyn, J. High Resolut. Chromatogr. 15 (1992) 741.

[50] A.G.J. Tredoux, MSc Thesis, University of Stellenbosch, 2000.

[51] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.

[52] C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, F. David, P. Sandra, J. Chromatogr. A 1094 (2005) 9.

[53] P. Serodio, J.M.F. Nogueira, Anal. Bioanal. Chem. 382 (2005) 1141.

[54] A. de Villiers, G. Vanhoenacker, F. Lynen, P. Sandra, Electrophoresis, 25 (2004) 664.

3

A Survey of Sample Preparation for Volatiles in Wine

3.1 Introduction

Analysis of the volatile constituents of wine is important for several reasons. Determination of these compounds serves as a means of quality control to ensure a consistent product, free of harmful contaminants, can be used for classification purposes, detection of adulteration, identification of compounds responsible for characteristic aroma nuances (and ideally improve production processes), or to identify compounds implicated in spoilage.

More than 800 volatiles have been identified in wine to date, making the complete analysis of these compounds an extremely complicated task. Theoretically, a standard capillary gas chromatic analysis under optimum conditions provides a peak capacity of ~1000, which means that for perfect selectivity, the same number of compounds can be separated. However, as was shown by Davis and Giddings, this number is significantly reduced when random distribution of analyte peaks across the separation window is assumed (as in real-life separations), to about 1/5 or 200 compounds [1,2]. Furthermore, this estimate assumes the efficient introduction of an 'ideal' sample, something which is virtually impossible when working with real samples because of matrix effects.

In order to approach the maximum 'practical peak capacity', most chromatographic analyses require a sample pretreatment step prior to instrumental analysis, a step that most often has a detrimental effect on the accuracy and validity of the results obtained. The pretreatment step in its simplest form can involve dilution, filtration or centrifugation. However, with the ongoing trend of investigation ever decreasing levels of analytes in increasingly complex matrices, sample preparation necessarily becomes a complex, expensive and multi-step process [3,4]. The analytes of interest determine the methodology and extent of sample pretreatment required.

Ferreira classified volatile compounds in wine into three broad classes from an analytical perspective: major volatiles, which are 'easy' to analyse, major as well as minor compounds that are somewhat problematic to analyse, and compounds which are exceedingly difficult to analyse (for example novel wine volatiles) [5]. Not surprisingly, the number of reports in the literature dealing with wine volatiles is overwhelming for the first group and becomes increasingly scarce towards the third group. Often in wine analyses the major compounds routinely analysed are the compounds that may be isolated from the wine

matrix with relative ease, yet may not have the highest impact on the perceived flavour. Conversely, compounds with significant sensory impact are often overlooked because their concentrations are too low, or they are too unstable or polar to isolate successfully. Recent examples of such compounds include a marker for the pepper aroma associated with Shiraz wines [6] and novel low-level sulphur compounds [7].

Sample preparation before chromatographic analysis remains therefore vital for sample clean-up and/or preconcentration purposes, despite the high sensitivity and robustness offered by modern analytical equipment. For 'easy to analyse' compounds a simple liquid extraction might suffice, whereas 'difficult to analyse compounds' may require the development and optimisation of a sophisticated, multi-step sample preparation procedure. The final goal of course remains keeping the analytical procedure as simple, cheap and environmentally friendly as viable.

The purpose of the current chapter is to provide an overview of recent sample preparation techniques available for analysis of volatiles found in wine. For the sake of simplicity, the discussion will be split in two, based on the compounds of interest: the major volatiles, and several groups of minor (target) compounds such as sulphur- and nitrogen-containing compounds, carbonyl compounds, terpenes and related compounds, volatile phenols, lactones, non-volatile phenolics and volatiles indicative of faulty wine. Because of the necessarily vague definitions involved in this classification, some overlapping is unavoidable.

3.2 Major volatile compounds

As stated above, grouping of wine volatiles is not straightforward. For the purpose of this review major volatiles will be considered as those compounds regularly reported in methods dealing with general analysis of wine volatiles - typically those present at higher concentration levels. Major volatiles therefore include the common acids (including fatty acids), their corresponding esters, alcohols, as well as additional compounds commonly included in screening methods (furfuryl type compounds and several varietal compounds such as C6 varietals and aromatic volatiles).

Regarding the possible flavour contribution of major wine volatiles, these compounds are present in most wines and responsible for the base of the flavour profiles of these wines. The higher fusel alcohols produce a negative effect when present in higher levels, although their effect can be positive at normal levels. Hexanol, for example, is characterized by a grassy herbaceous flavour [8], while β-phenylethanol produces a rose-like flavour [9,10]. Isoamyl alcohol (3-mehyl-1-butanol) has been described as providing fusel [11] and cheese [9,10] flavours. Acids are derived both from the grape and the yeast during fermentation. Volatile, low MW compounds such as formic-, butyric- and especially acetic acid are important contributers to the so-called "volatile" acidity; excess amounts are indicative of bacterial spoilage. Acetic acid provided a vinegar flavour to the wine [10,11]. Higher MW fatty acids such as hexanoic-, octanoic-, and decanoic acids, are yeast-derived and indirectly affect wine flavour by leading to the production of fatty acid esters, although octanoic acid has been described as being responsible for a fatty and unpleasant odour [9,10]. Esters, most commonly ethyl esters, are formed by either enzymatic or chemical esterification of organic acids and alcohols. Ethyl acetates of fatty acids are important compounds for the "base aroma" of wines, contributing mainly fruity aromas (for example ethyl- butyrate, hexanoate and octanoate) [9-11], but also flowery and rose flavours (β-phenethyl acetate) [10,11]. Isoamyl acetate produces a banana aroma important for especially young wines, whereas ethylphenyl acetate contributes floral characteristics. Ethyl esters of the main organic acids in wine (tartaric, malic, lactic, succinic, acetic and citric acid) are thought to contribute relatively little to wine aroma. Furfuryl compounds (furfuryl alcohol, furfural, and 5-hydroxymethyl furfural) are derived from wood ageing. Reduction of furfural to furfuryl alcohol (and further possible products) takes place during wine ageing. A similar process leads to the formation of 5-hydroxymethyl furfural from 5-methylfurfural.

Apart from screening methods which allow the obtainment of a volatile signature for wines, valuable information may be found by monitoring different stages of the wine-making process to provide information relevant to their optimization [4]. Often the data obtained by such methods are extremely useful in combination with statistical analysis (chemometrics), enabling the classification of wine according to cultivar, geographical origin or authenticity.

It should be noted that in most methods for analysis of major volatiles some minor volatiles are also detected.

The analysis of certain major volatiles present at very high concentrations is infrequently performed by direct injection of wine into the GC without any prior sample pretreatment [12]. Munoz *et al.* reported the injection 0.5 μL of wine directly after removal of tartaric acid (one of the main non-volatiles) for analysis of major volatiles by GC-FID [13]. More frequently alcoholic distillates such as tsipouroare, a distillate made from the wine press residue, is directly injected, being less problematic due to the high content of ethanol [14]. Souflerous *et al.* used direct injection to determine higher alcohols and glycerol (on a packed column) in kiwi wines [15]. The disadvantages of direct injection of aqueous samples are well known: the need for frequently cleaning or replacing injector components such as liners due to deposits of non-volatiles, lack in sensitivity and selectivity, regular column trimming and re-installation and the risk of column blocking. Furthermore, several GC columns are damaged by the introduction of water, while the high vapour volume and polarity of water often leads to chromatographic problems. Nevertheless, direct injection is still used for high-concentration compounds due to the simplicity of the method.

3.2.1 Liquid-liquid extraction

The most widespread technique for isolating volatiles from a wine matrix is liquid-liquid extraction (LLE) and recent variations or enhancements of this technique. The popularity of LLE can be explained by the ease of use and simplicity, despite drawbacks such as the use of environmentally harmful and costly solvents. Very often the same LLE procedure developed for major volatiles is simultaneously used for analysis of minor analytes – these will be dealt with under the relevant sections below.

The most critical aspect of LLE is the selection of a proper solvent. Solvents reported in literature vary widely, but the most common include dichloromethane (DCM) [4,16-20] diethyl ether [21,22], chloroform [16], and infrequently Freon [13,23] or combinations of the above solvents such as dichlorormethane/pentane [24,25], ether/pentane [26], etc.

DCM extraction of wine generally suffers from the formation of an emulsion (often not reported). In order to overcome this problem, various approaches have been reported, including the use of a special syringe filter for breaking the emulsion, performing the

extraction at low temperature [16,17,27], addition of a salt such as NaCl to aid the extraction by increasing the ionic strength of the sample [19,28], centrifugation and sonication [19,28] or using sonication to also enhance extraction [29]. The extracts obtained are normally dried on anhydrous Na_2SO_4 prior to GC analysis [19,28,30]. Often the solvent is evaporated before analysis to increase the concentration of the analytes, however this may lead to a loss of highly volatiles [17]. In addition to DCM, ether also extracts a wide number of compounds of different polarities. Concerns with this solvent include the fact that it has a low boiling point which precludes the efficient utilization of the solvent focusing effect, and cryo-cooling is not an option when using a wax column since these columns normally have a lower temperature limit of ~40^{o}C due to poor chromatographic performance below these temperatures and to avoid damage to the stationary phase. Freon was very popular in the past and proved to be highly efficient, but is to be avoided after its detrimental effects to the environment have been demonstrated.

In order to enhance the sensitivity of LLE, the use of continuous LLE for wine volatile analysis has been reported [28,31-33].

Another alternative to increase sensitivity and minimize solvent consumption, in which the amount of solvent used is typically 200 – 500 μL, is micro-LLE (μLLE) [4,9,27,34]. Although this technique has been proven successful at increasing sensitivity and avoiding an evaporation step, the recovery of such small amounts of solvents is practically difficult and suitable solvents are limited due to slight miscibility with water.

An interesting alternative to LLE is supercritical fluid extraction (SFE). This technique offers the possibility of excellent recovery and selectivity by optimizing several instrumental parameters, and has been applied to wine volatile analysis [35]. However, instrumentation is usually a problem (availability and simplicity); hence the technique is currently much less used for wine analysis.

As can be seen from the above, no ‘universal’ LLE method for wine volatiles exists, and optimization according to compounds of interest is frequently required. The techniques’ simplicity, versatility and the possibility of automation will, however, ensure its continued extensive use for wine volatile analysis.

3.2.2 Solid phase extraction

Solid phase extraction (SPE) is a popular alternative to LLE and is based on liquid chromatographic technology. Effective use of SPE involves both a sample clean-up and a concentration step. SPE is particularly advantageous for analysis of minor compounds because of high sensitivity achievable (this is due to the high phase ratios of the packings used in SPE). Furthermore, a wide range of stationary phases are available, adding to the techniques selectivity and versatility. By optimizing the loading, washing and elution steps, SPE can be fine-tuned to be very specific for compounds of interest. Alternatively, more generic methods for the extraction of most volatiles from aqueous samples can be developed. As was the case with LLE, these methods developed are often suitable for the extraction of major and some minor compounds simultaneously.

The most common SPE methods for wine volatile analysis are based on reversed phase fractionation, where apolar analytes are retained and polar interferences rinsed from the cartridge. By far the most common phases used for wine volatiles are C18 [36], styrene divinylbenzene (SDVB) [17,28,31] and Lichrolute EN [37]. A typical SPE procedure would involve loading of the sample (which may be diluted with water or pH adjusted if necessary), washing the stationary phase with water or a stronger solvent to remove interfering compounds or impurities, and finally elution of the target analytes with a suitable solvent. Generally aqueous solvents are used, but may contain organic phases such as methanol to remove less polar organic molecules such as esters, while DCM is commonly used for elution of the analytes [36].

Palemo *et al.* used a SPE procedure on a SDVB phase for the fractionation of free and glycosidically-bound major and minor volatile compounds (C6 alcohols, monoterpenes, poly-oxygenated terpenes, aromatic compounds, norisoprenoids and aliphatic compounds). The unbound volatiles were eluted from the cartridge with DCM/pentane, concentrated and analysed by GC-MS. The retained, more polar glycosidically bound volatiles were eluted by ethyl acetate. The ethyl acetate fraction was dried, re-dissolved in methanol, and after evaporation underwent enzymatic treatment. Finally, the volatile compounds were recovered by LLE, dried and derivatised with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) for GC-MS analysis [38].

In another application flavour precursors from grapes and their acid hydrolysis products were loaded onto a Lichrolut EN SPE cartridge, rinsed with water and then pentane/DCM and eluted with ethyl acetate/methanol. This was followed by acidic hydrolysis at high temperature, and another cleanup step on a Lichrolut EN cartridge before analysis. Major as well as minor volatiles were extracted and the authors compared different sorbents [39].

In an interesting application of SPE phases, major and minor volatiles were extracted by dynamic headspace sampling and subsequently trapped on LiChrolute EN resin. A purge and trap set-up was used for purging 80 mL wine mixed with 20 mL artificial saliva through the cartridge, and volatiles were subsequently eluted with dichloromethane for gas chromatography-olfactometric (GC-O) analysis [9].

From these examples, it can be concluded that SPE is a highly selective and sensitive sample preparation procedure which can be fine-tuned by careful selection of the stationary phase and solvents for highly specific applications. Compared to classical LLE, SPE offers increased sensitivity and selectivity with lower solvent consumption. SPE, as LLE, can also be partially or completely automated. However, if the interest is solely in major volatiles, LLE is well worth considering due to the simplicity and lower cost of the technique.

3.2.3 Sorptive techniques

Solid phase micro-extraction (SPME) is has become an extremely popular technique for analysis of wine volatiles and a vast amount of literature exist on this topic. Features making SPME such an attractive alternative sample preparation technique include a wide range of fiber coatings with varying characteristics for different selectivity, high sensitivity, ease of automation and the large amount of information that can be obtained in a single chromatographic analysis. Due to this a high number of compounds are extracted and often minor compounds can be analysed together with major volatiles. Conversely, methods for minor volatiles often yield several major compounds which may interfere with trace-level compounds.

SPME can be used either for headspace sampling (HS-SPME) [40] or for immersion mode sampling [41]. For wine analysis the headspace option is almost exclusively preferred due to less matrix interferences, at the same time providing a more representative extraction of

aroma compounds. A technique called multiple headspace SPME (MHS-SPME), where several consecutive HS extractions are performed while trapping desorbed analytes between extractions, has also been developed for increased sensitivity in trace analysis; this will be discussed under the minor compounds [42].

Several phases are commercially available and those used for wine volatile analysis include polydimethysiloxane, (PDMS) [12,43,44], PDMS/divinylbenzene (PDMS/DVB) [45], polyacrylate (PA), carboxen/PDMS (CAR/PDMS) [46] carbowax/DVB (CW/DVB) [45,47,48] and a three phase fiber DVB/CAR/PDMS [45].

Numerous authors have reported comparisons between the different fibers for their suitability for extraction of volatiles from wine. For example, Tat *et al.* evaluated different commercially available SPME fibers for HS analysis of major wine volatiles [49]. The fibers tested were PDMS, PDMS/DVB, PA, CAR, CW and PDMS/DVB/CW. They concluded that the three phase fiber yielded the best results for wine HS analysis. While CW/DVB showed high sensitivity towards the most volatile compounds, the large peak areas in the beginning of the chromatogram hampered resolution in this region. The PDMS phase was found to be least suited for this type of analysis [41,43]. For analysis of varietal C6 compounds, benzyl alcohol and 2-phenylethanol in the skin and berry pulp of Muscat grapes by HS-SPME-GC-MS, Sanchez-Palomo *et al*. investigated CAR/DVB/PDMS, CW/DVB and PDMS/DVB fibers. They reported the latter to be most suited for the determination of these compounds [45].

A novel sol-gel coating for SPME was developed by Lui *et al.*, who demonstrated high sensitivity for both polar and non-polar major volatiles including alcohols, esters and acids. A hydroxy-terminated silicone oil-butyl methacrylate-divinylbenzene (OH-TSO-BMA-DVB) copolymer was synthesized and used as a stationary phase with the aid of γ-methacryloxypropyltrimethoxylsilane as a bridge. Comparison to other fibers is also performed in this work [50].

Exhaustive optimization of influential operational parameters (often with the aid of experimental design) has been reported in the literature. Important experimental parameters evaluated in these studies include salt addition, pH adjustment, temperature of extraction and extraction time [41,43,45-48,51].

Stir bar sorptive extraction (SBSE), in which a stir bar covered with sorptive stationary phase is used for extraction of analytes from the sample matrix, was shown to be a viable alternative to SPME. The mechanism of extraction is the same as in SPME but due to a higher amount of stationary phase the recoveries are typically much higher.

Recently SBSE immersion mode was developed for analysis of 14 important wood compounds in wine. Compared to SPME, SBSE was found to be a more sensitive technique, as predicted by theory [52].

Sorptive extraction techniques (HS-SPME and SBSE) were applied for the sorption of major volatiles, mostly esters (SPME with a 100% PDMS fiber) and minor volatiles (SBSE) from Madeira wines. Once again, SBSE showed a marked sensitivity increase compared to SPME and a total of 42 compounds were detected [44].

Recently a novel headspace sampling technique termed headspace sorptive extraction (HSSE) was used for screening analysis of major and minor volatiles in wine. HSSE is the headspace version of SBSE in which compounds of interest are sorbed into a PDMS layer coated onto a magnetic stir bar placed in the headspace. This technique is similar to HS-SPME with the exception of a higher recovery due to the increased volume of PDMS coating on the stir bar [53].

For the analysis of major and minor volatiles (including terpenes) in synthetic grape juice, Cavin-Quantrill compared SBSE with simultaneous distillation extraction (SDE) using dichloromethane. These authors also reported the identification of 126 compounds in real grape juice with SBSE in immersion mode [54].

Both SPME and SBSE present solventless, highly sensitive and selective means of extraction of volatiles from wine. Especially SPME has been extensively studied and optimized for this type of analysis, and the simplicity, sensitivity and option for automation makes this an attractive technique for wine volatile analysis. In cases where ultra-trace levels of compounds need to be determined, SBSE provides the required sensitivity.

3.2.4 Miscellaneous techniques

The techniques outlined above represent the most common form of sample preparation applied to wine volatile analysis. However, several alternative methods have been proposed in the literature for this purpose.

Chalier *et al.* used static and dynamic headspace sampling to determine the effect of mannoprotein concentration on the headspace concentration of iso-amyl acetate, hexanol and ethyl hexanoate in model solutions [55] Steam distillation has also been shown to be a useful method for the isolation of major wine volatiles [56].

Another technique used for volatile analysis is adsorptive trapping. For the analysis of major and minor volatiles, the headspace of fermented cashew apple juice was swept through a Porepack Q trap for 2 hours and subsequently eluted with 300 μL of acetone. Separation of the eluted volatiles was performed by GC-FID and identification by GC-MS [57].

A novel sampling technique applied for the analysis of wine aroma compounds and beer headspace analysis is the SniffProbe, developed by Gordin *et al.* [58]. Here the sampling device is a short piece of 0.53 mm I.D. capillary column coated with various stationary phases and connected to a pump for dynamic air sampling. Trapped analytes are desorbed in a programmed temperature vaporizing (PTV) injector for conventional GC or GC-MS analysis. The authors, however, do not report identification or quantification of wine volatiles.

A novel handheld GC, called the zNose, was used in combination with purge and trap on Tenax for the analysis of four C6 compounds, namely hexanal, *cis*-2-hexen-1-ol, *cis*-3-hexen-1-ol and *trans*-2-hexenal, contributing to a grassy flavour of grapes. As detector a surface acoustic wave sensor was utilized. This method compared favourably to HS-SPME-GC-MS [59].

For a recent review on GC olfatometric (GCO) analysis, including different techniques for sample preparation, refer to [60].

3.3 Minor volatile compounds

3.3.1 Carbonyls

A wide number of aldehydes and ketones are found in wine and their origin and flavour contribution vary widely. Many carbonyl compounds have very characteristic odours and low odour threshold values and are therefore important for their contribution to wine flavour. For example 1-octene-3-one has a characteristic mushroom aroma with an

extremely low odour threshold in water (2 – 10 ng/L). Other carbonyls also present specific flavour characteristics including apple, citrus, nutty and buttery attributes. Some of the unsaturated C6 carbonyls are also associated with varietal character. In studying malolactic fermentation there is often a need to monitor the evolution of especially carbonyl compounds. Malolactic fermentation is a secondary fermentation process following alcoholic fermentation where malic acid is converted to lactic acid. At the same time the total acidic content of the wine is reduced, the mouthfeel and taste are improved, while some desirable flavour characteristics are added to the wine.

Analysis of carbonyl compounds is often hampered by their high volatility, polarity and instability. For these reasons these compounds are frequently derivatised prior to GC analysis [61].

Depending on the compounds of interest, aldehydes and ketones can either be analysed together with major wine volatiles [4,13,18,23,24,28,53,56] or their determination requires dedicated sample preparation techniques [9,61-63]. One exception is the determination of acetaldehyde, for which direct injection of wine or distillates [12,14,64] has been described.

LLE is frequently used for the extraction of carbonyl compounds from the wine matrix. Solvents used include DCM [4,18,19], Freon [23], ether/hexane [56] and DCM/pentane [24]. Continuous LLE using pentane/DCM [28,32]. has also been described.. Escudera *et al.* used a salting out technique followed by LLE with Freon [64] for these analyses.

The use of SPE applying a Lichrolut EN resin for the quantitation of several minor volatiles including aldehydes and ketones in wine has been reported by Campo *et al.* [9].

Rocha *et al.* used HS-SPME with a DVB/CAR fiber to determine the volatile signature of wines in combination with MS detection and chemometric methods [47]. HSSE has also been used for acetoin [53]. Several carbonyl compounds were detected using this technique.

Three SPME fibers have been evaluated for the analysis of methyl ketones in congac using HS-SPME, namely PDMS, PA and PDMS/DVB. The PDMS/DVB was found to display the highest sensitivity for these compounds [65]. In addition to SPME, HSSE has also been applied for the quantitation of acetoin together with other major volatiles [53].

1-octene-one has been determined following derivatisation with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA). Wine is loaded onto a LiChrolute-EN SPE phase and PFBHA is passed through for on-cartridge derivatization, followed by elution of

the derivatives with pentane [62,66]. A similar approach has been followed for the determination of several carbonyl compounds, where the derivatives are eluted with DCM [61]. Carlton *et al.* analysed acetaldehyde by SPME with on-fiber derivatisation using PFBHA [63].

In addition, nonanal in apple cashew wine has been analysed together with major volatiles using adsorptive trapping as outlined previously [57].

In conclusion, although carbonyl compounds can be extracted by LLE together with the major volatiles, SPE is more beneficial when the focus is only on these compounds. SPME has also been shown to be a suitable method for these determinations. Furthermore, several authors have included a derivatization step to eliminate several complications in the analysis of these compounds.

3.3.2 Terpenes and related compounds (including terpenols and C13 norisoprenoids)

Terpenes, sesquiterpenes, norisoprenoids and their derivatives have often been associated with the varietal character of wine [67]. Of the numerous monoterpenes known the most important in wine are believed to be linalool, geraniol, nerol, α-terpineol and β-damascenone, the latter compound believed to contribute to the berry-like aroma of some wines [68].

The grape berry-derived glycoconjugates of these compounds (especially monoterpenoids and norisoprenoids) have received a considerable amount of attention recently due to their important role as flavour precursors, contributing amongst others to the varietal character of wines. Particularly during storage the acid-catalysed degradation of these compounds is considered to make a significant contribution to the characteristic bouquet of bottle-aged wines [67].

As for the carbonyl compounds, certain terpenes and related compounds can be analysed together with major volatiles [18,23,26,28,29,44,45], while dedicated methods are required for others [32,46,69-73].

LLE is often applied to terpene analysis, using common solvents outlined previously such as DCM [18,29,74] ether/pentane [26], ether/hexane [73], pentane [75] and Freon [23]. Some authors have also reported the use of continuous LLE [6,32] for these analyses.

Various SPE methods for terpene analysis, employing SDVB [28,31,46], Lichrolut EN [37,46] and C18 [36] cartridges have been reported. Lukic *et al*. suggested that the C18 phase facilitates the extraction of polar, mid-polar and non-polar compounds [36]. Gycosidically bound and free have been successfully fractionated on C18 and Lichrolut EN SPE cartridgese. Typically, free terpenes are eluted with an apolar eluent such as DCM/pentane, while the glycosidically bound terpenes are retained under these conditions and subsequently eluted with methanol and/or ethyl acetate prior to their hydrolysis [38,39,70,76].

Numerous authors have utilized HS-SPME for the analysis of terpenes and related compounds. Typical experimental procedures are as outlined above for the major volatiles, making use of CW/DVB [17,47,48,72], PDMS/DVB [68,72], PDMS [44,72] and PA [72] fibers. Terpenes were analysed in the pulp and skin of Muscat grapes by HS-SPME followed by GC-MS. Three fibers were evaluated namely CAR/DVB/PDMS, CW/DVB and PDMS/DVB; the latter was found to be most suited for these compounds [45]. A HS-SPME method using a PA phase was developed for terpene analysis after evaluation of PDMS, PDMS/DVB CAR/DVB fibers [72]. Rocha *et al.* analysed monterpenoids in grapes by HS-SPME in combination with comprehensive two-dimentianal GC and time of flight MS [77]. Terpenes have also been analysed together with major volatiles in Madeira wines using HS-SPME and SBSE, where the latter technique was better suited for low-level determinations [44]. Other authors have reported the screening of several terpenes by SBSE in a synthetic mixture and grape juice [54].

Camara *et al.* used terpene data, obtained by HS-SPME-GC-MS, to characterize four Madeira wines (Boal, Malvazia, Sercial and Verdelho) by using principal component analysis (PCA) and linear discriminant analysis (LDA) [71].

In another study, ultrasound assisted extraction (UAE) using DCM was compared with direct immersion SPME (using a PDMS phase) for the analysis of monoterpenoids by GC-MS. Although the limits of detection were slightly lower with SPME (10-25 μg/L compared to 30-40 μg/L), the overall recovery was significantly better using UAE [78].

β-ionone was included in the study by Chalier *et al.* referred to previously where the effect of mannoprotein concentration on the headspace concentrations in model solution was studied [55].

Parker *et al.* used HS-SPME to quantify a novel tricyclic sesquiterpene, α-ylangene, identified as a marker for the "peppery" aroma of Shiraz wines [6].

Although LLE is often used for the analysis of terpene-like compounds, SPE, SPME and recently SBSE have been shown to be beneficial for the isolation of specifically terpene-like compounds and the number of dedicated methods developed using these techniques for these compounds exceeds those for the other groups of volatiles. These methods offer high sensitivity, specificity, ease of use and the option of automation. Both glycocydically bound and free terpenes can successfully be studied using these techniques, particularly SPE.

3.3.3 Sulphur compounds

Suphur compounds in wines, most notably thiols and sulfides, are generally characterized by very unpleasant odours even at extremely low concentrations, and therefore their analytical determination is of particular concern in the wine industry [79]. Recently some sulphur compounds have been shown to contribute to the characteristic fruitiness of wines [34] further underlining their importance. Sulphur compounds are also known to be extremely troublesome to isolate and analyse due to high volatility (boiling points as low as 50^{o}C), high polarity (which complicates both extraction and chromatographic analysis) and instability [61].

The levels of sulfur compounds in wine vary widely. Some of these compounds may be analysed together with major wine volatiles using techniques such as LLE [4,7,13,18,19,23,28], HS-SPME [41,47] and SBSE [54].

On the other hand, more selective techniques are required for trace-level quantification of sulfur compounds. For instance, 2-methyl-3-furanthiol and 3-mercaptohexyl acetate were extracted by HS-SPME using on-fiber derivatisation with pentafluorobenzyl bromide and tributyl amine. The fiber was exposed to the derivatisation reagents prior to HS sampling, and high sensitivity is reported for this method [80]. The same method has been applied for the analysis of polyfunctional mercaptans at ng/L levels [81].

Dimethylsulphide (DMS) was extracted from red wine by gas purging followed by adsorption on gold-coated glass wool. The analytes are normally thermally desorbed from the glass wool and the analytes were cryotrapped before re-injection for analysis by GC-atomic emission detection (AED) [82]. AED is known to be highly specific for sulphur compounds. DMS has also been determined by headspace sampling followed by trapping on Lichrolute EN and desorption with DCM [34].

Fang *et al.* reported the extraction of 11 volatile sulphur compounds from wine using HS-SPME with a CAR/PDMS fiber, followed by pulsed flame photometric detection (PFPD) [83].

The preceeding discussion highlights the fact that many sulphur compounds present analytical challenges, with the result that specific compounds are often analysed by SPME in combination with derivatisation as well as sulphur-specific detectors due to their extremely low levels of occurrence in wine samples.

3.3.4 Nitrogen compounds

The nitrogen-containing compounds also represent a group of analytes with notably potent flavour characteristics. Arguably the best-known, and most widely discussed, are the methoxypyrazines. The aroma characterisitics of methoxypyrazines have been described as green, grassy and herbaceous, and these compounds are known to play an important role in the flavour of especially Sauvignon blanc and Cabernet sauvignon wines when present at normal levels. In addition, if present at elevated levels their presence has been shown to be overwhelming and unpleasant [67].

Methoxypyrazines are basic compounds, and therefore their removal from the polar wine matrix is not straightforward. Nevertheless, most extraction methods employ the surprisingly hydrophobic nature of these molecules for this purpose. Relatively specific sample pretreatment procedures are required for methoxypyrazine analysis due to the low natural concentration of these compounds (typically < 20 ng/L).

Campo *et al.* employed a SPE method on a Lichrolut EN phase to analyse these compounds together with other minor volatiles. 50 mL wine was loaded onto the cartridge and eluted with 1.3 mL DCM prior to analysis by GC-MS [9]. A variation on this technique is to rinse the cartridge with basic aqueous methanol (50%) to remove interfering compounds [34].

Other authors have used LLE with DCM [74] and HS-SPME using a PDMS/DVB fiber with NPD [84] and/or TOF-MS detection for these compounds [85].

Although biogenic amines are more often analysed by HPLC following suitable derivatization, some authors have reported their analysis by GC. For the analysis of diamines (1,3-diaminopropane, putrescine and cadaverine), polyamines (spermidine and spermine) and aromatic amines (β-phenylethylamine and tyramine), extraction was performed using an ion-pairing reagent in chloroform followed by back-extraction with HCl. This extract was derivatised with heptafluorobutyric acid anhydride and analyzed by GC–MS [86].

3.3.5 Lactones

Lactones have received considerable attention in the literature due to their characteristic flavours. These compounds have also been associated with varietal character. Most lactones are derived from wood ageing, with the remainder originating from the grape or produced during fermentation. γ-butyrolactone for instance is formed during fermentation by internal lactonisation of γ-hydroxybutyric acid, while the whiskey lactones (*cis*- and *trans*- 3-methyl-γ-octalactone) are extracted from oak during wine ageing. The *trans* isomer is associated with a coconut flavour [11,87]. Other oak-derived lactones include γ-nonalactone.

Lactones are commonly analysed by LLE using Freon [13,23], DCM [4], or other solvents [24,28] often in combination with other major and minor compounds. Similarly, SPE on a Lichrlolut EN phase [9] and HS-SPME using a DVB/CAR fiber [47] have been used successfully to quantify lactones in wine and related samples.

The effect of type of oak used in wine-production on the volatile aroma composition of several wines was studied using LLE with DCM/pentane to quantify several wood-derived compounds including the lactones [25].

More recently SBSE in the immersion mode has also been reported for the analysis of 14 important wood compounds, including lactones [52]. Moreover, HSSE [53] has also successfully been applied for the determination of oak lactones.

3.3.6 Volatile phenols

Several volatile phenols are found in wine and these compounds add significantly to the complexity of wine aroma. Volatile phenols enter the wine during fermentation, are released from oak during ageing, or are produced by micro-organisms such as *Brettanomyces* and *Dekara*. Especially the 4-ethylphenol, 4-ethylguiacol, 4-vinylphenol and 4-vinylguaiacol are amongst the compounds with a characteristic "barnyard, sweaty" aroma. When present at acceptable concentrations these molecules contribute favourably to the wine aroma, but when present at elevated levels, they are considered to cause spoilage of the wine [88]. Guaiacol (2-methoxyphenol) and the eugenol isomers (*cis*- and *trans*-2-methoxy-4-(2-propenyl)-phenol) are directly extracted from oak. The latter two compounds are associated with spicy, smoky aromas. Vanillin (4-hydroxy-3-methoxy-benzaldehyde) enters the wine during barrel ageing and produces a distinctive vanilla aroma. Vanillin undergoes reduction during further ageing, leading to the production of vannilyl alcohol and further products.

Due to the important contribution of these compounds to wine flavour, numerous methods for their analytical determination have been reported in the literature. The predominant volatile phenols such as guaiacol, 4-ethyl-guaicol, eugenol, vanillin and 4-ethyl-phenol, may be analysed together with major wine volatiles using techniques such as LLE or continuous LLE [13,18,23,25,32,64,89,90], SPE [9,20,28,78], HS-SPME [91] and multiple HS-SPME [92] . A few dedicated methods are available, especially for spoilage compounds such as the volatile phenols produced by *brettanomyces*, namely 4-ethylphenol, 4-ethylguiacol, 4-vinylphenol and 4-vinylguaiacol. Caboni *et al.* reported the analysis of 4-ethylphenol and 4-ethylguiacol by direct injection LC-MS-MS and LC-DAD-Fluorescence [93].

Volatile phenols were also amongst the compounds quantified using SBSE [52] and HSSE [53] in two recent reports discussed previously.

It has been reported that some volatile phenols may also be glycosidically bound and thus can be liberated by methods for analysis of free and bound varietal compounds and their precursors. The method involves reversed phase SPE, where the free and bound fractions are eluted separately. The free fraction is then analysed and the bound fraction hydrolysed and analysed after sample clean-up [38,39,70].

It is evident that the predominant volatile phenols can be isolated for analysis by a wide variety of sample pretreatment techniques, often in combination with other major or minor volatiles.

3.3.7 Cork taint

Cork taint represents a serious concern in the wine industry, costing millions of dollars annually. The compounds responsible for cork taint are haloanisoles, mainly 2,4,6-trichloroanisole (TCA). TCA has an extremely low odour threshold and spoils wine by adding a musty, mouldy character. TCA and related compounds (tribromoanisole (TBA), tetrachloroanisole (TECA) and pentachloroanisole (PCA)) are formed by the biomethylation of their corresponding halophenols. There is a critical need to analysis both the halophenols and halo-anisoles compounds in corks and wine [94].

The most common way of analyzing the haloanisoles and halophenols is by derivatization (methylation) followed by HS-SPME and GC-ECD or GC-MS analysis [92,95-97]. However several other methods have been reported such as multiple HS-SPME with [98] and without [92] derivatisation (acetylation of the phenols). This technique compared favourably to pervaporation followed by cold-trapping and GC-MS analysis [42]. Pressured solvent extraction was compared to Soxhlet extraction and multiple HS-SPME by Gomez-Ariza *et al.* [99]. They found similar results using each of these techniques.[91]. A method based on SPE clean-up on Oasis HLB cartridges, followed by derivatisation (acetylation with acetic anhydride) and GC-ECD analysis has also been reported [100]. A more selective SPE method on Lichrolut EN cartridges has been used together with large volume injection (LVI) and GC-MS analysis [101]. Recently a promising HSSE method for two halophenols and 4 halo-anisoles has been descibed [102].

It can be conluded that for anisole determinations, sorptive pretreatment techniques prove most suitable. However, when the halophenols are also of interest, an additional derivatisation step and SPE clean-up is required.

3.3.8 Pesticides

Pesticide analysis is performed mainly to ensure the product safety. Due to the ultra-low concentrations at which these compounds represent potential health risks to humans,

sample pre-concentration is of utmost importance for these analysis. Although a few applications of liquid extraction can be found in the literature, this technique is often not sensitive enough and other forms of sample preparation are more common. Sorptive extraction techniques are very popular primarily due to the high sensitivity they offer. An overwhelming number of methods have been published for pesticides in foodstuffs.

Fungicides have been extracted from wine using DCM/ether and subsequently analysed by GC-ECD [103]. A SBSE method followed by thermal desorption GC-MS was described for the analysis of dicarboximide fungicides in wine [104]. The results were corroborated by employing liquid desorption and LC-MS analysis. For the analysis of zoxamide, a pesticide commonly used to prevent downy mildew, liquid extraction with hexane was performed prior to analysis by GC-ion trap MS [105].

3.3.9 Miscellaneous compounds

Several non-volatile phenolic compounds, although more commonly analysed by liquid chromatography [106], can be derivatised to increase their volatility and decrease their polarity, making them suitable for GC analysis. Citova *et al.* used ethyl- and methyl chloroformate derivatisation followed by in-liquid SPME to analyse vanillic, ferulic, caffeic, gallic, protacatechuic, p-coumaric and syringic acids in wine [107]. Similarly, *trans*-resveratrol was anaysed by HS-SPME followed by on-fiber trimethylsilyl derivatisation. For derivatization, the fiber was placed directly in the headspace of the derivatization agent [108].

Sotolon (4,5-dimethyl-3-hydroxy-2-furanone) is associated with botrytized wines. In addition, this compound was identified as an important aroma compound in Madeira wines and reported by Moreno *et al.* for use as age marker in sherry wines [23]. Sotolon was extracted from wine using LLE with Freon[23] and ether [109], as well as by SPE using a LiChrolute EN cartridge. In the latter method, sotolon was eluted with dichloromethane following a washing step with pentane/DCM [9].

Acrolein was proposed to be responsible for organoleptic defects in alcoholic beverages made from apples. 3-methylbenzothiazolone hydrazine was used to derivatise acrolein in freshly distilled cider and Calvados to form the corresponding azine. This compound was extracted with hexane for analysis by GC-NPD or GC-MS [110].

Although an exhaustive review of methods used to identify novel volatile compounds in wine falls outside the scope of this chapter, it can be pointed out that very selective sample preparation and/or analytical methods are required for this purpose. For example, to identify three novel ethyl esters (ethyl 2-, 3- and 4-methylpentanoate), wine was extracted using a modified purge and trap technique. LiChrolut EN resins were used to trap the purged volatiles. The trap was eluted with DCM, concentrated and injected by LVI for heart-cutting two-dimensional GC separation [111]. A similar approach has recently been used to identify additional aroma compounds [112,113].

3.4 Conclusions

From the above it is evident that sample preparation before wine analysis is indeed important. Furthermore, from the fact that the majority of the methods extracts several compounds from several groups it can be concluded that there is no universal method but that the method selected is only application specific when optimised for a particular group of compounds. That being said, it was also shown that a number of different methods can be used for analysis of the same compounds, especially for major volatiles.

3.5 References

[1] J.M. Davis, C.J. Giddings, Anal. Chem. 55 (1983) 418.

[2] D.R. Stoll, X. Li, X. Wang, P.W. Carr, S.E.G. Porter, S.C. Rutan, J. Chromatogr. A 1168 (2007) 3.

[3] H. sampleprep.

[4] C. Ortega, R. Lopez, J. Cacho, V. Ferreira, J. Chromatogr. A 923 (2001) 205.

[5] V. Ferreira, in 3rd International Enology and Viticulture Conference, South African Society for Enology and Viticulture, Somerset West, South Africa, 2007, p. 74.

[6] M. Parker, A.P. Pollnitz, D. Cozzolino, I.L. Francis, M.J. Herderich, J. Agric. Food Chem. 55 (2007) 5948.

[7] E. Sarrazin, S. Shinkaruk, T. Tominaga, B. Bennetau, E. Frerot, D. Dubourdieu, J. Agric. Food Chem. 55 (2007) 1437.

[8] Y. Ribereau-Gayon, A. Glories, Handbook of Enology and Viticulture - Volume 2 The Chemistry of Wine, Stabilization and Treatments, John Wiley & Sons, New York, 2000.

[9] E. Campo, V. Ferreira, A. Escudero, J.C. Marques, J. Cacho, Anal. Chim. Acta 563 (2006) 180.

[10] V. Ferreira, N. Ortin, A. Escudero, R. Lopez, J. Cacho, J. Agric. Food Chem. 50 (2002) 4048.

[11] A. Escudero, B. Gogorza, M.A. Melus, N. Ortin, J. Cacho, V. Ferreira, J. Agric. Food Chem. 52 (2004) 3516.

[12] M.A. Pozo-Bayon, E. Pueyo, P.J. Martin-Alvarez, M.C. Polo, J. Chromatogr. A 922 (2001) 267.

[13] D. Munoz, R.A. Peinado, M. Medina, J. Moreno, Food Chem. 100 (2007) 1188.

[14] A.A. Apostolopoulou, A.I. Flouros, P.G. Demertzis, K. Akrida-Demertzi, Food Control 16 (2005) 157.

[15] E.H. Soufleros, I. Pissa, D. Petridis, M. Lygerakis, K. Mermelas, G. Boukouvalas, E. Tsimitakis, Food Chem. 75 (2001) 487.

[16] T. Garde-Cerdan, M. Arias-Gil, A.R. Marselles-Fontanet, C. Ancin-Azpilicueta, O. Martin-Belloso, Food Control, In Press, Corrected Proof.

[17] S. Selli, A. Canbas, T. Cabaroglu, H. Erten, Z. Gunata, Food Chem. 94 (2006) 319.

[18] R. Perestrelo, A. Fernandes, F.F. Albuquerque, J.C. Marques, J.S. Camara, Anal. Chim. Acta 563 (2006) 154.

[19] A. Mallouchos, M. Komaitis, A. Koutinas, M. Kanellaki, Food Chem. 80 (2003) 109.

[20] P.J. Spillman, A.P. Pollnitz, D. Liacopoulos, K.H. Pardon, M.A. Sefton, J. Agric. Food Chem. 46 (1998) 657.

[21] C. Louw, D. La Grange, I.S. Pretorius, P. van Rensburg, J. Biotech., In Press, Corrected Proof.

[22] M. Lilly, M.G. Lambrechts, I.S. Pretorius, Appl. Environ. Microbiol. 66 (2000) 744.

[23] J.A. Moreno, L. Zea, L. Moyano, M. Medina, Food Control 16 (2005) 333.

[24] J. Ubeda, A. Briones, Lebensmittel-Wissenschaft und-Technologie 33 (2000) 408.

[25] T.G. Cerdan, C. Ancin-Azpilicueta, LWT - Food Sci. and Tech. 39 (2006) 199.

[26] A. Calleja, E. Falque, Food Chem. 90 (2005) 357.

[27] J.M. Oliveira, M. Faria, F. Sa, F. Barros, I.M. Araujo, Anal. Chim. Acta 563 (2006) 300.

[28] E.S. Palomo, M.C.D.-M. Hidalgo, M.A. Gonzalez-Vinas, M.S. Perez-Coello, Food Chem. 92 (2005) 627.

[29] S. Cabredo-Pinillos, T. Cedron-Fernandez, M. Gonzalez-Briongos, L. Puente-Pascual, C. Saenz-Barrio, Talanta 69 (2006) 1123.

[30] B. Fernandez de Simon, E. Cadahia, T. Hernandez, I. Estrella, Anal. Chim. Acta 563 (2006) 198.

[31] E.S. Palomo, M.C. Diaz-Maroto, M.A.G. Vinas, A. Soriano-Perez, M.S. Perez-Coello, Food Control In Press, Corrected Proof.

[32] E. Guchu, M.C. Diaz-Maroto, M.S. Perez-Coello, M.A. Gonzalez-Vinas, M.D.C. Ibanez, Food Chem. 99 (2006) 350.

[33] J.J. Mateo, M. Jimenez, A. Pastor, T. Huerta, Food Res. Int. 34 (2001) 307.

[34] A. Escudero, E. Campo, L. Farina, J. Cacho, V. Ferreira, J. Agric. Food Chem. 55 (2007) 4501.

[35] L.-K. Ng, Anal. Chim. Acta 465 (2002) 309.

[36] I. Lukic, M. Banovic, D. Persuric, S. Radeka, B. Sladonja, J. Chromatogr. A 1101 (2006) 238.

[37] N. Loscos, P. Hernandez-Orte, J. Cacho, V. Ferreira, J. Agric. Food Chem. 55 (2007) 6674.

[38] E.S. Palomo, M.S. Perez-Coello, M.C. Diaz-Maroto, M.A. Gonzalez Vinas, M.D. Cabezudo, Food Chem. 95 (2006) 279.

[39] M. Jesus Ibarz, V. Ferreira, P. Hernandez-Orte, N. Loscos, J. Cacho, J. Chromatogr. A 1116 (2006) 217.

[40] S. Francioli, J. Torrens, M. Riu-Aumatell, E. Lopez-Tamames, S. Buxaderas, Am. J. Enol. Vitic. 54 (2003) 158.

[41] P. Comuzzo, L. Tat, A. Tonizzo, F. Battistutta, Food Chem. 99 (2006) 217.

[42] J.L. Gomez-Ariza, T. Garcia-Barrera, F. Lorenzo, R. Beltran, J. Chromatogr. A 1112 (2006) 133.

[43] M. Riu-Aumatell, J. Bosch-Fuste, E. Lopez-Tamames, S. Buxaderas, Food Chem. 95 (2006) 237.

[44] R.F. Alves, A.M.D. Nascimento, J.M.F. Nogueira, Anal. Chim. Acta 546 (2005) 11.

[45] E. Sanchez-Palomo, M.C. Diaz-Maroto, M.S. Perez-Coello, Talanta 66 (2005) 1152.

[46] Z. Pineiro, R. Natera, R. Castro, M. Palma, B. Puertas, C.G. Barroso, Anal. Chim. Acta 563 (2006) 165.

[47] S.M. Rocha, P. Coutinho, A. Barros, I. Delgadillo, M.A. Coimbra, J. Chromatogr. A 1114 (2006) 188.

[48] I.G. Roussis, I. Lambropoulos, D. Papadopoulou, Food Chem. 93 (2005) 485.

[49] L. Tat, P. Comuzzo, I. Stolfo, F. Battistutta, Food Chem. 93 (2005) 361.

[50] M. Liu, Z. Zeng, Y. Tian, Anal. Chim. Acta 540 (2005) 341.

[51] E. Marengo, M. Aceto, V. Maurino, J. Chromatogr. A 943 (2002) 123.

[52] J. Marin, A. Zalacain, C. De Miguel, G.L. Alonso, M.R. Salinas, J. Chromatogr. A 1098 (2005) 1.

[53] B.T. Weldegergis, A.G.J. Tredoux, A.M. Crouch, J. Agric. Food Chem. 55 (2007) 8696.

[54] D.J. Caven-Quantrill, A.J. Buglass, J. Chromatogr. A 1117 (2006) 121.

[55] P. Chalier, B. Angot, D. Delteil, T. Doco, Z. Gunata, Food Chem. 100 (2007) 22.

[56] M. Gil, J.M. Cabellos, T. Arroyo, M. Prodanov, Anal. Chim. Acta 563 (2006) 145.

[57] D.S. Garruti, M.R.B. Franco, M.A.A.P. da Silva, N.S. Janzantti, G.L. Alves, LWT - Food Sci. and Tech. 39 (2006) 373.

[58] A. Gordin, A. Amirav, J. Chromatogr. A 903 (2000) 155.

[59] P. Watkins, C. Wijesundera, Talanta In Press, Corrected Proof.

[60] B. Plutowska, W. Wardencki, Food Chem. 107 (2008) 449.

[61] V. Ferreira, L. Cullere, N. Loscos, J. Cacho, J. Chromatogr. A 1122 (2006) 255.

[62] L. Cullere, J. Cacho, V. Ferreira, Anal. Chim. Acta 563 (2006) 51.

[63] W.K. Carlton, B. Gump, K. Fugelsang, A.S. Hasson, J. Agric. Food Chem. 55 (2007) 5620.

[64] A. Escudero, E. Asensio, J. Cacho, V. Ferreira, Food Chem. 77 (2002) 325.

[65] C.E.B. Vivian A Watts, J. of the Sci. Food Agric. 83 (2003) 1143.

[66] L. Cullere, J. Cacho, V. Ferreira, J. Agric. Food Chem. 55 (2007) 876.

[67] A.L. Waterhouse, S.E. Ebeler, Chemistry of Wine Flavor, ACS Symposium Series, New York, 1998.

[68] J. Marais, Wynboer July 2004 (2004).

[69] E. Coelho, S.M. Rocha, I. Delgadillo, M.A. Coimbra, Anal. Chim. Acta 563 (2006) 204.

[70] M. Esti, P. Tamborra, Anal. Chim. Acta 563 (2006) 173.

[71] J.S. Camara, M.A. Alves, J.C. Marques, Food Chem. In Press, Corrected Proof.

[72] J.S. Camara, M.A. Alves, J.C. Marques, Anal. Chim. Acta 555 (2006) 191.

[73] B. Pineau, J.C. Barbe, C. VanLeeuwen, D. Dubourdieu, J. Agric. Food Chem. 55 (2007) 4103.

[74] L.D. Falcao, G. deRevel, M.C. Perello, A. Moutsiou, M.C. Zanus, M.T. Bordignon-Luiz, J. Agric. Food Chem. 55 (2007) 3605.

[75] S. LaGuerche, B. Dauphin, M. Pons, D. Blancard, P. Darriet, J. Agric. Food Chem. 54 (2006) 9193.

[76] M. Vilanova, C. Sieiro, J. Food Comp. Anal. 19 (2006) 694.

[77] S.M. Rocha, E. Coelho, J. Zrostlikova, I. Delgadillo, M.A. Coimbra, J. Chromatogr. A 1161 (2007) 292.

[78] R.M. Pena, J. Barciela, C. Herrero, S. Garcia-Martin, Talanta 67 (2005) 129.

[79] V. Ferreira, N. Ortin, J.F. Cacho, J. Chromatogr. A 1143 (2007) 190.

[80] L. Mateo-Vivaracho, V. Ferreira, J. Cacho, J. Chromatogr. A 1121 (2006) 1.

[81] L. Mateo-Vivaracho, J. Cacho, V. Ferreira, J. Chromatogr. A 1146 (2007) 242.

[82] H.B. Swan, J. Food Comp. Anal. 13 (2000) 207.

[83] Y. Fang, M.C. Qian, J. Chromatogr. A 1080 (2005) 177.

[84] C. Sala, M. Mestres, M.P. Marti, O. Busto, J. Guasch, J. Chromatogr. A 953 (2002) 1.

[85] D. Ryan, P. Watkins, J. Smith, M. Allen, P. Marriott, J. Sep. Sci. 28 (2005) 1075.

[86] J.O. Fernandes, M.A. Ferreira, J. Chromatogr.. A 886 (2000) 183.

[87] J. Goode, in Wine Science, Octopus Publishing Group, New York, 2005, p. 187.

[88] R. Suarez, J.A. Suarez-Lepe, A. Morata, F. Calderon, Food Chem. In Press, Accepted Manuscript.

[89] P. Valentao, R.M. Seabra, G. Lopes, L.R. Silva, V. Martins, M.E. Trujillo, E. Velazquez, P.B. Andrade, Food Chem. 100 (2007) 64.

[90] A. Smit, R.R. CorderoOtero, M.G. Lambrechts, I.S. Pretorius, P. vanRensburg, J. Agric. Food Chem. 51 (2003) 4909.

[91] O. Ezquerro, A. Garrido-Lopez, M.T. Tena, J. Chromatogr.. A 1102 (2006) 18.

[92] C. Pizarro, N. Perez-del-Notario, J.M. Gonzalez-Saiz, J. Chromatogr. A 1166 (2007) 1.

[93] P. Caboni, G. Sarais, M. Cabras, A. Angioni, J. Agric. Food Chem. 55 (2007) 7288.

[94] P. Vlachos, A. Kampioti, M. Kornaros, G. Lyberatos, Food Chem. 105 (2007) 681.

[95] C. Pizarro, N. Perez-del-Notario, J.M. Gonzalez-Saiz, J. Chromatogr. A 1143 (2007) 26.

[96] A. Martinez-Urunuela, J.M. Gonzalez-Saiz, C. Pizarro, J. Chromatogr. A 1048 (2004) 141.

[97] S. Jonsson, T. Uusitalo, B. van Bavel, I.B. Gustafsson, G. Lindstrom, J. Chromatogr. A 1111 (2006) 71.

[98] A. Martinez-Urunuela, J.M. Gonzalez-Saiz, C. Pizarro, J. Chromatogr. A 1089 (2005) 31.

[99] J.L. Gomez-Ariza, T. Garcia-Barrera, F. Lorenzo, A.G. Gonzalez, Anal. Chim. Acta 540 (2005) 17.

[100] S. Insa, V. Salvado, E. Antico, J. Chromatogr. A 1047 (2004) 15.

[101] S. Insa, E. Antico, V. Ferreira, J. Chromatogr.. A 1089 (2005) 235.

[102] C. Lorenzo, A. Zalacain, G.L. Alonso, M.R. Salinas, J. Chromatogr. A 1114 (2006) 250.

[103] R.C. Calhelha, J.V. Andrade, I.C. Ferreira, L.M. Estevinho, Food Microbiol. 23 (2006) 393.

[104] P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.

[105] A. Angioni, A. Garau, P. Caboni, M.T. Russo, G.A. Farris, S. Zara, P. Cabras, J. Chromatogr. A 1097 (2005) 165.

[106] A. de Villiers, P. Majek, F. Lynen, A. Crouch, H. Lauer, P. Sandra, Eur. Food Res. Tech. 221 (2005) 520.

[107] I. Citova, R. Sladkovsky, P. Solich, Anal. Chim. Acta 573-574 (2006) 231.

[108] T. Luan, G. Li, Z. Zhang, Anal. Chim. Acta 424 (2000) 19.

[109] L. Dagan, R. Schneider, J.P. Lepoutre, R. Baumes, Anal. Chim. Acta 563 (2006) 365.

[110] J. Ledauphin, A. Lefrancois, N. Marquet, M. Beljean-Leymarie, D. Barillier, LWT - Food Sci. Tech. 39 (2006) 1045.

[111] E. Campo, V. Ferreira, R. Lopez, A. Escudero, J. Cacho, J. Chromatogr.. A 1122 (2006) 202.

[112] E. Campo, J. Cacho, V. Ferreira, J. Chromatogr. A 1140 (2007) 180.

[113] E. Campo, J. Cacho, V. Ferreira, J. Chromatogr. A 1137 (2006) 223.

4

Stir Bar Sorptive Extraction Gas Chromatography-Mass Spectrometry Combined with Chemometrics for Classification of South African Wines*

*Submitted for publication as "Stir bar sorptive extraction combined with GC-MS analysis and chemometric methods for the classification of South African wines according to the volatile composition", Andreas Tredoux, André de Villiers, Pavel Májek, Frédéric Lynen, Andrew Crouch, Pat Sandra, to J. Agric. Food Chem. November 2007

4.1 Introduction

Volatile and semi-volatile compounds present in wine determine the perceived flavour and aroma and have a definitive influence on the quality and therefore consumer acceptance of the final product [1]. As the content of aroma compounds in grapes and wine depend on many factors such as the climatic and geographical origin as well as viticultural and wine-making practices, the volatile composition may be used for purposes of quality control as well as for authentication and classification purposes [2].

Analysis of wine flavour compounds is commonly performed by gas chromatography (GC). As the influential volatiles exist in wine at levels ranging from ng/L (ppt) to mg/L (ppm), sample preparation prior to GC analysis is crucial. The most common methods of sample preparation reported in the literature for wine volatile analysis include liquid-liquid extraction (LLE) [3,4] solid phase extraction (SPE) [4-6] and solid phase micro-extraction (SPME) [7,8] either performed in the immersion but more commonly in the headspace mode.

Sorption-based sample preparation techniques such as SPME offer the advantages of solventless extraction, high sensitivity, limited matrix interference, ease of use and the option of automation [9]. As a result, these techniques have been applied extensively for wine analyses. Applications include target analysis such as the determination of off-flavours [10,11], oak-derived compounds [12], terpenoids [13], low molecular weight aldehydes [14], sulphur compounds [15], age markers [16], as well as screening of major volatiles in grapes and wine [17-20].

Relatively recently, stir bar sorptive extraction (SBSE) was developed as an alternative sorption-based sample preparation technique. SBSE offers increased sensitivity compared to SPME due to the increased amount of sorptive stationary phase [21]. SBSE has been applied successfully for the analysis of predominantly additives, off-flavours and contaminants in foods [22-26], as well as for target analysis of biological [27-29] and environmental [30-32] samples, amongst others. Application of SBSE to wine analysis has also been reported, including the determination of halo-anisoles and halo-phenols involved in cork taint [33-35], volatile phenols related to *Brettanomyces* spoilage [36], pesticides [37], oak-derived volatiles

[38], monoterpenes [39] and γ-butyrolactone [40]. Recent reports have also indicated the suitability of this technique for screening of a broad range of wine volatiles. Thus, SBSE has been applied for the characterization of Madeira wine [41] and general flavour analysis of wine and grape-derived products [42-48]. In addition, headspace sorptive extraction (HSSE) has recently successfully been applied for screening of wine volatiles [49].

Screening methods are typically used to quantify the major wine volatiles such as alcohols, esters and acids. Most of these compounds are common grape- and fermentation-derived products and as such are present in all wines and play a relatively minor role in determining the so-called varietal character [50]. Nevertheless, screening methods are often used for quality control and in authentication and classification studies due to the large amount of information provided in a single analysis. As vast quantities of data are typically generated in such studies, it is often problematic to meaningfully interpret the data and relate chemical composition to the desired wine properties [51,52]. For this purpose multivariate analysis methods have been extensively used as valuable aids for extracting relevant information from large data sets [53,54].

Exploratory analysis is frequently performed using principle component analysis (PCA). By extraction of latent variables based on the maximum explained variance in the data set, PCA allows the reduction of the dimensionality for visualization purposes [55]. Cluster analysis (CA) can also be used to evaluate similarity between samples. In this method, it is assumed that distances between the objects in an n-dimensional space (defined by n variables) bears relationship to their similarity [55,56]. PCA and CA are both unsupervised exploratory techniques, where the grouping of samples is not pre-determined. In contrast, supervised pattern recognition techniques are used to derive classification rules obtained from a set of similar objects for subsequent categorization of unknown samples. In the case of wine samples this can be a common denominator such as cultivar, age, origin, etc. In linear discriminant analysis (LDA) a set of latent variables, known as canonical variables, are derived as linear combinations of discernable variables. Canonical variables describe a multivariate space in

which pre-defined classes of samples are plotted, and classification of an unknown sample is based on the shortest distance to the centroid of a particular class [55].

The efficacy of multivariate methods for classification of wines has been demonstrated by several authors using diverse sets of chemical data, including volatiles [41,57-60] and non-volatiles such as amino acids and biogenic amines [61], phenolic- [62] and metallic compounds [63]. Chemometric methods in combination with chemical data can in this way be applied for quality control and authentication purposes by classification according to variety or cultivar, vintage and/or geographical origin [64]. To be able to unequivocally determine whether a specific wine is indeed of the claimed cultivar, origin, or even vintage, is of benefit to the numerous regulatory bodies. In addition, comprehensive data of volatile wine constituents may serve to identify causes of defects in wine and/or be used to provide insight into the effect of oenological practice. Specifically, comparison of volatile data for South African wines could provide insight into the unique climatological- and cultivar-dependant characteristics of these wines. In combination with sensorial data, the end result would ideally be to obtain information that would allow tuning of wine manufacturing, within the given constraints of climate, grape variety, etc, in such a way as to provide a product of given desirable properties.

The ability to perform statistical classification of wines based on relatively simple analytical techniques is therefore of significant interest. Within this context, the aim of the current study was in the first instance to develop a simple and robust method, based on SBSE, for the analysis of major wine volatiles and semi-volatiles. Subsequently, the developed method was utilised in the investigation of volatile composition of South African wines. The data were used to evaluate the possible differentiation of these wines according to cultivar, independent of geographical origin, vintage (age) or oenological practice.

4.2 Experimental

4.2.1 Materials

Polydimethylsiloxane (PDMS) coated stir bars (Twisters™) of 10 mm length and 0.5 mm film thickness were obtained from Gerstel, Mullheim a/d Ruhr, Germany. All standards were supplied by Sigma Aldrich (Steinheim, Germany), while hydrochloric acid was purchased from Merck (Darmstadt, Germany). Deionised water was obtained from a Millipore Elix water purification system (Supelco, Bellefonte, PA, USA).

A total of 43 red and 19 white wines of vintages ranging between 1996 and 2003 were analyzed (**Table 1**). Wines were either purchased commercially, obtained from the KWV (Paarl, South Africa) or the South African National Wine Show Association. The wines originated from most of the major wine-producing regions in South Africa. Samples were transferred under nitrogen from freshly opened bottles to completely filled amber vials for storage (4^{o}C) prior to analysis.

Table 1. Summary of the South African wines analysed in the current study.

Red wine cultivars	**Vintage (*number*)**	**White wine cultivars**	**Vintage (*number*)**
Cabernet Sauvignon	1996(*1*), 1997(*1*), 1998(*2*), 2003(*3*)	Chardonnay	1999(*1*), 2000(*1*), 2001(*3*), 2003(*6*)
Pinotage	1999(*2*), 2001(*3*), 2003(*2*)	Chenin Blanc	2000(*1*), 2003(*2*)
Merlot	1999(*1*), 2003(*6*)	Sauvignon Blanc	2000(*1*), 2001(*2),* 2003*(2)*
Ruby Cabernet	2003(*5*)		
Shiraz	1999(*1*), 2001(*5),* 2003 *(7)*		
Blends	1997 *(1)*, 1998(*1*), 1999(*1*), 2000(*2*)		

4.2.2 Sample preparation

The sample preparation procedure was optimised as outlined in the Results and Discussion section. The optimised procedure was as follows: to a 15 mL vial 10 mL of de-ionised water, 0.5 mL of wine (pH previously adjusted to 3.0 using 0.1 M hydrochloric acid) and 5 µL of an internal standard solution (500 mg/L 2-octanol in ethanol) are added. A preconditioned stir bar (coated with 55 uL PDMS) is introduced to the vial, which is covered with aluminium foil and stirred at 1200 rpm for 1 hour at a temperature 22^{o}C (thermostated room). Following extraction, the stir bar is removed, washed with a small amount of de-ionised water and dried with a lint free paper towel before being introduced in a thermal desorption tube (180 mm L, 4 mm OD, 3 mm ID, Gerstel). Stir bars were reconditioned (in a desorption tube installed in a GC oven) at 300^{o}C under a constant flow of nitrogen (100 mL/min) for 1 hour.

4.2.3 Chromatographic conditions

A 6890 GC coupled to a 5972 MS (Agilent Technologies, Palo Alto, CA, USA) equipped with a thermal desorption system (TDS2) and a programmed temperature vaporizing injector (CIS4) both from Gerstel. For thermal desorption the TDS was programmed as follows: 60^{o}C, held for 5 min, ramped to 300^{o}C (10^{o}C/min), held for 5 min. The TDS was operated in the solvent vent mode for the first 2 min and splitless mode thereafter. The transfer capillary temperature was kept constant at 300^{o}C. Analytes were trapped in the PTV cooled to -100^{o}C with liquid nitrogen and subsequently injected onto the column by ramping the injector to 280^{o}C at 600^{o}C/min (held at this temperature for 2 min). The PTV was kept in solvent vent mode during desorption and splitless mode (2 min) during injection. The split flow was adjusted to 50 mL/min. Separation was performed on an HP-INNOWAX capillary column (30 m L, 0.25 mm ID and 0.25 µm d_f, Agilent Technologies) with helium as carrier gas at a constant pressure of 50 kPa. The oven program was as follows: 40°C held for 8 min, ramped at 3°C/min to 60°C, 5°C/min to 200°C, and at 20°C/min to 250°C (held for 5 min). The transfer line to the MS was kept at 280^{o}C with the MS scanning from 30-350 m/z at a rate of 2.5 scans/s.

4.2.4 Data analysis

The relative peak areas of the 38 target analytes were used, after correction for the peak area of the internal standard, to construct matrices for red, white, and red and white wines together. All analytical data were autoscaled to produce variables with zero means and unit standard deviation [65]. ANOVA, PCA and LDA were performed using Statistica v.6.0 (Statsoft Inc., Tulsa, OK, USA).

4.3 Results and discussion

4.3.1 Optimization of the sample preparation procedure

Optimization was performed using real wine samples instead of model solutions in order to take into account possible matrix effects commonly encountered with sorptive sample preparation methods [9]. The influence of the stirring time, sorption temperature, sample pH, sample dilution and salt addition on 38 selected volatile compounds in wine was investigated. The same red wine sample was used for all optimization experiments and the stirring speed was kept constant at 1200 rpm throughout. All analyses were performed in triplicate using preconditioned stir bars and mean values are presented. Note that since most of the influential parameters are well-known from extensive SPME investigations, a full experimental design was omitted.

Due to the relatively large amount of sorptive phase used for SBSE, the method offers enhanced sensitivity compared to SPME [21]. A consequence of this increased sensitivity is that for undiluted wine samples a number of solutes are extracted at levels which result in overloading of the column, often obscuring other trace level volatile compounds. We therefore evaluated different dilution ratios of the wine samples. It was found that diluting 0.5 mL of wine with 10 mL de-ionised water provided the largest number of identifiable compounds without overloading the column. As an added benefit, matrix effects are reduced and dilution increases the lifetime of the stir bars. Stir bars become □iscoloured following extended use and together with increased detection of PDMS degradation products, this indicates that a particular stir bar is no longer usable. We have found that use of undiluted wine samples speeds up this

degradation, probably due to small amounts of non-volatile compounds (for instance sugars) remaining on the surface of the stir bar even after rinsing with water. In agreement with our findings for SBSE, previous reports utilizing both SPME and SPE [15] have indicated that dilution of wine samples often produces better results, due to both the reduction of interference from matrix components and ethanol concentration (thereby improving extraction by apolar phases).

The optimal extraction time was determined using a diluted sample adjusted to pH 3. Extraction times of 30, 60 and 120 min were evaluated at a temperature of 22^{o}C. The results for 7 compounds representative of the different chemical classes analysed (i.e. acids, esters, alcohols, phenols, aldehydes, ketones and lactones) are summarized in **Figure 1a**. For most compounds equilibrium was reached between one and two hours. The exceptions are furfural, 1-hexanol and Z-whiskeylactone for which the extracted amount decreased after 1 hour sampling, most probably due to competition effects. Less polar compounds taking longer to partition into the PDMS may reduce the extraction of more polar compounds that easily migrate into the PDMS during the initial extraction phase. Accordingly, it seems that longer extraction times favour less volatile compounds (larger molecules) at the expense of highly volatile analytes**.** A stirring time of 60 min was selected since this is sufficient for the establishment of equilibrium for the majority of wine volatiles, also taking into account practical considerations such as the total analysis time.

Using a fixed extraction time of 60 min, extraction efficiency was evaluated at pH values of 2.5, 3.0, 3.5 and 4.0. Higher pH was not considered in order to avoid deprotonation of the acidic compounds which would reduce their partitioning into the PDMS phase. As is evident from **Figure 1b**, recovery for most compounds was optimal at pH 3.0, whereas a reduction in peak areas for certain compounds, notably the volatile organic acids, was observed at higher pH values. For this reason, and since most wines have pH values between 3.1 and 4.0, it was decided to standardize the pH to 3.0 for all samples.

Extraction at elevated temperature was evaluated by comparing stirring at 22ºC (thermostated room) and at 50ºC in a GC oven. Sorption at elevated temperature, as illustrated in **Figure 2a**, had a minor positive effect on mainly larger molecules. These results are in agreement with the

effect of extraction time and may presumably be ascribed to faster extraction kinetics at higher temperature. However, a negative effect on some polar and low molecular weight components was also observed (phenols, ketones and acids). The slight benefit for selected compounds was not considered sufficient to warrant sampling at elevated temperature, especially taking into account practical implications for the routine application of the method.

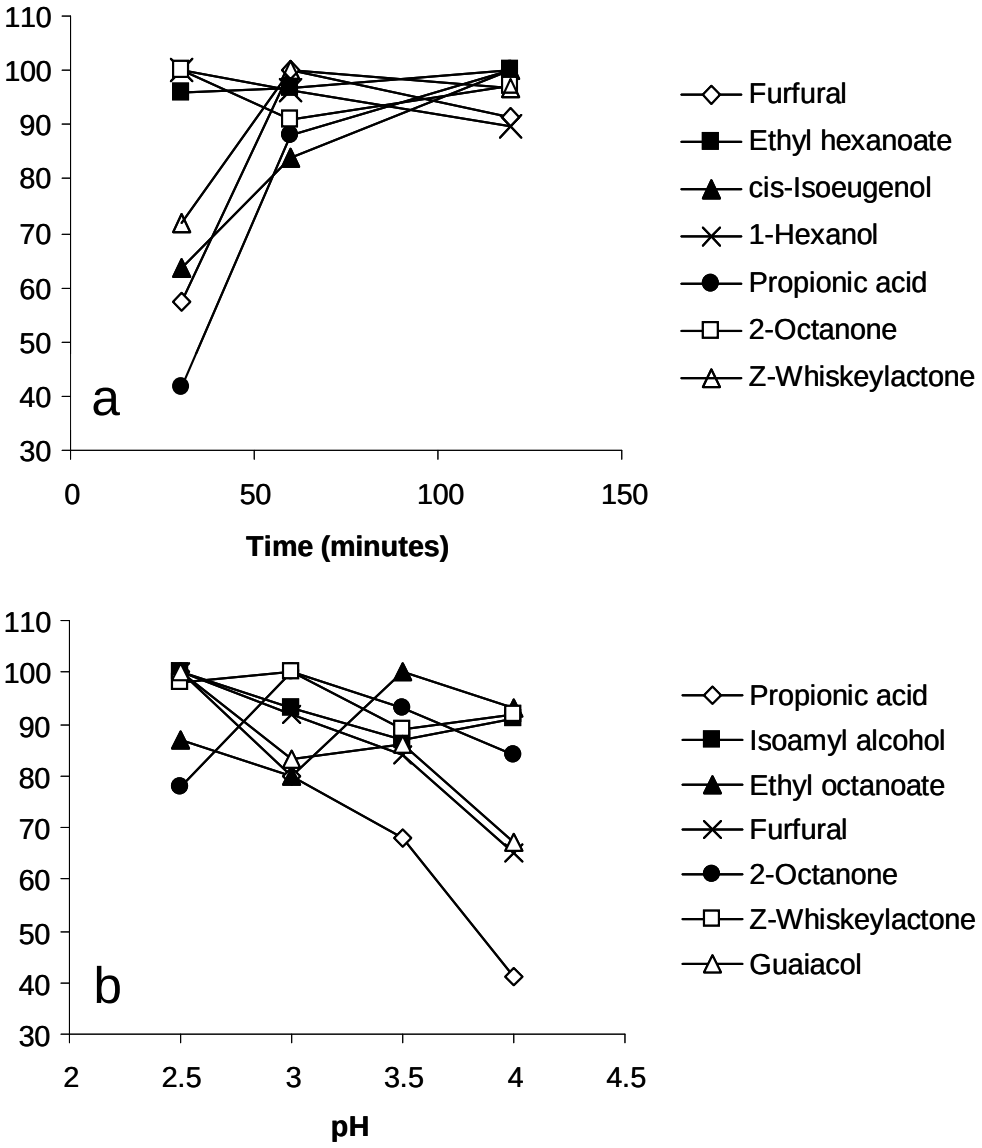

Figure 1. Effect of sampling time (a) and sample pH (b) on SBSE extraction of selected wine volatiles.

The effect of saturating the sample with 2 g of NaCl prior to extraction was evaluated by sampling for 60 min at 22°C using a diluted sample adjusted to pH 3.0. Minor positive and negative effects were observed, depending on the analyte under investigation (**Figure 2b**), and may be explained in terms of the change in the PDMS/matrix distribution constants for the compounds, as reported previously for SPME [8]. This effect was not considered prominent enough to include the addition of salt in the sample preparation procedure.

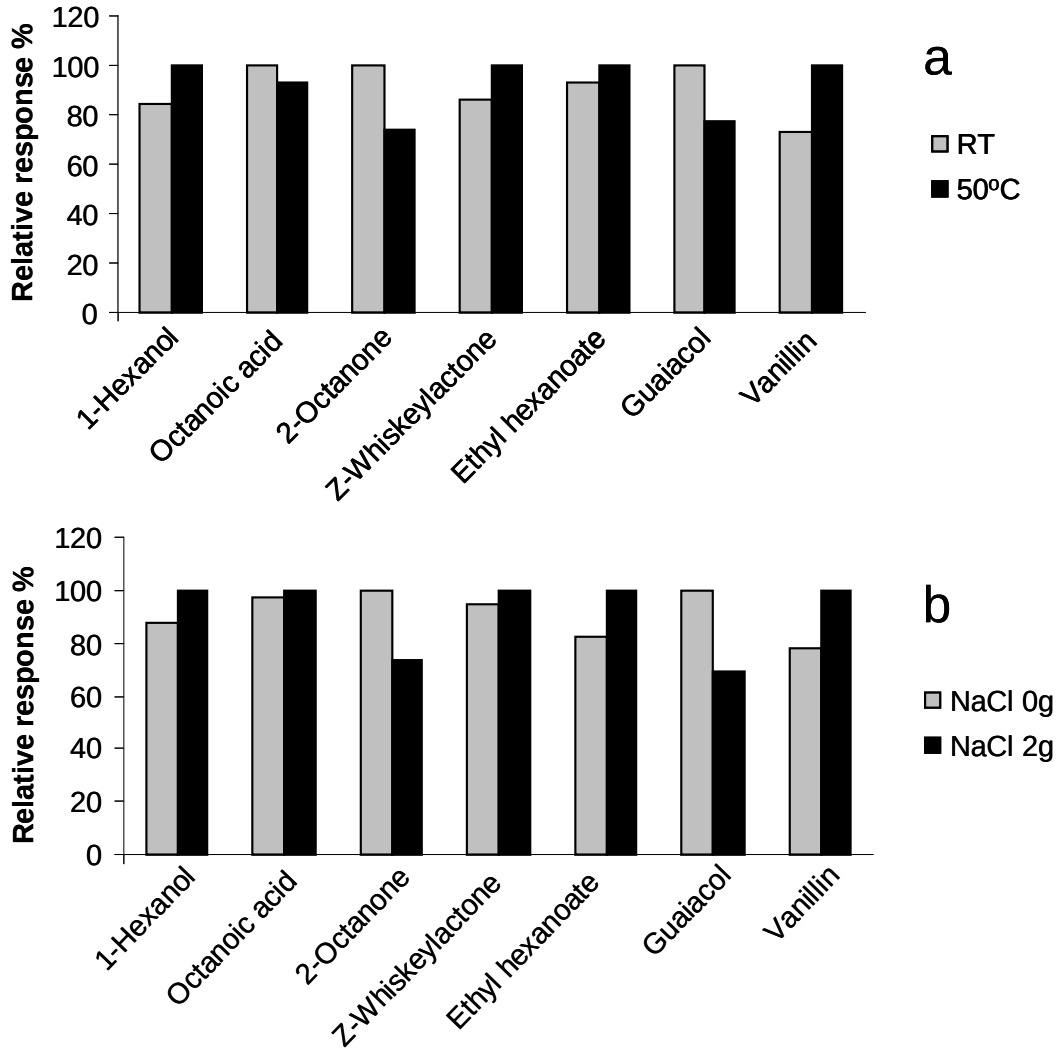

Figure 2. Effect of sampling temperature (a) and addition of salt (b) on SBSE extraction of selected wine volatiles.

4.3.2 Optimisation of thermal desorption and injection parameters

Following optimization of the sample preparation step, the thermal desorption and injection parameters were fine-tuned. For thermal desorption, it was found that using a trapping temperature of -100ºC instead of -150ºC greatly improved the peak shapes for early eluting compounds. It is believed that faster heating of the liner to the injection temperature leads to reduced injection times and therefore less band broadening. The occasional occurrence of distorted and even split peaks, especially for ethyl esters, was observed. This is thought to be due to a small amount of water remaining on the stir bar or between the PDMS layer and the glass sleeve after drying with a paper towel. In order to avoid this, a 'solvent venting' step was performed by raising the TDS temperature to 60^{o}C in the solvent vent (2 min) mode prior to thermal desorption. The loss of some highly volatile compounds was considered of less significance compared to the benefit of avoiding split peaks.

4.3.3 Evaluation of the SBSE-TD-GCMS method

In **Figure 3** a typical total ion chromatogram obtained for the SBSE-TD-GCMS analysis of red wine is presented. Identification of volatile compounds was performed using authentic standards, NIST 98 and Wiley 275 mass spectral databases and correlation with retention indices (RI's) reported in literature [66,67].

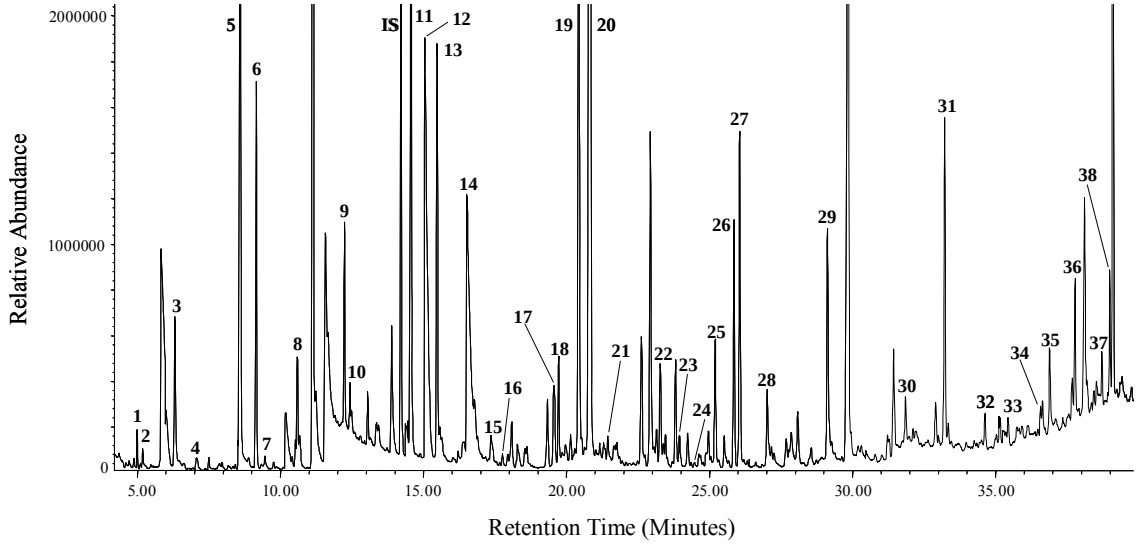

Figure 3. Total ion chromatogram for the SBSE-TD-GC-MS analysis of a South African red wine (Shiraz, 2000). Peak numbers correspond to Table 2. For experimental details, refer to experimental section.

38 positively identified compounds, representing the bulk of the major wine volatile constituents were selected for quantitative and chemometric analyses (**Table 2**). Peak areas relative to the IS (2-octanol) were used for quantitation as reported previously, since certain reference standards were unavailable [68,69]. Also, for statistical techniques absolute concentrations are not required. Attempts to find a suitable IS for each of the different classes of compounds were unsuccessful due to failure to obtain sufficient separation for the complex wine extracts obtained by SBSE. 2-octanol was therefore selected as IS based on the fact that it elutes in the middle of the chromatogram and possesses polar as well as non-polar properties.

The method demonstrated good repeatability as reflected by relative standard deviations (%RSD, n = 5) ranging from 2.3% for ethyl octanoate to 9.8% for formic acid. These results demonstrate that SBSE provides acceptable sensitivity and good repeatability for a wide range of wine volatiles and therefore presents a viable alternative to SPME analysis. Comparison of the proposed methodology to published SPME methods for wine volatile analysis is complicated by the fact that results are highly dependent on sampling conditions. Nevertheless, both SPME and SBSE screening methods are applicable to the same compounds (i.e. the major

wine volatiles). As mentioned earlier, the inherent sensitivity of SBSE for especially non-polar analytes may be exploited by diluting wine samples prior to extraction, thereby reducing matrix concerns. On the other hand SPME offers the possibility of selecting a stationary phase based on the chemical properties of the compounds of interest (alternative phases for SBSE are not commercially available as yet). Both techniques offer the advantages of solventless extraction and higher sensitivity compared to LLE and both benefit from ease of use, although the sampling step for SBSE cannot be fully automated as is the case for SPME. In productivity terms this is not a limitation, bearing in mind that multiple simultaneous SBSE extractions can be performed prior to subsequent automated injection. We have recently reported a headspace sorptive extraction (HSSE) method for the analysis of wine [49]. The volatiles extracted in both the headspace and immersion modes using PDMS-coated stir bars are similar. SBSE compares favourably to the HSSE approach in terms of reduced matrix effects, repeatability and sensitivity, while advantages of the proposed SBSE method include reduced extraction time and ease of use (no salt addition or specialised glassware is required).

Table 2. Summary of the wine volatiles quantified by SBSE-TD-GC-MS, together with repeatability data and ions used for quantitation.

Number[a]	Compound	%RSD (n = 5)	Quantitation ions (m/z)
1	ethyl butyrate[b,c]	8.2	88
2	ethyl isovalerate[b,c]	5.6	88
3	isoamyl acetate[b,c]	4.3	87
4	1-butanol[b,c]	6.2	56
5	isoamyl alcohol[b,c]	5.9	55
6	ethyl hexanoate[b,c]	3.4	88
7	hexyl acetate[b,c]	4.6	84
8	2-octanone[c]	6.4	99
9	ethyl lactate[b,c]	3.7	75
10	1-hexanol[b,c]	7.9	56
11	ethyl octanoate[b,c]	2.3	88
12	acetic acid[b,c]	9.0	60

13	furfural[b,c]	2.9	96
14	formic acid[b,c]	9.8	46
15	propanoic acid[b,c]	8.2	74
16	1-octanol[b,c]	7.1	84
17	γ-butyrolactone[c]	5.6	86
18	ethyl decanoate[b,c]	4.5	88
19	furfuryl alcohol[b,c]	4.7	98
20	diethyl succinate[b,c]	2.5	101
21	ethyl-9-decenoate[c]	5.5	88
22	ethylphenyl acetate[c]	6.4	91
23	phenylethyl acetate[b,c]	3.1	91
24	ethyl dodecanoate[b,c]	6.7	88
25	guaiacol[b,c]	4.6	109
26	ethyl isopentyl succinate[c]	5.8	101
27	phenylethyl alcohol[b,c]	4.0	91
28	(Z)-whiskey lactone[c]	5.1	99
29	octanoic acid[b,c]	4.1	60
30	4-vinylguaiacol[b,c]	4.6	150
31	decanoic acid[b,c]	5.8	60
32	cis-isoeugenol[c]	6.9	164
33	trans-isoeugenol[c]	7.3	164
34	dodecanoic acid[b,c]	8.2	60
35	5-hydroxymethyl furfural[c]	6.4	97
36	vanillin[b,c]	4.1	152
37	ethyl vanillate[c]	5.2	151
38	acetovanillone[c]	5.1	151

a Compound number as assigned in Figure 3.
b Identified with comparison of retention times of authentic standards.
c Identified with comparison to mass spectral databases and retention indices.

In terms of the compounds quantified using the described SBSE-TDS-GCMS method (**Table 2**), these represent the common wine volatiles, present in most wines and responsible for the base of the flavour profiles of wines. Regarding alcohols, all red wines contain relatively high amounts of isoamyl- and β-phenethyl alcohol. As fermentation products, their levels are

largely determined by fermentation conditions. In terms of flavour contribution, the higher (or fusel) alcohols, mostly quantified in the current study, produce a negative effect when present at high levels, although their effect can be positive at normal levels. Furfuryl alcohol is produced by reduction of furfural, itself derived from wood cooperage.

Wine acids are derived both from the grape and the yeast during fermentation. Volatile, low molecular weight compounds such as formic-, butyric- and especially acetic acid are important contributors to the so-called "volatile" acidity; excess amounts are indicative of bacterial spoilage [50,70]. Higher molecular weight fatty acids including hexanoic-, octanoic-, decanoic- and dodecanoic acids quantified in the current study, are yeast-derived. These compounds indirectly affect wine flavour by leading to the production of fatty acid esters, although octanoic acid has been described as being responsible for a fatty and unpleasant odour [50,71].

Esters are formed by either enzymatic or chemical esterification of organic acids and alcohols, and in wine the most common are ethyl esters. Levels normally increase with age as chemical esterification occurs. However, for some ethyl wax esters (such as ethyl- hexanoate, octanoate and decanoate), levels decrease with age as the excess fatty acid esters formed by yeast are hydrolyzed during ageing. Fatty acid esters contribute mainly to fruity aromas (ethyl- butyrate, hexanoate and octanoate) [50,70,71], but also to flowery and rose flavour notes (β-phenethyl acetate) [50,70] to the base aroma of wines. Isoamyl acetate produces a banana aroma important for especially young wines. Ethyl esters of the main organic acids in wine (tartaric, malic, lactic, succinic, acetic and citric) are formed in all wines during ageing. These compounds are thought to contribute little to the improvement of wine aroma. The exception to this observation is ethyl lactate, the formation of which is related to malolactic fermentation.

Furfuryl compounds such as furfuryl alcohol, furfural, and 5-hydroxymethyl furfural analysed in the current study are derived from wood ageing. Reduction of furfural to furfuryl alcohol (and further possible products) takes place during wine ageing. A similar process leads to the formation of 5-hydroxymethyl furfural from 5-methylfurfural. Thus the content of these three compounds is strongly determined by wine-making practice (i.e. wood ageing) and by inference by wine age [72]. Aside from the furfural-derived products, vanillin (4-hydroxy-3-

methoxy-benzaldehyde), also analysed in the current study, enters wine during barrel ageing and produces a distinctive vanilla aroma. Vanillin undergoes reduction during further ageing, leading to the formation of vannilyl alcohol and further products. The vanillin-derived compounds ethyl vanillate and acetovanillone were also quantified in the current study.

γ-butyrolactone is produced during fermentation by internal esterification of γ-hydroxybutyric acid. In contrast, whiskey lactones (*cis*- and *trans*- 3-methyl-γ-octalactone) are released from oak during wine ageing and the *trans* isomer is associated with a coconut flavour [70,73]. The content of volatile phenols is also associated with wood ageing. Guaiacol (2-methoxyphenol) and the isoeugenol isomers (cis- and trans- 2-methoxy-4-(2-propenyl)-phenol) are directly extracted from oak. The latter two compounds contribute spicy, smoky aromas to wine, although these compounds are also associated with 4-vinyl- and 4-ethyl-phenols, the latter compounds being linked to largely negative aromatic properties [70,73,74]

4.3.4 Classification of South African wines according to cultivar based on the selected volatile compounds

The developed SBSE-TDS-GCMS method was applied to the quantitative analysis of 62 South African wines (Table 1). For quantitative data, peak areas relative to the internal standard were used for reasons outlined above. In order to study the suitability of volatile data for the classification of wines according to cultivar, the results were separated into three data sets containing volatile information for red, white, and red and white wines, respectively. Matrices of the autoscaled data were constructed containing the wine samples (objects) as rows, and the chemical compounds (variables) as columns. These data sets were subsequently investigated using chemometric methods as outlined below.

4.3.4.1 Red wines

Analysis of variance (ANOVA)

Quantitative results for the red wine volatiles are summarised according to the different classes of compounds in **Table 3**. As a first exploratory step analysis of variance (ANOVA) was

carried out to determine which compounds display significant differences between cultivars. 15 of the 38 quantified analytes showed significant differences between red wine cultivars at the 95% level (Table 3).

Table 3. ANOVA results for the volatile compounds quantified in red wines. Mean values for each variety are listed together with calculated F ratios. F ratios above the critical value ($F_{5,44,0.05}$ = 2.47) are presented in bold.

Peak no.[a]	Variety (*n*[b])	Blend (5)	Cabernet Sauvignon (7)	Merlot (7)	Pinotage (6)	Ruby Cabernet (5)	Shiraz (13)	F_{calc}
	Alcohols							
5	isoamyl alcohol	2.72	3.52	3.23	1.60	3.09	2.89	**4.76**
4	1-butanol	0.17	0.13	0.11	0.16	0.14	0.14	0.84
10	1-hexanol	0.56	0.51	0.46	0.50	0.81	0.52	**2.97**
16	1-octanol	0.91	0.99	0.66	0.93	0.76	0.98	0.88
27	β-phenylethyl alcohol	6.84	12.91	7.92	2.77	9.42	7.66	**5.76**
19	furfuryl alcohol	2.55	3.74	2.20	3.02	2.98	3.21	0.62
	Phenols							
25	guaiacol	0.29	0.28	0.26	0.31	0.24	0.36	0.40
30	4-vinylguaiacol	0.37	0.39	0.36	0.43	0.34	0.47	0.32
32	cis-isoeugenol	0.08	0.08	0.05	0.08	0.06	0.09	0.77
33	trans-isoeugenol	0.23	0.24	0.28	0.32	0.23	0.33	0.30
	Aldehydes							
13	furfural	18.92	23.69	18.61	21.37	18.97	23.93	0.37
35	5-hydroxymethyl furfural	0.45	0.62	0.61	0.67	0.66	0.70	0.42
36	vanillin	0.39	0.50	0.50	0.56	0.45	0.57	0.27
	Ketones							
8	2-octanone	0.51	0.60	0.54	0.59	0.59	0.56	1.77
38	acetovanillone	0.29	0.34	0.31	0.37	0.29	0.36	0.20
	Acids							
14	formic acid	18.68	34.04	25.23	29.95	24.89	36.29	0.80
12	acetic acid	42.35	43.72	43.67	48.22	32.35	53.54	0.44
15	propanoic acid	1.33	1.79	1.06	1.79	1.42	2.32	1.72
29	octanoic acid	0.26	0.25	0.29	0.35	0.38	0.26	**2.57**
31	decanoic acid	0.30	0.40	0.53	0.58	0.63	0.37	**2.98**
34	dodecanoic acid	0.01	0.03	0.03	0.03	0.05	0.02	**3.48**
	Esters							
3	isoamyl acetate	0.10	0.18	0.23	0.27	0.31	0.18	1.29
7	hexylacetate	0.03	0.06	0.09	0.17	0.17	0.09	**2.65**

22	ethylphenyl acetate	0.25	0.21	0.09	0.07	0.09	0.12	**15.73**
23	β-phenylethyl acetate	0.12	0.30	0.23	0.18	0.40	0.27	1.56
9	ethyl lactate	10.26	7.09	4.13	6.55	4.22	5.06	**4.17**
1	ethyl butyrate	0.06	0.06	0.07	0.08	0.08	0.06	1.40
2	ethyl isovalerate	0.16	0.14	0.08	0.06	0.03	0.08	**5.19**
6	ethyl hexanoate	3.03	3.229	3.68	3.97	4.30	2.30	1.51
11	ethyl octanoate	7.17	8.967	11.19	12.34	12.28	7.86	2.31
18	ethyl decanoate	1.16	2.177	2.67	2.69	3.68	1.65	**4.52**
21	ethyl-9-decenoate	0.02	0.016	0.05	0.06	0.04	0.04	1.13
24	ethyl dodecanoate	0.02	0.107	0.13	0.06	0.32	0.06	**3.72**
20	diethyl succinate	170.56	146.664	81.77	62.04	94.91	105.50	**3.02**
26	ethyl isopentyl succinate	16.19	25.026	15.17	5.76	19.93	16.40	**4.20**
37	ethyl vanillate	0.22	0.17	0.09	0.13	0.08	0.19	**3.12**
	Lactones							
17	γ-butyrolactone	0.20	0.158	0.11	0.15	0.15	0.19	1.43
28	(Z)-whiskey lactone	0.11	0.108	0.10	0.12	0.10	0.13	0.25

a Compound number as assigned in Figure 3.
b Number of samples per cultivar.

The following alcohols displayed significant variation between cultivars: β-phenethyl alcohol (**27**), isoamyl alcohol (**5**) and hexanol (**10**). Especially β-phenethyl- and isoamyl alcohol may be used to differentiate Pinotage wines from the rest of the cultivars as this cultivar is characterised by significantly lower amounts of these compounds. Regarding the acids, dodecanoic acid (**34**), decanoic acid (**31**) and octanoic acid (**29**) were found to vary significantly. The ester ethylphenyl acetate (**22**) showed the highest variation between cultivars, with Cabernet Sauvignon wines containing on average more of this compound than the other cultivars. In fact, the content of this compound differs significantly between Cabernet Sauvignon and the rest of the single-cultivar wines, as well as between the blended wines and each of the single-cultivar wines. This latter distinction of blends can probably be ascribed to the fact that blended wines invariably contain Cabernet Sauvignon as the predominant wine. Other esters that showed significant variation between cultivars include ethyl isovalerate (**2**), hexyl acetate (**7**), ethyl decanoate (**18**) and ethyl dodecanoate (**24**). For the last two compounds, differences in mean amounts between cultivars mirror the behaviour of the corresponding acids, decanoic and dodecanoic acid: Ruby Cabernet contains on average the

highest levels of these compounds, while Shiraz, Cabernet Sauvignon and the blended wines contain on average the lowest. This variation might be ascribed to differences in the average age of each of these classes of wine, as their levels in wine decrease with age. The average age of each of the classes of red wine at the time of analysis (2003) were: 5 years for blends, 3 years for Cabernet Sauvignon, one year for Merlot, Pinotage and Shiraz and less than one year for Ruby Cabernet (Table 1). Both esters derived from succinic acid (ethyl isopentyl succinate (**26)** and diethyl succinate (**20**)) displayed similar variations in the analysed wines: Pinotage and Cabernet Sauvignon wines displayed the lowest and highest mean values of these compounds, respectively. As part of this study, the organic content of the same wines was quantified using an ion-exclusion HPLC method [75]. These data indicate that the content of succinic acid also varies significantly between the cultivars, with Cabernet Sauvignon wines containing significantly higher mean levels than Pinotage (results not shown). It thus seems reasonable that the variation in the succinic acid between these cultivars is responsible for the measured variations in the volatile ester derived from this compound. Similarly, significant variation in ethyl lactate levels (**9**) can partially be correlated to the content of lactic acid in the analysed wines: the lowest mean value of lactic acid was measured in Merlot wines, as is the case for ethyl lactate. High levels of lactic acid in turn are associated with increased incidence of malolactic fermentation. Moreover, the content of these so-called acid-esters have been shown to generally increase during wine ageing [76]. Thus differences in the average ages of wines of each cultivar as outlined above may serve to obscure cultivar-related differences.

The amount of ethyl vanillate (**37**), a compound associated with wood contact, was found to vary significantly between the wines, with lower mean values in Merlot and Ruby Cabernet wines compared to the other three wines. This might be explained by common wine-making practice in South Africa, where Cabernet Sauvignon, Shiraz and Pinotage are more often exposed to wood ageing in order to produce wines with ageing potential. In contrast to this observation, however, the content of none of the analyzed phenols, lactones or ketones were found to differ significantly between the cultivars. Any trends might then be concealed by the varied oenological practices necessarily associated with the diverse wines analysed in the current study.

The picture for the blended red wines is slightly unclear: the highest content of certain compounds (ethyl vanillate, ethylphenyl acetate, ethyl lactate, ethyl isovalerate, diethyl succinate, γ-butyrolactone) is measured in the blends, while for other compounds the content is lower in the blended wines than any of the single cultivar wines (furfuryl alcohol, trans-isoeugenol, 5-hydroxymethyl furfural, vanillin, 2-octanone, formic acid, decanoic acid, dodecanoic acid, isoamyl acetate, hexyl acetate, β-phenyl acetate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate). As alluded to above, the concentration of especially the neutral esters decreases significantly with ageing [74,76] whereas concentrations of acid esters such as ethyl lactate and diethyl succinate increase with age. In light of this observation the differences in volatile content for the blended wines can likely be ascribed to the higher average age of the blended red wines.

Principal component analysis (PCA)

Application of PCA to the volatile data set for red wines revealed that 83.34 % of total variance is extracted by the first seven principal components. PC1 accounts for 30.80 % of the variance and correlates positively with all the analysed phenols, lactones and furfural compounds, as well as vanillin and acetovanillone. All of these compounds are derived from oak cooperage. Therefore it seems that wood ageing practices are responsible for most of the variation in the volatile composition of the analysed wines. Although ANOVA indicated that none of these compounds varied significantly between cultivars, it should be noted that PCA is an unsupervised exploratory technique used to maximise the variance in the complete data set (i.e. not according to pre-defined classes). This underscores the fact that wood exposure, present for some of the wines of each cultivar, is responsible for most of the variation in the data set. The expectation would thus be that this factor might overshadow variatal differences in major volatile content, thereby complicating their classification according to cultivar.

In addition to the wood-derived products, the low molecular weight organic acids (formic-, acetic- and propanoic acid) also show high loading factors on PC1. PC2, responsible for 20.53 % of the total variance, and PC3 (9.24%), describe the behaviour of the remaining acids and

esters. The content of these compounds are known to vary with wine age, and were also shown by ANOVA to differ significantly between cultivars. PCs 2 and 3 therefore seem to reflect variations due to wine age. In conclusion, PCA results indicate that the principal variations in the volatile data for the analysed red wines can be related to corresponding variation in wine age and wood maturation, and not cultivar.

Cluster analysis (CA)

Cluster analysis (CA) largely corroborated the conclusions reached from PCA data. Using Ward's method of agglomeration and Manhattan distances to measure the similarity between variables, two main clusters can be discerned (**Figure 4**). The cluster on the left contains the wood-related compounds and low molecular weight acids, the same compounds highly correlated with PC1. The second group contains the remainder of the acids and esters, correlated with PC2 and PC3, again indicating that wood ageing and wine age are largely responsible for the variation in the current data set.

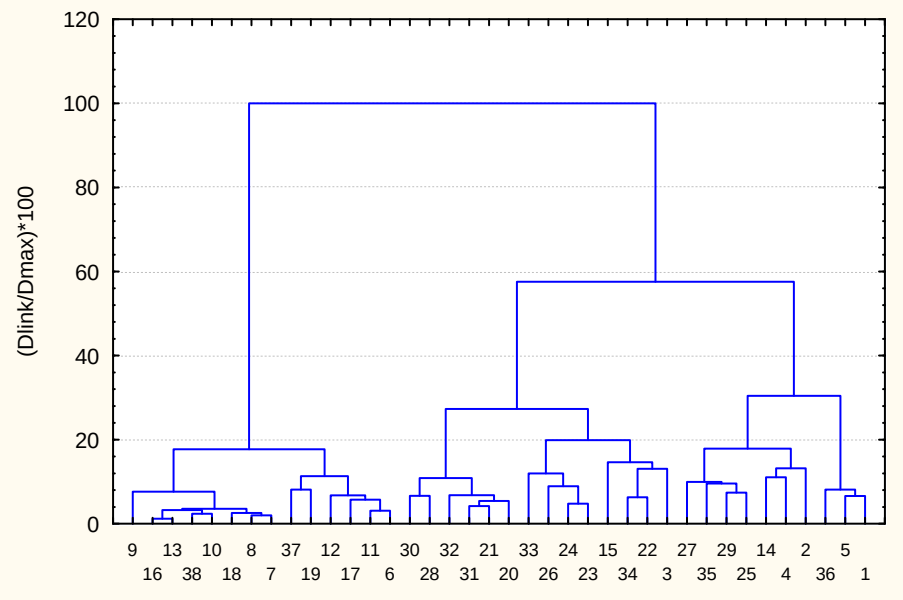

Figure 4. Tree diagram obtained for the volatile compounds in red wines using Ward's method and Manhattan distances. Numbers refer to the compounds specified in Figure 3 and Table 2.

Linear discriminant analysis (LDA)

To achieve a classification of red wines according to cultivar, stepwise standard linear discriminant analysis (LDA) was used. Blended red wines were omitted for this study as the aim was to classify according to grape variety. 14 variables were used in the classification function, including alcohols (isoamyl alcohol, 1-hexanol, β-phenylethyl alcohol), phenols (cis- and trans-isoeugenol), aldehydes (furfural and ethyl vanillate), acids (propanoic acid and octanoic acid) and esters (isoamyl acetate, ethylphenyl acetate, ethyl lactate, ethyl dodecanoate and diethyl succinate). Most of the compounds were shown to vary significantly between cultivars by ANOVA. Also, these compounds broadly reflect wood ageing (eugenols, furfural, ethyl vanillate), fermentation practices (alcohols, acids, esters) as well as wine age (esters). It was further found that four canonical roots accounted for 100% of the properties of the data set.

Due to the relatively limited number of samples, the complete set of samples was used as training data to derive the classification function in LDA. Accordingly, the same data set was also used to evaluate the recognition ability of the model, thus the *posterior* probabilities were calculated. The classification function in this manner provided correct prediction of all wine samples according to cultivar. This promising result is obtained in spite of the contribution of other parameters such as age and wood maturation to the variability in the volatile data. **Figure 5** presents the scatter plot of red wines on the first two canonical roots, where relatively good discrimination between the different red wine samples according to cultivar is evident in the 2-dimensional space.

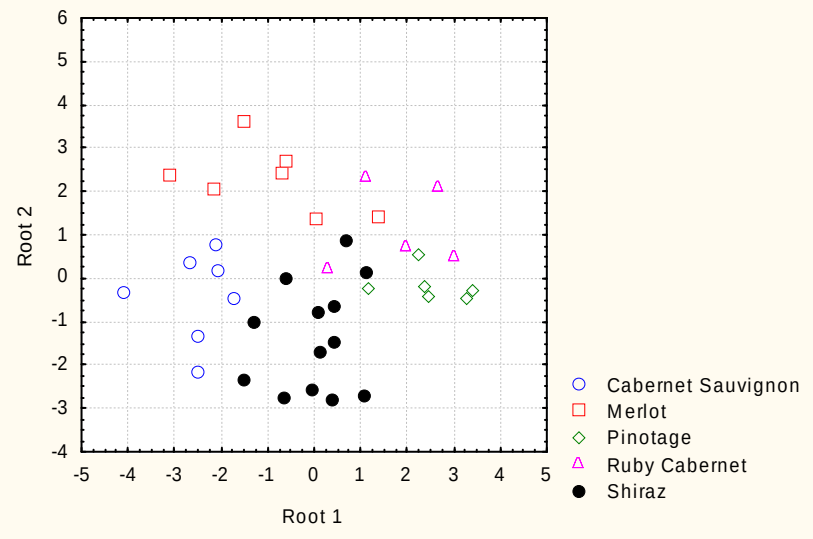

Figure 5. Two-dimensional scatter plot of the scores for red wine samples depicted on canonical roots 1 and 2.

4.3.4.2 White wines

A similar statistical procedure as outlined above was applied to white wines. ANOVA indicated that only 3 esters displayed significant differences between the three cultivars ($F_{2,18,0.05}$ = 3.63). These compounds, ethyl lactate (**9**, F_{calc}= 4.47), diethyl succinate (**20**, F_{calc}= 4.86) and ethyl isopentyl succinate (**26**, F_{calc} = 5.75), are all esters of principal wine organic acids. As alluded to earlier, the formation of especially ethyl lactate can be related to fermentation practices. ANOVA applied to the (unpublished) results for the organic content of the same wines indicated a significant (F = 6.16, $F_{2,37,0.05}$ = 3.27) difference in the lactic acid content of the three cultivars. Specifically, Chardonnay wines contained significantly higher levels of lactic acid. This is related to the increased incidence of malolactic fermentation for these wines, as also indicated by significantly higher pH-levels (F = 5.76) and lower levels of malic acid (F = 2.76) measured for Chardonnay wines. It therefore seems reasonable that these differences in fermentation practice are also reflected in the content of related volatile esters for the same wines. No difference in the content of succinic acid was observed from the organic acid data, although this might be related to the co-elution of an unkown compound with succinic acid in the HPLC method utilized [77].

As was the case for red wine, PC1 was highly correlated to all compounds associated with wood ageing (1-butanol, guaiacol, 4-vinylguaiacol, cis- and trans-isoeugenol, furfural, 5-hydroxymethyl furfural, vanillin, acetovanillone and (Z)- whiskey lactone), while PC2 was correlated to fatty acids and esters. In agreement with the discussion for the red wines, these two PCs, describing most of the variation in the analysed wines, can be related to wood contact and wine age. In addition, cluster analysis (data not shown) reveals two distinct groups of variables. The first contains wood-derived phenols, alcohols, aldehydes and ketones as well as the low molecular weight acids and the acid esters. The presence of the acid esters (ethyl isopentyl succinate, diethyl succinate and ethyl lactate) can probably be ascribed to the fact that wooded wines on average are of older vintages (these wine have more ageing potential).

Standard LDA applied to the white wine data provided a function containing 8 variables, once again providing 100% correct prediction for all white cultivars according to posterior probabilities. The compounds used in the classification of white wines include: 1-butanol, 4-vinylguaiacol, furfural, 2-octanone, ethylphenyl acetate, ethyl-9-decanoate, diethyl succinate and ethyl isopentyl succinate. Note that ethyl lactate is not included in this model, which is unexpected considering the higher incidence of malolactic fermentation of Chardonnay wines referred to above. Nevertheless, two canonical roots cover 100% of the properties for white wines. In **Figure 6** a scatter plots of the canonical scores for the white wines on these two roots are depicted, again illustrating good discrimination between white wines according to cultivar.

Interestingly, wood ageing was shown to be largely responsible for variance in the volatile content of red wines by PCA, but did not vary significantly between cultivars (ANOVA). In contrast, for white wines differences in volatile content related to fermentation and wood ageing are responsible both for significant variation (PCA), but are also useful for classification purposes. This can be ascribed to higher incidence of wood ageing and malolactic fermentation for one of the white cultivars in our data set (Chardonnay).

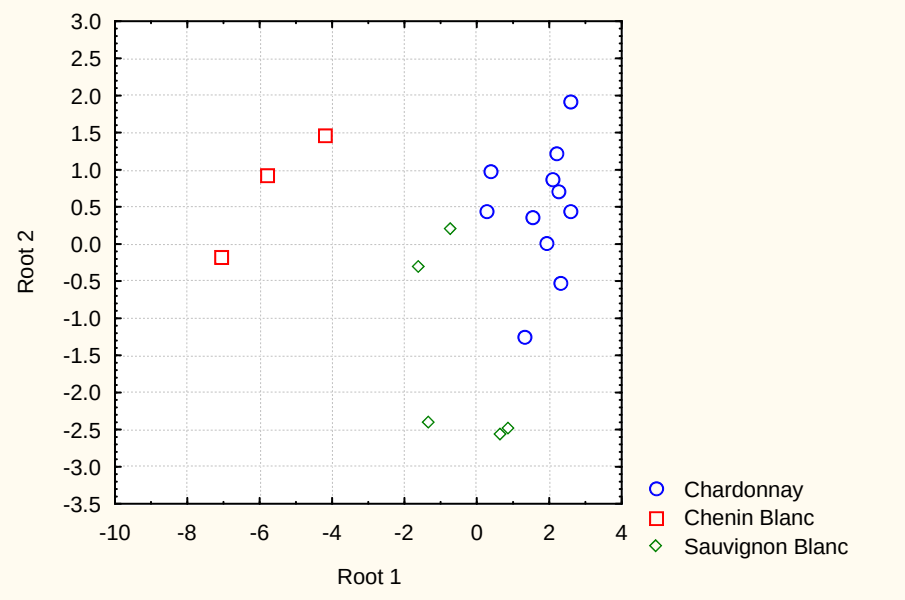

Figure 6. Scatter plot of the canonical scores for the white wines on the two canonical roots obtained by standard LDA.

4.3.4.3 Red and white wines

The presented results clearly demonstrate that the content of major volatile compounds can be used for the classification of both red and white wines according to cultivar, despite significant variation in the data set due to extraneous factors such as wood ageing, vintage and geographical origin. However, of more practical importance would be a classification function that would allow simultaneous categorization of both red and white wines (and eventually also special cases such as rosé wines) according to cultivar. This was attempted using the complete data set of all analysed wines. Use of the complete data set allows the option to remove a wine sample from the training set and to obtain a classification function which can subsequently be used to categorize the unknown sample.

To this end, a random wine sample was removed from the data set, and stepwise standard LDA was performed. Seven roots that cover 95.2% of the data properties and formed by 20 volatile components were obtained (**Table 4**). This classification function once again provides 100% correct posterior prediction of all red and white wines (excluding the omitted sample) according to cultivar.

The discriminant function allows the calculation of the coordinates of each group (or cultivar) centroid on each of the canonical roots from the coefficients of the discriminant functions and values of each variable. A three-dimensional scatter plot of the cultivar centroids plotted on the first three discriminant roots is presented in **Figure 7**, where the red and white varieties are separated to differently scaled sections for clarity purposes.

The variables of the unknown wine were subsequently inserted into the discriminant function and the resultant scalar values compared to the centroid coordinates for each of the cultivars. The unknown wine is then assigned to the group to which the Euclidian distance is shortest, in this case the Chardonnay wines (Euclidean distance 1.39, compared to other white wines > 4.73 and red wines > 6.12).

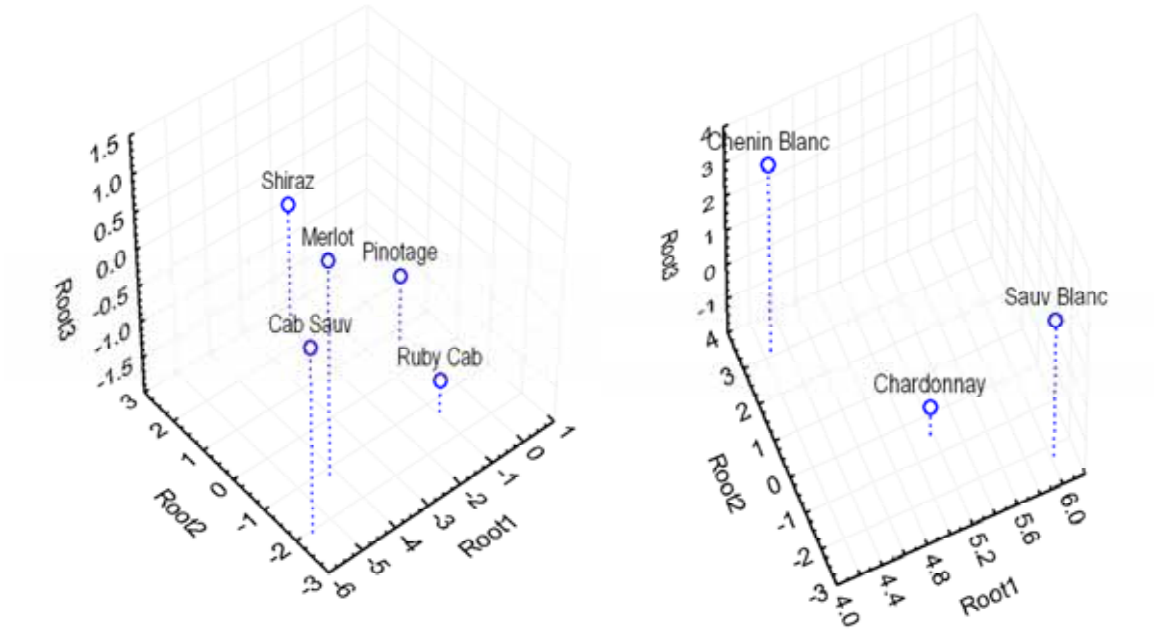

Figure 7. Scatter 3D plot of group centroids for red (left) and white (right) wine cultivars. The red and white wines are separated for the sake of clarity.

To determine the probability of this assignment, the variance radii for each centroid was calculated using Fisher's F-statistics and the risk value (α) determined. From the risk factor, the probability P can be calculated ($P = 1 - \alpha$). Using this method, the unknown wine sample is identified as Chardonnay with a 97.3 % probability. (The probability for Sauvignon Blanc is 2.0%, and less than 0.1% for the other cultivars). Following this prediction, the unknown sample was inserted into the modeling data set and new classification was performed. From this stepwise standard LDA the posterior probability of this wine being a Chardonnay was calculated as 99.99%, thereby confirming the previous prediction.

In conclusion, a summary of the volatile compounds used in each of the classifications presented above is depicted in **Table 4**. *trans* isoeugenol and isoamyl acetate were used for the classification of red wines, but not the complete data set. The content of the former is on average higher, and for the latter lower, in red wines compared to white wines. Similarly, 4-vinyl-guaicol, ethyl isopentyl succinate were used to classify white wines, but were not used for the complete data set. Mean values for the latter compound were on average lower in white wines, while for the former levels were similar for red and white wines. These observations can be ascribed to significant variation in levels of these compounds within the complete data set, which serves to obscure any cultivar-related differences and thereby precludes their utility in an overall classification function for red and white wines.

Other volatile compounds were used in the classification function of the complete set of wines, although they were not used to classify either red or white wines separately. These compounds generally display differences in mean levels between red and white cultivars. This increases the discriminatory power of these compounds for all wine cultivars, even though they might not be suitable to differentiate between only red or only white cultivars. Compounds included in this class are acetovanillone (higher levels in red), decanoic acid (higher in white), hexyl acetate (higher in white), ethyl isovalerate (higher in red) and formic acid (higher in Chardonnay and Sauvignon Blanc).

Table 4. Summary of volatile compounds utilized as variables in discriminant analyses of red and white wines.

Compound	white	red	red + white	Compound	white	red	red + white
alcohols				**acids**			
isoamyl alcohol		X	X	formic acid			X
1-butanol	X		X	acetic acid			
1-hexanol		X	X	propanoic acid		X	X
1-octanol				octanoic acid		X	X
β-phenylethyl alcohol		X	X	decanoic acid			X
furfuryl alcohol				dodecanoic acid			
phenols				**esters**			
guaiacol				isoamyl acetate		X	
4-vinylguaiacol	X			hexylacetate			X
cis-isoeugenol		X	X	ethylphenyl acetate	X	X	X
trans-isoeugenol		X		β-phenyl ethyl acetate			
aldehydes				ethyl lactate		X	X
furfural	X	X	X	ethyl butyrate			
5-hydroxymethyl furfural				ethyl isovalerate			X
vanillin				ethyl hexanoate			
ketones				ethyl octanoate			
2-octanone	X		X	ethyl decanoate			
acetovanillone			X	ethyl-9-decenoate	X		X
lactones				ethyl dodecanoate		X	X
γ-butyrolactone				diethyl succinate	X	X	X
(Z)-whiskey lactone				ethyl isopentyl succinate	X		
				ethyl vanillate		X	X

The following compounds were not used in any of the LDA functions: γ-butyrolactone, (Z)-whiskey lactone, furfuryl alcohol, guaiacol, 5-hydroxymethyl furfural, vanillin, acetic acid, dodecanoic acid, β-phenyl ethyl acetate, ethyl-butyrate, ethyl-hexanoate, ethyl-octanoate and ethyl-decanoate. With the exception of ethyl decanoate and dodecanoic acid, none of these compounds showed significant differences between cultivars by ANOVA for red or white wines. This indicates that, at least for the current data set, fewer compounds may be quantified while still providing successful differentiation of wine samples according to cultivar. Contrary

to our findings for red and white wines, ethyl decanoate has previously been used to successfully classify Nebbiollo-based Italian wines according to geographical origin by SLDA [76] while ethyl octanoate has been used to classify white and Rosé Spanish wines according to cultivar [78].

It is important to note that the content of major wine volatiles analysed in the current study do not necessarily have a significant bearing on the perceived flavour characteristics of the wines. A number of these compounds are typically present at levels below their odour threshold values, in other words with odour activity values (OAVs) below 1. Depending on the nature of wine sample, examples of compounds with OAVs below 1 include β-phenylethyl acetate, 1-hexanol, guaiacol, ethyl vanillate, acetovanillone, decanoic acid, furfural, furfuryl alcohol and diethyl succinate. Examples of compounds typically present above their threshold values include isoamyl alcohol, ethyl decanoate, ethyl butyrate, ethyl hexanoate, isoamyl acetate, ethyl octanoate, hexanoic acid, butyric acid, octanoic acid, hexanoic acid, 4-vinylguaiacol, Z-whiskey lactone and vanillin [71,79]. Relation of aroma characteristics to chemical composition is further complicated by the fact that high OAVs do not guarantee an impact on wine flavour. Aroma model and emission experiments indicate that especially compounds such as fusel alcohols, acids, esters and some volatile phenols (ie. the majority of the compounds analysed here) often do not contribute to wine aroma individually, even though they are present at levels significantly above their thresholds [50]. Rather, it is commonly accepted that cultivar-specific flavour can often be ascribed to trace-level "varietal" aroma compounds such as monoterpenes (Muscat wines), norisoprenoids [80], pyrazines [81] (Sauvignon Blanc, Cabernet Sauvignon and Cabernet Franc), thiols and mercaptans [68,82]. However, analysis of these so-called impact odorants is significantly more labour- and time intensive and expensive [68,82]. From this perspective, the simple screening method presented here should prove advantageous in studies where the aim is to classify large numbers of wine samples according to cultivar, and as such competes with screening methods utilizing SPME and LLE.

The same set of wines used in the current study has previously been used to classify wine cultivars by anthocyanin- [83] and non-coloured phenolic content [62]. Compared to

classification according to non-volatile phenolics, the current method based on the major wine volatiles offers the advantages of simple (although time-consuming) sample preparation, straightforward quantitation, and provides a better overall classification of wines according to cultivar.

In conclusion, the principal value of the current classification lies in the fact that wine samples were not selected according to pre-defined criteria in order to reduce variability due to age, oenological practice or geographic origin. It is well-known that each of these factors significantly affects the volatile composition of wines. In fact, PCA and CA have shown that most of the variability in the volatile data for the selected set of wines can be related to wood ageing, fermentation practice and wine age. It should furthermore be noted that in South Africa, by law, wine may be labelled as a single cultivar if it contains at least 85% of the specified cultivar. Therefore any number of the analysed wines may contain up to a maximum of 15% of a different variety, which may logically serve to hamper attempts at classifying these wines according to cultivar. However, despite these contributions to variability, we have shown that it is possible to extract the information from the data set to allow successful classification of wine samples according to cultivar. It would seem that the major volatile composition contains a substantial amount of information that can fruitfully be employed in combination with chemometric methods. Further work is required to investigate the suitability of major volatile data for the classification of wines according to alternative criteria (geographical origin, vintage, sensory data, detection of adulteration).

4.4 Conclusions

A SBSE-TD-GC-MS method suitable for the routine analysis of major wine volatile- and semi-volatile components has successfully been developed. The method is characterised by good sensitivity, repeatability and robustness for the analysis of a variety of volatiles of diverse physico-chemical properties, and as such presents an attractive alternative to liquid-liquid extraction and other sorption based methods for the routine screening of major wine volatiles. Following quantitation of 38 volatile compounds in South African red and white wines using

the described method, statistical investigation of the generated data highlighted some interesting aspects. Un-supervised pattern recognition techniques (PCA and CA) indicated that the variation in volatile content in the analysed wines could largely be ascribed to corresponding variations in wine age and fermentation practices. Despite these findings, however, discriminant analysis demonstrated that sufficient cultivar-related differences are contained in the volatile data set to allow correct classification of wine samples according to cultivar. Importantly, this classification is obtained irrespective of the effects of vintage, geographical origin, oenological practices or other differentiating factors. The combination of simple and reliable screening methods as developed in this paper with chemometric methods of analysis therefore provides a powerful tool to study the volatile composition of wine samples.

4.5 References

[1] A.L. Waterhouse, S.E. Ebeler (Eds), Chemistry of Wine Flavor, Oxford University Press, New York, 1998, pp 31-80.

[2] L.M. Reid, C.P. O'Donnell, G. Downey, Trends Food Sci. Technol. 17 (2006) 344.

[3] A. Mallouchos, M. Komaitis, A. Koutinas, M. Kanellaki, Food Chem. 80 (2003) 109.

[4] C. Ortega, R. Lopez, J. Cacho, V. Ferreira, J. Chromatogr. A 923 (2001) 205.

[5] V. Ferreira, I. Jarauta, L. Ortega, J. Cacho, J. Chromatogr. A 1025 (2004) 147.

[6] E. Campo, J. Cacho, V. Ferreira, J. Chromatogr. A 1140 (2007) 180.

[7] S.A. Wercinski (Ed.), Solid Phase Microextraction: A Practical Guide Marcel Dekker Inc., New Cork, 1999, pp 177.

[8] J. Pawliszyn, Solid Phase Microextraction: Theory and Practice Wiley VHC, Inc., New York, 1997, pp 97.

[9] E. Baltussen, C. Cramers, P. Sandra, Anal. Bioanal. Chem. V373 (2002) 3.

[10] J.L. Gomez-Ariza, T. Garcia-Barrera, F. Lorenzo, R. Beltran, J. Chromatogr. A 1112 (2006) 133.

[11] A. Martinez-Urunuela, J.M. Gonzalez-Saiz, C. Pizarro, J. Chromatogr. A 1089 (2005) 31.

[12] J.D. Carrillo, A. Garrido-Lopez, M.T. Tena, J. Chromatogr.. A 1102 (2006) 25.

[13] J.S. Camara, M.A. Alves, J.C. Marques, Anal. Chim. Acta 555 (2006) 191.

[14] Q. Wang, J. O'Reilly, J. Pawliszyn, J. Chromatogr. A 1071 (2005) 147.

[15] R. Lopez, A.C. Lapena, J. Cacho, V. Ferreira, J. Chromatogr. A 1143 (2007) 8.

[16] S. Francioli, J. Torrens, M. Riu-Aumatell, E. Lopez-Tamames, S. Buxaderas, Am. J. Enol. Vitic. 54 (2003) 158.

[17] E. Sanchez-Palomo, M.C. Diaz-Maroto, M.S. Perez-Coello, Talanta 66 (2005) 1152.

[18] K.L. Howard, J.H. Mike, R. Riesen, Am. J. Enol. Vitic. 56 (2005) 37.

[19] J. Aronson, S.E. Ebeler, Am. J. Enol. Vitic. 55 (2004) 13.

[20] S. Rocha, V. Ramalheira, A. Barros, I. Delgadillo, M.A. Coimbra, J. Agric. Food Chem. 49 (2001) 5142.

[21] E. Baltussen, P. Sandra, F. David, C.Cramers, J. Microcolumn Sep. 11 (1999) 737.

[22] A.G.J. Tredoux, H.H. Lauer, T, Heideman, P. Sandra, J. High Resolut. Chromatogr. 23 (2000) 644.

[23] N. Ochiai, K. Sasamoto, S. Daishima, A.C. Heiden, A. Hoffmann, J. Chromatogr. A 986 (2003) 101.

[24] N. Ochiai, K. Sasamoto, M. Takino, S. Yamashita, S. Daishima, A. Heiden, A. Hoffmann, Anal. Bioanal. Chem. 373 (2002) 56.

[25] C. Bicchi, C. Cordero, P. Rubiolo, P. Sandra, Eur. Food Res. Technol. 216 (2003) 449.

[26] P. Sandra, B. Tienpont, F. David, J. Chromatogr. A 1000 (2003) 299.

[27] A. Stopforth, A. Tredoux, A. Crouch, P.v. Helden, P. Sandra, J. Chromatogr. A 1071 (2005) 135.

[28] B. Tienpont, F. David, K. Desmet, P. Sandra, Anal. Bioanal. Chem. 373 (2002) 46.

[29] A. Stopforth, B.V. Burger, A.M. Crouch, P. Sandra, J. Chromatogr. B 856 (2007) 156.

[30] C. Yu, B. Hu, J. Chromatogr. A 1160 (2007) 71.

[31] E. Van Hoeck, F. David, P. Sandra, J. Chromatogr. A 1157 (2007) 1-9.

[32] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.

[33] C. Lorenzo, A. Zalacain, G.L. Alonso, M.R. Salinas, J. Chromatogr. A 1114 (2006) 250.

[34] H. Yoji, M. Kevin, A.B. Gayle, L.T. Randell, P.P. Alan, Anal. Bioanal. Chem. V375 (2003) 948.

[35] R.M. Callejon, A.M. Troncoso, M.L. Morales, Talanta 71 (2007) 2092.

[36] J. Diez, C. Dominguez, D.A. Guillen, R. Veas, C.G. Barroso, J. Chromatogr. A 1025 (2004) 263.

[37] P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.

[38] J. Marin, A. Zalacain, C. De Miguel, G.L. Alonso, M.R. Salinas, J. Chromatogr. A 1098 (2005) 1.

[39] F. Luan, A. Mosandl, M. Gubesch, M. Wust, J. Chromatogr. A 1112 (2006) 369.

[40] F. Tateo, M. Bononi, J. Food Compos. Anal. 16 (2003) 721.

[41] R.F. Alves, A.M.D. Nascimento, J.M.F. Nogueira, Anal. Chim. Acta 546 (2005) 11.

[42] A. Juan-Garcia, J. Manes, G. Font, Y. Pico, J. Chromatogr. A 1050 (2004) 119.

[43] A. Zalacain, J. Marin, G.L. Alonso, M.R. Salinas, Talanta 71 (2007) 1610.

[44] M.J. Gomez-Miguez, J.F. Cacho, V. Ferreira, I.M. Vicario, F.J. Heredia, Food Chem. 100 (2007) 1464.

[45] D.J. Caven-Quantrill, A.J. Bouglass, Flav. Frag. J. 2007 206.

[46] D.J. Caven-Quantrill, A.J. Buglass, J. Chromatogr. A 1117 (2006) 121.

[47] Y. Fang, M.C. Qian, J. Agric. Food Chem. 54 (2006) 8567.

[48] M.R. Salinas, A. Zalacain, F. Pardo, G.L. Alonso, J. Agric. Food Chem. 52 (2004) 4821.

[49] B.T. Weldegergis, A.G.J. Tredoux, A.M. Crouch, J. Agric. Food Chem. 55 (2007) 8696.

[50] A. Escudero, B. Gogorza, M.A. Melus, N. Ortin, J. Cacho, V. Ferreira, J. Agric. Food Chem. 52 (2004) 3516.

[51] N. Boudaoud, L. Eveleigh, J. Agric. Food Chem. 51 (2003) 1530.

[52] K.S. Howell, D. Cozzolino, E.J. Bartowsky, G.H. Fleet, P.A. Henschke, FEMS Yeast Research 6 (2006) 91.

[53] L.S. Wu, R.E. Bargmann, J.J. Powers, J. of Food Sci. 42 (1977) 944.

[54] W.O. Kwan, B.R. Kowalski, J. of Food Sci. 43 (1978) 1320.

[55] B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, in Handbook of Chemometircs and Qualimetrics: Part B, Elsevier, 1997, p. 87.

[56] I.E. Frank, B.R. Kowalski, Anal. Chim. Acta 162 (1984) 241.

[57] A. Calleja, E. Falque, Food Chem. 90 (2005) 357.

[58] M. Gil, J.M. Cabellos, T. Arroyo, M. Prodanov, Anal. Chim. Acta 563 (2006) 145.

[59] J.S. Camara, M.A. Alves, J.C. Marques, Food Chem, Food Chem. 101 (2007) 475.

[60] J.L. Aleixandre, V. Lizama, I. Alvarez, M.J. Garcia, J. Agric. Food Chem. 50 (2002) 751.

[61] K. Heberger, E. Csomos, L. Simon-Sarkadi, J. Agric. Food Chem. 51 (2003) 8055.

[62] A. de Villiers, P. Majek, F. Lynen, A. Crouch, H. Lauer, P. Sandra, Eur. Food Res. Technol. 221 (2005) 520.

[63] S. Frias, J.E. Conde, J.J. Rodriguez-Bencomo, F. Garcia-Montelongo, J.P. Perez-Trujillo, Talanta 59 (2003) 335.

[64] I.S. Arvanitoyannis, M.N. Katsota, E.P. Psarra, E.H. Soufleros, S. Kallithraka, Tr. Food Sci. Technol. 10 (1999) 321.

[65] B.R. Kowalski, C.F. Bender, J. Am. Chem. Soc. 94 (1972) 5632.

[66] S. Selli, T. Cabaroglu, A. Canbas, H. Erten, C. Nurgel, J.P. Lepoutre, Z. Gunata, Food Chem. 85 (2004) 207.

[67] S.J. Lee, A.C. Noble, J. Agric. Food Chem. 51 (2003) 8036.

[68] V. Ferreira, N. Ortin, J.F. Cacho, J. Chromatogr. A 1143 (2007) 190.

[69] L. Setkova, S. Risticevic, J. Pawliszyn, J. Chromatogr. A 1147 (2007) 213.

[70] E. Campo, V. Ferreira, A. Escudero, J.C. Marques, J. Cacho, Anal. Chim. Acta 563 (2006) 180.

[71] V. Ferreira, N. Ortin, A. Escudero, R. Lopez, J. Cacho, J. Agric. Food Chem. 50 (2002) 4048.

[72] P.J. Spillman, A.P. Pollnitz, D. Liacopoulos, K.H. Pardon, M.A. Sefton, J. Agric. Food Chem. 46 (1998) 657.

[73] J. Goode, in Wine Science, Octopus Publishing Group, New York, 2005, p. 187.

[74] Y. Ribereau-Gayon, A. Glories, Handbook of Enology and Viticulture - Volume 2 The Chemistry of Wine, Stabilization and Treatments, John Wiley & Sons, New York, 2000, pp209-254.

[75] A. de Villiers, F. Lynen, A. Crouch, P. Sandra, Chromatographia 59 (2004) 403.

[76] E. Marengo, M. Aceto, V. Maurino, J. Chromatogr. A 943 (2002) 123.

[77] A. de Villiers, F. Lynen, A. Crouch, P. Sandra, Eur. Food Res. Technol. 217 (2003) 535.

[78] M.A. Pozo-Bayon, E. Pueyo, P.J. Martin-Alvarez, M.C. Polo, J. Chromatogr. A 922 (2001) 267.

[79] L. Cullere, A. Escudero, J. Cacho, V. Ferreira, J. Agric. Food Chem. 52 (2004) 1653.

[80] B. Pineau, J.C. Barbe, C. VanLeeuwen, D. Dubourdieu, J. Agric. Food Chem. 55 (2007) 4103.

[81] C. Sala, M. Mestres, M.P. Marti, O. Busto, J. Guasch, J. Chromatogr. A 953 (2002) 1.

[82] L. Mateo-Vivaracho, J. Cacho, V. Ferreira, J. Chromatogr. A 1146 (2007) 242.

[83] A. de Villiers, G. Vanhoenacker, P. Majek, P. Sandra, J. Chromatogr. A 1054 (2004) 195.

5

Stir Bar Sorptive Extraction Applied to the Determination of Dicarboximide Fungicides in Wine*

*Published as "Stir Bar Sorptive Extraction Applied to the Determination of Dicarboximide Fungicides in Wine", P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.

5.1 Introduction

Fungicides are intensively used in the wine industry and they are typically dosed close to or post harvest [1,2]. Iprodione, procymidone and vinclozolin are not fully removed or metabolized during the winemaking process and residues are distinctively present in wine and distillates [1-3]. Iprodione, procymidone and vinclozolin are found to act as androgen receptors and show xeno-endocrine disrupting properties in rats [4,5] and monkeys [6]. It was stated that it is very likely that humans would adversely be affected if the human fetus is exposed to sufficient levels during critical stages of neonatal life.

The analysis of dicarboximide fungicides has been described by many groups and is also incorporated in the Food and Drug Administration (FDA) Pesticide Residue Monitoring Program of food samples. To the best of our knowledge, there are no regulations within the European Community. For the analysis of aqueous food products like wine and cherry juice, sample preparation mainly consists of liquid-liquid extraction (LLE) or solid phase extraction (SPE). LLE with *n*-hexane [7] or acetone-dichloromethane (1:1) [8] followed by capillary GC analysis has been described. Selectivity and sensitivity is obtained by using electron capture detection (ECD) or mass selective detection (MSD). Limits of detection are in the order of 1 µg/L for vinclozolin. A valid alternative is enrichment on SPE cartridges packed with C_{18} or carbon black [11,12]. Using capillary GC/ion trap mass spectrometry (CGC-ITD) determinations in the ng/L range for vinclozolin and in the µg/L range for iprodione could be reached. The advantage of SPE is that the sample preparation can be fully automated [13]. Another very elegant and solvent free enrichment technique for aqueous samples is solid-phase micro-extraction (SPME) [14,15]. In SPME, solutes are (ad)sorbed into a specific layer coated onto a fused silica fiber. SPME has been applied to the analysis of fungicides in water and wine samples [16-18]. Fibers coated with 100 µm polydimethylsiloxane (PDMS) (approximately 0.5 µL) were used in combination with splitless thermal desorption of the fiber in the GC injector allowing quantitative transfer of the enriched analytes into the capillary column. On PDMS, solute enrichment is by partitioning between the polymer and the aqueous phase and the enrichment is controlled by the distribution coefficients. To increase recovery

rates, modified fibers (PDMS-divinylbenzene 65 µm) or fibers with polar coatings (polacrylate 85 µm and carbowax-divinylbenzene 65 µm) were applied. Limits of detection were in the order of 50 ng/L with good linearity up to 10 µg/L level.

Recently, a novel sorptive extraction technique for aqueous samples namely stir bar sorptive extraction (SBSE) was described [19]. In SBSE, a magnetic rod encapsulated in a glass jacket and coated with a relatively high amount (25 to 125 µL) of PDMS is placed in the aqueous sample and stirred for a given time. The stir bar is then thermally desorbed on-line with capillary GC-MS. The stir bars, commercialised under the name 'Twister' (Gerstel GmbH, Mülheim a/d Ruhr, Germany) allow a 500-fold increase in enrichment, and thus sensitivity, compared to SPME with 100 µm PDMS fibers. SBSE was used for the analysis of contaminants in wine like phthalates, nonylphenols, organochloro pesticides [20] and 2,4,6-trichloroanisole [21], for the determination of benzoic acid in soft drinks [22], for the analysis of polychlorobiphenyls (PCBs) in sperm [23], etc.

In this contribution the analysis of procymidone, vinclozolin and iprodione in spiked water samples and in several white wines by SBSE in combination with thermal desorption and on-line capillary GC-MS analysis is reported. Iprodione, a thermolabile fungicide, was measured through its degradation product 3,5-dichlorophenyl hydantoin. To verify the accuracy of this method, SBSE followed by liquid desorption and analysis by liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy (SBSE-LD-LC-APCI-MS) was also applied.

5.2 Experimental

5.2.1 Sample preparation

10 mL ChromaSolv water (Riedel-de Haën, Seelze, Germany) or 10 mL undiluted wine was poured into a headspace vial of 20 mL. For recovery studies and standard addition quantification, an appropriate amount of a methanol solution of procymidone, vinclozolin and iprodione (Sigma-Aldrich, Bornem, Belgium) was added. A twister containing 25 µL PDMS

was stirred in the sample for 40 min at a speed of 1400 rpm. After sampling, the twister was rinsed in distilled water and residual water droplets were removed with tissue paper. For thermal desorption (TD), the stir bar was put into a glass tube of 187 mm L, 6 mm O.D. and 4 mm I.D.). Blank runs of the stir bar were done before and after each analysis and no memory effects occurred for the target solutes. For liquid desorption (LD), the stir bar was extracted with 1 mL acetonitrile in an ultrasonic bath for 15 min. 5 µL of the extract was injected for LC-MS analysis.

5.2.2 Instrumental set-up

5.2.2.1 Thermal desorption-capillary GC-MS (TD- CGC-MS)

A TDS-2 thermodesorption unit (Gerstel GmbH, Mülheim a/d Ruhr, Germany) was mounted on a 6890 Agilent GC (Agilent Technologies, Litlle Falls, DE). The analytes were cryofocused in a programmed temperature vaporizing (PTV) injector (CIS-4, Gerstel) at –150°C with liquid nitrogen prior to injection. An empty baffled liner was used in the PTV. For splitless thermal desorption, the TDS-2 was ramped from 30°C to 300°C at a rate of 60°C/min and the upper temperature was held for 10 min. Splitless injection (2.5 min) was performed by ramping the PTV from –150° to 300°C at a rate of 600°C/min. Capillary GC analyses were performed on a 30 m L × 0.25 mm I.D., 0.25 µm d_f HP-5MS column (Agilent Technologies) with helium as carrier gas. The oven was sequentially programmed from 70°C (2.5 min) to 150°C at a rate of 25°C/min, to 200°C at a rate of 3°C/min and to 300°C at a rate of 8°C/min. The Agilent 5973 mass spectrometric detector was operated in the scan mode (m/z 50-300) or in the selected ion monitoring mode with a dwell time of 100 ms and 1.44 cycles/s.

5.2.2.2 Liquid chromatography-atmospheric pressure chemical ionization mass spectroscopy (LC-APCI-MS).

LC-APCI-MS analyses were carried out on a benchtop HP1100 Series LC-MSD instrument (Agilent Technologies, Waldbronn, Germany). A Phenomenex Luna C18 column, 250 mm L × 4.6 mm I.D., 5 µm particle size (Bester, Amstelveen, The Netherlands) was used. The mobile phase consisted of water (solvent A) and 10% tetrahydrofuran in methanol (solvent B). A

gradient from 70% B at 0 min to 80% B at 20 min was applied. The flow rate was 1 mL/min and the analyses were performed at 22°C. The injection volume was 5 µL. Atmospheric pressure chemical ionisation (APCI) was carried out in the negative ion mode at a mass range between m/z 200-350. The fragmentor voltage was set to 70 V. The nitrogen drying gas was at 350°C with a flow rate of 5 L/min. The nebulizer pressure was 60 psig. The capillary voltage was 4000 V and the corona current was 25 µA. Analyses in the selected ion monitoring (SIM) mode for iprodione were carried out at m/z 242.9, 245.0 and 246.8.

5.3 Results and discussion

5.3.1 SBSE-TD-CGC-MS analysis of spiked water samples

Water samples were spiked in a concentration range between 0.5 and 100 µg/L for SBSE-TD-CGC-MS analysis. **Figure 1** shows the extracted ion chromatogram at m/z 187 (iprodione and degradation product), 283 (procymidone) and 285 (vinchlozolin) at the 10 µg/L level. The recorded spectra are shown in **Figure 2** together with the structures of the fungicides. Vinclozolin (**1**) and procymidone (**2**) can easily be identified, whereas iprodione (**4**) shows a relatively low abundance. It is known that iprodione (1-isopropylcarbamoyl-3-(3,5-dichlorophenyl) hydantoin) shows sample decomposition in capillary GC at temperatures >200°C [24]. The carbamate-like compound is degraded for 90% to the more stable (3,5-dichlorophenyl) hydantoin (**3**). The degradation rate is expressed as the ratio of the peak areas (extracted ion at m/z 187) of the degradation product versus those of the sum of iprodione and (3,5-dichlorophenyl) hydantoin. Decomposition not only occurs during thermal desorption of the stir bar at 300°C and during transfer of the analyte in the hot transfer line (300°C) but additionally the solute only elutes at a temperature of 245°C (retention time = 28 min) and is therefore also degraded in the capillary column itself. The ratio of the peak areas of iprodione and its degradation product was constant for all SBSE-TD-CGC-MS analyses performed and quantification could be done on (3,5-dichlorophenyl) hydantoin.

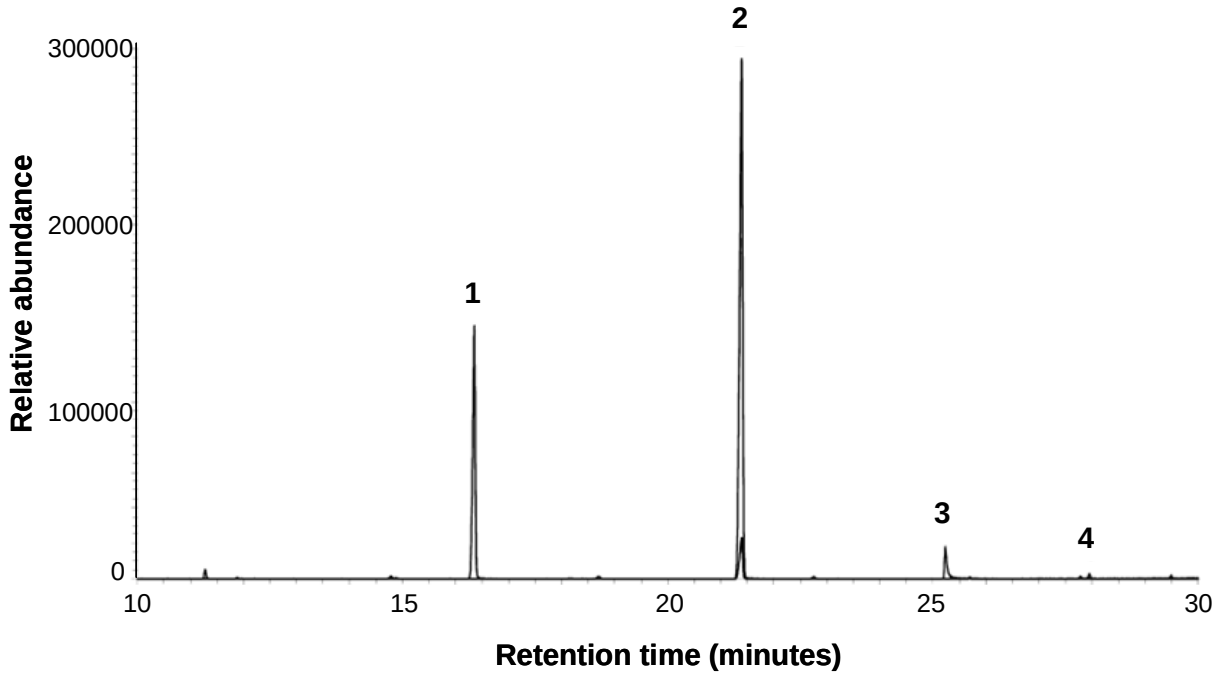

Figure 1. Extracted ion chromatogram at m/z 187, 283 and 285 of a SBSE-TD-CGC-MS analysis of water spiked at 10 µg/L with vinclozolin (**1**), procymidone (**2**) and iprodione (**4**). Peak **3** 3,5-dichlorophenyl) hydantoin is the degradation product of iprodione.

Recoveries of the target solutes by SBSE were calculated by comparing the peak areas with those of a direct analysis of a standard solution spiked on glass wool placed in a thermal desorption tube (**Table 1)**. Theoretical recoveries were calculated using the theory described by Baltussen *et al*. [19]. Octanol-water distribution coefficients ($K_{o/w}$) of the analyzed compounds were calculated with the *SRC-KOWWIN* software package (Syracuse Research Corporation, Syracuse, NY, USA) according to a 'fragment constant' estimation methodology [25].

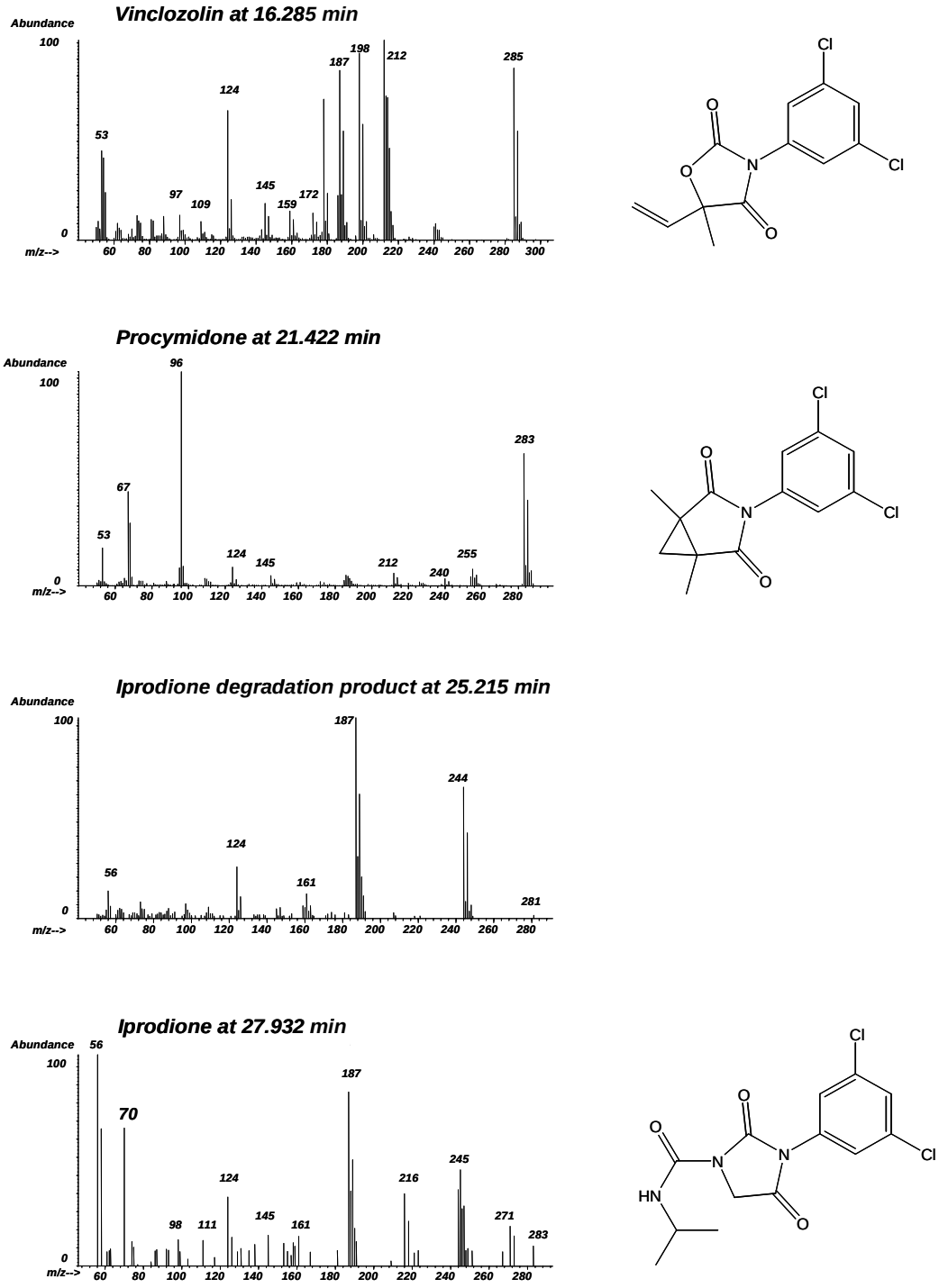

Figure 2. Mass spectra recorded at 10 µg/L (Figure 1) and structures of the fungicides.

Theoretical recoveries are somewhat higher than the experimental recoveries. This indicates that equilibrium of the solutes between the PDMS coating and the sample is not yet attained after 40 min sampling. However, reaching equilibrium conditions is not stringent as long as the sampling conditions are kept constant for calibration. The difference between the theoretical and real recovery of iprodione is relatively high and this can be explained by its unequal degradation rate in thermal desorption from an inert PDMS stir bar (SBSE) or a plug of glass wool (injection standard). Repeatability of SBSE-CGC-MS analysis was verified by analyzing six samples spiked at the 10 µg/L level. Integration of the peaks was done in the extracted ion mode at m/z 187, 283 and 285. Relative standard deviations on the peak areas were 2% and 1% for vinclozolin and procymidone, respectively. For iprodione the precision was 7% RSD. Limits of detection (LOD) for full scan MS were 0.2 µg/L for vinclozolin and procymidone and 2 µg/L for iprodione. The limits of quantification were set at 0.5 µg/L and 5 µg/L, respectively. When operating the mass spectrometer in the ion monitoring mode (m/z 187, 283 and 285) the limits of detection were in the order of 2 ng/L for vinclozolin and procymidone and of 50 ng/L for iprodione. Linearity was tested in a concentration range between 0.5 and 100 µg/L using the MSD in the full scan mode and correlation coefficients were all above 0.997 (**Figure 3,** full line).

5.3.2 SBSE-TD-CGC-MS analysis of wines

SBSE can be used to profile flavour compounds in wine [26], to dose µg/L amounts of contaminants [20] and ng/L concentrations of off-flavours [21]. This illustrates the very versatile and universal character of sorptive extraction.

White wines and sparkling wines of different origin (France, Italy, South Africa) were analyzed for the presence of vinclozolin, procymidone and iprodione. The relative large amount of PDMS (25 µL) allows, even for trace analysis, to use the mass spectrometer in the full scan mode. **Figure 4** shows the ion extracted chromatogram (m/z 187, 283 and 285) of an Italian sparkling wine. The three target fungicides can easily be detected. As for the water sample, the iprodione degradation product (**3**) is much larger than iprodione (**4**). Degradation rates were relatively constant and ranged between 89 and 91% for all white wines. Quantification of iprodione was thus done using the peak areas of the degradation product.

Table 1. SBSE of fungicides: quantification ions, log *Ko/w*, recoveries (%) and repeatability (n = 6) for vinclozolin, procymidone and iprodione in water and wine. Iprodion is measured as its degradation product 3,5-dichlorophenyl hydantoin.

Fungicide	Log $K_{o/w}$	Quant. Ion[a]	Theoretical Recovery[b]	Recovery water[b]	Repeat-ability water[b]	Recovery wine[b]	Repeat-ability wine[b]
Vinclozolin	3.03	285	76	51	2	35	2
Procymidone	2.59	283	53	41	1	15	3
Iprodione	2.85	187	67	31	7	7	5

[a] Quantitative ion.
[b] Recovery and repeatability expressed as % and %RSD, respectively.

Repeatability of SBSE-TD-CGC-MS was tested by spiking 6 sub-samples of a South Africa blank white wine at the 10 µg/L level. Relative standard deviations of the peak areas never exceeded 5 % (**Table 1**). Sorptive enrichment is equilibrium driven and is therefore subjected to changes in sampling conditions like sampling time and temperature but also to matrix effects [22]. In the case of SBSE sampling of wine the reduction in recovery compared to that in water was already demonstrated for organochloro pesticides [20]. Therefore, quantification of the target compounds was done using standard addition. The three pesticides were added to 10 mL of each wine sample in concentrations between 1 and 100 µg/L by spiking 10 µL of the corresponding standard solutions in methanol. Quantification of the three target fungicides was done in the extracted ion mode at m/z 187, 283 and 285. Linear regression was performed and correlation coefficients were higher than 0.99 for vinclozolin and procymidone and higher than 0.98 for iprodione. This is illustrated in **Figure 3 (**dashed lines) showing the standard addition curves for the Italian sparkling wine. The fungicide recoveries were calculated for the South Africa blank white wine and are reduced to 35%, 15% and 7% for vinclozolin, procymidone and iprodione, respectively (**Table 1**). The slopes of the standard addition curves, however, are relatively constant for all white wines. This means that calibration can be done by spiking a blank reference wine. However, attention should be paid when red wines or very sweet wines, containing rather high amounts of polyphenolic polymers or saccharides, respectively, are analysed. Matrix effects should be evaluated in this case before quantification is performed.

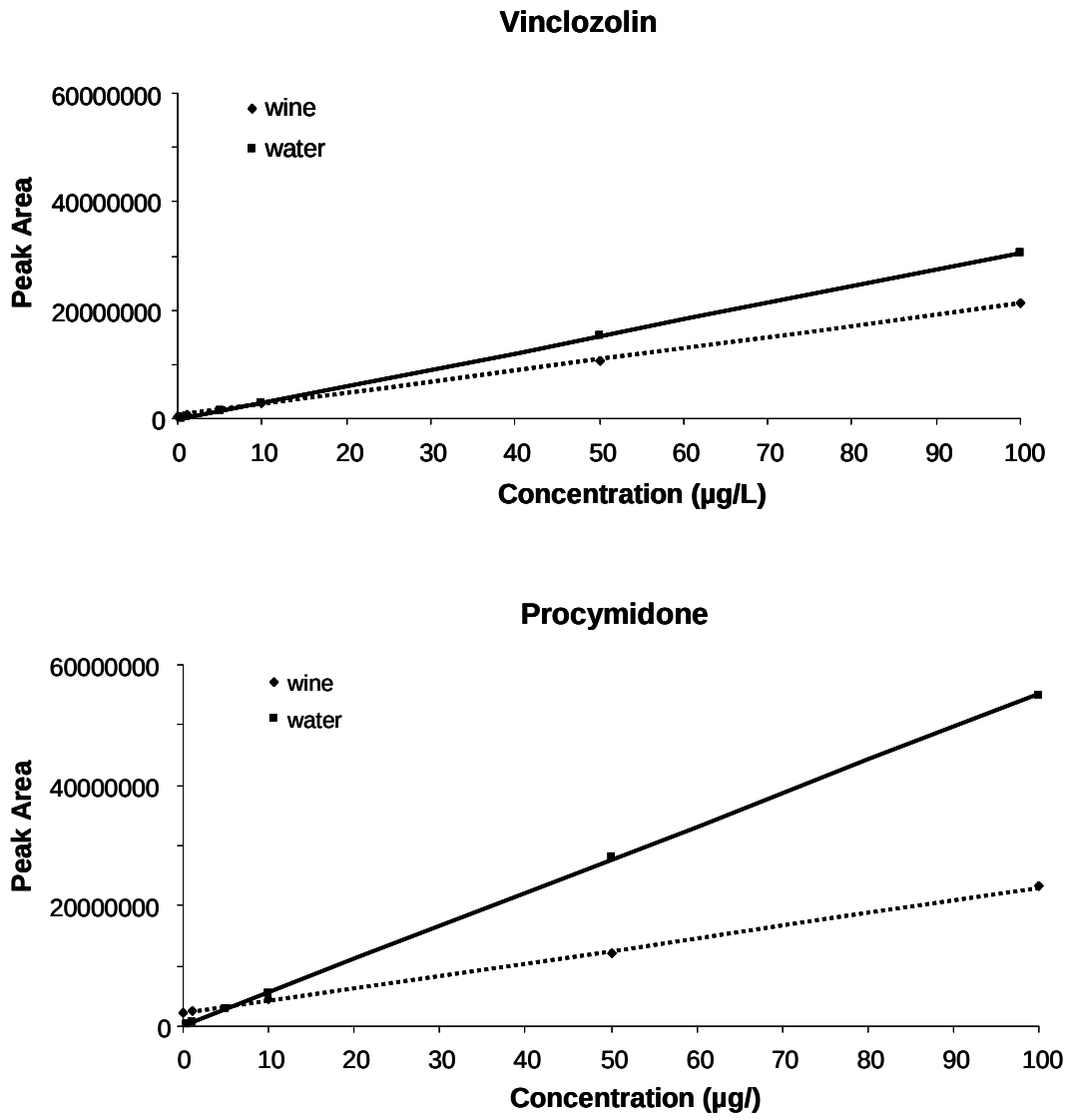

Figure 3. Linearity of SBSE-TD-CGC-MS of vinclozolin and procymidone spiked in water (full lines) and wine (dashed lines).

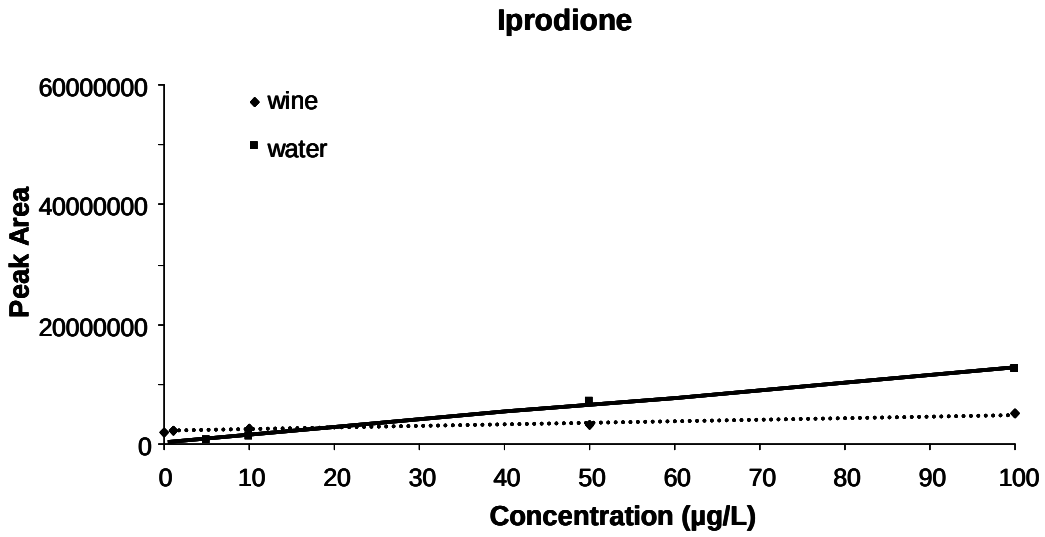

Figure 3 (continued). Linearity of SBSE-TD-CGC-MS of iprodione spiked in water (full lines) and wine (dashed lines). Iprodione was quantified through its degradation product.

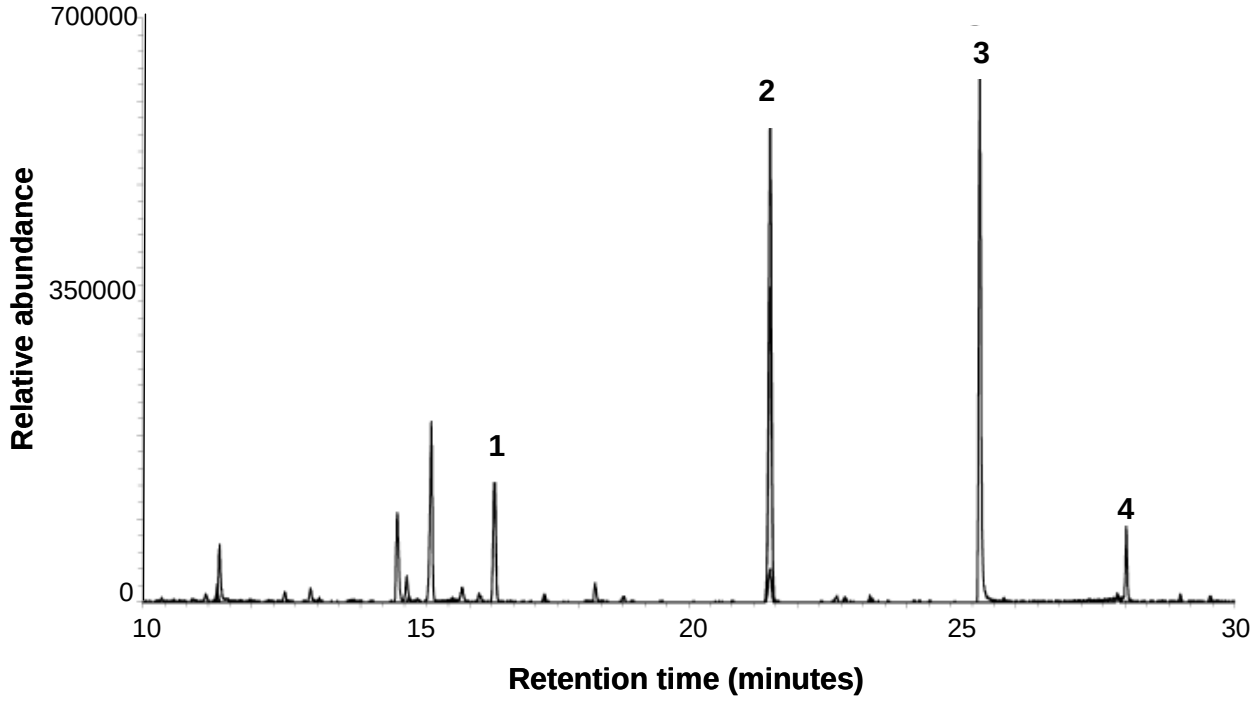

Figure 4. Extracted ion chromatogram at m/z 187, 283 and 285 of the SBSE-TD-CGC-MS analysis of Italian sparkling wine; vinclozolin (**1**), procymidone (**2**) 3,5-dichlorophenyl hyndatoin (**3**) and iprodione (**4**).

The carboximide fungicide concentrations in different positive white and sparkling wines were calculated using the standard addition curves and are listed in **Table 2**. Vinclozolin was only found in the Italian sparkling wine in low concentration (2.6 µg/L). Procymidone and iprodione are more abundant and their concentrations vary between 5 and 65.0 µg/L. In an Italian white wine, procymidone was present in higher concentration (61.3 µg/L) than iprodione (16.1 µg/L) while in the Italian sparkling wine iprodione (65.0 µg/L) was much higher in concentration than procymidone (10.7 µgL).

Table 2. Detected levels of the three fungicides in different white wines and blank wine used for spiking.

Wine	Vinclozolin (µg/L)	Procymidone (µg/L)	Iprodione (µg/L)
White wine, 1997, France	< 0.5	10.8	5.6
Champagne, 2000, France	< 0.5	5.5	3.7
Sparkling wine, 1995, Italy	2.6	10.7	65.0
White wine, 2000, Italy	< 0.5	61.3	16.1
Sparkling wine, 1998, South Africa	< 0.5	< 0.5	< 0.5

5.3.3 SBSE-LD-LC-MS analysis of wine

The accuracy of the SBSE-TD-CGC-MS method for the iprodione determination via the degradation product was verified by analyzing the Italian sparkling wine with SBSE-LD-LC-MS. The LC separation of procymidone, vinclozolin and iprodione was optimised on a C18 column using a gradient of water (solvent A) and 10% tetrahydrofuran in methanol (solvent B) as mobile phase. The mass spectrometric detector was used in the negative ion APCI mode. **Figure 5** shows the total ion chromatogram and **Figure 6** the mass spectra of a 30 mg/L standard mixture of the target fungicides. Interesting to note is that under the LC conditions applied vinclozolin (MW 285 dalton) and procymidone (MW 283 dalton) give ions at $(M+CH_3OH-H)^-$ while iprodione (MW 329 dalton) gives an $(M-CONHCH(CH_3)_2)^-$ ion. This illustrates the thermolabile character of iprodione because it decomposes under chemical ionization conditions. On the other hand, negative chemical ionization was giving much better

ionization and robustness than positive chemical ionization and positive and negative electrospray ionization. For quantification of iprodione, the MSD was used in the selected ion monitoring mode at m/z 242.9, 245.0, and 246.8. The linearity of LC-MS analysis for iprodione was tested in a concentration range between 10 µg/L and 10 mg/L. The correlation coefficient was >0.99 (**Figure 7**). Iprodione in the Italian sparkling wine was quantified by standard addition in the range 20 to 100 µg/L to a 10 mL sample. After SBSE sampling, the stir bar was liquid desorbed in 1 ml acetonitrile and the extract was injected for LC-MS analysis. This is the first application of SBSE followed by liquid desorption and this principle broadens the applicability of sorptive extraction on PDMS to non-volatile solutes. The correlation coefficient of the standard addition curve was >0.99 (**Figure 8**). The calculated concentration of iprodione in the Italian sparkling wine was 66 µg/L (RSD 4.6% for triplicate analysis). This proves that SBSE-TD-CGC-MS and SBSE-LD-LC-MS gives comparable data for iprodione.

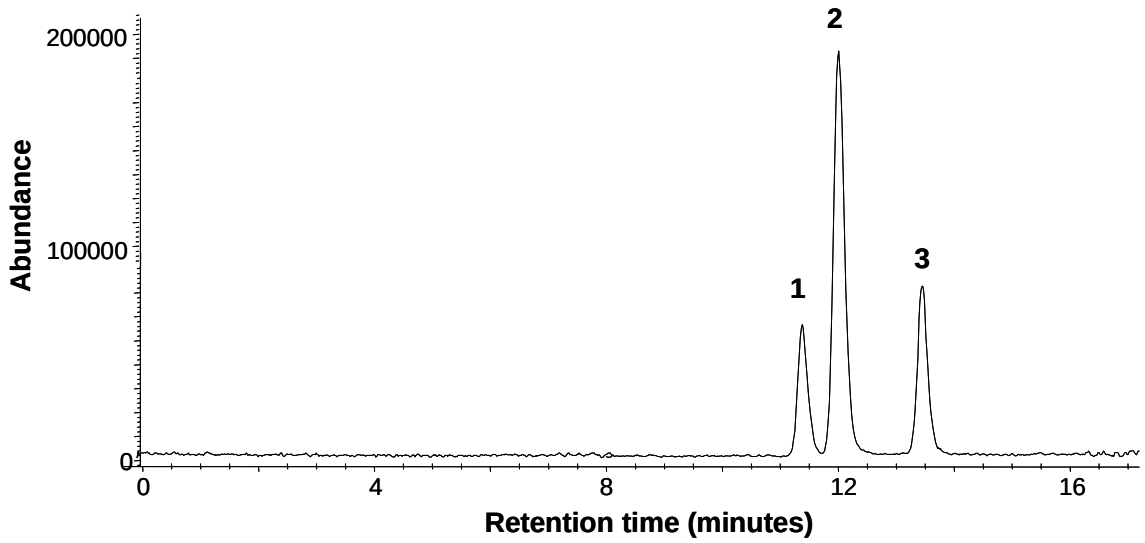

Figure 5. LC-APCI-MS chromatogram of a 30 mg/L standard mixture; procymidone (**1**), iprodione (**2**) and vinclozolin (**3**).

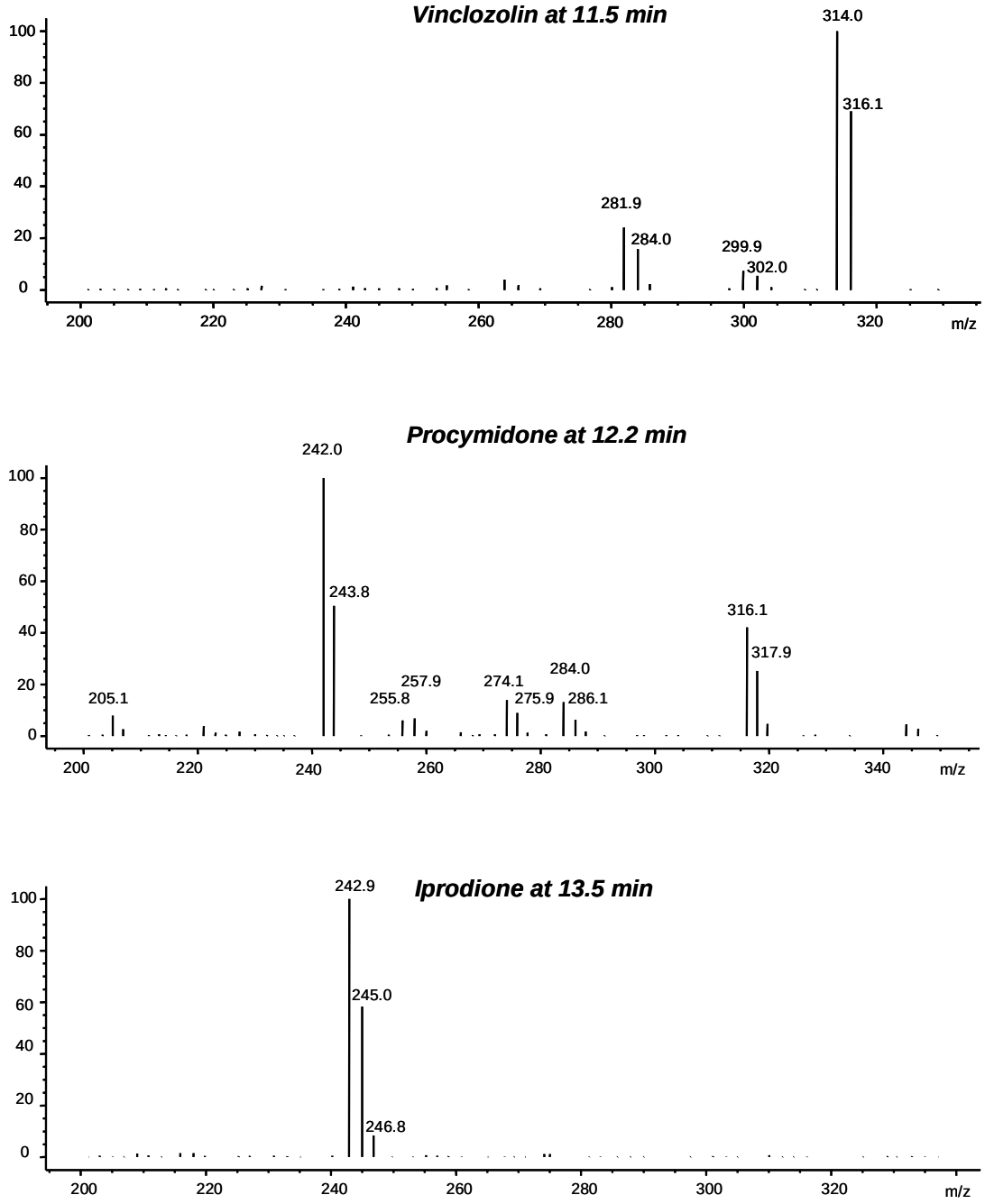

Figure 6. Mass spectra, LC-negative ion APCI-MS of procymidone (**1**), iprodione (**2**) and vinclozolin (**3**).

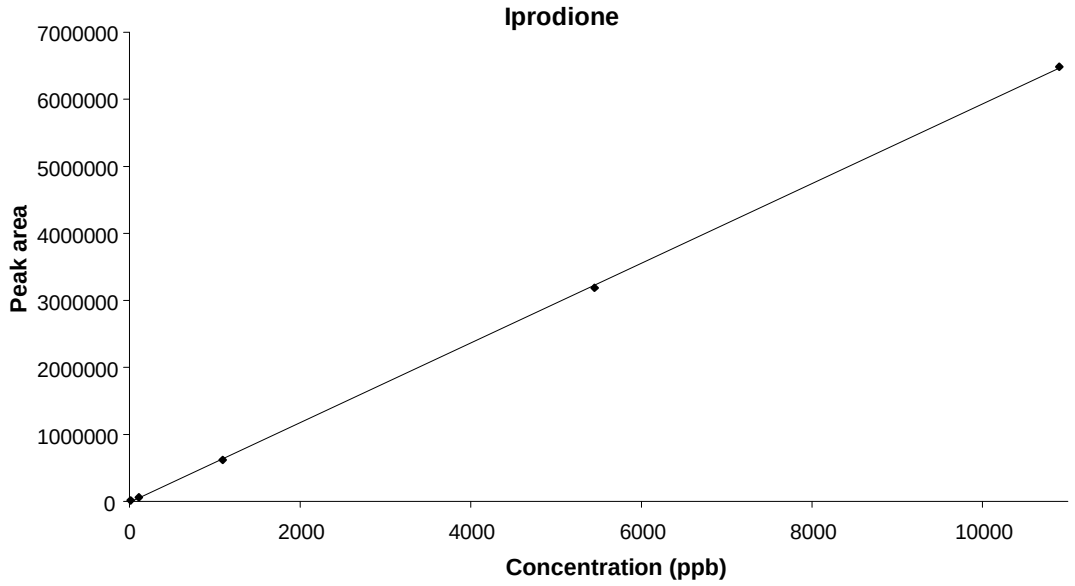

Figure 7. Linearity of LC-APCI-MS analysis of iprodione.

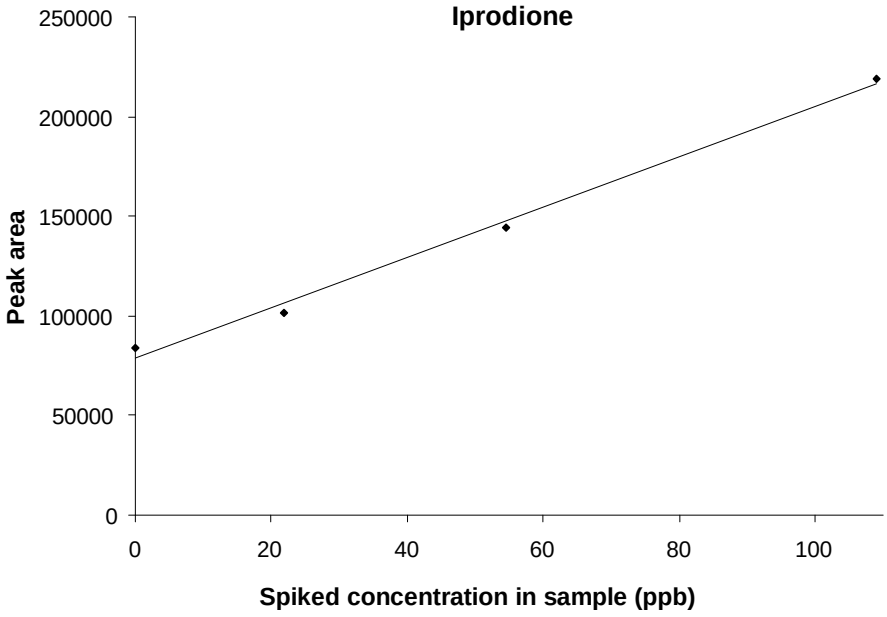

Figure 8. Standard addition curve of the SBSE-LD-LC-MS analysis of iprodione in Italian sparkling wine.

5.4 Conclusions

Stir bar sorptive extraction (SBSE) in combination with TD-CGC-MS is a simple, fast and sensitive method for the analysis of vinclozolin, procymidone and iprodione in wine samples. Iprodione was measured as its degradation product 3,5-dichlorophenyl hydantoin. The accuracy of SBSE-TD-capillary GC-MS for iprodione was verified using SBSE followed by liquid desorption and analysis by liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy (SBSE-LD-LC-APCI-MS). The techniques gave comparable data. Liquid desorption of the stir bars broadens the applicability of sorptive extraction on PDMS to non-volatile solutes.

5.5 References

[1] B.D. Ripley, L.I. Lissemore, P.D. Leishman, M.A. Denomme, L. Ritter, J. AOAC Int. 83 (2000) 196.

[2] P. Cabras, A. Angioni, J. Agric. Food Chem. 48 (2000) 967.

[3] P. Cabras, A. Angioni, V.L. Garau, F.M. Pirisi, G.A. Farris, G. Madau, G. Emonti, J. Agric. Food Chem. 47 (1999) 3854.

[4] L.E. Gray, Toxicol. Letters 103 (1998) 331.

[5] L.E. Gray, C. Wolf, C. Lambright, P. Mann, M. Price, R.L. Cooper, J. Ostby, Toxicol. Ind. Health 15 (1999) 94.

[6] J. Ostby, W.R. Kelce, C. Lambright, C.J. Wolf, P. Mann, L.E. Gray, Toxicol. Ind. Health 15 (1999) 80.

[7] C. Sala, O. Busto, J. Guasch, Chromatographia 44 (1997) 320.

[8] J. Oliva, S. Navarro, A. Barba, G. Navarro, J. Chromatogr. A 833 (1999) 43.

[9] J.S. Gandara, P.P. Losada, V.G. Rodriguez, A.R. Rodriguez, J. Agric. Food Chem. 41 (1993) 674.

[10] G.J. Soleas, J. Yan, K. Hom, D.M. Goldberg, J. Chromatogr. A 882 (2000) 205.

[11] A. Prieto, G. Etienne, D. Medina, I. Buscema, G. Gonzalez, L. Araujo, Food Addit. Contam. 16 (1999) 57.

[12] E. Matisova, L. Kakalikova, J. Lesko, J. De Zeeuw, J. Chromatogr. A 754 (1996) 445.

[13] A. Kaufmann, J. AOAC Int. 80 (1997) 1302.

[14] C.L. Arthur and J. Pawliszyn, J. Anal. Chem., 19 (1990) 2145.

[15] D.W. Potter and J. Pawliszyn, J. Chromatogr. A, 625 (1992) 247.

[16] D.A. Lambropoulo, I.K. Konstantinou, T.A. Albanis, J. Chromatogr. A 893 (2000) 143.

[17] L. Urruty, M. Montury, M. Braci, J. Fournier, J.M. Dournel, J. Agric. Food Chem. 45 (1997) 1519.

[18] R.W. Hu, B. Hennion, L. Urrruty, M. Montury, Food Addit. Contam. 16 (1999) 111.

[19] E. Baltussen, P. Sandra, F. David, C.A. Cramers, J. Microcolumn Sep. 11 (1999) 737.

[20] F. David, A. Tredoux, E. Baltussen, A. Hoffmann, P. Sandra, *in* "Proceedings of the 23th International Symposium on Capillary Chromatography", P. Sandra (Ed.), I.O.P.M.S., Kortrijk, Belgium (Pub.), CD-Rom paper M 35, 2000.

[21] A. Hoffmann, W.R. Sponholz, F. David, P. Sandra, *in* "Proceedings of the 23th International Symposium on Capillary Chromatography", P. Sandra (Ed.), I.O.P.M.S., Kortrijk, Belgium (Pub.), CD-Rom paper D 35, 2000.

[22] A. Tredoux, H.H. Lauer, T. Heideman, P. Sandra, J. High Resolut. Chromatogr. 23 (2000) 644.

[23] T. Benijts, J. Vercammen, R. Dams, Hai Pham Tuan, W. Lambert, P. Sandra, J. Chromatogr. B 755 (2001) 137.

[24] F. David, P. Sandra, S.S. Stafford, B. Slavica, Agilent Technologies Application Note 228-272, Publication Number (23) 5962-9424 E, April 1994.

[25] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83.

[26] A. Hoffmann, A. Heiden, E. Pfannkoch, *in* "Proceedings of the 23th International Symposium on Capillary Chromatography", P. Sandra (Ed.), I.O.P.M.S., Kortrijk, Belgium (Pub.), CD-Rom paper M 32, 2000.

6

The Determination of Benzoic Acid in Lemon Flavored Beverages by Stir Bar Sorptive Extraction GC-MS*

*Published as "The Determination of Benzoic Acid in Lemon Flavored Beverages by Stir Bar Sorptive Extraction-CGC-MS", A.G.J. Tredoux, H.H. Lauer, Th. Heideman, P. Sandra, J. High Resolut. Chromatogr. 23 (2000) 644.

6.1 Introduction

In recent years, much attention has been devoted to the miniaturization of sample preparation techniques and several new methods have been introduced e.g. solid phase extraction (SPE), solid phase microextraction (SPME), micro-liquid-liquid extraction (μLLE) followed by large volume (LV) programmed temperature vaporization (PTV) injection, etc. [1].

Recently a novel solventless and simple technique for pre-concentration of organic solutes from aqueous matrices, namely stir bar sorptive extraction (SBSE), was developed by Sandra et al. [2]. SBSE approaches or equals the high enrichment factors of packed sorptive beds [3,4] but with the application range and simplicity of SPME [5,6]. In SBSE, a stir bar coated with polydimethylsiloxane (PDMS) is placed in the sample and stirred for a predetermined time. The stir bar is then thermally desorbed on-line with a capillary GC-MS system. The applicability of SBSE for pre-concentration of the polar preservative benzoic acid in lemon flavored beverages is demonstrated.

6.2 Experimental

6.2.1 Samples

The samples consisted of three soft drinks and a lemon flavored herbal tea. Two of the soft drinks were cloudy (sample 1 and 2) and one was clear (sample 3). The tea was prepared by adding 200 mL boiling distilled water to one tea bag and stirring for 5 min (sample 4). Before SBSE extraction, the pH of the samples was adjusted to 2 with 0.1 N HCl. Calibration graphs were obtained by adding 1 to 1000 mg/L (ppm) sodium benzoate (95%, Merck, Darmstadt, Germany) to sample 1. A calibration was also made in distilled water adjusted to pH 2.

6.2.2 Sampling

A stir bar consisting of a magnetic core sealed inside a glass tube with a length of 1.2 cm, an outer diameter (OD) of 1.2 mm and coated with 50 mg PDMS was used. The stir bars are manufactured by Gerstel (Mülheim a/d Ruhr, Germany) under the name Twister™. Prior to

use, the stir bar was conditioned in a desorption tube (178 mm length, 6 mm OD, 4 mm ID glass tube) of a thermal desorption unit (Gerstel TDS-2) at 300°C for two hours.
The conditioned stir bar is placed in a 25 mL sample and stirred at 1400 rpm for 40 min at ambient temperature. The stir bar is then removed from the sample, washed with a small amount of distilled water and dried with a paper cloth. After positioning the stir bar in the middle of the heated zone of the desorption tube, the desorption program is started

6.2.3 Thermal desorption-capillary GC-MS

The instrumental set-up for TD-CGC-MS has been described by Baltussen et al. [7]. It consists of a Gerstel TDS-2 thermal desorption system mounted on a HP 6890 GC – HP 5972 MS combination (Hewlett Packard, Little Falls, USA). For thermal desorption the following conditions were applied: desorption temperature program 40°C at 25°C/min to 250°C (15 min), carrier gas helium at 100 kPa constant pressure, flow mode splitless and transfer line temperature set at 300°C. A Gerstel CIS-4 PTV injector was used for cryogenic focusing of the released analytes. The PTV was cooled to -100°C using liquid nitrogen. For the CGC-MS analysis the conditions were: column 30 m × 0.25 mm i.d. × 0.25 µm FFAP (home-made), injection PTV in the sample remove mode, injection temperature program from -100°C at 600°C/min to 250°C (2 min), oven temperature program from 40°C (1 min) at 5°C/min to 250°C (2 min) and detection in the full scan mode from 40-400 amu at 2.5 scans/s.

6.3 Results and discussion

In a first series of experiments, a calibration graph for benzoic acid in water was made to determine the linear range of the SBSE technique. As expected, the recovery of benzoic acid was much higher at low than at high pH. Benzoic acid is a polar compound with a pKa of 4.21 while the PDMS coating is apolar. In order to increase the affinity of benzoic acid for PDMS, the pH of the sample was adjusted to 2 to have benzoic acid in the protonated form. The log $K_{(o/w)}$ for benzoic acid is 1.87 [8] which should, according to Baltussen et al. [2], correspond to a recovery at equilibrium of *ca.* 40%. When benzoic acid is present in the anionic, dissociated form the log $K_{(o/w)}$ is –2.27 [8] which corresponds to zero recovery. The linear range in water

was found to be 1 to 1000 ppm (**Figure 1**). A similar standard addition of benzoic acid to an undiluted soft drink (sample 1) was performed to determine the benzoic acid concentration. In this case the graph was only linear up to *ca.* 400 ppm (Figure 1). The reason for the deviation from linearity is because of the matrix effect of the complex soft drink sample which influences the partitioning process. To reduce the matrix-effects standard addition to a 1 to 10 diluted sample was performed. The graph is again linear up to 1000 ppm, allowing a more accurate determination especially at high concentrations (Figure 1). For this soft drink (sample 1) the benzoic acid concentration calculated by the standard addition procedure was found to be 247 ppm. The determination was repeated six times and the mean value was 255 with a %RSD of 4.8. Concerning the sensitivity of the method, the limit of quantification (LOQ) at S/N 10 is 8 μg/L (ppb) in the full scan mode. It should be noted that the FDA considers 1000 ppm safe for human consumption [9]. Sample 2 and 3 contained 228 and 195 ppm, respectively, while the concentration in the tea (sample 4) was 43 ppm. Compared to the official method for the determination of benzoic acid in soft drinks [10] which is based on ether extraction, successive partitionings into a sodium hydroxide solution and dichloromethane, followed by convertion of the acid in the trimethylsilyl (TMS) ester and analysis by GC, the presented SBSE-TD-CGC-MS method is much faster with less risk for contamination.

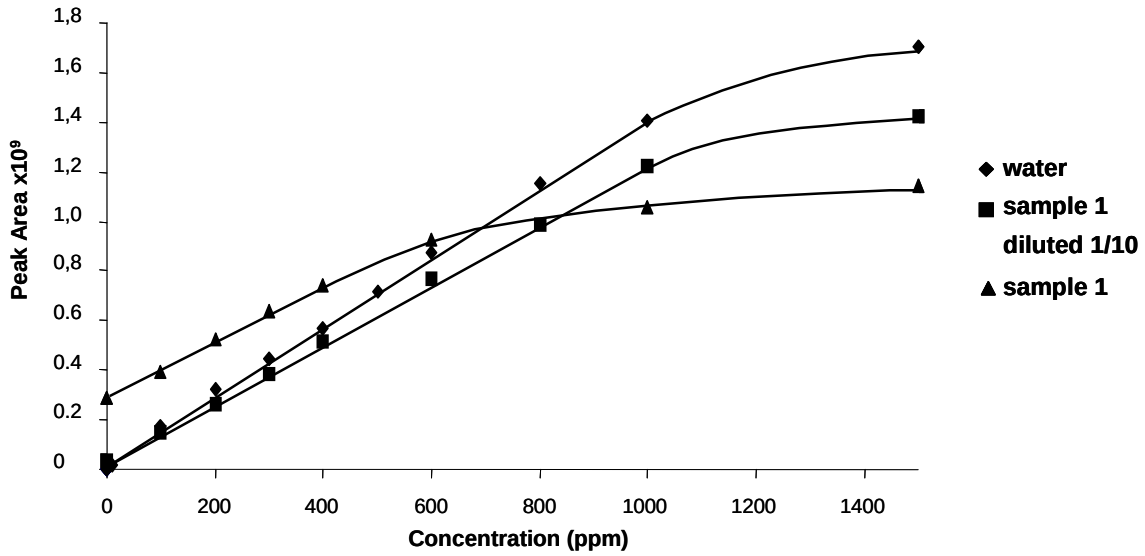

Figure 1. Calibration graphs for benzoic acid in water, undiluted and diluted (1/10) soft drink sample 1.

Figure 2A and B show the SBSE chromatograms of soft drink samples 2 and 3, respectively, and it is obvious that other important conclusions related with the lemon oil quality can be drawn from these chromatograms. Some of the main components are listed in **Table 1**. The lemon oil used in sample 2 (Figure 2A) is characterised by a high concentration of monoterpenes (peaks 1 to 5) while in sample 3, the lemon oil seems to have been demonoterpenised, probably by distillation. This is based on the presence of β-farnesene (peak 25 in Figure 2B) which is a sesquiterpene that should have been removed as well if fractionation based on polarity has been carried out. Peaks 20, 21 and 23 are typical for the carbohydrates added to the soft drinks. Although it was not the aim of this study, it is clear that SBSE can also be used to elucidate the flavor compounds in soft drinks.

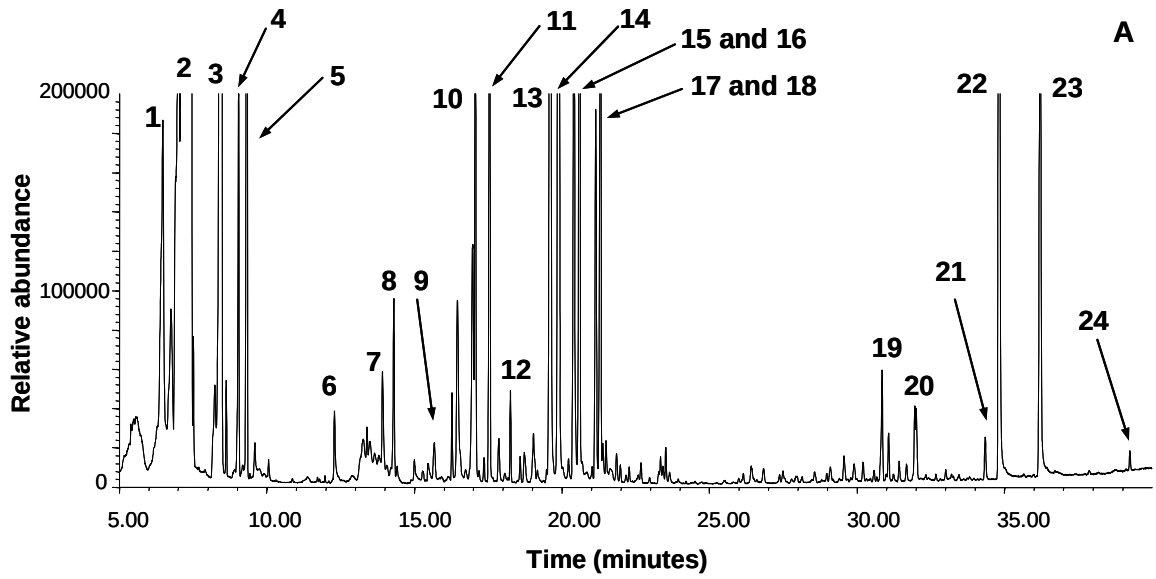

Figure 2A. SBSE-TD-CGC-MS analyses of sample 2.

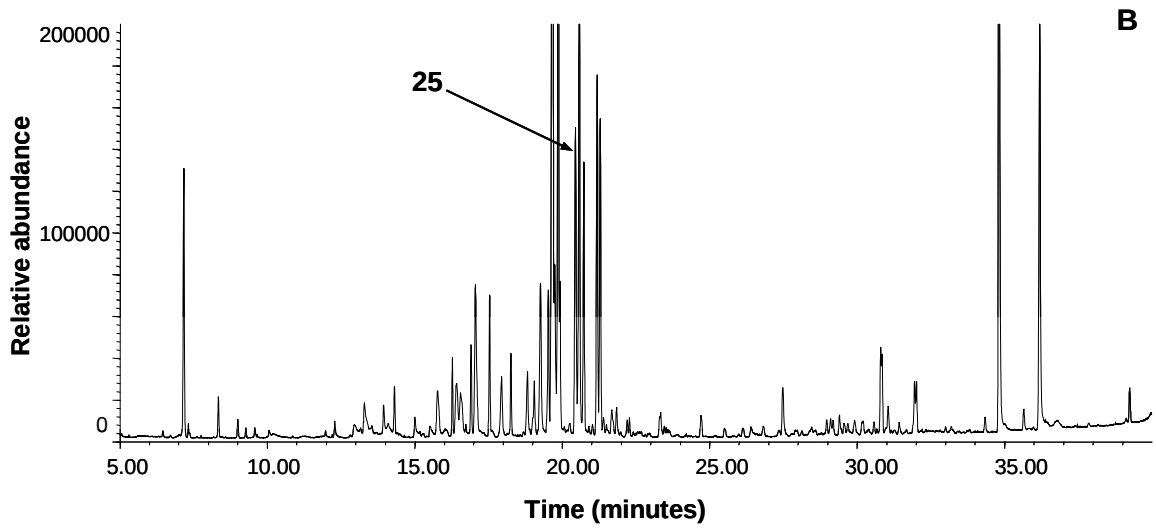

Figure 2B. SBSE-TD-CGC-MS analyses of sample 3.

Table 1: Identification of compounds in Figure 2.

Nr	Compound	Nr	Compound
1	β-pinene	14	α-terpineol
2	limonene	15	neral
3	γ-terpinene	16	geranial
4	p-cymene	17	neryl acetate
5	α-terpinolene	18	geranyl acetate
6	n-nonanal	19	α-bisabolol
7	acetic acid	20	2,3-dihydro-3,5-dihidroxy-6-methyl-4H-pyran-4-one
8	furfural	21	1,4:3,6-dianhydro-,alpha,-d-glucopyranose
9	linalool	22	benzoic acid
10	fenchol	23	5-(hydroxymethyl)-2-furancarboxaldehyde
11	terpinen-4-ol	24	dibutylphthalate
12	β-terpineol	25	β-farnesene
13	linalyl propionate		

6.4 Conclusion

SBSE is a simple and reliable technique for the analysis of benzoic acid and flavor compounds in lemon flavoured beverages.

6.5 References

1. A.J. Handley (Ed.), Extraction Methods in Organic Analysis, Sheffield Academic Press, London, 1999, pp 5-231.
2. E. Baltussen, P. Sandra, F. David and C.A. Cramers, J. Microcolumn Sep. 11 (1999) 737.
3. E. Baltussen, F. David, P. Sandra, H.-G. Janssen, C.A. Cramers, J. Chromatogr. A 805 (1998) 237.
4. E. Baltussen, F. David, P. Sandra, H.-G. Janssen, C.A. Cramers, J. High Resolut. Chromatogr. 21 (1998) 645.
5. J. Pawliszyn, *Solid Phase Microextraction. Theory and Practice*, Wiley-VCH, Weinheim, Germany, 1997.
6. J. Pawliszyn (Ed.), *Applications of Solid Phase Microextraction.* The Royal Society of Chemistry, Cambridge, England, 1999.
7. E. Baltussen, H.-G. Janssen, P. Sandra, C.A. Cramers, J. High Resolut. Chromatogr. 20 (1997) 385.
8. W.M. Meylan and P.H. Howard, J. Pharm. Sci., 84 (1995) 83. Software KowWIN Version 1.66, SRC-LOGKOW, SRC-ESC, Syracuse, USA, 2000.
9. US Food and Drug Administration, Title 21, Section 184.1021, FDA Website, 2000.
10. JAOAC 60 (1983) 775.

7

Selected Applications of Stir Bar Sorptive Extraction for the Analysis of Foodstuffs

7.1 Introduction

Increased awareness by consumers regarding the quality and safety of the foodstuffs they consume has necessitated the development of fast, accurate and sensitive analytical methods for the determination of compounds linked to safety and quality. Especially concerning safety issues, governmental regulatory bodies are responsible for determining maximum levels at which certain substances, for instance carcinogens or endocrine disruptors, may be present in products intended for human consumption. The use of preservatives also needs to be indicated on product labeling, requiring analysis methods for confirmation and monitoring purposes. Since the sensation of taste and flavour involves the combination of numerous compounds, not only their respective levels but also the relative ratio's in which these compounds occur are of importance. Therefore important flavour compounds often need to be monitored for quality control purposes.

Due to the varied chemical nature and levels of these compounds and in a variety of sample matrices, sample enrichment and / or clean-up step(s) prior to instrumental analysis is often required. Stir bar sorptive extraction (SBSE) is a relatively new sorption-based sample enrichment technique in which the stationary phase is coated on a glass encapsulated magnet [1]. The stir bar is typically introduced in the sample or sample headspace, and removed after a predetermined time followed by thermal desorption and GC-MS analysis. Especially for non-polar to medium polar analytes, SBSE has been proved to be considerably more sensitive than solid phase micro extraction (SPME) due to a larger amount of sorptive phase [2]. Several applications of SBSE for foodstuff analysis have been reported to date [1,3-15].

In this chapter various applications of SBSE for foodstuff analysis in both alcoholic and non-alcoholic beverages, for the determination of contaminants, flavours and preservatives, are described. The applicability of SBSE for diverse classes of compounds is demonstrated as a viable alternative sample preparation technique in comparison with conventional techniques.

7.2 Experimental

7.2.1 Samples and chemicals

Wine and beer samples were purchased from local retailers. Yoghurt samples consisted of six different flavoured varieties from one manufacturer, as well as plain (unflavoured) yoghurt from three different manufacturers, supplied by a local distributor. Drinking water was obtained from a tap in a local bathroom (University of Stellenbosch). Concentrated HCl was obtained from Merck (Darmstadt, Germany) and diluted in-house to 1 N with Milli-Q water.

7.2.2 Sampling

Stir bars obtained from Gerstel (Mülheim a/d Ruhr, Germany), consisting of a magnetic core sealed inside a glass tube with a length of 1.2 cm, an outer diameter (OD) of 1.2 mm and coated with 50 mg PDMS, were used. Prior to use, the stir bar was conditioned in a thermal desorption unit (Gerstel TDS-2) at 300^{o}C for two hours under a flow of He (50 mL/min), in the split mode.

For SBSE, a conditioned stir bar was placed in 25 mL sample and stirred at 1400 rpm for 40 min at ambient temperature. The stir bar is then removed from the sample, washed with a small amount of distilled water and dried with a paper cloth. For headspace sorptive extraction (HSSE) the stir bar was exposed to the headspace of a diluted sample (9 mL water in 1 mL sample) for 1h at ambient temperature, after which any condensation is removed with a paper cloth.

For analysis of benzoic acid in yoghurt the following procedure was followed: to a 40 mL yoghurt sample, 80 mL of distilled water was added together with 20, 40, 60 or 80 μL of a 10000 mg/L (ppm) solution of benzoic acid in water in order to obtain a addition of 5, 10, 15 and 20 mg/L respectively (in cases where higher standard addition levels were required the amount was modified accordingly). The mixture was shaken briefly and centrifuged to separate any solids from the aqueous phase. This step was necessitated because as the pH is lowered, glomerates are formed, which reduce the efficiency of stirring. 40 mL of the supernatant was gently poured into the sampling vessel and the pH adjusted to 2 with 1 N HCl. Stirring was performed as described above.

In all analyses, following sampling the stir bar is positioned in the middle of the heated zone of a thermal desorption tube (178 mm length, 6 mm OD, 4 mm ID glass tube) and the desorption program is started.

7.2.3 Thermal desorption-capillary GC-MS

The instrument used was an HP 6890 GC equipped with an HP 5972 MSD (Agilent, Little Falls, USA) and a thermal desorption unit (TDS-2, Gerstel) connected to a PTV injector (CIS-4, Gerstel). For all analyses the carrier gas was helium at 100 kPa constant pressure. During thermal desorption the TDS was set to splitless flow and the PTV was in solvent vent mode, whilst during injection the TDS was set to split flow and the PTV in splitless mode. Liquid nitrogen was used for cryogenic cooling. The MS was operated in the full scan mode scanning from from 40-400 amu at 2.5 scans/s. A summary of the capillary columns and thermal desorption- as well as separation temperature programs for each analysis is presented in Table 1.

Table 1. Summary of instrumental conditions.

Matrix	Analyte(s)	Mode	TDS, PTV[a]	Oven temperature program	Analytical column[b]
Wine	2,4,6-TCA	SBSE	1	40°C, 1min, 5°C/min to 300°C, 2min	1
Water	Contaminants	SBSE	1	40°C, 1min, 20°C/min to 300°C, 5min	1
Wine	Screening	SBSE	2	40°C, 2min, 12°C/min to 250°C, 2min	2
Beer	Screening	SBSE	2	40°C, 2min, 12°C/min to 250°C, 2min	2
Wine	Screening	HSSE	2	40°C, 5min, 4°C/min to 250°C, 2min	2
Yoghurt	Benzoic acid	SBSE	3	40°C, 1min, 10°C/min to 240°C, 2min	3

[a] TDS and PTV conditions:
1: TDS: 40°C, 25°C/min to 300°C, 10 min, PTV: -100°C, 1 min, 600°C/min to 300°C, 2 min
2: TDS: 40°C, 25°C/min to 250°C, 10 min, PTV: -100°C, 1 min, 600°C/min to 250°C, 2 min
3: TDS: 40°C, 25°C/min to 250°C, 15 min, PTV: -100°C, 1 min, 600°C/min to 250°C, 2 min

[b] Analytical columns: 1: HP5MS (Agilent), 2: HP-INNOWAX (Agilent), 3: free fatty acid phase FFAP (home-made), all of dimensions 30 m × 0.25 mm i.d. × 0.25 µm d_f.

7.3 Results and Discussion

7.3.1 SBSE applied the analysis of contaminants in foodstuffs

We have previously demonstrated the successful analysis of contaminants such as pesticides [10-13], nonylphenols and phthalates [5,12] in wine, as well as chlorobenzenes in drinking water [12]. Furthering these applications, SBSE was used for determination of 2,4,6-trichloroanisole (TCA) in a red wine blend. TCA is considered one of the principle compounds responsible for cork taint in wine. This compound is formed by the biomethylation of 2,4,6-trichlorophenol, a widely used microbiocide, by microbial action [16]. **Figure 1** presents the full scan MS chromatogram for the analysis of TCA in a red wine sample. It should be noted that TCA analysis is commonly performed by selected ion monitoring (SIM) because of the enhanced sensitivity and selectivity of this mode of detection. Analysis of TCA and other haloanisoles is routinely performed by SPME [17,18] and large volume injection (LVI) [19], although applications using SBSE and HSSE have recently been reported [4,7]. Although the method presented here is not optimised for quantitation purposes, figure 1 clearly indicates the potential of SBSE for the routine analysis of this compound in wine.

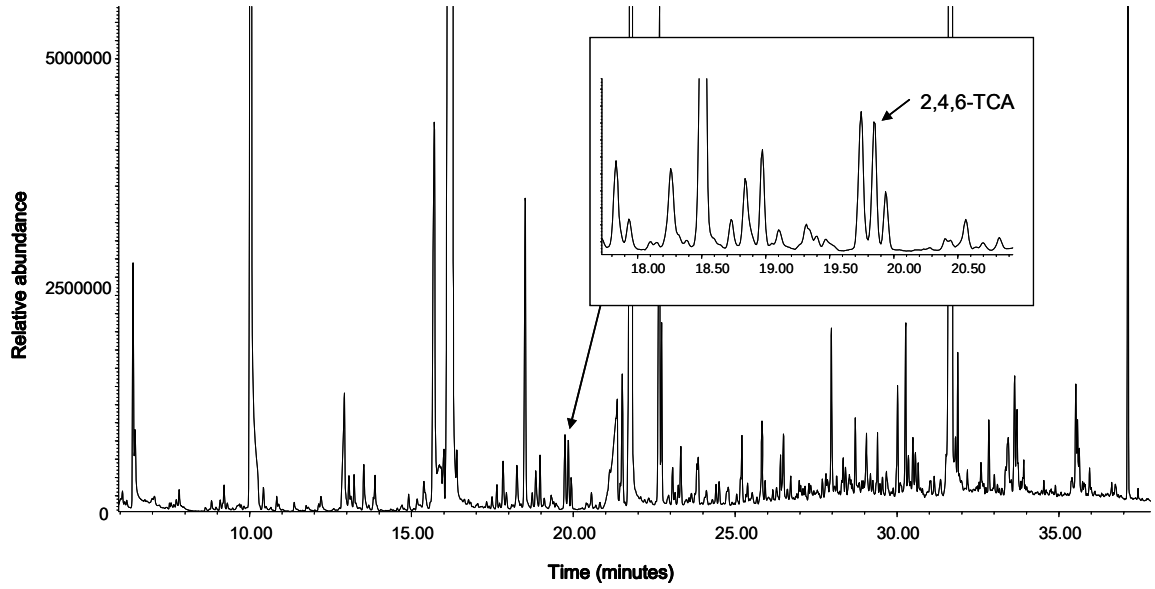

Figure 1. SBSE-TD-GC-MS analysis of TCA in red wine.

In **Figure 2** a chromatogram of the analysis of tap-water is presented. Two tri-halogenated methanes (dichlorobromomethane and dibromochloromethane) were detected together with nonanal, acetic acid and two phthlates. The halogenated methanes originate from algae activity in water disinfected by the addition of chlorine. These compounds are known mutagens and in he US the total amount of these compounds may not exceed 80 μg/L as stipulated by EPA regulations [20,21]. Phthalates are suspected endocrine disruptors [22] and are commonly used as plasticisers and as a result of extensive use are prevalent in the environment. The origin of nonanal and acetic acid is unclear. The halogenated methanes presented in Figure 2 are commonly analysed by direct injection GC-MS or liquid-liquid extraction (LLE) followed by GC-ECD [23]. Compared to LLE-GC-ECD the SBSE method described here presents an easy to use, sensitive and completely solventless alternative. Moreover, unlike LVI, no retention gap and less frequent injector maintenance is required. It should be noted though that the sensitivity of the ECD is superior to an MS in scan mode for halogenated compounds, allowing lower detection limits using the former detector.

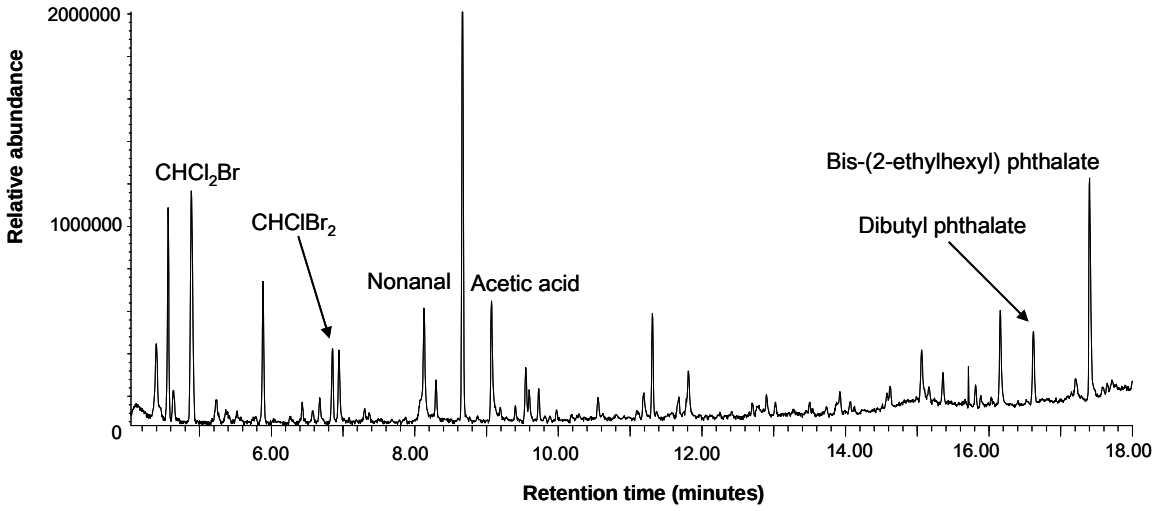

Figure 2. SBSE-TD-GC-MS chromatogram obtained by the analysis of municipal drinking water.

7.3.2 SBSE and HSSE applied to flavour profiling of alcoholic beverages

Flavour profiling is important for several reasons, including the detection of defects originating from the production processes or during storage (as is commonly seen with beer), quality control, authentication of products and relating chemical composition to flavour. Sorptive extraction techniques, including SPME [24,25] and more recently SBSE [3,6,26] have been applied for the flavour profiling of wine samples. **Figure 3** shows the volatile profiles obtained by SBSE-TD-GC-MS analysis of a) Cabernet Sauvignon, b) Merlot and c) Shiraz wines. Identification of the compounds can be found in Table 2. These compounds represent mainly the major volatile constituents (primarily originating from fermentation processes) present in all wines, including esters, alcohols and acids. This screening technique was extensively covered in Chapter 4, but the example shown here indicates the versatility of the technique and that the chromatographic analysis of these screening methods are performed in half the time of the method developed in Chapter 4.

In beer analysis the focus is more commonly to analyse specific target compounds such as flavour active esters, contributing to 'fruit' aroma characteristics [27], sulphur compounds, which may contribute positively but when present in higher concentrations produce undesirable flavours [28] and carbonyl compounds responsible for stale flavours [8]. Screening of major volatile composition of beer samples has recently been reported using SPME [29] and SPME in combination with sol-gel technology [30]. The flavour profiles of 3 different beers obtained by SBSE are presented in **Figure 4**. The compounds identified by means of spectral data (**Table 3**) represent many of the major volatiles originating from the brewing process and are either yeast derived or originate from the raw starting materials used. Noticeably more terpenes or terpene derivatives are detected in the beer compared to wine samples. These compounds are known to be present in hop oil and therefore originate from the hops added during brewing [31]. In both the case of wine and beer, clear qualitative and quantitative differences between the different samples are noticeable, demonstrating the potential of SBSE for volatile screening purposes. Further application of the technique of wine volatile analysis has been described in Chapter 4.

Stir bars can also be used for the selective and sensitive analysis of aroma compounds in the headspace, a technique called headspace sorptive extraction (HSSE). The potential advantages of this technique include less interference from the sample matrix, a more

representative analysis of aroma compounds and better extraction of highly volatile compounds. In **Figure 5** a chromatogram obtained by HSSE-TD-GC-MS of a Shiraz wine is shown. The relevant regions in the chromatogram are enlarged for clarity in **Figure 6**. Compared to the SBSE analysis interesting differences are observed. HSSE seems to extract more volatile polar compounds and less high boiling compounds. This difference compared to SBSE can be explained by the fact that high boiling compounds do not easily migrate to the headspace. Possibly less interference from the ethanol present in the sample is responsible for better extraction of the highly volatiles. We have recently reported an HSSE method for quantitative analysis of major volatiles in wine [14]. In comparison with this method as well as the SBSE method presented previously for contaminant analysis, it is clear that by altering a combination of different sampling and analysis conditions it is possible to design a method suitable for analysis of specific compounds of interest. This illustrates the versatility of the stir bar technology. In terms of sensitivity and reproducibility, both SBSE and HSSE present viable alternatives for the analysis of wine volatiles; the mode selected will largely be dictated by the compounds of interest. In terms of ease of use, SBSE offers the advantage of simpler and faster experimental procedures and does not require specialised glassware.

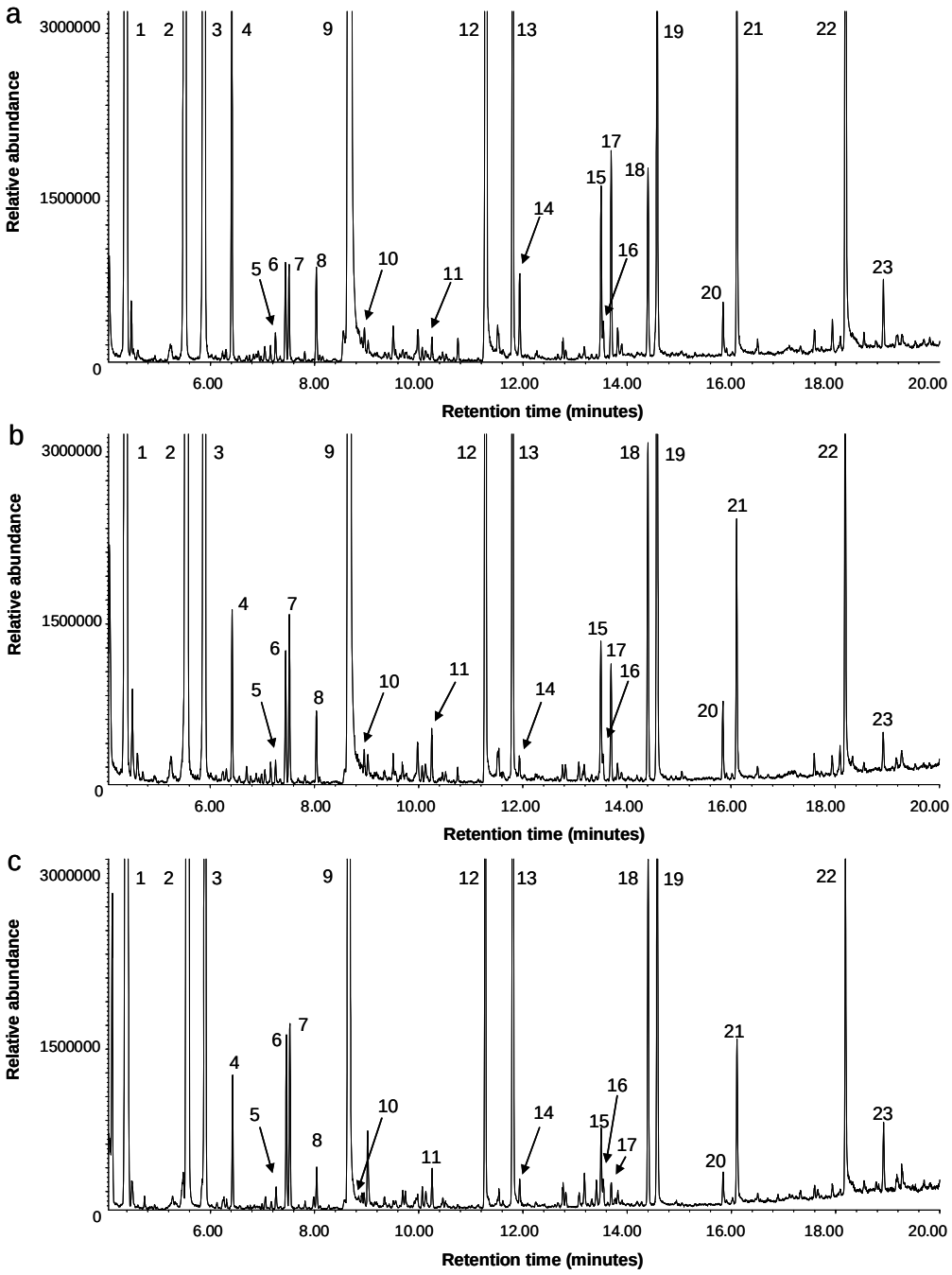

Figure 3. Volatile profiles obtained by SBSE-TD-GC-MS for a) Merlot, b) Cabernet Sauvignon and c) Shiraz wines. For peak identification refer to Table 2.

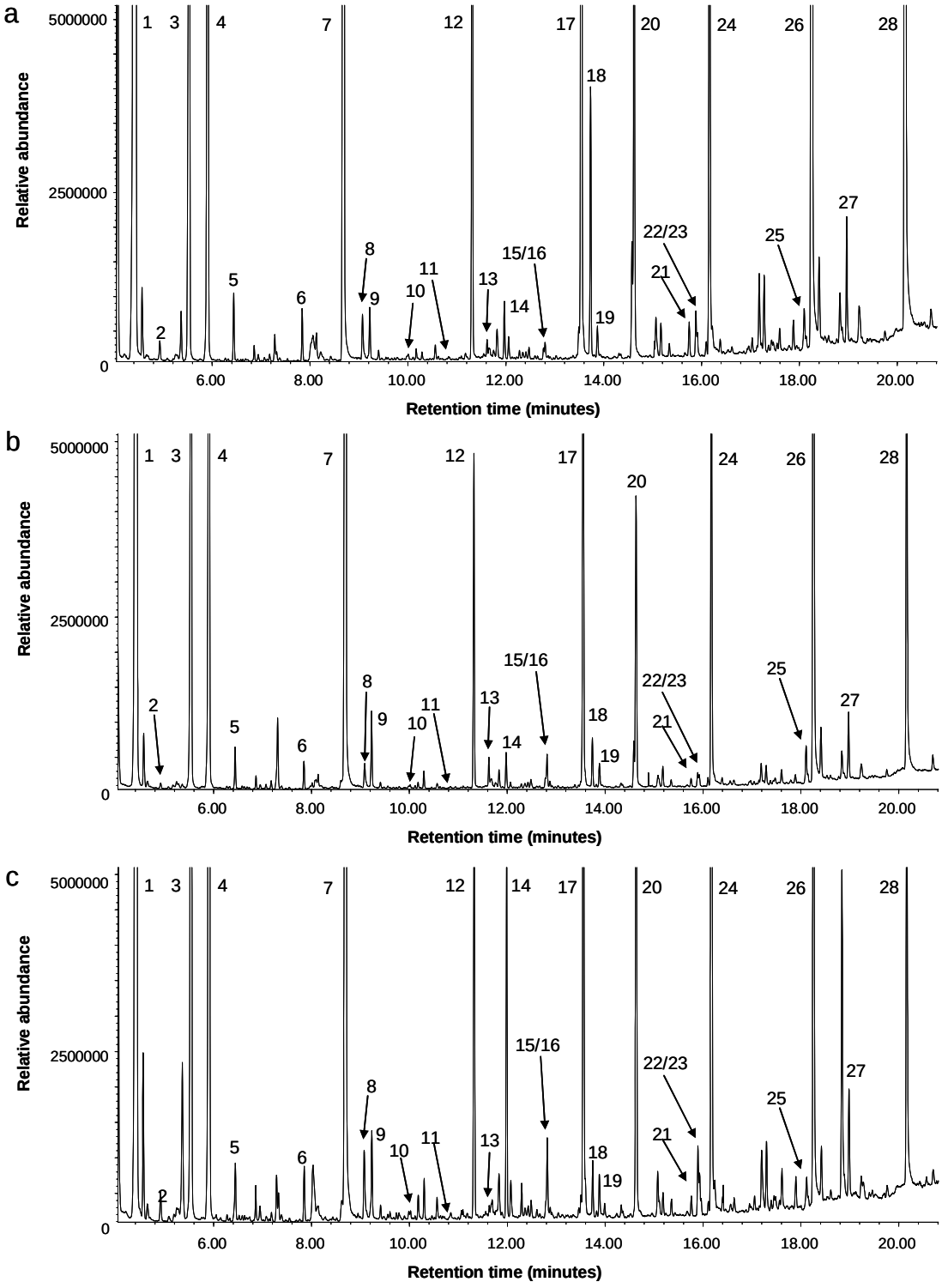

Figure 4. Chromatograms obtained from the analysis of three beer samples by SBSE-TD-GC-MS. For peak identification, refer to Table 3.

Table 2. Major wine volatiles identified tentatively by SBSE-TD-GC-MS (Figure 3).

Nr	Compound	Nr	Compound
1	isoamyl acetate	13	diethyl succinate
2	Isoamyl alcohol	14	ethyl-9-decenoate
3	ethyl hexanoate	15	β-phenylethyl acetate
4	hexyl acetate	16	hexanoic acid
5	ethyl heptanoate	17	ethyl dodecanoate
6	ethyl lactate	18	ethyl-3-methyl butanedioate
7	1-hexanol	19	β-phenylethyl alcohol
8	methyl octanoate	20	unidentified terpene
9	ethyl octanoate	21	octanoic acid
10	acetic acid	22	decanoic acid
11	1-octanol	23	unidentified terpene
12	ethyl decanoate		

Table 3. Volatile compounds identified tentatively in beer samples by SBSE-TD-GC-MS (Figure 4).

Nr	Compound	Nr	Compound
1	isoamyl acetate	15	geranyl acetate
2	β-myrcene	16	1-decanol
3	isoamyl alcohol	17	β-phenylethyl acetate
4	ethyl hexanoate	18	ethyl dodecanoate
5	hexyl acetate	19	hexanoic acid
6	heptyl acetate	20	β-phenylethyl alcohol
7	ethyl octanoate	21	α-humulene
8	octyl acetate	22	nerolidol
9	acetic acid	23	γ-octalactone
10	linalool	24	octanoic acid
11	1-octanol	25	unidentified terpene
12	ethyl decanoate	26	decanoic acid
13	citronallyl acetate	27	farnesol
14	ethyl-9-decenoate	28	dodecanoic acid

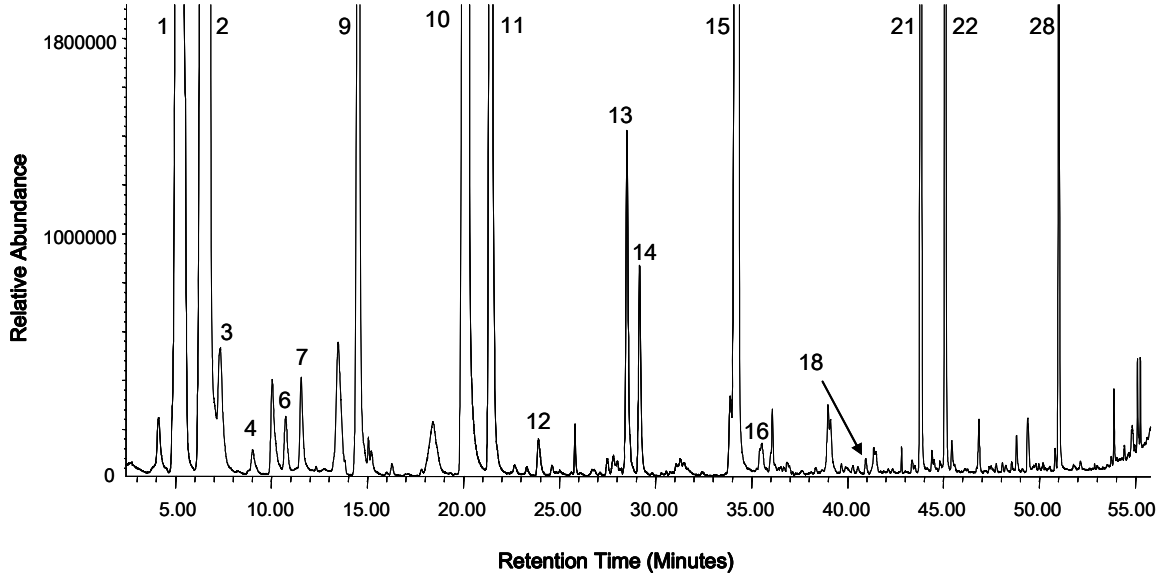

Figure 5. Chromatogram obtained from a Shiraz wine sample by HSSE-TD-GC-MS.

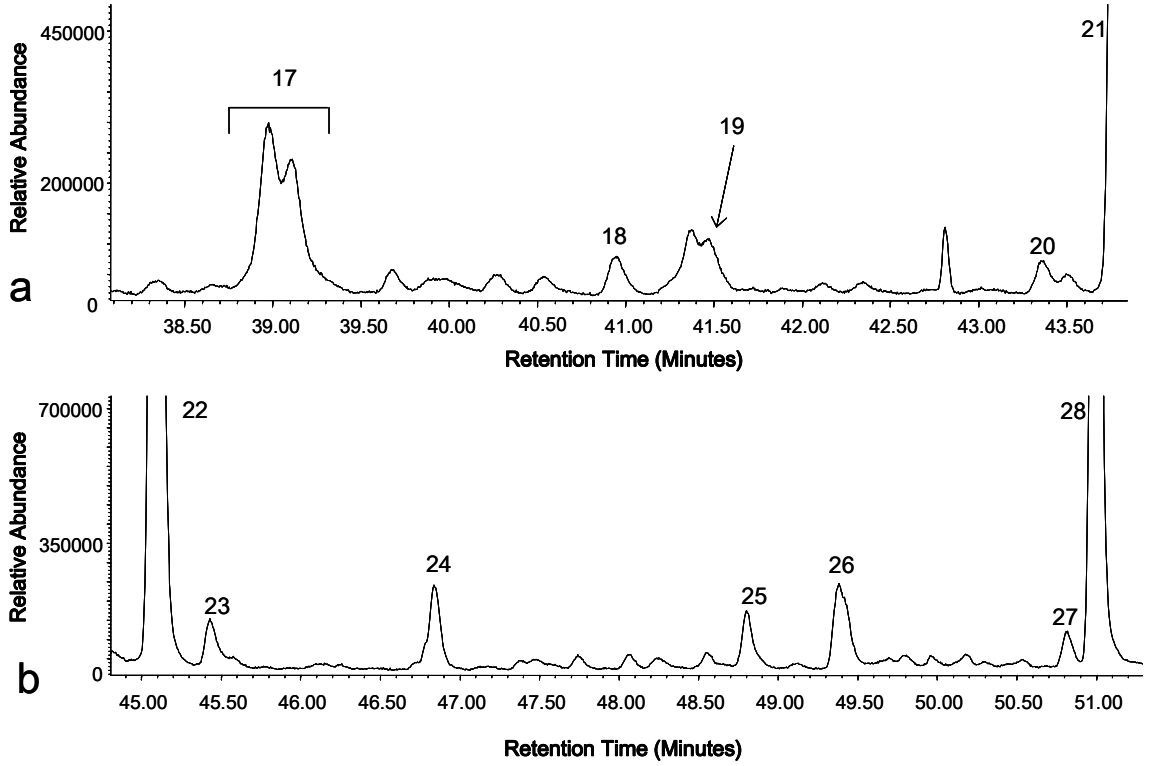

Figure 6. Detail of Figure 5. Time interval 38 – 44 min (3a) and 45 – 51 min (3b), respectively. Compound identification – see table 4.

Table 4. Wine volatiles identified tentatively by HSSE-TD-GC-MS (Figure 5 and 6).

Nr	Compound	Nr	Compound
1	ethyl acetate	15	ethyl octanoate
2	ethanol	16	acetic acid
3	ethyl isobutyrate	17	vitispirane (2 isomers)
4	isobutyl acetate	18	1-octanol
5	ethyl butyrate	19	5-methyl furfural
6	ethyl 2-methylbutyrate	20	γ-butyrolactone
7	ethyl isovalerate	21	ethyl decanoate
8	isobutanol	22	diethyl succinate
9	isoamyl acetate	23	ethyl-9-decenoate
10	isoamyl alcohol	24	3,4-dihydroionene
11	ethyl hexanoate	25	β-phenylethylacetate
12	hexyl acetate	26	ethyl dodecanoate
13	ethyl lactate	27	ethyl isopentyl succinate
14	1-hexanol	28	β-phenylethyl alcohol

7.3.3 Determination of preservatives in foodstuffs.

Preservatives, such as benzoic acid, are commonly added to foodstuffs to increase their shelf-life. The allowable levels of preservatives in foodstuffs are determined by the type of food/drink in the European Union and allowable levels for benzoic acid range from 150 to 1000 mg/L [32]. Therefore accurate methods of analysis are needed for regulatory purposes. Previously we have described the quantification of benzoic acid in lemon flavoured softdrinks [13]. Quantitation was performed by means of standard addition. Calibration of benzoic acid in water was shown to be linear up to 1000 ppm although for the soft-drinks containing high amounts of benzoic acid (100-400 ppm) as a preservative, calibration was only linear up to 400 ppm due to matrix effects. This limitation was overcome by diluting the soft-drink with water prior to sampling. Alternative methods for the analysis of preservatives such as benzoic acids include various GC or HPLC methods,

often following sample cleanup and concentration by SPE [33,34] (also in fatty matrices), [35] as well as sorption based methods such as SPME and SBSE more recently [9,36].

Yoghurt represents a challenging matrix due to the presence of proteins and fats. The method described previously [13] was modified to allow analysis of benzoic acid in yoghurts by means of standard addition. In order to overcome these problems, samples had to be diluted, acidified and centrifuged to obtain an aqueous phase suitable for SBSE.

As mentioned in Chapter 6, it is necessary to adjust the sample pH in order to ensure benzoic acid is present in the protonated form. For yoghurt we also found the recovery of benzoic acid to be much higher at a pH of 2. Theoretically, based on the octanol/water partition coefficient ($K_{(o/w)}$) of 1.87 [37] the recovery of benzoic acid is predicted to be 40% [1]. As a result of the polarity of benzoic acid, a stirring time of 60 minutes was necessary in order to reach equilibrium and produce repeatable results. The repeatability of the method was found to be acceptable (< 5% RSD (n=4)). Concerning the sensitivity of the method, the limit of detection is 50 μg/kg (ppb) in the full scan mode. This value is relatively high compared to the previous method reported for soft drinks [13] due to the complexity of the matrix and resulting requisite dilution. Nevertheless, this was sufficient for determination of benzoic acid in all yoghurt samples (**Table 5**).

A typical chromatogram obtained by SBSE-TD-GC-MS analysis of a yoghurt sample using the optimized conditions outlined in the experimental section is shown in **Figure 7**. Yoghurt benzoic acid levels were found to be rather low - the maximum amount of benzoic acid added for standard addition was 80 ppm. In most cases, however, it was sufficient to add only up to 20 ppm. In **Figure 8** a typical standard addition graph for a yoghurt sample is shown. The concentration of benzoic acid in yoghurt samples varied from 300 ppb to 33.5 ppm. Quantitative results for 9 different yoghurt samples are summarised in Table 5.

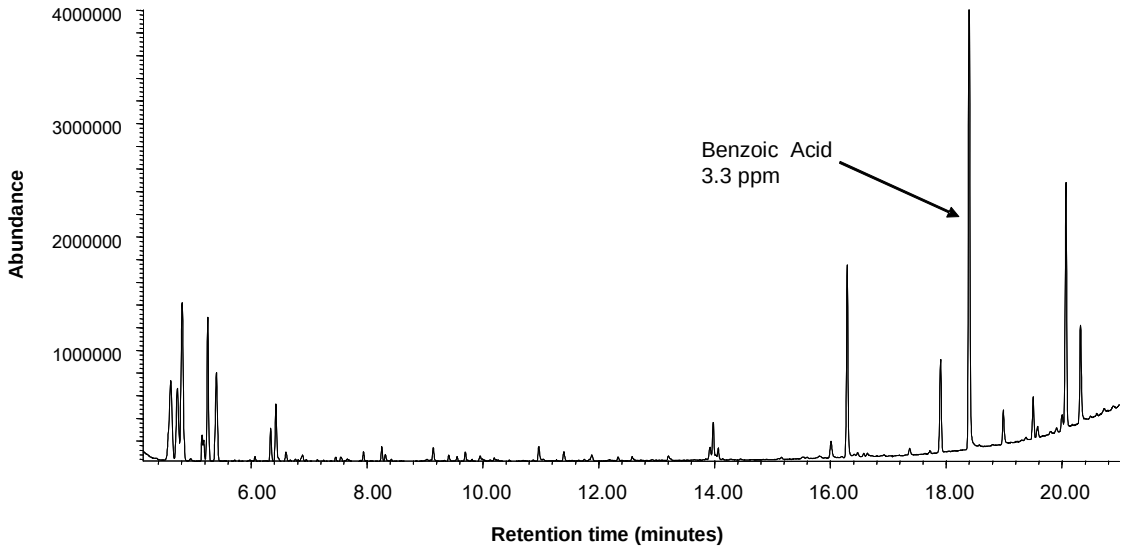

Figure 7. Chromatogram obtained for the analysis of benzoic acid in a yoghurt sample containing 3.3 ppm of the preservative.

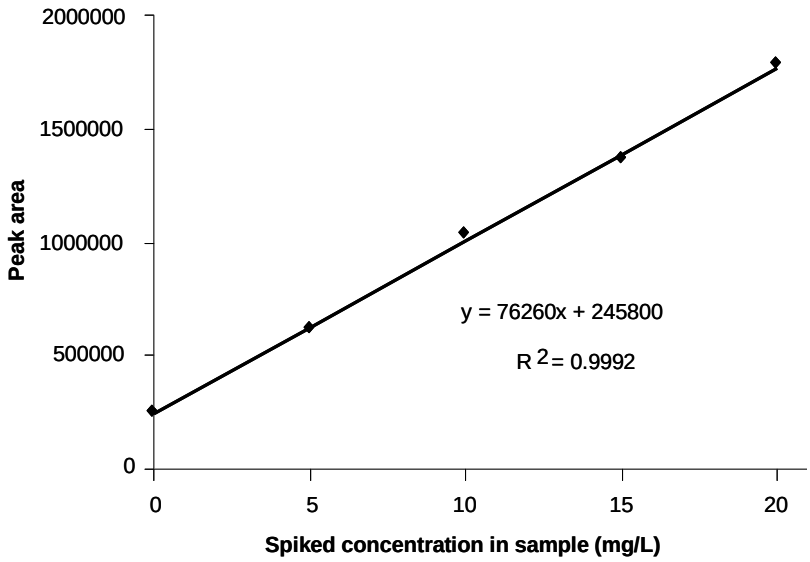

Figure 8. Standard addition graph for analysis of benzoic acid in a yoghurt sample.

Table 5. Summary of results for the 9 yoghurt samples investigated.

Sample Nr.	Variety	Conc. Benzoic Acid (ppm)	R^2 value of std. addition graph
1	Chocolate[a]	0.3	0.9993
2	Caramel[a]	3.0	0.9971
3	Peach & Mango[a]	3.3	0.9992
4	Plain Low Fat[a]	2.6	0.9970
5	Plain Fat Free[a]	16.2	0.9964
6	Bulgarian Fat Free[a]	5.8	0.9969
7	Plain Low Fat[b]	33.5	0.9981
8	Plain Low Fat[b]	6.7	0.9972
9	Plain Low Fat[b]	7.4	0.9983

[a] Samples from the same manufacturer.
[b] Samples from 3 other manufacturers.

7.4 Conclusions

SBSE has been shown to be a powerful sample preparation technique with high sensitivity and characterised by excellent performance for the determination of a wide range of compounds in a variety of foodstuffs. The linear working regions ranged from trace analysis (ng/L) to the determination of relatively high concentrations (mg/L) of analytes. Compared to conventional techniques, sample-preparation is significantly simplified, thus implying that the method is less time-consuming and labour intensive with a smaller risk of contamination. Importantly, the use of toxic solvents is eliminated.

7.5 References

[1] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcolumn Sep. 11 (1999) 737.

[2] E. Baltussen, C. Cramers, P. Sandra, Anal. Bioanal. Chem. V373 (2002) 3.

[3] R.F. Alves, A.M.D. Nascimento, J.M.F. Nogueira, Anal. Chim. Acta 546 (2005) 11.

[4] R.M. Callejon, A.M. Troncoso, M.L. Morales, Talanta 71 (2007) 2092.

[5] F. David, A. Tredoux, E. Baltussen, A. Hoffmann, P. Sandra, *in* "Proceedings of the 23th International Symposium on Capillary Chromatography", P. Sandra (Ed.), I.O.P.M.S., Kortrijk, Belgium (Pub.), CD-Rom paper M 35, 2000.

[6] M.J. Gomez-Miguez, J.F. Cacho, V. Ferreira, I.M. Vicario, F.J. Heredia, Food Chem. 100 (2007) 1464.

[7] C. Lorenzo, A. Zalacain, G.L. Alonso, M.R. Salinas, J. Chromatogr. A 1114 (2006) 250.

[8] N. Ochiai, K. Sasamoto, S. Daishima, A.C. Heiden, A. Hoffmann, J. Chromatogr. A 986 (2003) 101.

[9] N. Ochiai, K. Sasamoto, M. Takino, S. Yamashita, S. Daishima, A. Heiden, A. Hoffmann, Anal. Bioanal. Chem. 373 (2002) 56.

[10] P. Sandra, B. Tienpont, F. David, J. Chromatogr. A 1000 (2003) 299.

[11] P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.

[12] A.G.J. Tredoux, MSc Thesis, University of Stellenbosch, 2000 p 70-76

[13] A.G.J. Tredoux, H.H. Lauer, T. Heideman, P. Sandra, J. High Resolut. Chromatogr. 23 (2000) 644.

[14] B.T. Weldegergis, A.G.J. Tredoux, A.M. Crouch, J. Agric. Food Chem. 55 (2007) 8696.

[15] A. Zalacain, J. Marin, G.L. Alonso, M.R. Salinas, Talanta 71 (2007) 1610.

[16] P. Vlachos, A. Kampioti, M. Kornaros, G. Lyberatos, Food Chem. 105 (2007) 681.

[17] C. Pizarro, N. Perez-del-Notario, J.M. Gonzalez-Saiz, J. Chromatogr. A 1166 (2007) 1.

[18] C. Pizarro, N. Perez-del-Notario, J.M. Gonzalez-Saiz, J. Chromatogr. A 1143 (2007) 26.

[19] L. Zhang, R. Hu, Z. Yang, Water Research 40 (2006) 699.

[20] R. Soniassy, P. Sandra, C. Schlett, *in* 'Water Analysis', Agilent Technologies, 1997, p 45-72.

[21] Environmental Protection Agency, Office of Water *in* 'Stage 1 Disinfectants and Disinfection Byproducts Rule: A Quick Reference Guide', E. 816-F-01-010, United States, 2001 p1-2.

[22] B. Tienpont, F. David, E. Dewulf, P. Sandra, Chromatographia 61 (2005) 365-370.

[23] Y.-C. Ma, C.-Y. Chiang, J. Chromatogr. A 1076 (2005) 216.

[24] S. Rocha, V. Ramalheira, A. Barros, I. Delgadillo, M.A. Coimbra, J. Agric. Food Chem. 49 (2001) 5142.

[25] K.L. Howard, J.H. Mike, R. Riesen, Am. J. Enol. Vitic. 56 (2005) 37.

[26] Y. Fang, M.C. Qian, J. Agric. Food Chem. 54 (2006) 8567.

[27] K.J. Verstrepen, G. Derdelinckx, J.-P. Dufour, J. Winderickx, J.M. Thevelein, I.S. Pretorius, F.R. Delvaux, J. Biosc. Bioeng. 96 (2003) 110.

[28] Q. Xiao, C. Yu, J. Xing, B. Hu, J. Chromatogr. A 1125 (2006) 133.

[29] O. Pinho, I.M.P.L.V.O. Ferreira, L.H.M.L.M. Santos, J. Chromatogr. A 1121 (2006) 145.

[30] M. Liu, Z. Zeng, B. Xiong, J. Chromatogr. A 1065 (2005) 287.

[31] K. Helmja, M. Vaher, T. Pussa, K. Kamsol, A. Orav, M. Kaljurand, J. Chromatogr. A 1155 (2007) 222.

[32] European Parliament and Council Directive *in* 'On Food Additives other than Colours and Sweeteners, No 95/2/EC, OJ L 61, 18.3.1995, p 19-23.

[33] I. Techakriengkrai, R. Surakarnkul, J. Food Com. Anal. 20 (2007) 220.

[34] M. Gonzalez, M. Gallego, M. Valcarcel, J. Chromatogr. A 823 (1998) 321.

[35] M. Gonzalez, M. Gallego, M. Valcarcel, J. Chromatogr. A 848 (1999) 529.

[36] C. Dong, Y. Mei, L. Chen, J. Chromatogr. A 1117 (2006) 109.

[37] W.M. Meylan and P.H. Howard, J. Pharm. Sci., 84 (1995) 83. (Software KowWIN Version 1.66, SRC-LOGKOW, SRC-ESC, Syracuse, USA, 2000.)

8

Conclusions

Conclusions

The primary goal of this study was do develop new technologies for the analysis of volatile compounds in wine and beverages. This investigation took the form of the evaluation of a recently developed technique, stir bar sorptive extraction (SBSE), for sample pre-concentration prior to chromatographic analysis, utilizing thermal desorption gas chromatography-mass spectrometry (TD-GC-MS).

In Chapter 2 the relevant background pertaining to the analytical techniques used are presented. These include brief discussions on basic chromatographic theory, gas chromatographic separations and instrumental aspects of GC and mass spectrometry, thermal desorption, as well as qualitative and quantitative analysis. In addition, background information is provided regarding the most relevant sample preparation techniques generally used for volatile analysis of foodstuffs. These include liquid liquid extraction (LLE), solid phase extraction (SPE), purge and trap sampling, and sorptive techniques such as solid phase micro extraction (SPME) and SBSE.

Chapter 3 presents a survey of recent advances in sample preparation specifically used for wine volatile analysis. From this discussion it is evident that a vast number of technologies have, with varying success, been applied for the analysis of both major and minor wine volatile constituents. The most prevalent sample preparation method is LLE, normally employed for the analysis of major volatiles. Recent trends indicate increasing utilization of SPE and SPME, most often for the extraction of specific groups of compounds, although also suited for screening purposes. When major volatiles are determined, it may be possible to monitor simultaneously a few minor compounds. On the other hand, for specific methods targeting a specific group of minor compounds, additional interfering compounds should often be removed. From this chapter, it can be concluded that truly specific sample pretreatment methods for comprehensive wine volatile analysis do not exist.

Results for the main part of this study are discussed in Chapter 4. Here the outcome was twofold. Firstly, a SBSE-TD-GC-MS method was developed for the screening of volatiles in South African wines. This completely solventless method was shown to be sensitive, robust, repeatable and easy to use, and as such presents a viable alternative to SPME and LLE. It was further illustrated that compounds from a large number of groups are

successfully extracted, including alcohols, esters and acids, volatile phenols and compounds related to wood cooperage such as the furfuryl derivatives, lactones and carbonyls. The second part of the study was to employ the developed analytical method for the volatile screening of 60 South African wines. 38 volatile compounds were quantified in these wines and subsequently this dataset was investigated using statistical techniques (chemometric methods). Principle component analysis (PCA) and cluster analysis (CA) indicated that the most important variations in the volatile data could be related to wine age, while discriminant (DA) analysis allowed discrimination of these wines according to cultivar based on their volatile composition. This discrimination is obtained notwithstanding differentiating factors such as geographical origin, vintage, or wine-making practice.

In Chapter 5 the applicability of SBSE-TD-GC-MS for the analysis of three commonly used fungicides, vinclozolin, procymidone and iprodione, in wine was demonstrated. In order to validate the accuracy of the SBSE method the same three compounds were analysed by liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy (SBSE-LD-LC-APCI-MS) following liquid desorption of the stir bar with acetonitrile. The data obtained by both techniques was comparable indicating the suitability of the SBSE technique for analysis of these fungicides. This study also demonstrated the applicability of liquid desorption following SBSE, extending the possible use of the technology to analysis of non-volatiles.

In order to evaluate the performance of SBSE for polar compounds, Chapter 6 illustrates the application of SBSE-TD-GC-MS for the analysis of benzoic acid in lemon flavoured soft drinks. It was found that if the sample is diluted and acidified the linear range is extended up to 1000 ppm, while the method was characterized by a relative standard deviation of less than 5%. The method was found to be simple, robust and sensitive, and importantly does not require derivatisation prior to GC-MS analysis. It was simultaneously shown that the SBSE method can be used to analyse the terpenes originating from the lemon oil used and that differentiation between the type of oil is possible in this manner.

In Chapter 7 a number of diverse applications of the SBSE technology are shown. These include the analysis of trichloroanisole (TCA) in wine, contaminants in drinking water and furthering the method developed in Chapter 6, the analysis of benzoic acid in yoghurt

samples. Additional screening methods for wine and beer by SBSE are presented. The main advantage of these methods is that the chromatographic analysis time is about half of that of the method presented in Chapter 4. An application of stir bar sampling performed in the headspace, HSSE, believed to be a more representative analysis of the aroma of wine, is also presented. Each of these methods were demonstrably robust, simple, and repeatable.

From the results presented in this thesis, it can broadly be concluded that SBSE technology can advantageously be applied for the analysis of a diverse range of compounds in beverages, ranging in polarity from the non-polar fatty acids and esters to the polar acetic- and benzoic acids. In terms of the molecular weight (MW) range of the analytes, it was possible to apply the method to low MW esters such as ethyl butyrate and high MW compounds such as acetovanillone. Concerning concentration ranges, compounds varying in concentration between low ng/L (pesticides) and high mg/L (benzoic acid) could successfully be quantified. Thus it can be concluded that SBSE is an extremely powerful analytical technique characterized by ease of use and good repeatability, sensitivity and robustness.

Future extension of the work presented here should include the exploration of SBSE for additional target analysis in wine samples and exploiting the inherent sensitivity of the technique to elucidate novel volatiles in wine. Furthermore, wine characterization according to additional relevant criteria such as geographical origin and vintage should be investigated using an extended dataset. SBSE will also benefit from selective phases other than polydimethylsiloxane (PDMS), currently the only commercially available phase. Another application worth exploring is the use of SBSE as a simple extraction method prior to GC-olfactometry (GC-O) in order to establish relations between chemical composition and sensory attributes of beverages.

Appendix A

Cabernet Sauvignon	Vintage
CC Cab S 96	1996
CC Cab S 97	1997
CC Cab S 98	1998
Stel'ryck Cab S 98	1998
YW 48/2[b]	2003
YW 48/3[b]	2003
YW 48/4[b]	2003

Merlot	Vintage
Plaisir Merlot 99	1999
YW 49/2[b]	2003
YW 49/3[b]	2003
YW 49/4[b]	2003
YW 49/5[b]	2003
YW 49/7[b]	2003
YW 49/8[b]	2003

Pinotage	Vintage
K'kop Pinot	1999
Lutzv Pinot 99	1999
M Vilj Pinot	2001
WK Pinot B16/t55[a]	2001
KWV Pinot A06/t69[a]	2001
YW 46/2[b]	2003

Shiraz	Vintage
Villiera Shiraz	2001
Eikdl Shiraz	2001
Boland Shiraz	1999
Neil J Shiraz 01	2001
Landsk Shiraz 00	2001
WK Shiraz B16/52[a]	2001
YW 55/1[b]	2003
YW 55/2[b]	2003
YW 55/3[b]	2003
YW 55/5[b]	2003
YW 55/8[b]	2003
YW 55/9[b]	2003
YW 55/10[b]	2003

Ruby Cabernet	Vintage
YW 57/2[b]	2003
YW 57/3[b]	2003
YW 57/4[b]	2003
YW 57/5[b]	2003
YW 57/6[b]	2003

Red Blends	Vintage
RdB 97	1997
RdB 98	1998
RdB 99	1999
RdB 00	2000
Simonsvlei	2000

[a]Tank samples obtained from KWV

[b]Wines from the Young Wine Show 2003

Table A1. The red wines analysed in this study.

Chardonnay	Vintage
CC Chard 99	1999
CC Chard 00	2000
CC Chard 01	2001
KWV Chard 01	2001
YW 9/1[a]	2003
YW 9/2[a]	2003
YW 9/5[a]	2003
YW 9/6[a]	2003
YW 9/7[a]	2003
YW 9/10[a]	2003
Simonsvlei	2001

Sauvignon Blanc	Vintage
KWV S Bl 01	2000
KWV S Bl 00	2001
YW 8/2[a]	2003
YW 8/3[a]	2003
Gecko Ridge	2001

Chenin Blanc	Vintage
KWV Ch Bl 01	2001
YW 15/1[a]	2003
YW 15/2[a]	2003

[a]Wines from the Young Wine Show 2003

Table A2. The white wines analysed in this study.

Appendix B

Compound	Abbreviation	Boland Shiraz 99	Eikdl Shiraz 99	K'kop pinot 99	YW46/2	YW46/3	YW48/2
Alcohols							
isoamyl alcohol	isoam.oh	2.5737	5.1817	1.5783	1.9421	1.6557	3.9559
1-butanol	but.oh	0.1270	0.1203	0.1432	0.1113	0.3085	0.1891
1-hexanol	hex.oh	0.5592	0.6368	0.3527	0.3272	0.4674	0.3682
1-octanol	oct.oh	1.4575	0.4422	0.7999	1.2763	0.7966	0.9181
β-phenylethyl alcohol	phenet.oh	6.8218	5.5176	2.5338	3.1894	2.7350	18.3855
furfuryl alcohol	furf.oh	4.5292	2.2667	0.6629	4.1137	4.4662	4.5214
Phenols							
guaiacol	g.col	0.7848	0.1842	0.0807	0.3432	0.3569	0.3708
4-vinylguaiacol	4vg	1.0377	0.2804	0.0913	0.4353	0.4899	0.4860
cis-isoeugenol	c-eug	0.9042	0.1993	0.0607	0.2940	0.3446	0.2775
trans-isoeugenol	t-eug	0.1966	0.0759	0.0528	0.0552	0.0653	0.0554
Aldehydes							
furfural	furf	40.9885	17.3261	5.1791	27.7030	28.6799	28.2066
5-hydroxymethyl furfural	5-HMF	1.3920	0.6306	0.1670	0.8025	0.8413	0.4889
vanillin	vanil	1.3417	0.3914	0.1166	0.5547	0.6335	0.5536
Ketones							
2-octanone	2-oct.one	0.5532	0.6773	0.5516	0.6873	0.6648	0.5820
acetovanillone	acet.van.one	0.8452	0.2465	0.0543	0.3635	0.4251	0.3884
Acids							
formic acid	form.ac	61.8449	30.1405	4.2179	54.8122	50.1679	58.2059
acetic acid	acet.ac	122.2407	31.8434	13.8253	53.1464	57.3985	51.1414
propanoic acid	prop.ac	5.9670	2.3612	0.7249	3.6030	2.0015	2.3813
octanoic acid	oct.ac	0.3247	0.2338	0.2807	0.4826	0.4269	0.3859
decanoic acid	dec.ac	0.4166	0.3942	0.3602	0.9100	0.7930	0.8694
dodecanoic acid	dodec.ac	0.0379	0.0252	0.0253	0.0671	0.0457	0.0269
Esters							
isoamyl acetate	isoam.act	0.0842	0.3076	0.0598	0.8467	0.2876	0.3145
hexylacetate	hex.act	0.0437	0.1490	0.0116	0.3659	0.3247	0.1744
ethylphenyl acetate	eth.phen.act	0.1675	0.1244	0.0934	0.0304	0.0642	0.1493
β-phenylethyl acetate	phen.eth.act	0.1462	0.2360	0.0441	0.3668	0.2762	0.5926
ethyl lactate	eth.lact	7.4185	9.7916	9.9872	5.3752	5.4520	2.5118
ethyl butyrate	eth.but	0.0736	0.0809	0.0565	0.1152	0.0742	0.0671
ethyl isovalerate	eth.isoval.	0.1241	0.2012	0.0898	0.0423	0.0774	0.0393
ethyl hexanoate	eth.hex	3.8016	5.5032	2.4678	5.7068	4.9638	4.1117
ethyl octanoate	eth.ocy	8.7296	10.5658	5.9371	21.7443	16.3030	17.6655
ethyl decanoate	eth.dec	1.4263	1.9863	1.0900	4.8770	3.5041	5.3322
ethyl-9-decenoate	eth-9-dec	0.0412	0.1209	0.0360	0.0688	0.1327	0.0459
ethyl dodecanoate	eth.dodec	0.0171	0.0222	0.0199	0.1002	0.0532	0.0743
diethyl succinate	dieth.suc	163.2157	65.7028	77.2783	56.9708	23.3369	108.1441
ethyl isopentyl succinate	eth.ispen.suc	14.0796	7.1695	5.4624	9.6632	3.1823	38.0709
ethyl vanillate	eth.vanil	0.1030	0.0742	0.1013	0.0441	0.0486	0.0460
Lactones							
γ-butyrolactone	γ-butlact	0.1968	0.1081	0.0734	0.2311	0.1569	0.2859
(Z)-whiskey lactone	w-lact	0.3086	0.0816	0.0395	0.1124	0.1345	0.1030

Table B1. Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	YW48/3	YW48/4	YW49/2	YW49/3	YW49/4	YW49/5	YW49/7
Alcohols							
isoam.oh	4.3287	4.2741	3.3187	4.7780	2.2956	2.2508	3.2857
but.oh	0.2255	0.0581	0.0961	0.0980	0.0732	0.0767	0.1220
hex.oh	0.5121	0.6236	0.3898	0.8206	0.1456	0.5075	0.4581
oct.oh	1.3371	0.8303	0.7798	0.6467	0.3537	0.6351	0.6497
phenet.oh	17.8828	17.5488	8.0293	5.1567	6.8861	5.5828	11.3855
furf.oh	4.0972	4.4679	2.7783	2.2052	0.9911	0.7393	1.9309
Phenols							
g.col	0.2734	0.3614	0.3481	0.1627	0.0843	0.0622	0.1728
4vg	0.3780	0.5369	0.4508	0.2334	0.1099	0.0965	0.2209
c-eug	0.2491	0.3358	0.3774	0.1469	0.0673	0.0539	0.1437
t-eug	0.0507	0.0673	0.0678	0.0276	0.0135	0.0117	0.0267
Aldehydes							
furf	27.0030	29.6923	23.2601	17.3266	8.0589	6.7037	15.6272
5-HMF	0.6599	0.9276	0.6754	0.4532	0.4349	0.1745	0.6062
vanil	0.5520	0.6616	0.6964	0.3283	0.1356	0.1409	0.2770
Ketones							
2-oct.one	0.5913	0.6589	0.5621	0.5955	0.5492	0.4073	0.5607
acet.van.one	0.3522	0.4494	0.4134	0.2038	0.0991	0.0849	0.1950
Acids							
form.ac	51.7596	56.3109	36.4071	26.5795	4.5398	10.6004	19.8607
acet.ac	53.8411	49.1849	65.3868	28.0426	11.5319	11.2160	27.4834
prop.ac	2.1946	2.3097	1.6319	0.7562	0.7414	0.7724	0.9400
oct.ac	0.1634	0.3045	0.3388	0.2657	0.2056	0.3345	0.2576
dec.ac	0.2804	0.5778	0.5088	0.4814	0.5712	0.5745	0.4389
dodec.ac	0.0177	0.0419	0.0323	0.0204	0.0138	0.0477	0.0290
Esters							
isoam.act	0.2553	0.2360	0.5772	0.4655	0.2123	0.1009	0.1289
hex.act	0.0761	0.0819	0.2278	0.2136	0.0810	0.0596	0.0337
eth.phen.act	0.2162	0.2323	0.0724	0.0610	0.0672	0.0700	0.1312
phen.eth.act	0.4903	0.3905	0.5220	0.2253	0.3456	0.1063	0.1892
eth.lact	2.4368	3.4025	4.0772	4.4920	2.3581	2.4939	4.0669
eth.but	0.0451	0.0686	0.0748	0.0876	0.0348	0.0739	0.0623
eth.isoval.	0.0951	0.0847	0.0620	0.1031	0.0435	0.0667	0.0835
eth.hex	2.2712	4.2124	3.6083	5.6453	2.2912	4.0700	2.2472
eth.ocy	5.3893	10.3575	11.9488	11.0967	6.7783	12.8974	9.3647
eth.dec	1.3885	2.9350	2.3962	2.4018	2.0490	3.4901	3.0374
eth-9-dec	0.0163	#VALUE!	0.1288	0.0199	0.0598	0.0125	0.0560
eth.dodec	0.0549	0.1159	0.0551	0.1100	0.0335	0.2663	0.2958
dieth.suc	68.2036	56.0895	19.8648	47.2146	76.7000	24.9785	65.5058
eth.ispen.suc	28.9021	16.9053	4.7828	8.7326	18.5686	3.8734	16.9773
eth.vanil	0.1471	0.0546	0.0843	0.0249	0.0381	0.0810	0.0989
Lactones							
γ-butlact	0.1830	0.1875	0.1268	0.1175	0.0489	0.0696	0.1016
w-lact	0.0965	0.1452	0.1070	0.0615	0.0422	0.0197	0.0671

Table B1 (continued). Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	YW49/8	YW55/1	YW55/10	YW55/2	YW55/3	YW55/5	YW55/8
Alcohols							
isoam.oh	3.3880	2.5013	3.6563	2.4822	2.6009	3.0637	2.7651
but.oh	0.1176	0.2712	0.1168	0.1372	0.1510	0.0583	0.1263
hex.oh	0.4866	0.6211	0.4738	0.6845	0.4684	0.4226	0.6555
oct.oh	0.5830	2.6226	0.6921	1.4433	0.5397	0.6571	0.6925
phenet.oh	10.1261	6.1921	14.1704	2.7208	6.3779	10.9412	5.9995
furf.oh	6.2347	1.9477	5.4448	0.1694	3.4674	1.1158	2.4394
Phenols							
g.col	0.9273	0.2551	0.5174	0.0947	0.2558	0.1663	0.2348
4vg	1.3166	0.3342	0.6662	0.1994	0.3920	0.2426	0.3424
c-eug	1.1136	0.2041	0.4676	0.0835	0.2050	0.1816	0.2371
t-eug	0.1959	0.0664	0.1118	0.0301	0.0730	0.0609	0.0578
Aldehydes							
furf	52.9693	17.0244	36.7428	8.4961	24.6774	10.5579	22.8116
5-HMF	1.6852	0.4254	0.8930	0.2753	0.7823	0.3073	0.5835
vanil	1.7636	0.3637	0.8402	0.1705	0.4773	0.2716	0.4814
Ketones							
2-oct.one	0.4971	0.6995	0.6029	0.4578	0.5180	0.6161	0.5089
acet.van.one	1.0858	0.2438	0.5249	0.1190	0.3108	0.1732	0.2872
Acids							
form.ac	74.5421	29.4592	77.3464	9.6049	42.2223	11.1251	35.2825
acet.ac	146.6177	36.6042	80.3765	20.0079	39.7693	22.6765	52.6376
prop.ac	1.7987	2.1696	3.0398	0.7410	1.9454	0.8714	1.4515
oct.ac	0.2359	0.1886	0.2085	0.2035	0.1463	0.2773	0.2859
dec.ac	0.3622	0.3631	0.3045	0.2649	0.1301	0.5306	0.3658
dodec.ac	0.0139	0.0393	0.0174	0.0184	0.0068	0.0166	0.0167
Esters							
isoam.act	0.0735	0.0482	0.1352	0.1950	0.2104	0.3544	0.2226
hex.act	0.0269	0.0495	0.0363	0.0936	0.1279	0.1550	0.1332
eth.phen.act	0.0775	0.0516	0.1151	0.0796	0.0795	0.1014	0.1002
phen.eth.act	0.1005	0.0709	0.2031	0.0918	0.3716	0.7245	0.2427
eth.lact	2.6767	4.2102	3.0187	6.4791	1.8668	3.2296	3.8807
eth.but	0.0373	0.0654	0.0428	0.0756	0.0361	0.0733	0.0910
eth.isoval.	0.0410	0.0168	0.0497	0.0218	0.0359	0.0663	0.0811
eth.hex	2.5641	2.3723	2.0690	2.5848	1.2334	2.6689	3.9789
eth.ocy	6.8175	7.4002	6.8732	6.5636	1.5800	8.8329	8.6458
eth.dec	1.7077	2.1699	1.7734	1.4824	0.2573	2.1282	1.6757
eth-9-dec	0.0086	0.0247	0.0141	0.0160	0.0036	0.1287	0.0507
eth.dodec	0.0854	0.2572	0.1321	0.1595	0.0028	0.0230	0.0500
dieth.suc	67.0098	165.6534	73.8511	32.8331	60.0779	67.6601	94.4910
eth.ispen.suc	16.4089	22.3018	26.3049	5.7837	13.5070	24.3894	14.3705
eth.vanil	0.0846	0.3276	0.2221	0.3568	0.2506	0.1245	0.0794
Lactones							
γ-butlact	0.2202	0.2415	0.2748	0.0866	0.1350	0.1026	0.1302
w-lact	0.3676	0.0770	0.1651	0.0376	0.1060	0.0643	0.0908

Table B1 (continued). Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	YW55/9	YW57/2	YW57/3	YW57/4	YW57/5	YW57/6	Landsk Shiraz 00
Alcohols							
isoam.oh	3.8499	3.6019	3.7344	3.4717	1.9396	2.6897	2.4837
but.oh	0.1985	0.1511	0.0748	0.2074	0.1418	0.1290	0.1265
hex.oh	0.6756	1.3126	0.5760	0.8961	0.4972	0.7421	0.4880
oct.oh	0.6503	0.5954	0.5754	0.9250	0.9652	0.7471	0.8597
phenet.oh	16.9041	8.8609	16.1226	9.9063	4.8490	7.3472	6.0689
furf.oh	6.2287	1.0901	1.2279	3.7283	4.9694	3.8864	4.6535
Phenols							
g.col	0.4862	0.1421	0.1290	0.2616	0.3405	0.3101	0.4637
4vg	0.6651	0.2102	0.1768	0.3718	0.4855	0.4451	0.6713
c-eug	0.4180	0.1376	0.1355	0.2420	0.3286	0.3168	0.5097
t-eug	0.1234	0.0341	0.0532	0.0635	0.0696	0.0775	0.1457
Aldehydes							
furf	35.4842	9.7319	8.7460	21.2856	31.5349	23.5531	29.0914
5-HMF	1.2122	0.2958	0.2985	0.7953	1.1675	0.7502	0.8505
vanil	0.7944	0.2327	0.2658	0.5156	0.6693	0.5735	0.9429
Ketones							
2-oct.one	0.5941	0.5991	0.5663	0.5662	0.6494	0.5792	0.5200
acet.van.one	0.5193	0.1644	0.1512	0.3296	0.4267	0.3566	0.5433
Acids							
form.ac	60.3718	9.5884	10.4606	29.1959	40.1001	35.1082	39.3606
acet.ac	61.5025	19.6861	16.7786	37.0198	47.7036	40.5764	81.1883
prop.ac	2.9468	1.1580	0.8996	1.5434	1.9720	1.5264	2.2589
oct.ac	0.2690	0.3023	0.2292	0.3221	0.6163	0.4072	0.2395
dec.ac	0.3957	0.5719	0.3515	0.5717	1.1526	0.5115	0.2838
dodec.ac	0.0191	0.0832	0.0171	0.0282	0.0351	0.0784	0.0099
Esters							
isoam.act	0.2651	0.4624	0.2353	0.4058	0.2006	0.2584	0.0689
hex.act	0.1627	0.3123	0.0672	0.2487	0.1335	0.0740	0.0262
eth.phen.act	0.2216	0.0855	0.1745	0.0637	0.0417	0.0862	0.1631
phen.eth.act	0.7704	0.4480	0.5312	0.5979	0.1659	0.2723	0.1107
eth.lact	2.8123	4.0855	4.3526	2.9849	4.4734	5.2173	5.2397
eth.but	0.0307	0.0940	0.0472	0.0759	0.0990	0.0788	0.0494
eth.isoval.	0.0763	0.0318	0.0632	0.0228	0.0242	0.0248	0.1177
eth.hex	2.4389	4.5050	2.3676	3.7441	6.5244	4.3447	2.3710
eth.ocy	7.9916	11.0710	6.2806	12.3561	19.7297	11.9470	5.3973
eth.dec	2.0041	4.4965	1.3366	3.5479	4.4462	4.5688	0.9761
eth-9-dec	0.0333	0.1212	0.0324	0.0429	0.0060	0.0076	0.0261
eth.dodec	0.0496	0.5999	0.0235	0.1835	0.0632	0.7035	0.0022
dieth.suc	77.1551	110.2629	59.7371	125.5219	72.4210	106.6259	178.7079
eth.ispen.suc	26.1962	24.8882	24.6614	21.0340	11.9534	17.1081	16.6029
eth.vanil	0.0920	0.1179	0.0740	0.1168	0.0321	0.0552	0.3456
Lactones							
γ-butlact	0.2873	0.1026	0.0817	0.1693	0.1960	0.1833	0.2656
w-lact	0.1870	0.0657	0.0588	0.0991	0.1348	0.1202	0.1583

Table B1 (continued). Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	Lutzv pinot 99	Plaisir Merl 99	M Vilj pinot01	CC Cab S96	CC Cab S 97	CC Cab S 98	Neil J Shiraz 01
Alcohols							
isoam.oh	1.0819	3.2699	1.7435	3.1119	3.2132	3.1429	2.5119
but.oh	0.1507	0.2151	0.1493	0.1014	0.1500	0.0956	0.1341
hex.oh	0.2719	0.3936	0.5383	0.5617	0.5897	0.4057	0.3523
oct.oh	0.5351	0.9499	1.1929	0.9165	1.2235	0.8827	1.5509
phenet.oh	2.1206	8.2623	3.2096	8.0349	10.3717	10.1481	8.3405
furf.oh	5.1858	0.4977	3.8472	2.6651	2.1467	1.0674	3.1922
Phenols							
g.col	0.8599	0.0832	0.6903	0.2273	0.1956	0.1309	0.2659
4vg	0.7461	0.1203	0.9735	0.2966	0.2617	0.1658	0.4050
c-eug	0.4817	0.0730	0.7862	0.1748	0.1641	0.1206	0.2603
t-eug	0.0788	0.0313	0.1799	0.0712	0.1047	0.0839	0.0845
Aldehydes							
furf	36.1847	6.3474	32.7417	16.5526	14.7712	7.5615	22.5924
5-HMF	0.5391	0.2167	1.0896	0.3103	0.7139	0.3214	0.6973
vanil	0.6461	0.1746	1.1477	0.3529	0.3852	0.2392	0.5267
Ketones							
2-oct.one	0.5226	0.6118	0.5309	0.5358	0.5826	0.6348	0.5621
acet.van.one	0.4151	0.0630	0.7654	0.2601	0.2566	0.1489	0.3537
Acids							
form.ac	49.1005	4.0735	33.7359	15.9578	12.7486	5.8421	20.2922
acet.ac	90.0240	15.4043	95.1991	35.1073	25.8377	20.5163	38.9890
prop.ac	4.4793	0.8066	1.5923	1.0964	1.0970	0.5636	1.0103
oct.ac	0.3161	0.4165	0.3921	0.2065	0.2437	0.2298	0.3566
dec.ac	0.2621	0.7650	0.6105	0.2405	0.2638	0.3074	0.5904
dodec.ac	0.0050	0.0270	0.0154	0.0076	0.0352	0.0470	0.0251
Esters							
isoam.act	0.0558	0.0727	0.1066	0.1155	0.1279	0.1341	0.1033
hex.act	0.0275	0.0130	0.0333	0.0288	0.0324	0.0218	0.0208
eth.phen.act	0.0632	0.1665	0.0613	0.1783	0.2471	0.2406	0.1394
phen.eth.act	0.0605	0.1037	0.0839	0.1452	0.1717	0.1754	0.1381
eth.lact	5.6703	8.7399	11.5835	11.0450	10.6537	8.7051	4.5816
eth.but	0.0393	0.0843	0.0898	0.0521	0.0661	0.0563	0.0671
eth.isoval.	0.0528	0.1742	0.0324	0.1504	0.2635	0.2381	0.0887
eth.hex	2.5928	5.3461	4.2067	2.4886	3.8643	2.8956	3.6293
eth.ocy	7.5595	19.4390	11.2410	5.9972	9.7872	7.1422	11.8601
eth.dec	0.8660	3.6275	2.6074	1.0224	1.7923	1.8456	2.6928
eth-9-dec	0.0546	0.0354	0.0149	0.0039	0.0319	0.0047	0.0332
eth.dodec	0.0040	0.0419	0.0538	0.0200	0.1860	0.2898	0.0648
dieth.suc	91.8392	271.1033	64.5196	155.8199	293.6019	175.3691	146.6949
eth.ispen.suc	4.6322	36.8753	5.7572	14.9240	37.9956	24.9510	16.8660
eth.vanil	0.0635	0.1834	0.2105	0.2276	0.3205	0.1740	0.1764
Lactones							
γ-butlact	0.3383	0.0830	0.1537	0.1831	0.1366	0.0924	0.1681
w-lact	0.1846	0.0241	0.2878	0.0904	0.0957	0.0662	0.1204

Table B1 (continued). Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	Simonsvl 00	RdB 98	KWV Pinot A06/T69	Pinotage B16/T55	RdB 00	RdB 98	RdB 99
Alcohols							
isoam.oh	1.9308	2.6045	1.1128	1.5689	2.7593	3.2523	3.0810
but.oh	0.1250	0.1475	0.1377	0.1193	0.2203	0.0991	0.2667
hex.oh	0.4614	0.5508	0.5024	0.7920	0.5188	0.6769	0.5857
oct.oh	0.8214	0.7538	0.8028	0.7048	1.1870	0.9571	0.8326
phenet.oh	6.2684	6.4052	2.0655	2.9046	7.0225	7.0713	7.4223
furf.oh	3.8313	2.2376	2.2039	2.8281	1.7118	1.0214	3.9499
Phenols							
g.col	0.3166	0.2361	0.1923	0.2133	0.2948	0.2049	0.4403
4vg	0.4636	0.3343	0.2757	0.2957	0.3226	0.2509	0.4805
c-eug	0.2882	0.2187	0.1938	0.2154	0.1517	0.1947	0.2907
t-eug	0.0877	0.0695	0.0519	0.0471	0.0607	0.0747	0.0951
Aldehydes							
furf	23.3354	19.8833	15.9551	17.9873	15.9625	10.0757	25.3314
5-HMF	0.6888	0.1149	0.4869	0.6526	0.4986	0.3173	0.6287
vanil	0.4871	0.4092	0.4104	0.4657	0.2483	0.3052	0.5168
Ketones							
2-oct.one	0.4712	0.5276	0.5730	0.5207	0.5578	0.5293	0.4698
acet.van.one	0.3407	0.2738	0.2582	0.2888	0.2167	0.2259	0.4014
Acids							
form.ac	26.4876	13.3640	15.7325	21.0164	18.0958	5.1310	30.3098
acet.ac	43.5938	37.0604	32.2831	37.4662	42.4762	29.4815	59.1491
prop.ac	0.8972	1.0648	1.5425	1.2717	1.7791	0.7660	2.1261
oct.ac	0.2548	0.2317	0.2368	0.2865	0.2719	0.2927	0.2519
dec.ac	0.3310	0.2509	0.3414	0.4362	0.3239	0.3059	0.3070
dodec.ac	0.0151	0.0132	0.0170	0.0343	0.0089	0.0176	0.0107
Esters							
isoam.act	0.0928	0.0882	0.1587	0.1674	0.1053	0.1096	0.1022
hex.act	0.0262	0.0230	0.1431	0.1668	0.0301	0.0280	0.0296
eth.phen.act	0.2124	0.2100	0.1433	0.0319	0.2295	0.2650	0.3222
phen.eth.act	0.1238	0.1017	0.1657	0.1123	0.1302	0.1276	0.1326
eth.lact	7.5693	10.1076	5.1238	1.7653	9.4181	12.6821	11.5114
eth.but	0.0494	0.0527	0.0362	0.0812	0.0626	0.0673	0.0600
eth.isoval.	0.1216	0.1213	0.0697	0.0340	0.1583	0.1747	0.1977
eth.hex	2.5234	3.0473	2.5299	3.9352	2.8863	3.6924	2.9832
eth.ocy	6.4517	7.0292	8.3900	10.4470	6.9622	8.2565	7.1292
eth.dec	1.0244	1.1984	1.7649	2.3052	1.1807	1.1984	1.1984
eth-9-dec	0.0164	0.0257	0.0068	0.0765	0.0195	0.0263	0.0204
eth.dodec	0.0137	0.0227	0.0536	0.0910	0.0120	0.0106	0.0147
dieth.suc	159.1515	160.6300	78.1697	71.9394	161.0649	204.3534	167.5769
eth.ispen.suc	16.0829	13.5981	5.1578	5.3082	16.2572	18.8264	16.2010
eth.vanil	0.2023	0.1987	0.1565	0.2278	0.2220	0.2601	0.2281
Lactones							
γ-butlact	0.1752	0.1309	0.1180	0.1655	0.2920	0.1069	0.2715
w-lact	0.1266	0.1082	0.0768	0.0791	0.1159	0.0804	0.1214

Table B1 (continued). Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	Stel'ryck Cab S 98	Vil Shriaz 01
Alcohols		
isoam.oh	2.5935	2.8203
but.oh	0.0644	0.1175
hex.oh	0.4758	0.4796
oct.oh	0.8176	0.6476
phenet.oh	7.9672	7.3544
furf.oh	7.2102	1.0538
Phenols		
g.col	0.4066	0.1432
4vg	0.5837	0.1708
c-eug	0.3436	0.0925
t-eug	0.1084	0.0473
Aldehydes		
furf	42.0215	9.1600
5-HMF	0.9404	0.5015
vanil	0.7610	0.2128
Ketones		
2-oct.one	0.5869	0.4980
acet.van.one	0.5346	0.1429
Acids		
form.ac	37.4481	5.6215
acet.ac	70.4262	18.1591
prop.ac	2.8662	0.8542
oct.ac	0.2396	0.3371
dec.ac	0.2756	0.5299
dodec.ac	0.0091	0.0137
Esters		
isoam.act	0.0734	0.2910
hex.act	0.0199	0.1624
eth.phen.act	0.1988	0.0843
phen.eth.act	0.1346	0.3781
eth.lact	10.8788	7.6233
eth.but	0.0271	0.0823
eth.isoval.	0.1173	0.0796
eth.hex	2.7608	3.6993
eth.ocy	6.4274	10.1138
eth.dec	0.9239	1.9548
eth-9-dec	0.0117	0.0258
eth.dodec	0.0049	0.0229
dieth.suc	169.4184	153.6675
eth.ispen.suc	13.4338	20.9573
eth.vanil	0.1870	0.3068
Lactones		
γ-butlact	0.0386	0.1246
w-lact	0.1583	0.0767

Table B1 (continued). Relative quantitative data obtained for the red wines. All data is reported as relative peak areas after correction with the internal standard.

Abbreviation	Geck ridge	YW15_1	YW15_2	YW8_2	YW8_3	YW9_1	YW9_10
isoam.oh	2.7802	1.1512	2.2162	1.4422	1.2091	1.6089	1.5448
but.oh	0.0930	0.1192	0.1328	0.5210	0.4746	0.4592	0.2165
hex.oh	0.8960	0.7604	0.6482	0.5891	0.8848	0.9183	0.7265
oct.oh	0.5135	0.3137	0.4753	1.0839	0.7432	0.7663	0.5761
phenet.oh	1.6531	1.1473	3.7927	2.2929	2.3347	2.9562	2.4344
furf.oh	1.3046	0.7661	0.8859	3.4804	2.0153	7.6749	0.7914
g.col	0.0565	0.2498	0.1914	0.2437	0.0590	0.1210	0.1786
4vg	0.1529	0.1520	0.2196	0.4256	0.3108	0.8998	0.4011
c-eug	0.1259	0.0840	0.1462	0.2451	0.1351	0.4941	0.0932
t-eug	0.0232	0.0289	0.0257	0.0431	0.0218	0.0906	0.0202
furf	10.0979	8.2674	12.6915	30.2409	19.7892	57.1844	10.5317
5-HMF	0.0668	0.3365	0.2640	0.2702	0.1740	0.2425	0.1702
vanil	0.2508	0.1529	0.2393	0.2270	0.3710	0.9513	0.2355
2-oct.one	0.3696	0.4454	0.3962	0.3766	0.3679	0.3756	0.4686
acet.van.one	0.1291	0.1096	0.1451	0.3374	0.2524	0.6385	#VALUE!
form.ac	17.6316	12.8354	11.7548	55.4243	31.2803	60.3817	8.4366
acet.ac	24.4613	23.0440	24.7970	67.8210	43.7052	117.8279	24.0332
prop.ac	1.6729	1.7867	1.1073	4.9282	3.2149	7.1098	1.7468
oct.ac	0.6905	0.7957	1.1074	1.6737	1.4138	1.0406	1.1665
dec.ac	0.8135	2.1083	2.2040	4.7430	4.4400	2.9851	6.1243
dodec.ac	0.0210	0.0930	0.0988	0.5109	0.2427	0.1606	0.2126
isoam.act	0.0869	0.1642	0.3394	0.7878	0.5682	0.3268	1.1525
hex.act	0.1144	1.2311	1.2513	2.7708	4.4974	0.4743	4.0399
eth.phen.act	0.1301	0.0775	0.2522	0.0322	0.0432	0.0158	n.d.
phen.eth.act	0.0434	0.1592	0.4297	0.7124	0.7459	0.5184	0.5294
eth.lact	1.2299	0.2641	0.4541	1.0336	0.5589	4.8438	3.2587
eth.but	0.3558	0.1212	0.1256	0.1786	0.1830	0.1264	0.1679
eth.isoval.	0.1080	0.0628	0.0350	0.0365	0.0354	0.0231	0.0020
eth.hex	10.5008	7.1897	11.3159	14.7408	14.0336	8.2471	10.8263
eth.ocy	22.2391	25.2180	41.6915	66.5448	70.1522	44.3457	63.0520
eth.dec	2.8351	8.2128	9.5279	17.8584	15.1771	11.5485	29.0923
eth-9-dec	0.0039	0.1360	0.0305	0.0032	0.0774	0.2127	0.0391
eth.dodec	0.0162	0.2037	0.0652	0.0528	0.1333	0.0722	0.4675
dieth.suc	11.9723	8.5806	8.6395	12.4898	6.4011	51.8940	15.9104
eth.ispen.suc	0.9168	0.6712	1.3142	1.7104	0.8742	4.2976	2.8649
eth.vanil	0.0810	0.0389	0.1177	0.0674	0.0305	0.0791	0.2621
γ-butlact	0.1029	0.0860	0.0912	0.4483	0.2259	0.3568	0.0594
w-lact	0.0201	0.0270	0.0451	0.0882	0.0450	0.1702	0.0232

Table B2. Relative quantitative data obtained for the white wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	YW9_2	YW9_5	YW9_6	YW9_7	KWV Ch Bl 01	KWV Chard 01	KWV S Bl 01
Alcohols							
isoam.oh	1.3065	0.9642	1.6318	1.5895	1.4450	1.8974	1.1160
but.oh	0.3046	0.8054	0.4941	0.3586	0.2641	0.4887	0.9019
hex.oh	0.5112	0.4297	0.7844	1.1638	0.6224	0.5531	0.7554
oct.oh	1.3892	1.2150	0.6723	1.7539	0.7629	0.7711	1.2076
phenet.oh	2.3014	1.8300	2.0459	2.7340	2.8025	2.7334	2.7626
furf.oh	1.8549	4.0501	1.7038	19.8663	1.8231	1.1873	4.1367
Phenols							
g.col	0.0663	0.1164	0.3403	0.0903	0.2384	0.3246	0.0583
4vg	0.3902	0.5770	0.2958	0.7564	0.2554	0.1963	0.5642
c-eug	0.1588	0.3362	0.1875	0.3610	0.1272	0.1271	0.3176
t-eug	0.0293	0.0604	0.0323	0.0700	0.0212	0.0300	0.0628
Aldehydes							
furf	16.7282	37.1127	25.7467	52.8497	13.6537	16.1621	48.0687
5-HMF	0.1101	0.1229	0.4849	0.1194	0.4670	0.3402	0.0867
vanil	0.3850	0.6462	0.4014	0.3178	0.3698	0.2039	0.7558
Ketones							
2-oct.one	0.3067	0.4128	0.3981	0.3794	0.4351	0.3484	0.4099
acet.van.one	0.2263	0.4265	0.2291	0.5194	0.2114	0.1306	0.4832
Acids							
form.ac	33.4546	70.3882	33.9736	97.7535	27.6582	19.7295	65.0274
acet.ac	34.7869	71.0970	39.9840	239.6929	30.6385	36.3591	67.9823
prop.ac	1.7331	5.5166	2.6080	13.3993	2.0913	2.9212	5.4051
oct.ac	1.4940	1.2322	2.0829	1.1851	1.5227	1.2457	1.7101
dec.ac	4.5967	5.3018	7.9695	4.1814	5.6789	4.4093	6.1911
dodec.ac	0.1452	0.1529	0.2047	0.1796	0.2206	0.1478	0.1697
Esters							
isoam.act	0.7528	0.5328	0.8420	0.5573	0.7323	1.3566	0.6007
hex.act	2.7966	1.9060	4.0657	0.6714	3.8447	2.9049	3.4443
eth.phen.act	0.0339	0.0496	0.1267	0.0220	0.0369	0.0320	0.0447
phen.eth.act	0.8771	0.5820	0.6247	0.5226	1.1893	1.2284	0.6695
eth.lact	0.7290	0.4203	0.6373	4.6715	0.3941	2.9396	0.3731
eth.but	0.2118	0.1362	0.2131	0.1770	0.1726	0.1610	0.1082
eth.isoval.	0.0254	0.0511	0.0394	0.0028	0.0208	0.0528	0.0830
eth.hex	16.1826	15.2954	18.0931	11.6864	17.7293	10.5401	14.7414
eth.ocy	89.0814	81.5394	97.6367	60.8144	69.3240	48.6154	70.7794
eth.dec	21.9939	31.6447	35.5844	17.0174	21.0472	16.3206	23.1640
eth-9-dec	0.0788	0.0457	0.0183	0.1038	0.2124	0.0383	0.0300
eth.dodec	0.0933	0.2816	0.4424	0.1275	0.2728	0.2299	0.2957
dieth.suc	4.4173	17.4477	7.9501	59.5050	2.9798	16.4869	8.0137
eth.ispen.suc	0.8586	1.2781	1.1531	6.6926	0.7063	2.2541	1.2122
eth.vanil	0.2854	0.0996	0.0736	0.0592	0.0257	0.2765	0.1467
Lactones							
γ-butlact	0.1824	0.4502	0.1968	2.2368	0.1203	0.2390	0.3711
w-lact	0.0532	0.1226	0.0605	0.1362	0.0295	0.0421	0.1219

Table B2 (continued). Relative quantitative data obtained for the white wines. All data is reported as relative peak areas after correction with the internal standard.

Compound	KWV S Bl 00	CC Chard 99	CC Chard 00	CC Chard 01	Simonsvl 01
Alcohols					
isoam.oh	1.5736	1.4783	1.4264	1.4265	1.5432
but.oh	0.2482	0.2412	0.1485	0.3806	0.3042
hex.oh	0.7799	0.6499	0.4336	0.4830	0.6093
oct.oh	0.4124	1.0012	0.4442	0.4325	0.8873
phenet.oh	2.2180	2.2489	2.1031	2.4090	2.6149
furf.oh	2.3096	3.3900	1.0940	5.0355	1.3945
Phenols					
g.col	0.1591	0.0916	0.2216	0.1346	0.2064
4vg	0.3542	0.4194	0.1838	0.6294	0.3059
c-eug	0.2271	0.2413	0.1161	0.3584	0.1835
t-eug	0.0413	0.0849	0.0600	0.0899	0.0440
Aldehydes					
furf	21.7653	26.7919	12.1692	32.5329	18.4195
5-HMF	0.1241	0.1286	0.2755	0.1948	0.1994
vanil	0.4963	0.5064	0.2188	0.7133	0.3604
Ketones					
2-oct.one	0.3590	0.4253	0.3157	0.3839	0.3737
acet.van.one	0.3117	0.3236	0.1310	0.4778	0.2236
Acids					
form.ac	31.7435	48.8575	16.5744	58.4039	17.0750
acet.ac	38.8773	48.2849	21.0514	63.0937	27.8672
prop.ac	2.1688	4.2890	2.3649	3.3578	1.5436
oct.ac	1.7401	0.8219	0.7711	1.1423	1.1216
dec.ac	4.5619	2.1857	1.9077	2.7829	2.7919
dodec.ac	0.0973	0.0458	0.0341	0.0702	0.0891
Esters					
isoam.act	0.3631	0.0536	0.0808	0.2269	0.3360
hex.act	1.2054	0.0344	0.0654	0.2685	0.6856
eth.phen.act	0.1189	0.0824	0.1027	0.0467	0.0446
phen.eth.act	0.3201	0.0523	0.0775	0.2702	0.3763
eth.lact	1.0539	10.1804	10.6776	7.7919	1.3877
eth.but	0.1576	0.0901	0.1063	0.1432	0.1280
eth.isoval.	0.0609	0.0834	0.0968	0.0415	0.0360
eth.hex	13.5028	7.2416	7.1986	10.0015	12.3143
eth.ocy	59.8289	30.1990	30.1300	39.8584	47.8211
eth.dec	14.5073	6.9447	8.3846	10.8267	11.2269
eth-9-dec	0.0905	0.0116	0.0230	0.0140	0.0486
eth.dodec	0.0906	0.0529	0.0515	0.0893	0.1055
dieth.suc	13.9218	88.0857	70.1843	76.6289	29.8737
eth.ispen.suc	1.8450	4.2488	3.9643	3.7397	2.5319
eth.vanil	0.1821	0.1392	0.1618	0.1252	0.1776
Lactones					
γ-butlact	0.1309	0.2472	0.1583	0.3107	0.1205
w-lact	0.0852	0.1124	0.0532	0.1464	0.0716

Table B2 (continued). Relative quantitative data obtained for the white wines. All data is reported as relative peak areas after correction with the internal standard.