

# A Rapid and Quantitative HPLC Method for Determination of Diethylene Glycol

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**A method was developed for determining diethylene glycol (DEG) by HPLC. Good separation was achieved by analysis of standard mixtures containing normal wine components and DEG. Addition of DEG to wine samples proved that DEG did not elute with any of the known constituents. Recovery studies proved the method to have a good repeatability. A selection of South African and imported wines were analysed and it was found that none contained any DEG.**

Since the start of the Austrian wine "scandal" (Freihold, 1985; Krieghäuser, 1985; Lord, 1985) it has become important to be able to determine the presence of diethylene glycol (DEG) in wines. Freihold (1985) and Pfeiffer and Radler (1985) recently gave account of the properties, toxicity and need to determine DEG. Published methods for the detection and identification of DEG include the use of packed column gas chromatography (GC) (Wagner & Kreuzer, 1985), capillary column GC and GC-mass spectrometry (Bandion, Valenta & Kohlmann, 1985; Haase-Aschoff & Haase-Aschoff, 1985) and thin-layer chromatography (Anon., 1985; Lehmann & Ganz, 1985). Bertrand (1985) used a complicated, time consuming extraction technique and capillary column GC to quantify DEG determination in wine.

We found the slightly modified HPLC method reported by Schwarzenbach (1982), which we used for quantitative determination of organic acids in wines, to be suitable for the detection of small amounts of DEG. A similar finding was reported by Pfeiffer & Radler (1985) on completion of this study.

This paper concerns the quantitative HPLC determination of DEG and the DEG-status of a number of South African and imported wines.

## MATERIALS AND METHODS

### Wine samples:

One hundred wine samples were obtained from the Sub-Directorate: Quality Control of which 22 were imported from Germany and Italy, ranging from the vintages 1977 to 1983. The remaining 78 wines were submitted to the South African Wine and Spirit Board for certification, of which 18 were intended for export and the remaining 60 for local distribution.

All imported wines contained less than 30 g/l residual sugar. Wines intended for export included 7 dry white (less than 4 g/l residual sugar), 5 semi-sweet and late harvest (more than 4 but less than 30 g/l residual sugar), 6 special late harvest and noble late harvest (more than 20 but less than 50 g/l residual sugar for special late harvest and more than 50 g/l residual sugar for noble late harvest) wines. The wines intended for local distribution included 26 dry, 29 semi-sweet and late harvest, 4 special late harvest and noble late har-

vest and 2 dessert wines (sweet, fortified).

### Standards:

Standard solutions containing 20, 15, 10, 5, and 1 g/l of diethylene glycol (Merck 803131) in freshly distilled and deionized water were prepared. A mixture of 20 g/l each of glucose and fructose (Merck 8342 and 5323), 9 g/l glycerol (May & Baker 73969), 0,5 g/l acetic acid (Riedel De Haen AG 33209), 1 g/l 2,3 butylene glycol (Fluka 18970), diethylene glycol (Merck 822329) and 10% (v/v) ethanol (redistilled, NCP) was also prepared.

For the recovery study of diethylene glycol (DEG) from different wine types 20, 15, 10, 5 and 1 g/l of DEG was added to respectively a semi-sweet, a late harvest, a special late harvest and a sweet fortified dessert wine.

To determine the sensitivity of the method, three wine types viz. a special late harvest, a late harvest and a semi-sweet wine were spiked with respectively 1 g/l, 0,1 g/l and 0,01 g/l of DEG. A 100 ml aliquot was concentrated fourfold by evaporation on a boiling water bath.

### Chromatography:

Except for the auto sampler (Spark Holland), a Knauer High Performance Liquid Chromatography system (HPLC) was used. This included a Model 64.00 pump and a Model 98.00 differential refractometer. Integration was done with an Apple IIe fitted with Chromatograph (Interactive Microwave, Inc. PA 16801) chromatography software.

The column used was an Aminex HPX -87H 300 x 7,8 mm ion exclusion column (Bio Rad 1250140) fitted with a Microguard ion exclusion column 40 x 4,6 mm (Bio Rad 1250129).

### Operating Conditions:

Mobile phase, freshly distilled water was deionized to 17 Megohms at 20°C by means of a Nanopure (Barnstead) and acidified to 0,013 N with H<sub>3</sub>PO<sub>4</sub> (Merck 565); column temperature, 50°C (constant); flow rate, 1,0 ml/min; injection volume, 20 µl, differential refractometer, x8; run time, 16 min.

### Sample Preparation:

Five ml aliquots of wine and standard samples were passed through a Waters C18-Sep Pak clean up precolumn (Subden *et al.*, 1979), followed by a 0,2 µm mem-

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brane filtration into separate auto sampler vials which were then sealed by capping.

### RESULTS AND DISCUSSION

The HPLC chromatogram of a standard mixture is shown in Fig. 1A. From Fig. 1B it can be seen that no other normal wine constituents elute at the retention time for DEG in Fig. 1A. Fig. 1C shows the position of the DEG peak in relation to the normal wine constituents. Pfeiffer and Radler (1985) presented a similar chromatogram by using an HPLC method with the same column. These methods differ in mobile phase composition, flow speed, column temperature and sample volume. At a column temperature of 50°C a satisfactory separation of glucose and fructose was obtained and the column pressure was reduced to an acceptable level at a flow speed of 1 ml/min.

Recovery of DEG in different wine types is shown in

Table 1. These data show good recovery with a variation of 1,5% and less over the range 1 to 20 g/l of DEG added to different wine types. Coefficients of variation for the recovery of DEG from standard solutions in water ranged from 3,7% for 20 g/l to 3,4% for 1 g/l.

The minimum detectable concentration of DEG as determined by addition to three different wine types was found to be 0,1 g/l. A concentration as low as 0,01 g DEG/l can be successfully detected after a four-fold concentration of the sample. Pfeiffer and Radler (1985) do not mention sensitivity data. It is possible to detect in the order of 10 mg DEG/l by means of capillary gas chromatography (Bandoni, Valenta & Kohlmann, 1985; Bertrand, 1985; Haase-Aschoff & Haase-Aschoff, 1985).

The different wine types that were analysed for the presence of DEG, are shown in Table 2. These wines

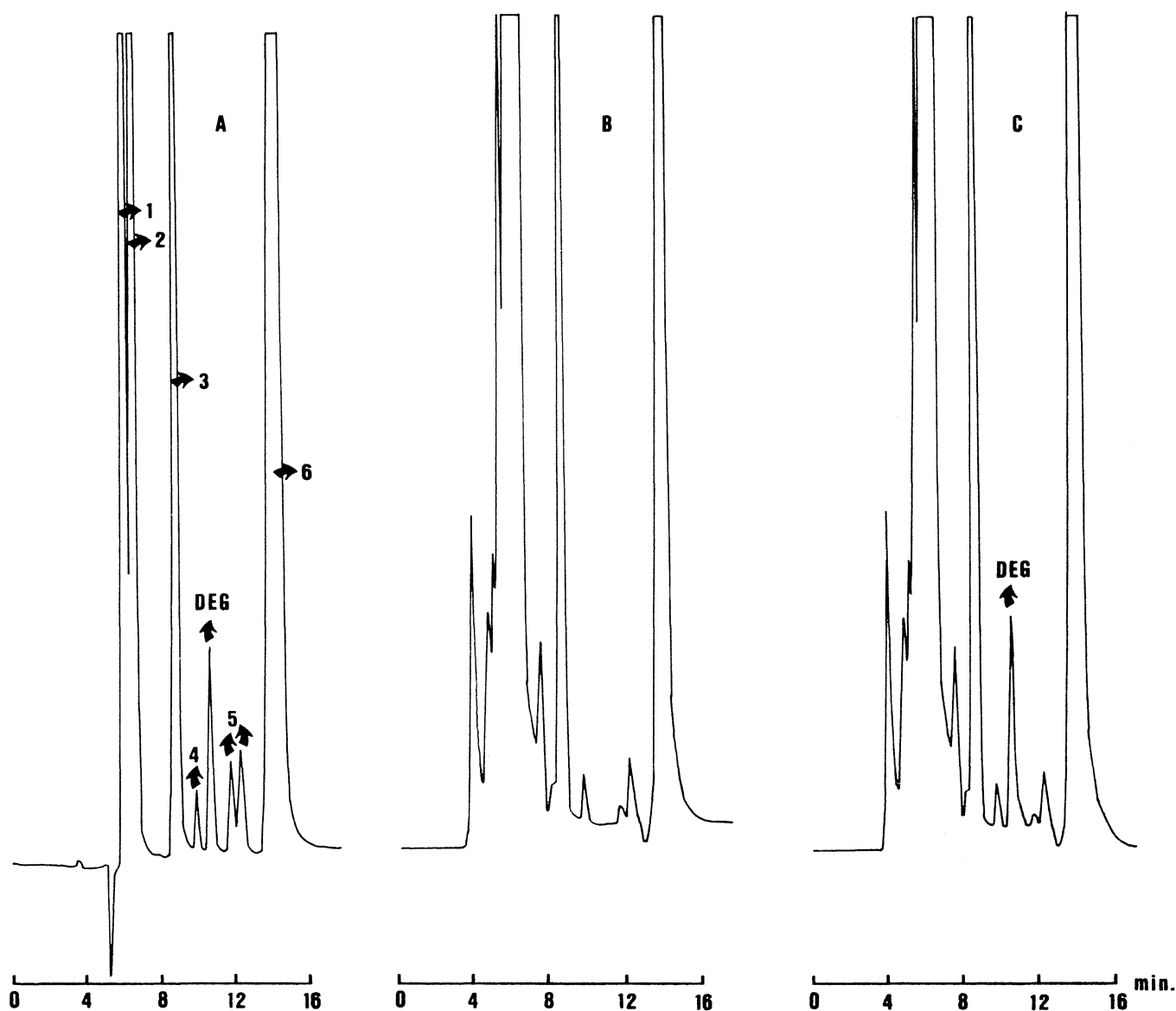


FIG. 1

Chromatograms of A: Standard mixture of wine components (1 - glucose, 2 - fructose, 3 - glycerol, 4 - acetic acid, DEG - RT = 10,79 min., 5 - 2,3-buthylene glycol, 6 - ethanol), B: wine without, and C: wine with 1 g/l DEG added.

TABLE 1

Recovery<sup>1)</sup> of DEG added to different wine types

Wine type	Amount of DEG added (g/l)	Recovery $\bar{X} \pm S.D.$ (g/l) <sup>2)</sup>	C.V. <sup>3)</sup> (%)
Semi-sweet	20	20,66±0,21	1,00
Semi-sweet	15	15,46±0,06	0,36
Late Harvest	10	10,41±0,10	0,98
Special Late Harvest	5	5,40±0,08	1,50
Sweet, fortified dessert	1	1,25±0,09	0,75

1) Represents the average recovery of five analyses

2) Average  $\pm$  standard deviation

3) Coefficient of variation

represented 27 different producers and ranged from cooperatively produced to estate produced wines. In no wine that was analysed could the presence of any DEG be detected. Because this method relies on the use of characteristic retention times for identification of the compounds, it would be advisable to make use of another method, such as mass spectrometry, for positive identification of DEG (Pfeiffer & Radler, 1985) in the event of DEG being detected in a wine sample.

TABLE 2

Selection of wine types analyzed for the presence of DEG

Wine type	Vintage	Destination	Number
Semi-sweet white	1977-1983	Import	19
Semi-sweet red	—	Import	3
Dry white	1984	Export	7
Semi-sweet and Late Harvest	1981-1984	Export	8
Special and Noble Late Harvest	1981-1983	Export	3
Dry white	1984-1985	Local	26
Semi-sweet and Late Harvest	1984-1985	Local	29
Special and Noble Late Harvest	1984-1985	Local	3
Dessert	1983	Local	2

## CONCLUSIONS

The HPLC method developed in this investigation has proved to be rapid (less than 20 min. per sample including sample preparation), reliable and repeatable. Although it would seldom be necessary to determine minimal amounts of DEG (Pfeiffer & Radler, 1985), this method can be utilized for quantitative determinations of DEG between 20 and 1 g/l. Successful detection of 0,1 g/l and as low as 0,01 g/l of DEG in wine (after fourfold concentration of the sample) can be achieved by this method.

No DEG was detected in any of the South African and imported wines analysed in this study.

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