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Trace determination of methanol in water–ethanol solution by derivatization and high-performance liquid chromatography

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Abstract

A simple and sensitive high-performance liquid chromatographic method has been established for the determination of methanol in water–ethanol solution. The method is based on the transfer of the methoxide anion, which is formed from methanol under strong alkaline treatment in aqueous solution, by benzalkonium chloride into the dichloromethane organic phase for derivatization with 3-bromomethyl-7-methoxy-1,4-benzoxazin-2-one. The derivative obtained was separated on a LiChrospher diol column with *n*-hexane–dichloromethane (9:1, v/v) as the mobile phase. Several parameters affecting the partition/derivatization of methanol were investigated. The linear range for the determination of methanol was 2–20 μmol/ml; the detection limit (signal-to-noise ratio=5; sample size, 10 μl) of methanol was about 0.10 μmol/ml (R.S.D.=16%, n=3). The method has been satisfactorily applied to the assay of methanol in spiked commercial liquors.

Keywords: Derivatization, LC; Liquors, commercial; Methanol

1. Introduction

Methanol is one of the most popular organic solvents and finds extensive application in industries and household use. It is rapidly and well absorbed by inhalation, and by oral and topical exposure. Although poisonings have been reported after absorption through the skin and inhalation of air containing as little as 0.2%, most disastrous methanol intoxications are related to ingestion of methanol itself or methanol containing beverages. Deaths have been reported in several cases of mass poisoning from methanol. Since methanol resembles ethanol in odor and taste and is tax-free, it has been used as an adulterant in alcoholic beverages which caused accidental and intentional intoxications [1]. Therefore, the availability of analytical methods suitable for the determination of trace quantities of methanol in ethanol aqueous solutions is of the highest importance.

A number of methods, including enzymatic [2,3], colorimetric [4,5], gas chromatographic (GC) [6–16] and high-performance liquid chromatographic (HPLC) [17–20] techniques, have been reported for the determination of methanol in various matrices. The one-carbon simple structure of methanol and its transparency to UV radiation limit the detection sensitivity in GC or HPLC. Although head-space GC

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is powerful for the determination of traces of methanol in aqueous solution, it is not quite satisfactory for methanol trace analysis in the presence of high ethanol content and volatile esters especially in the commercial liquors.

Several types of chemical derivative of methanol have been devised for increasing analytical sensitivity in GC [21,22] or HPLC [17,20], but any attempt to apply these methods in an alcoholic beverage has to face, at least, two problems: first, in spite of a slightly stronger acidity, the reactivity of methanol with most derivatizing agents much resembles that of ethanol, because of the same functional group they both possess. Second, in real samples, the amount of methanol, under usual conditions, is relatively small in comparison with ethanol posing relevant problems to the development of a selective determination. In the present study, a HPLC method with UV detection based on a selective derivatization is described. The selectivity is based on the chemical derivatization of methanol with 3-bromomethyl-7-methoxy-1,4-benzoxazin-2-one in a heterogeneous system, using benzalkonium chloride as phase transfer agent.

2. Experimental

2.1. Material and reagents

3-Bromomethyl-7-methoxy-1,4-benzoxazin-2-one (Br-MBX), benzalkonium chloride (benzyldimethyl-*n*-tetradecylammonium chloride) (BAC), tetraethylammonium chloride (TEAC), and tetraheptylammonium bromide (THPAB) (TCI, Tokyo, Japan), hexadecyltrimethylammonium bromide (HDTMAB) (Sigma, St. Louis, MO, USA), tetrabutylammonium chloride (TBAC), and tetrahexylammonium bromide (THAB) (Fluka, Buchs, Switzerland), potassium hydroxide, methanol and ethanol (Merck, Darmstadt, Germany), dichloromethane, *n*-hexane and other reagents were of analytical reagent grade. Solutions of 1-nitronaphthalene (internal standard, I.S.) and Br-MBX (derivatizing agent) were prepared in dichloromethane. Solutions of methanol at various concentrations were prepared in water–ethanol mixture (1:1, v/v). Phase transfer agents were prepared by dissolving appropriate amounts of BAC, TEAC, HDTMAB, TBAC, THAB and THPAB in distilled and deionized water. Various concentrations of the alkaline solution were prepared by dissolving potassium hydroxide in distilled and deionized water.

2.2. HPLC instrumentation and conditions

A HPLC system (Waters-Millipore, Milford, MA, USA) composed of a U6K injector, a Model 510 pump and a Model 486 UV–Vis detector was used. A LiChrospher diol column (250×4.0 mm I.D., 5 μm) (Merck) and a mixture of *n*-hexane–dichloromethane (9:1, v/v) as the mobile phase at a flow-rate of 1.2 ml/min at room temperature were used. The column eluate was monitored at 350 nm wavelength. The solvent was filtered with filter (Millipore, HV, 0.45 μm) under vacuum for degassing before use.

2.3. Mass spectrometry and nuclear magnetic resonance spectrometry

The Quattro 5022 mass spectrometer (VG Biotech. UK) was operated in the electron impact mode at 70 eV with an ionization source temperature of 200°C. ^{1}H NMR data were obtained from a Varian Gemini-200 (Varian, CA, USA) instrument. NMR was operated with 200 MHz and tetramethylsilane (TMS) as I.S. and $C^{2}HCl_{3}$ as solvent.

2.4. Optimization of the derivatization procedures

In order to establish the optimum conditions for methanol analysis, several parameters, including organic solvent, the concentration of potassium hydroxide, phase transfer agent, derivatizing agent and reaction time, that affect the partition/derivatization of methanol were investigated at a methanol concentration of 20 μmol/ml in water– ethanol (1:1, v/v) solution. The effects of those parameters were evaluated by the peak-area ratio of methanol derivative to 1-nitronaphthalene (I.S.).

2.5. Optimized derivatization procedures

A 0.1-ml aliquot of methanol solution at various concentrations in water–ethanol (1:1, v/v) or in commercial liquors was added to a 10-ml glass-stoppered test tube containing 0.1 ml of 0.05 *M*

potassium hydroxide and 0.1 ml of 0.1 *M* BAC aqueous solutions. Then 0.3 ml of 160 μ*M* 1-nitronaphthalene (I.S.) and 0.2 ml of 20 m*M* Br-MBX, both in dichloromethane, were added. The reaction mixture was shaken mechanically at 30°C in a thermostated water bath for 2 h. At the end of the reaction, 3.0 ml of water was added to the reaction vessel with gentle shaking, to stop the reaction. After the separation of the organic phase, a 5-μl aliquot of the dichloromethane layer was injected.

3. Results and discussion

3.1. Optimization of derivatization

3.1.1. Effect of organic solvent

With the view of quantitation, the derivatization of methanol with Br-MBX (a primary alkyl halide) described in present study is expected to proceed by SN2, rather than SN1, mechanism. As we know, reaction by SN2 is favored by solvents of low polarity or aprotic ones. Water-immiscible organic solvents, including dichloromethane, chloroform and toluene, were tested for their suitability as reaction solvents for the derivatization procedure. In the preliminary studies, that higher reaction temperature speeds up the derivatization rate was noted. To prevent the solvents from boiling, the reaction temperatures were set below the respective boiling point of the tested solvents. Results are shown in Fig. 1. The solvent effect on the yield of methanol derivative revealed in this way reflects not only its effect on the derivatization rate but also the partition coefficient of the complex consisting of phase transfer agent (quaternary ammonium cation) and the ultimate species of methanol, methoxide anion, between the organic solvents and aqueous solution. Among these solvents tested, dichloromethane was found to be the best one for the derivatization of methanol.

3.1.2. Effect of base

The effect of 0.1 ml of potassium hydroxide at various concentrations ranging from 0 to 0.05 *M* on the peak area ratio was studied and the results are shown in Fig. 2. In the absence of the KOH, no derivative was detected. The yield of methanol

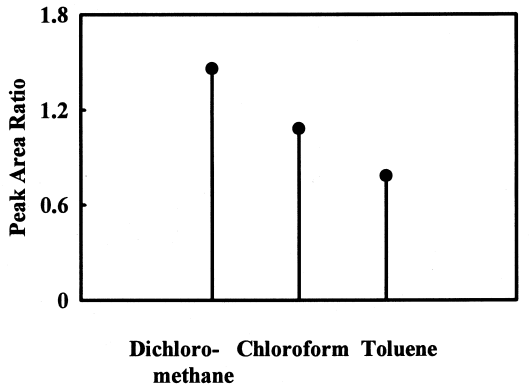

Fig. 1. Effect of organic solvents on the formation of methanol derivatives. Reactions were carried out at a temperature below the respective boiling point of the tested solvents for 2 h (30°C for dichloromethane, 50°C for chloroform and 85°C for toluene) in the presence of 0.1 ml of 0.05 *M* KOH, with 0.2 ml of 20 m*M* Br-MBX (in the specified solvent), 0.1 ml of 0.1 *M* BAC aqueous solution and 0.3 ml of 160 μ*M* 1-nitronaphthalene (in the specified solvent) as derivatizing agent, phase transfer agent and internal standard, respectively.

derivative increased almost linearly at higher concentration of base. It can probably be ascribed to the hydroxide ion mediated withdrawal of proton from the hydroxyl group of methanol, resulting in methoxide (CH_3O^-) formation, which is then transferred by

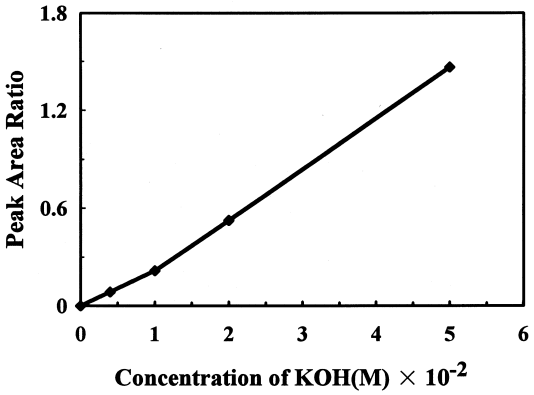

Fig. 2. Effect of potassium hydroxide on the formation of methanol derivative. Reactions were carried out at 30°C for 2 h in the presence of 0.1 ml of 0–0.05 *M* KOH with 0.2 ml of 20 m*M* Br-MBX dichloromethane solution, 0.1 ml of 0.1 *M* BAC aqueous solution and 0.3 ml of 160 μ*M* 1-nitronaphthalene dichloromethane solution as derivatizing agent, phase transfer agent and internal standard, respectively.

BAC in a complex form into the dichloromethane layer for further derivatizing reaction.

Although methoxide shows the following equilibrium with water:

$$CH_3O^- + H_2O \rightleftarrows CH_3OH + OH^-$$

the transfer of methoxide by BAC into the organic phase on its formation drives the reaction toward the left during the derivatization.

When the concentration of KOH was above 0.05 *M*, an additional peak with the similar retention time to that of methanol derivative was observed in the chromatogram and interfered with methanol determination. Therefore, 0.05 *M* KOH was chosen as the optimum alkali concentration for the determination of methanol.

3.1.3. Effect of phase-transfer agent

The effect of quaternary ammonium compounds, including BAC, HDTMAB, TBAC, TEAC, THAB and THPAB, at the concentration of 0.1 *M* (0.1 ml) on the transfer of methanol from alkaline aqueous phase to the dichloromethane organic phase for reacting with Br-MBX was studied. Shown in Table 1, the transfer effectiveness, evaluated by the yield of methanol derivative, of the tested catalysts, resulted: BAC > THAB > HDTMAB > THPAB > TBAC > TEAC. Hence, BAC was chosen. It appeared that the ability to transfer the methoxide anion is related to the length or bulk of the alkyl group of quaternary ammonium salts. The effect of BAC at various concentrations (0–0.5 *M*) on the transfer of CH_3O^- was also examined. The results, shown as Fig. 3 indicate that BAC (0.1 ml) was required at concentration higher than 0.1 *M* to achieve a plateau in the formation of the methanol derivative.

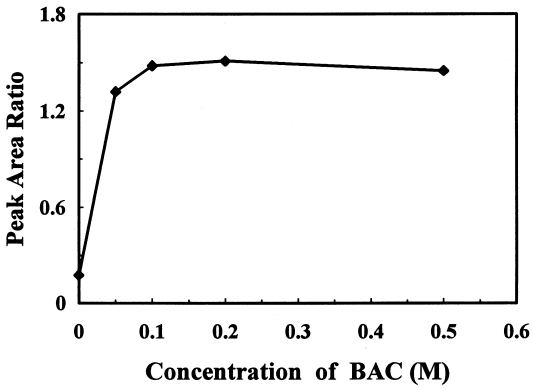

Fig. 3. Effect of the concentration of BAC on the formation of methanol derivative. Reactions were carried out at 30°C for 2 h in the presence of 0.1 ml of 0.05 *M* KOH with 0.2 ml of 20 m*M* Br-MBX dichloromethane solution, 0.1 ml of 0–0.5 *M* BAC aqueous solution and 0.3 ml of 160 μ*M* 1-nitronaphthalene dichloromethane solution as derivatizing agent, phase transfer agent and internal standard, respectively.

3.1.4. Effect of reaction time

The effect of reaction time at 30°C on the derivatization of methanol was studied. The results revealed that 2 h of reaction time was needed for the derivatization to reach the plateau; therefore, the reaction time for determination of methanol in this study was set at 2 h.

3.1.5. Effect of amount of derivatizing agent

For the purpose of establishing the optimum amount of derivatizing agent for the derivatization of methanol in water–ethanol solution (2 μmol in 0.1

Table 1
Effect of phase transfer agents on the yield of methanol derivative

Phase transfer agent	Peak-area ratio
Benzalkonium chloride (BAC)	1.46
Tetrahexylammonium bromide (THAB)	1.21
Hexadecyltrimethylammonium bromide (HDTMAB)	0.94
Tetraheptylammonium bromide (THPAB)	0.91
Tetrabutylammonium chloride (TBAC)	0.48
Tetraethylammonium chloride (TEAC)	0.09

Reactions were carried out at 30°C for 2 h in the presence of 0.1 ml of 0.05 *M* KOH with 0.2 ml of 20 m*M* Br-MBX dichloromethane solution, 0.1 ml of 0.1 *M* the specified quaternary ammonium compound in aqueous solution and 0.3 ml of 160 μ*M* of 1-nitronaphthalene dichloromethane solution as derivatizing agent, phase transfer agent and I.S., respectively. Each value is an average of six replicate analyses.

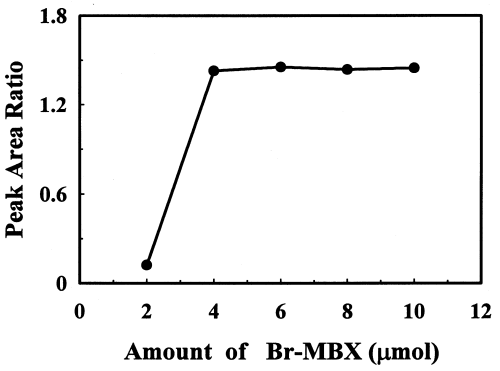

Fig. 4. Effect of the amount of Br-MBX on the formation of the methanol derivative. Reactions were carried out at 30°C for 2 h in the presence of 0.1 ml of 0.05 *M* KOH with 0.2 ml of 10–50 m*M* Br-MBX dichloromethane solution, 0.1 ml of 0.1 *M* BAC aqueous solution and 0.3 ml of 160 μ*M* 1-nitronaphthalene dichloromethane solution as derivatizing agent, phase transfer agent and internal standard, respectively.

ml) at 30°C for 2 h, different concentrations of Br-MBX in dichloromethane were tried. As shown in Fig. 4, more than 4 μmol (0.2 ml of 20 m*M*) of Br-MBX was needed to achieve a plateau formation of the derivative.

3.2. Analytical calibration

On the basis of the above optimized conditions, we formulated the analytical procedure for methanol determination as described in Section 2. To validate the quantitative application of the method, five different concentrations of methanol over the range 2–20 μmol/ml were evaluated. The linear regression equations were obtained as follows: $y=(-0.005\pm0.007)+(0.074\pm0.001)x$ for intra-day ($n=6$; $r=0.999$) and $y=(-0.008\pm0.005)+(0.074\pm0.001)x$ for inter-day ($n=8$; $r=0.999$); y indicates the peak area ratio of the methanol derivative to 1-nitronaphthalene; x, the concentration of methanol (in μmol/ml) and r, the correlation coefficient. The data indicate good linearity of the proposed method. The detection limit (signal-to-noise ratio = 5) of methanol in water–ethanol solution was 0.1 μmol/ml in 10 μl of injection (R.S.D. = 16%, $n=3$).

The precision (relative standard deviation) of the proposed method was assessed at three different concentrations of methanol (5, 10 and 20 μmol/ml in water–ethanol solution) and values of 3.0, 1.4 and 1.1% in intraday tests ($n=6$) and 3.8, 2.4 and 1.2% day-to-day ($n=8$), respectively, were found.

The stability of the derivative of methanol after derivatization was studied over a period of 24 h, no significant change of the peak area ratio was found.

3.3. Structural identification of the derivative

The typical HPLC chromatogram from determination of methanol in water–ethanol solution (20 μmol/ml) is presented in Fig. 5a. Peaks 1 and 3 represent the I.S. and the methanol derivative, respectively.

The structure of the methanol derivative of peak 3 in Fig. 5a was identified as 3-methoxymethyl-7-methoxy-1,4-benzoxazin-2-one by comparing the retention time with that of authentic sample, which was synthesized by a procedure similar to that described in Section 2.5. The resulting product was examined by Quattro 5022 mass spectrometry. The

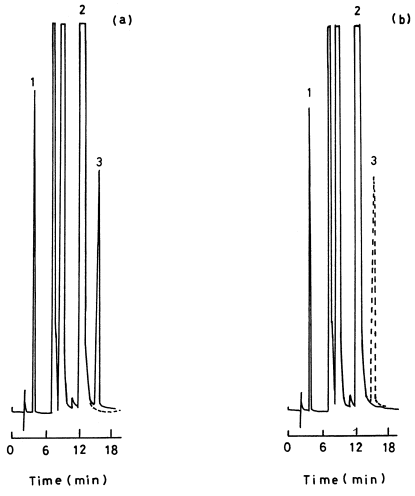

Fig. 5. Superimposed HPLC chromatograms from (a) analysis of water–ethanol solution containing 20 μmol/ml of methanol (solid line) and a reagent blank (dotted line), (b) analysis of a commercial liquor with labeled ethanol content of 41% (solid line) and the same liquor spiked with 20 μmol/ml of methanol (dotted line). Peaks: 1, 1-nitronaphthalene (I.S.); 2, derivative of ethanol; 3, derivative of methanol. HPLC conditions: LiChrospher diol column (250×4 mm I.D.; 5 μm); mobile phase, *n*-hexane–dichloromethane (9:1, v/v); flow-rate, 1.2 ml/min; UV detection, 350 nm.

molecular ion of the methanol derivative was found at m/z 221. The ion fragment at m/z 162 corresponds to the molecular ion minus both the methoxyl and carbonyl groups. Furthermore, by comparing the ^{1}H NMR (C^2HCl_3) spectrum of the derivative of methanol with those of the derivatizing agent (Br-MBX), a downfield shift from δ 4.54 of 3-methylene proton on Br-MBX to δ 4.92 of 3-methylene on the derivative of methanol was observed.

The derivative of ethanol, equivalent to peak 2 in Fig. 5a, was elucidated as 3-ethoxymethyl-7-methoxy-1,4-benzoxazin-2-one by the mass spectrometry and ^{1}H NMR(C^2HCl_3).

3.4. Applied analysis

Several kinds of commercial liquor with labeled ethanol content ranging 40–43% and 16–20%, respectively, were spiked with three different concentrations of methanol at 5, 10, 20 μmol/ml to examine the applicability of the proposed method. The chromatograms, shown in Fig. 5b, were obtained from the derivatized sample of a commercial liquor (solid line) and a spiked the same liquor (containing 20 μmol/ml of methanol) (dotted line). Table 2 presents the relative recoveries of spiked methanol in the commercial liquors with labeled ethanol content ranging from 40% to 43% and the average recovery was 101% with a R.S.D. of 3.2%. As shown in Table 3, the average recovery was 101% with a R.S.D. of 5.7% for the spikes in the commercial liquors with labeled ethanol content ranging from 16% to 20%. No significant difference was found for the applicability of the present method to the commercial liquors with those two levels of labeled ethanol content even though the analytical procedure was established in a standard solution containing ethanol as high as 50%. The proposed method shows a considerable potential for methanol analysis in the quality control of commercial liquors.

Table 2
Relative recoveries of the analysis of methanol spiked in the commercial liquors with labeled ethanol content ranging from 40% to 43%

Sample no.	Concentration spiked (μmol/ml)	R.S.D.[a] (%)	Recovery (%)
1[b]	0	–	–
	5	0.2	98
	10	1.4	105
	20	2.6	99
2[b]	0	–	–
	5	2.8	100
	10	2.4	100
	20	1.9	100
3[c]	0	–	–
	5	4.6	108
	10	2.4	100
	20	2.1	105
4[d]	0	–	–
	5	2.9	98
	10	4.1	102
	20	0.4	99

[a] The results are the average of three replicate analyses.
[b,c,d] Represent sample liquors with 40%, 41% and 43% labeled ethanol content, respectively.

Table 3
Relative recoveries of the analysis of methanol spiked in the commercial liquors with labeled ethanol content ranging from 16% to 20%

Sample no.	Concentration spiked (μmol/ml)	R.S.D.[a] (%)	Recovery (%)
1[b]	0	–	–
	5	3.4	110
	10	4.8	102
	20	3.9	99
2[b]	0	–	–
	5	2.7	90
	10	2.1	109
	20	1.6	99
3[c]	0	–	–
	5	1.8	102
	10	3.5	93
	20	3.4	103
4[d]	0	–	–
	5	2.6	102
	10	2.4	105
	20	3.3	98

[a] The results are the average of three replicate analyses.
[b,c,d] Represent sample liquors with 16%, 17.5% and 20% labeled ethanol content, respectively.

4. Conclusions

A HPLC method based on the selective derivatization of methanol with Br-MBX in a heteroge-

neous system, using benzalkonium chloride as phase transfer agent has been established and optimized.

Validation of the method for quantitation of methanol in water–ethanol solution showed that the method has excellent precision, accuracy and both intraday and interday reproducibility.

The application to spiked commercial liquors characterized by high ethanol content revealed high selectivity and sensitivity of the method. Because of the stability of the methanol derivative, the method can be easily applied for routine analysis of large number of samples in batches using an automated sampling system.

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