Determination of Diethylene Glycol in Wines by High-Resolution Gas Chromatography/Mass Spectrometry

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A simple and sensitive quantitative method was developed to determine diethylene glycol (DEG) in wines. The method involves no extraction but instead uses decolorization by charcoal followed by dilution with acetonitrile and filtration. The recovery was calculated to be about 90%. By HRGC/MS and selected ion monitoring, a minimum detection limit of 0.1 mg/L (ppm) can be reached easily. Repetitive scanning EI and isobutane CI methods can be used to confirm the identity of DEG in wines at 1 mg/L or above. Other confirmation approaches were studied. Three internal standards were evaluated, and 3-methoxy-1,2-propanediol was found to be the best. The method was validated by two series of spiking studies. Results were linear at least up to 100 mg/L and agreed very well with the levels spiked. The method is well suited for routine analysis.

Recently, it was found that diethylene glycol (DEG), one of the major ingredients in antifreeze solution for spinkler solution (Merck Index, 1983), had been illegally added to wines in order to improve their sweetness and smoothness and thus increase their commercial value. Adulteration with DEG was found in Austrian, German, and Italian wines. Although DEG is not considered to be highly toxic, it is suspected to be linked to liver and kidney damage when consumed in large quantities [fatal dosage for humans 1–2 g/kg (Gosselin et al., 1984; Tatken and Lewis, 1983)]. It is prohibited from use as a food additive in Canada and in other countries. Health and Welfare Canada recently set an interim "actionable level" at 10 mg/L (ppm) on the basis of evaluation of the toxicity data and current analytical capabilities.

A search of the literature has indicated that there are only a few papers published in this area. HPLC with refractive index detection can be used to measure DEG reliably only down to 20–50 mg/L (Bonn, 1985; Day et al., 1985), much higher than the Canadian limit. Recent methods (Kaplan et al., 1982; Holzer, 1985; Bandion et al., 1985; Boneva and Dimov, 1981) utilizing capillary gas chromatography provide enough sensitivity, but the direct injection of wine results in a variety of chromatographic problems such as rapid degradation of column performance, contamination of the injector liner, adsorption, and poor reproducibility. In addition, the resulting chromatograms from direct analysis on polar columns are usually very complex and thus make the identification and quantitation difficult. This situation was not improved even when sequentially coupled columns were employed (Kaiser and Rieder, 1985). Several cleanup methods using solvent extraction (Bertrand, 1985), precipitation by barium hydroxide–acetone (Wagner and Kreutzer, 1985), and an experimental cartridge packed with Carbopack C (Caccamo et al., 1986) have been described. Trimethylsilyl (Fuehrling and Wollenberg, 1985; Arancibia and Catoggio, 1980) and heptafluorobutyl (Brooks et al., 1984) derivatives of DEG were examined by GC and GC/MS, but both derivatization methods require nonaqueous preparation. None of these authors reported the percentage recovery of their extraction methods. It has been reported that quantitative extraction of DEG from aqueous solutions by conventional methods is extremely difficult (Kaplan et al., 1982) and results in very low recovery. In this paper, we report a simple and rapid nonextractive cleanup procedure followed by a quantitative determination by capillary GC/MS.

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EXPERIMENTAL SECTION

Materials. 1,4-Butanediol (1,4-BDO), 3-methoxy-1,2-propanediol (MPD), and 2,2′-thiodiethanol (TDE) were used as internal standards were purchased from Aldrich Chemical Co. and used without further purification. A stock solution of diethylene glycol (DEG, reagent grade, Fisher Scientific) was prepared by dissolving a known weight of DEG in 10% (v/v) absolute ethanol in distilled water. The final working standard solutions (for calibration purposes) were prepared by mixing 1 volume of standard solutions (containing 10 mg/L of 1,4-BDO, 10 mg/L of MPD, 20 mg/L of TDE, and various concentrations of DEG) with 4 volumes of acetonitrile.

All solvents were glass distilled and obtained from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada). Decolorizing carbon (charcoal, Darco G-60) was obtained from J. T. Baker Chemical Co. The syringe filter units (Millex-GV, 0.22 μm) were products of Millipore Corp. All wine samples were obtained directly from local liquor stores.

Spiking Studies. In order to evaluate which one of the three internal standards (MPD, 1,4-BDO, TDE) was most suitable for the DEG analysis, 100 μL of a mixture of all three internal standards (1000 mg/L each in absolute ethanol) and various amounts of DEG (in 10% ethanol) was diluted with wine to 10 mL in volumetric flasks. Final concentrations: 10 mg/L of 1,4-BDO; 10 mg/L of MPD; 20 mg/L of TDE; 0, 0.1, 0.5, 1, 5, 10, 100 mg/L of DEG.

Charcoal Cleanup Method. Approximately 30 mg of decolorizing carbon was added to 1 mL of wine, and the resultant mixture was vortexed and allowed to settle. An aliquot of 200 μL of the supernatant was diluted with 800 μL of acetonitrile, cooled in an ice–water bath for 10–15 min, and then filtered through a Millipore Millex-GV syringe filter unit. One microliter of the filtrate was injected directly onto the GC column.

GC/MS Analysis. Only the conventional mass spectrometer section of a VG Analytical 7070EQ hybrid MS/MS system was used in these studies. A Varian 6000 GC equipped with a capillary column (J & W Scientific; 0.25 mm (i.d.) × 30 m DBWAX with 0.15-μm film thickness) and a splitless injector was directly interfaced to the mass spectrometer. The head pressure was 120 kPa (17 psi), and the injector temperature was 180 °C. The following temperature program was used: an initial hold of 60 °C for 2.5 min followed by a ramp of 50 °C/min to 110 °C and then a ramp of 10 °C/min to a final temperature of 180 °C held until elution was completed.

The mass spectrometer conditions were as follows: ionization by electron impact at 70 eV, trap current of 100 μA, source temperature of 180 °C, all reentrant and

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transfer lines at 200 °C. Resolution was 1000 (10% valley definition) for repetitive scanning and 1000 or 4000 for selected ion monitoring (SIM). The repetitive scanning speed was 0.6 s/decade between m/z 40 and 200. In the computer-controlled SIM mode, the following ions were monitored by voltage switching using a dwell time of 120 ms each and a 10-ms delay time between switching among ions: m/z 71.05, 72.06, 75.04, 76.05. Methane and isobutane were used as reagent gases for chemical ionization (CI) at ion source pressure gauge readings of 5×10^{-5} and 7.5×10^{-5} Torr, respectively. CI conditions were optimized with use of amyl acetate as a model compound. For chloride attachment negative ion CI, methylene chloride was bled into the ion source through the septum reference inlet.

It was found that matrix materials in the wine sample deactivated some sites in the GC system and enhanced the response of the standards. Thus, it was our routine procedure to inject several old spiked wine samples at the beginning of the day until the responses from the compounds of interest were stabilized.

Quantitation. Quantitation was based on the peak area (or height) from the m/z 75 and/or 76 mass chromatograms in the SIM mode. A calibraion curve was constructed by plotting various concentrations of DEG vs. response ratios between DEG and the internal standard MPD (fixed at 10 mg/L). The concentration of DEG in unknown samples was determined directly from the calibration curve after the response ratio between DEG and MPD peaks was measured.

RESULTS AND DISCUSSION

Charcoal and Protein Precipitation Method. In our preliminary experiments, the use of disposable solid-phase extraction (SPE) tubes (under the trade names SEP-PAK from Waters Associates and Baker 10 SPE from J. T. Baker Chemical Co.) was evaluated as an one-step cleanup method to remove relatively less polar materials from wines. Using 10% aqueous EtOH as an eluting solvent and collecting the fraction at retention volumes 0.5–1.5 mL, it was noted that some coeluting materials (such as sugars and/or proteins) in the eluate led to deterioration of column performance (mainly in the form of peak splitting) after approximately 30 splitless injections. This problem prompted us to undertake another approach.

It was found that decolorizing carbon removed virtually all colored compounds in wine. Experiments showed that no additional loss of DEG was observed when the wine was in contact with charcoal for periods up to 6 h. Dilution of wine with acetonitrile (1:4) also precipitated some proteins and/or sugars. The precipitation is more effective when the solution is placed in an ice–water bath. This method involves no extraction step and is very simple and reliable. The whole procedure takes less than 20 min, and several samples can be processed simultaneously. It seems that the filtrate prepared in this way does not create a problem in the injector area. To date, over 200 samples have been injected into our splitless injector, and no deterioration of chromatographic performance (such as peak splitting, peak tailing, loss of sensitivity, etc.) has been experienced. Later experiments (Lawrence et al., 1986) indicated that the same filtrate could be analyzed satisfactorily by GC with FID detection; however, the sensitivity was lower that than of the GC/MS method.

Figure 1A shows a total ion current (TIC) trace of a direct injection of the filtrate obtained from a red wine spiked with 50 mg/L of DEG according to this cleanup procedure (the addition of acetonitrile resulted in a fivefold dilution of the original wine sample). The sample was also spiked with three proposed internal standards: 10 mg/L of MPD, 10 mg/L of 1,4-BDO, 20 mg/L of TDE. DEG and internal standards were readily identified by comparing the retention times and mass spectra with that of the standards (Figure 1B). Reconstructed mass chromatograms from a set of ions (Table I) characteristic of each compound of interest improve the signal to noise ratio and facilitate the identification. Semiquantitative results can be obtained from the integration of the peak area or from the peak height in one of the representative mass chromatograms. On the basis of detection of all characteristic ions with correct relative intensity ratios, the detection limit of DEG using the reconstructed mass chromatogram approach was estimated to be 1 mg/L.

Table I. 70-eV Electron Impact Mass Spectra of DEG, MPD, 1,4-BDQ, and TDE in the range m/z 40–200 [Similar Spectra Reported in EPA/NIH Mass Spectral Library (1978, 1980)]

fragment ions, m/z	RI, %	proposed structure[a]
(1) Diethylene Glycol (DEG), MW 106		
76	13	$[M - CH_2O]^{\cdot+}$
75	25	$CH_2{=}O^+CH_2CH_2OH$
45	100	
(2) 3-Methoxy-1,2-propanediol (MPD), MW 106		
88	5	$[M - H_2O]^{\cdot+}$
76	10	$[M - CH_2O]^{\cdot+}$
75	36	$[M - CH_2OH]^+$
61	20	$HOCH_2CH{=}O^+H$
45	100	$CH_2{=}O^+CH_3$ or 
44	29	$[CH_2{=}CHOH]^{\cdot+}$
43	16	$[C_2H_3O]^+$
(3) 1,4-Butanediol (1,4-BDO), MW 90		
72	7	$[M - H_2O]^{\cdot+}$
71	21	$CH_2{=}CHCH_2CH{=}O^+H$
62	3	$[M - C_2H_4]^{\cdot+}$
60	4	$[M - CH_2O]^{\cdot+}$
57	9	$CH_2{=}CHCH{=}O^+H$
44	75	$[CH_2{=}CHOH]^{\cdot+}$
42	100	$[CH_3CH{=}CH_2]^{\cdot+}$
(4) 2,2′-Thiodiethanol (TDE), MW 122		
122	5	$[M]^{\cdot+}$
104	32	$[M - H_2O]^{\cdot+}$
91	28	$CH_2{=}S^+CH_2CH_2OH$
75	3	$CH_3CH_2S^+{=}CH_2$
73	3	$CH_2{=}CHS^+{=}CH_2$
61	100	$[C_2H_5S]^{\cdot+}$
47	28	$H\text{-}S^+{=}CH_2$
45	50	

[a] All structures are tentative assignments and will require further confirmation by other techniques such as high-resolution accurate mass measurements, stable isotopic labeling, metastable ion studies, collision-induced dissociation, etc.

Quantitative Analysis in Selected Ion Monitoring Mode. All quantitative analyses were performed in the SIM mode for better sensitivity and accuracy. Because the accelerating voltage switching used in magnetic sector instruments imposed a restriction on the mass range in the SIM mode, only four ions were monitored: m/z 71, 72, 75, 76. The first two ions are characteristic of 1,4-DBO whereas the latter two are common ions for MPD and DEG. The use of TDE as an internal standard was dropped due to its late elution on polar columns. In our earlier experiments, a resolution of 4000 was used. However, with the objective of developing a general procedure for all GC/MS instruments (including low-resolution

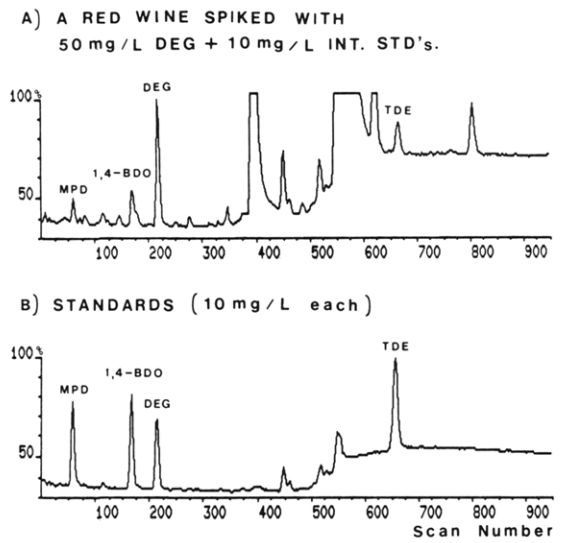

Figure 1. Total ion current traces from direct injections of (A) a red wine spiked with 50 mg/L of DEG, 10 mg/L of MPD, 10 mg/L of 1,4-DBO, and 10 mg/L of TDE and (B) a standard containing 50 mg/L each of DEG, 1,4-BDO, DEG, and TDE.

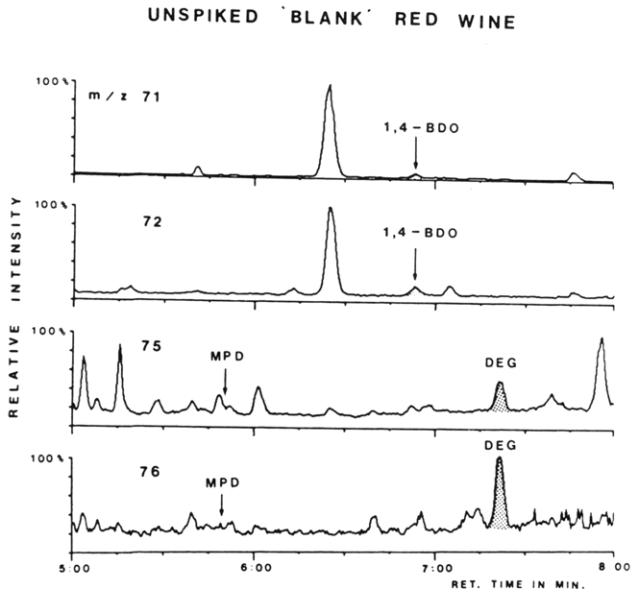

Figure 2. Mass chromatograms obtained from a unspiked blank red wine sample containing 0.23 mg/L of DEG. Intensities were normalized to the largest peak of each ion within the retention time range.

quadrupole instruments), the resolution was reduced to 1000. Although more GC peaks were observed at lower resolution, no significant interfering peak was detected in the vicinity of the peaks of interest (i.e., MPD, 1,4-BDO, DEG). Figure 2 displays typical mass chromatograms from a blank red wine sample, whereas Figure 3 shows the same wine spiked with 5 mg/L of DEG, 10 mg/L of MPD, and 10 mg/L of 1,4-BDO. The peak at retention time 6 min 25 s in both m/z 71 and 72 ion traces is derived from the reagent blank. This red wine plus other wines that we have surveyed (total number over 26) contained trace amounts of 1,4-butanediol. Therefore, 1,4-BDO is not recommended for the use as an internal standard. Around 0.23 mg/L of DEG was also detected in this blank wine. As shown in Table II, there appears to be a background level of DEG in certain wines. This is the first report indicating the presence of a very minute quantity of naturally occurring DEG in wines. It is not certain whether or not the four

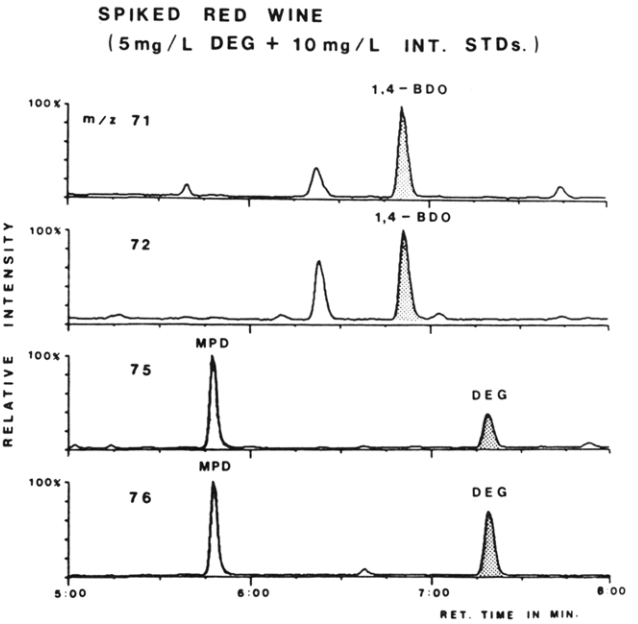

Figure 3. Mass chromatograms obtained from the same red wine sample as in Figure 2 but spiked with 5 mg/L of DEG, 10 mg/L of MPD, and 1,4-BDO. Intensities were normalized to the largest peak of each ion within the retention time range.

Table II. Spiking Studies: DEG in Red and White Wines

wine no.	blank wine	level spiked, mg/L	found, mg/L	% rec[a]
W-1	0.80			
W-2	0.16			
W-3	0.16			
W-4	0.21			
W-5	0.16			
W-6	0.13	8.0	8.35	103
W-7	0.10	21.0	18.5	88
W-8	1.04	18.0	16.9	88
W-9	0.14	45.0	39.5	87
W-10	0.20	3.0	3.36	105
W-11	6.37	31.0	34.2	90
W-12	0.24	0.1	0.31	70
		0.5	0.81	114
		1.0	1.01	77
		5.0	4.69	89
		10.0	10.4	102
		100.0	91.4	91
W-13	3.80			
W-14	0.24			
R-1	0.23	0.5	0.55	64
		1.0	1.16	93
		5.0	5.05	96
		10.0	10.3	101
		50.0	46.8	93
R-2	0.20			
R-3	0.22			
R-4	0.17			
R-5	0.19	13.0	12.3	93
R-6	0.22	22.0	20.0	90
R-7	0.14	36.0	31.4	87
SP3327	0.31	2.0	2.00	85
482453	0.30			
482315	0.44			
482316	0.20			
482317	3.80			
				mean = 91%
				SD = 11%
				n = 21

[a] Percentages of recoveries were calculated by the formula (DEG found in spiked wine − DEG in blank wine)/DEG spiked × 100%.

blank wine samples (Table II) containing DEG levels >1 mg/L (but lower than the Canadian guideline of 10 mg/L)

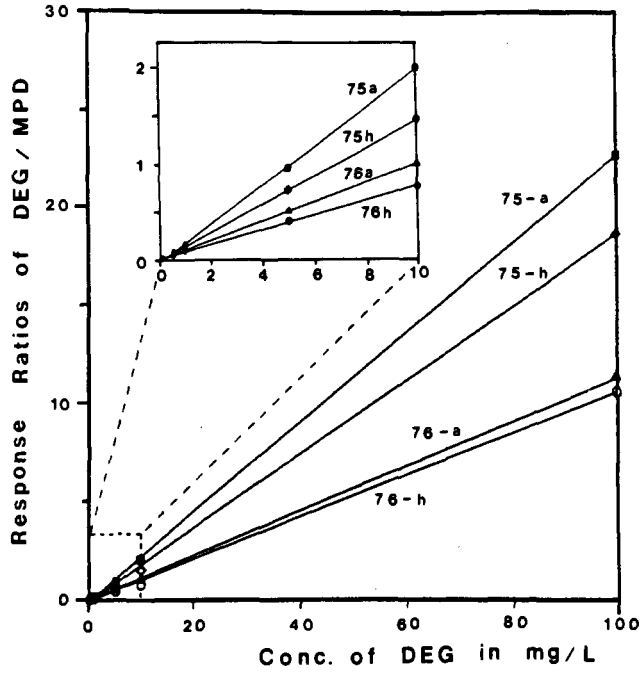

Figure 4. Calibration curves plotting response (in height and in area) ratios between DEG and MPD at masses 75 and 76 vs. concentrations of DEG. The concentration of MPD remained constant at 10 mg/L in all cases.

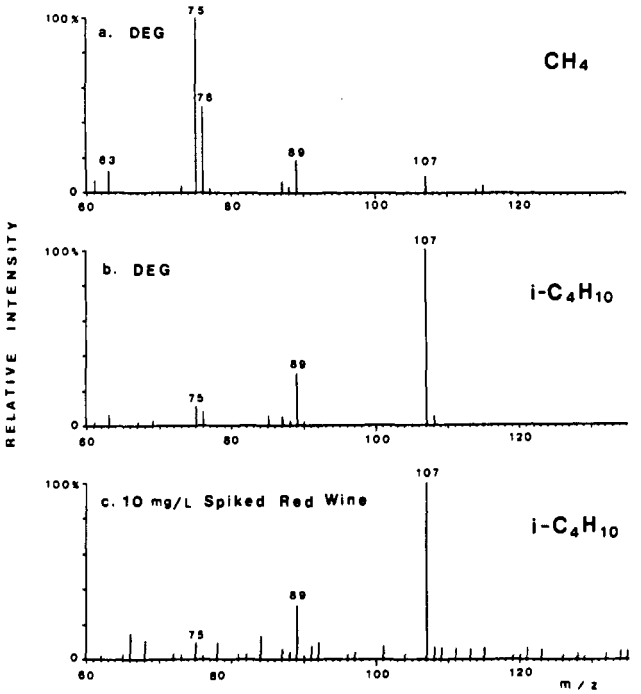

Figure 5. Positive-ion CI mass spectra of DEG: (a) using methane as reagent gas; (b) using isobutane as reagent gas; (c) using isobutane as reagent gas, in a red wine sample spiked with 10 mg/L of DEG.

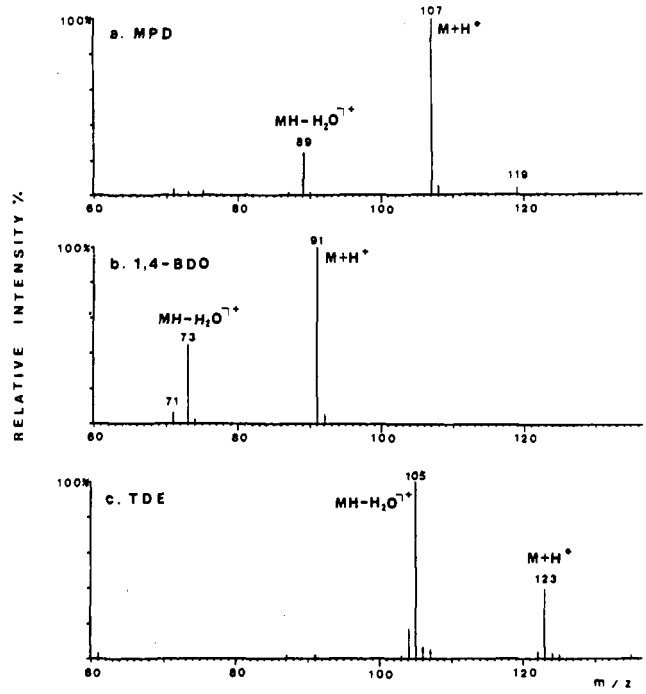

Figure 6. Isobutane positive-ion CI mass spectra of internal standards: (a) 3-methoxy-1,2-propanediol (MPD); (b) 1,4-butanediol (BDO); (c) 2,2′-thiodiethanol (TDE).

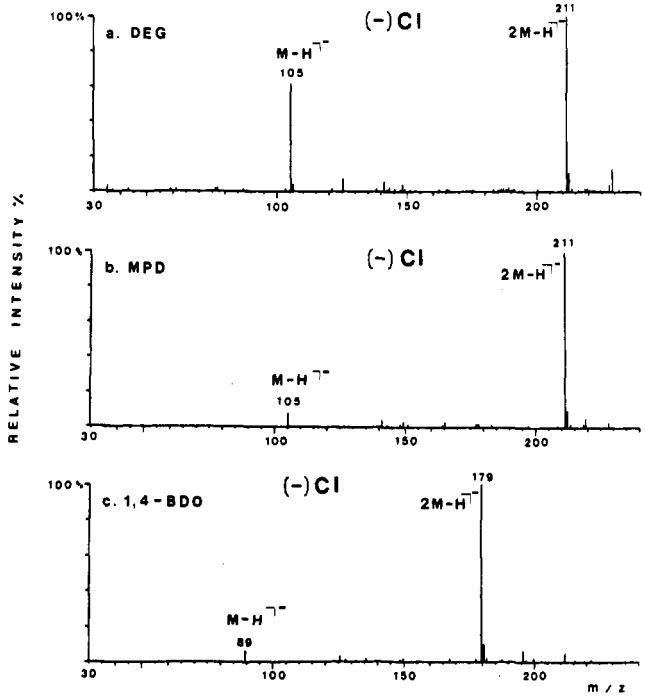

Figure 7. Electron-capture negative-ion CI mass spectra of DEG, MPD, and 1,4-BDO.

are in fact the results of fortification, blending, or contamination. Contamination can be from natural sources such as the cork used to seal the bottle (Merck Index, 1983). Fortification and blending to improve the commercial value of product are expected to cause higher levels of DEG, especially if older contaminated stocks are used.

Both m/z 75 and 76 can be used as quantitation ions for MPD and DEG. The calibration curve indicates that responses using either peak height or peak area are linear at least up to 100 mg/L (Figure 4). Correlation coefficients in all cases are better than 0.999.

The method was validated by analyzing several red and white wine samples spiked with various amounts (between 0 to 100 mg/L) of DEG and a constant level of 10 mg MPD/L. Results based on the internal standard method are summarized in Table II. The average percentage recovery for the spiked samples was calculated to be 91% with standard deviation of 11%. Very good correlations between spiked levels and concentrations found were obtained in both types of wine. The minimum detection limit was estimated to be around 0.1 mg/L, which is more than adequate for regulatory purposes.

Table III demonstrates the precision of the method. The standard deviation from eight determinations (all separate

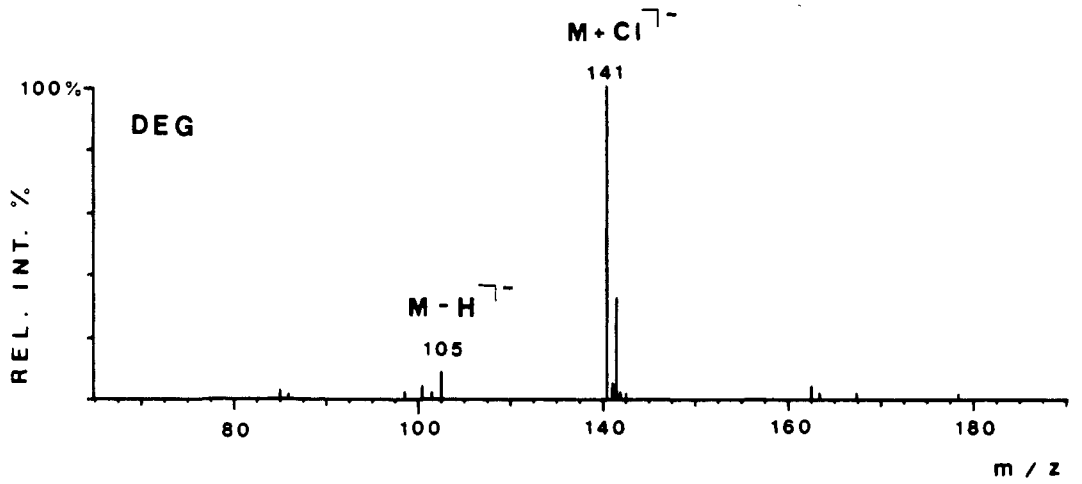

Figure 8. Chloride attachment negative-ion CI mass spectrum of DEG.

Table III. Replicate Analyses (mg/L) of DEG from a Red Wine Spiked with 5 mg/L of DEG[a]

4.99	5.05	5.06
4.88	4.99	5.01
4.94	4.92	

mean = 4.98 (n = 8)
SD = 0.06
RSD = 1.3%

[a] All samples were prepared separately.

sample preparations) of a red wine spiked with 5 mg/L was calculated to be 0.06 mg/L (RSD = 1.3%).

Other Confirmation Methods. As far as specificity and sensitivity are concerned, GC/MS offers the best detection method for this type of analysis. It is our belief that a combination of retention time and the 70-eV EI mass spectrum (in repetitive scanning mode) will provide enough specificity to meet the requirements at the 10 mg/L "actionable level" set by Health and Welfare Canada. However, the absence of molecular ions for DEG and MPD in EI mode may be a handicap, and it is therefore desirable to develop supplementary techniques for regulatory purposes.

(*1*) *Positive-Ion Chemical Ionization (CI).* Methane positive-ion CI does not provide significant improvement in DEG identification (Figure 5a). In addition, the sensitivity is much lower than that in the EI mode. On the other hand, isobutane CI produces a strong protonated DEG molecule at m/z 107 (Figure 5b) and other ions at m/z 89 ($[M + H - H_2O]^+$), 76, 75, and 63 ($[HOCH_2CH_2OH + H]^+$). Figure 5c shows the isobutane CI positive-ion mass spectrum of DEG obtained from a 10 mg/L spiked red wine. In general, the sensitivity seems to be slightly lower than that in the EI mode but is deemed to be satisfactory for confirmation tests. The CI (i-C_4H_{10}) mass spectra for the other internal standards are shown in Figure 6. Similar to DEG, the protonated molecules (m/z 107, 91, and 123 for MPD, 1,4-BDO, and TDE, respectively) and their corresponding dehydrated ions (m/z 89, 73, 105) are the major peaks in the positive-ion spectra.

(*2*) *Negative-Ion Chemical Ionization.* Using isobutane as the moderator gas and the water-cooled solid probe for sample introduction, the electron-capture negative CI mass spectra of the three compounds of interest are shown in Figure 7. It is interesting to observe that the $[2M - H]^-$ ions (m/z 211 for DEG and MPD, 179 for 1,4-BDO) are more predominant than the $[M - H]^-$ ions. It is unlikely that the dimer formation was attributed to the local high sample concentration in the ion source because similar spectra were obtained when the solid probe was withdrawn and only residue of sample remained in the ion source.

Chloride attachment negative CI can also be used to confirm the identity of DEG by its $[M + Cl]^-$ ion (Figure 8). However, both electron-capture negative CI and chloride attachment CI have much lower sensitivity than the EI mode and can only be applicable to heavily contaminated wines.

CONCLUSIONS

It has been demonstrated that the method based on decolorization by charcoal and protein/carbohydrate precipitation by the addition of acetonitrile followed by filtration is well suited for both routine qualitative and quantitative determination of DEG in wines by GC/MS. The method proved to be simple and fast and will be particularly useful in situations where large numbers of samples have to be monitored continuously.

From the three internal standads evaluated, MPD was selected. TDE (a sulfur analogue of DEG) resembles the structure of DEG most, but its late elution together with possible high column bleed background at that temperature makes it least suitable for the use as an internal standard. Although 1,4-BDO elutes very near the retention time of DEG, the presence of a trace amount of 1,4-BDO in amost all the wines that we analyzed forbids its use as well. The internal standard approach should be able to compensate for some common analytical problems such as absorption, sample volume changes, injection, variations in instrumentation response, and matrix effect.

Using the internal standard method, the extensive spiking studies further proved that the method is reliable and precise. Positive samples with DEG concentrations at or above the 10 mg/L limit can be confirmed by repetitive scanning in EI or isobutane CI mode. Other alternatives would include the use of a second GC column with different polarity or high-resolution MS.

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Interaction of Synthetic Proanthocyanidin Dimer and Trimer with Bovine Serum Albumin and Purified Bean Globulin Fraction G-1

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To evaluate the type of binding involved, thermodynamic analysis of the temperature dependence of proanthocyanidin binding to bovine serum albumin (BSA) and bean glycoprotein G-1 (G-1) was investigated. Binding was analyzed with tritiated proanthocyanidin by ultrafiltration to separate free ligand and protein-bound ligand. Binding constants were determined from Scatchard plots. Van't Hoff plots indicated proanthocyanidin binding to BSA was spontaneous and entropy driven. Analysis with *cis*-parinaric acid supported the conclusion drawn from the thermodynamic analysis that the binding of proanthocyanidin to BSA was a hydrophobic interaction. Van't Hoff plots indicated proanthocyanidin binding to native G-1 protein was also spontaneous but, in contrast to BSA, enthalpy driven. Analysis with *cis*-parinaric acid confirmed the hydrophilic character of proanthocyanidin binding to native G-1. Evaluation of proanthocyanidin binding to heat-denatured G-1 with *cis*-parinaric acid indicated hydrophobic interactions.

Proanthocyanidins or condensed tannins are polymeric plant phenolics that interact with protein, decreasing protein digestibility (Feeny, 1969; Aw and Swanson, 1985). The mechanism of the interaction has received substantial attention, and several mechanisms have been proposed. The two major noncovalent binding mechanisms proposed were hydrogen bonding and hydrophobic interactions. Gustavson (1956), Loomis and Battaile (1966), Loomis (1974), and van Sumere et al. (1975) have proposed hydrogen bonding as the probable mechanism. Loomis and Battaile (1966) concluded on the basis of the interaction of condensed tannin with polymers like polyvinylpyrrolidone (PVP), gelatin, collagen, and nylon that the mechanism was hydrogen bonding. Haslam (1974) evaluated the association of proanthocyanidin dimer and trimer with glucosidase. He concluded that hydrogen bonding occurred between phenolic hydroxyl groups and peptide bond carbonyl groups. Hagerman and Butler (1981) enhanced hydrogen bonding of condensed tannin to formamide by alkyl substitution on the amide N adjacent to the carbonyl. Formamide had less affinity for condensed tannin than the substituted derivatives *N*-methylformamide or *N,N*-dimethylformamide. Electron-donating methyl groups on the N adjacent to the carbonyl increased the electronegativity of the oxygen on the carbonyl group. They suggested this increased the strength of the hydrogen bonds between the carbonyl and the proanthocyanidin hydroxyls.

There is also evidence of hydrophobic interaction between proanthocyanidin and protein. Detergents (Goldstein and Swain, 1965) as well as organic solvents (Loomis, 1969) can partially dissociate tannin from protein. Calderon et al. (1968) found ethanol decreased the affinity of tannin for protein while NaCl increased the affinity of tannin for protein. Oh et al. (1980) indicated that the predominant mode of interaction was hydrophobic based on several observations: (1) protein was eluted from a column of immobilized tannin with detergents; (2) interaction between tannin and protein was enhanced with increased ionic strength and temperature; (3) tannins were absorbed readily onto uncharged polystyrene columns. Butler et al. (1984) suggested that interactions between tannin and protein involve hydrogen bonding and hydrophobic associations.

The type of interaction has not been completely elucidated. Thermodynamic analysis provides a means for rigorously determining the type(s) of interactions involved. Hydrophobic interactions are also called entropy-driven interactions because they are characterized by a positive change in entropy and enthalpy. On the other hand, hydrophilic interactions are characterized by a decrease in enthalpy. To obtain estimates for the entropy and enthalpy changes upon binding, Van't Hoff plots are often

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