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QUANTITATIVE ANALYSIS OF 68 POLAR COMPOUNDS FROM TEN CHEMICAL CLASSES BY DIRECT AQUEOUS INJECTION GAS CHROMATOGRAPHY

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SUMMARY

Porous polymer packings have been used successfully in many applications of direct aqueous injection gas chromatography. We have expanded the use of aqueous injection to the quantitative analysis of 68 alcohols, acetates, ketones, ethers, sulfides, aldehydes, diols, diones, nitriles and amides on a glass column packed with unmodified Tenax GC using a flame-ionization detector. Analysis of single- and multi-component mixtures, accurate standard preparation, assessment of analytical errors, limitations of the method and problems encountered are discussed. Peak ghosting and tailing were not serious problems. The analytical error between chemical classes expressed as the coefficient of variation between duplicate samples ranged from 0.81% for nitriles ($n = 3$) to 7.09% for ethers ($n = 5$). The method described is fast, precise and accurate, requires little sample preparation and is applicable to a wide variety of compounds.

INTRODUCTION

Our laboratory has been involved in an aquatic toxicology acute testing program with polar organic compounds. Routine monitoring of bioassay water required a convenient, fast and accurate gas chromatographic (GC) method suitable for alcohols, ketones, acetates, ethers, sulfides, aldehydes, diols, diones, nitriles and amides. Compounds selected for testing from the above classes included C_1–C_{10} straight-chain, branched and cyclic compounds with various substituents.

The compounds used were appreciably soluble in water, making solvent extraction techniques difficult. Therefore, direct aqueous injection on the porous polymer Tenax GC (Applied Science Labs., State College, PA, U.S.A.) with a flame-ionization detector was the chosen method of analysis. The properties of Tenax GC that make it suitable for direct aqueous injection of polar organics and its ability to separate multi-component mixtures have been thoroughly described[1–3]. The problems associated with direct aqueous injection include ghosting[4,5], peak tailing[2] and effects from excess water on the column packing[6]. Aqueous injection has been successfully

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applied to volatile halogenated organics[7], polyamines[8], C_1–C_4 alcohols[9–11], C_2–C_6 monocarboxylic acids[12] and phenols[13,14]. Tenax GC modified with polymetaphenoxylene was used on the 1975 Viking Spacecraft, which resolved a variety of organic compounds in the presence of excess water[15].

This paper demonstrates the application of direct aqueous injection on Tenax GC for 68 compounds from ten chemical classes. The analysis of single- and multi-component mixtures, accurate standard preparation, an assessment of analytical error, limitations of the method and problems encountered are discussed.

EXPERIMENTAL

Gas chromatographic conditions

Analysis was performed on a Hewlett-Packard Model 5730A gas chromatograph equipped with dual flame-ionization detectors. The instrument was also equipped with 91 cm × 2 mm I.D. and 120 cm × 2 mm I.D. glass columns packed with 60–80-mesh Tenax GC. The detector and inlet temperatures were 300 and 200–250°C, respectively. Nitrogen (15–25 ml/min) was used as the carrier gas and hydrogen (15–25 ml/min) and air (240 ml/min) were used for the flame operation. Peak-area calculations were performed by a Hewlet-Packard Laboratory Automation Data System (Model 3354). The column was operated isothermally for single-component analysis. Multi-component analyses were performed isothermally or with temperature programming, depending on the mixture composition. On-column injections of 1.0 μl were used for all samples and standards.

Columns were pre-conditioned with a nitrogen flow (15–25 ml/min) for 1 h at 30°C, then programmed at 4°C/min to 300°C and held for 1 h.

Standard preparation

Stock solutions were prepared in methanol, acetone, distilled water or test exposure water, depending on the approximate solubility of the particular compound in water. Estimated water solubility values used to prepare standards were obtained from either the literature or simple laboratory experiments where an excess of the compound in water was stirred, filtered and analyzed. Stock solutions of compounds with water solubilities greater than approximately 500 mg/l were prepared in distilled or test exposure water. Stock solutions of compounds with water solubilities less than approximately 500 mg/l were prepared in methanol or acetone. Four working standards were prepared for each compound or mixture by diluting stock solutions to the desired concentration range with distilled or test water. When using non-aqueous stocks, the concentration of solvent in the working standards was kept below 5.0% (v/v) to represent aqueous exposure samples better. Calibration graphs for compound quantification were established by linear regression analysis of the four standards. Standards of furan, diisopropyl ether and di-*n*-butyl ether were prepared daily because of concentration changes due to their volatility. Hexyl ethanoate and ethyl hexanoate were also prepared daily owing to degradation in water. Methyl chloroacetate and methyl dichloroacetate were not chemically stable in water and we were unable to prepare quantitative aqueous standards. Standards for all other compounds were stable and stored at 20–25°C for 5–7 days. All samples were analyzed immediately upon collection.

Spikes and duplicate samples

The stability and accuracy of the standards of each test compound were checked daily by preparing a spiked sample. Compounds with water solubilities, greater than 500 mg/l were added directly to the test water by weighing of the pure compound. Compounds with water solubilities less than 500 mg/l were prepared by adding the solvent stock, or intermediate stock solution, to the test water to achieve the desired concentration. Samples and duplicates were collected in glass GC vials using a disposable pasteur pipet.

Statistical analysis

The accuracy of the method was evaluated by calculating the means and standard deviations of 3–6 spike recovery determinations per compound. The precision of the method was assessed by calculating the coefficient of variation (CV) of 3–9 duplicate pairs per compound. The mean CV was then calculated for each class of compound. One-way analysis of variance was performed to test for a significant difference in the CV for individual compounds within a class and also to compare the CV between classes.

RESULTS AND DISCUSSION

The formation of gaps in the Tenax GC column packing after several weeks of use has been demonstrated and discussed previously[2]. The formation of column packing gaps also occurred during our study but seemed to have a minimal effect on column performance, possibly owing to their small size. Tightly packing the columns appeared to reduce gap formation, but we have not been able to unpack a Tenax GC column successfully.

Proper selection of the standard preparation technique was critical for obtaining reliable standards. Compounds with estimated solubilities greater than 500 mg/l provided few problems in preparing reliable aqueous standards. Aqueous standards of compounds with estimated solubilities less than 500 mg/l were unreliable. Stock solutions were then prepared in methanol or acetone to ensure complete dissolution. Methanol was preferred to acetone because it produced a lower flame-ionization detector response. Standards prepared in water from solvent stock solutions were stable and produced linear calibration graphs ($r > 0.999$); correlation coefficients lower than 0.999 were investigated and new working standards prepared if necessary.

The method used for stock solution preparation and the GC retention time, isothermal GC temperature, working range and analytical error for each compound are listed in Table I. The working range is defined as the concentration range in which we quantitatively analyzed each compound based on our research needs, and does not reflect the chromatographic capabilities. The detection limit for all compounds was approximately 1.0 μg/ml. The retention time varied inversely with the weight injected, which is common with porous polymer-type packings[2], and the retention times listed in Table I serve only as a guide. Newly packed columns will also give slightly different retention times. In general, peak symmetry was good for most of the compounds tested.

Peak ghosting or carry-over of a compound from one injection to the next was not a serious problem except for all ketones, which exhibited an intermittent

TABLE I

ANALYTICAL PARAMETERS, ACCURACY AND PRECISION FOR INDIVIDUAL COMPOUNDS ANALYZED BY AQUEOUS INJECTION GAS CHROMATOGRAPHY

Class	*Compound*	*Analytical parameters*				*Accuracy and precision***	
		Isothermal GC temp. (°C)	*Retention time (min)**	*Standard preparation technique***	*Working range (mg/l)*	*Mean recovery ± S.D. (%) (n)*	*Mean CV (%) of duplicates (n)*
Alcohols	Methanol	80	1.1	DW	2900–24,000	101.5 ± 2.7 (5)	1.32 (5)
	Ethanol	100	1.0	DW	2500–22,000	99.3 ± 1.2 (3)	1.44 (5)
	1-Propanol	100	1.8	DW	1000–12,000	101.8 ± 2.9 (5)	0.86 (5)
	2-Propanol	100	1.2	DW	2400–15,800	101.6 ± 5.4 (5)	1.44 (4)
	1-Butanol	130	2.8	DW	300–10,000	ND	1.77 (6)
	1-Hexanol	180	1.1	DW	10–400	103.6 ± 9.1 (5)	2.65 (5)
	1-Octanol	185	3.5	DW	3–40	94.6 ± 4.9 (5)	1.29 (6)
	1-Nonanol	180	3.5	M	0.5–10	99.7 ± 2.7 (3)	2.89 (5)
	1-Decanol	205	3.9	A	0.5–10	104.6 ± 6.2 (5)	2.08 (5)
	4-Decanol	190	3.0*	M	0.5–30	93.1 ± 7.9 (5)	3.64 (5)
	2-Chloroethanol	140	1.1	DW	10–200	95.2 ± 12.1 (6)	1.48 (5)
	2,3-Dibromopropanol	205	2.7	DW	5–200	99.4 ± 10.6 (5)	4.25 (5)
	2,2,2-Trichloroethanol	180	1.6	DW	30–500	94.8 ± 9.0 (5)	2.01 (5)
	1-Chloro-2-propanol	150	2.2	DW	80 800	104.8 ± 5.9 (4)	2.80 (5)
	1-Amino-2-propanol	160	1.9	DW	400–5000	96.0 ± 10.0 (5)	1.61 (5)
	2-Methyl-1-propanol	140	1.1	DW	50–2000	95.8 ± 4.6 (5)	3.85 (5)
	Cyclohexanol	180	1.7	DW	70–1000	100.7 ± 1.0 (5)	0.95 (4)
	2-Phenoxyethanol	225	2.1	DW	20–600	102.8 ± 3.9 (5)	3.80 (5)
	3-Furylmethanol	150	2.7	DW	50–1000	101.4 ± 1.7 (5)	3.20 (5)
	3-(3-Pyridyl)-1-propanol	220	2.4	DW	100–1000	97.0 ± 4.5 (5)	3.16 (5)
	2-(2-Ethoxyethoxy) ethanol	180	3.0	DW	1000–27,000	100.0 ± 1.7 (5)	1.78 (7)
	Phenol	180	1.9	DW	5–70	117.7 ± 13.0 (5)	0.77 (4)
Ketones	Acetone	100	1.1	DW	1000–12,000	103.7 ± 1.1 (5)	0.70 (9)
	2-Butanone	130	1.6	DW	500–5000	110.8 ± 4.1 (5)	1.13 (5)
	3-Pentanone	150	2.0*	DW	100–1100	101.6 ± 1.1 (5)	1.16 (5)
	2-Octanone	180	2.1	DW	10–200	121.0 ± 4.4 (4)	7.87 (5)
	5-Nonanone	200	2.0*	A	1–50	99.6 ± 6.1 (5)	2.96 (5)
	2-Decanone	200	1.7	M	0.5–15	101.0 ± 6.1 (5)	3.72 (4)
	Cyclohexanone	180	2.0	DW	50–1300	100.3 ± 3.9 (6)	1.23 (4)
	Acetophenone	210	2.4*	DW	10–300	99.3 ± 6.8 (5)	2.68 (5)
	3-Methyl-2-butanone	150	1.7*	DW	50–2100	106.4 ± 3.2 (5)	1.09 (5)

	4-Methyl-2-pentanone	150	1.4	DW	100–1100	104.4 ± 3.0 (4)	0.25 (4)
	5-Methyl-2-hexanone	165	2.0	DW	10–300	122.4 ± 2.8 (4)	5.61 (5)
	6-Methyl-5-hepten-2-one	200	1.6*	DW	10–200	112.2 ± 5.2 (5)	0.89 (5)
	2-Butanone oxime	145	3.4*	DW	200–2700	106.9 ± 2.8 (4)	0.14 (5)
	3,3-Dimethyl-2-butanone	170	1.1*	DW	50–800	100.6 ± 2.0 (5)	1.86 (5)
Acetates	Methyl ethanoate	95	1.9	DW	40–800	102.0 ± 5.9 (6)	1.29 (5)
	Ethyl ethanoate	110	1.8	DW	30–500	103.7 ± 3.9 (5)	2.50 (5)
	Propyl ethanoate	130	1.8	DW	10–150	105.7 ± 5.5 (4)	1.14 (4)
	Butyl ethanoate	145	2.0	DW	1–1100	100.6 ± 7.1 (5)	3.26 (5)
	Hexyl ethanoate	160	2.3	DW	1–100	ND	7.68 (4)
	Ethyl hexanoate	170	2.4	M	2–12	104.6 ± 5.1 (4)	3.62 (5)
	Methyl chloroacetate	140	2.1	DW	1–20	ND	ND
	Methyl dichloroacetate	150	2.0	DW	0.5–10	ND	12.3 (5)
	2-Bromoethyl acetate	150	4.0	DW	5–40	100.6 ± 1.5 (4)	2.49 (4)
	2-Ethoxyethyl acetate	150	4.0	DW	7–110	100.2 ± 1.8 (4)	0.34 (4)
	Diethyl malonate	175	3.1	DW	5–150	98.8 ± 3.8 (4)	0.63 (5)
Aldehydes	Ethanal	80	1.6*	DW	10–400	104.4 ± 1.7 (5)	0.50 (5)
	Butanal	120	2.3	10% M	1–60	99.4 ± 2.5 (5)	3.81 (5)
	Hexanal	140	3.5	M	5–100	117.2 ± 8.3 (4)	11.0 (4)
	Benzaldehyde	200	2.2*	DW	2–50	98.8 ± 2.8 (5)	1.62 (5)
	Isopropylbenzaldehyde	205	3.0	M	2–10	95.8 ± 2.2 (4)	2.13 (5)
Ethers	Diisopropyl ether	140	1.1	DW	10–600	102.7 ± 11.2 (12)	3.43 (7)
	Di-*n*-butyl ether	160	3.7	DW	10–140	123.8 ± 18.5 (6)	4.87 (4)
	Furan	85	2.1	M	10–100	109.2 ± 6.4 (5)	21.0 (3)
	Tetrahydrofuran	120	1.6	DW	200–4500	101.1 ± 3.5 (5)	1.12 (5)
	2,4-Dinitroanisole	220	14.5	M	10–50	106.9 ± 4.6 (4)	5.06 (5)
Nitriles	Acetonitrile	90	1.5	DW	350–3500	104.9 ± 5.6 (5)	1.00 (5)
	Chloroacetonitrile	130	1.6	M	1–15	ND	ND
	Allyl cyanide	125	2.0	DW	40–400	102.6 ± 2.4 (5)	1.02 (5)
	o-Tolunitrile	185	2.9	M	10–100	100.8 ± 1.8 (5)	0.41 (5)
Sulfides	Di-*n*-butyl sulfide	180	2.8	M	ND	ND	ND
	Di-*n*-propyl sulfide	165	1.9	M	5–75	104.6 ± 7.9 (5)	1.27 (5)
Diols	Triethylene glycol	215	3.5	DW	10,000–50,000	100.1 ± 2.8 (7)	1.08 (10)
	2-Methyl-2,4-pentanediol	200	1.0	DW	2000–20,000	100.3 ± 1.3 (4)	0.80 (5)
Amides	N,N-dimethylformamide	160	1.6	DW	1700–17,000	100.2 ± 0.45 (6)	2.41 (5)
	N,N-Dibutylformamide	215	2.1	DW	15–300	102.5 ± 8.7 (5)	2.04 (5)
Diones	2,4-Pentanedione	165	1.8	DW	5–400	108.5 ± 13.8 (5)	4.92 (5)

* Retention times marked with an asterisk were chromatographed on a 120 cm × 2 mm I.D. column; all others were chromatographed on a 91 cm × 2 mm I.D. column.

** DW = distilled water; M = methanol; A = acetone.

*** ND = not determined.

carry-over of 0.7% or less. The intermittent nature of the carry-over problem may be related to column age or the accumulation of charred deposits within the injection port area[4,5], and can be reduced by periodically replacing the glass-wool at the injector end of the column. In addition, N,N-dimethylformamide, 2-methyl-1-propanol, 3-methyl-2,4-pentanediol and 3-furylmethanol had a carry-over of 0.7–1.0%, and 1-amino-2-propanol, 2-phenoxyethanol, phenol, 3-(3-pyridyl)-1-propanol, N,N-dibutylformamide and diethyl malonate had a carry-over of 1–5%. The carry-over problems described were considered acceptable, and no corrective action was taken.

Researchers must be aware of the possible existence and extent of carry-over when working with aqueous injection GC. If severe carry-over is encountered, various technique have been described for overcoming the problem[4,5,15]. Peak tailing also occurred with compounds that exhibited carry-over, but posed little problem except in severe cases.

2-Chloroethanol and chloroacetonitrile co-eluted with an interfering peak which made quantitative analysis difficult at levels below 10 μg/ml. After the Tenax GC column had been in use for a few months, an unknown peak eluted when operating isothermally between 110 and 140°C. At 130°C the retention time of the interferent was 1.6 min and exhibited good peak synmmetry with a consistent peak area. The cause or source of the interference has not yet been determined.

The spike recovery determinations were very important in confirming that reliable results were being generated, and they should be included in all aqueous injection work. Daily preparation of spikes was used to check the accuracy of the original standard preparation and allowed the detection of changes due to compound degradation and/or volatility. The mean spike recoveries and standard deviations for each compound are listed in Table I.

A one-way analysis of variance of the CV of the duplicate data within each class of compound indicated that the ketones, aldehydes, ethers and acetates contained compounds with significantly ($p < 0.05$) more difference in the CV. Further examination of the data showed 2-octanone, hexanal, furan, methyl dichloroacetate and hexyl acetate as outliers with individual CVs of 7.87, 11.0, 21.0, 12.3 and 7.68%, respectively. All other compounds had CVs less than 5.0%, except 5-methyl-2-hexanone (5.6%) and 2,4-dinitroanisole (5.1%).

Comparison of the CVs of the chemical classes by one-way analysis of variance showed no significant ($p < 0.05$) difference (Table II). The mean CVs for the different chemical classes ranged from 0.81% for nitriles to 7.09% for the ethers. The volatile nature of the ether group may have contributed to the large CV. All chemical classes except for the ethers exhibited CVs < 5.0%.

In addition to quantitative analysis of the individual compounds listed in Table I, quantitative multi-component analysis was successfully performed. Fig. 1 shows a typical chromatogram of a multi-component standard. Component concentrations in toxicity exposure tanks were simultaneously monitored for hexanol, 2-octanone, 1-octanol and acetophenone with a single injection of standards and samples. Other multi-component systems that were monitored included benzaldehyde and 2-octanone, benzaldehyde and 1-octanol, 1-octanol and 2-chloroethanol and 2-octanone and 2,4-pentanedione.

From experience we have found that aqueous injection could be applied to a

TABLE II

NUMBER OF COMPOUNDS AND MEAN COEFFICIENT OF VARIATION (%) FOR DUPLICATE SAMPLES OF THE TEN CHEMICAL CLASSES ANALYZED BY DIRECT AQUEOUS INJECTION

Class	*No. of compounds*	*Mean CV (%)**
Alcohols	22	2.23
Ketones	14	2.23
Aldehydes	5	3.81
Amides	2	2.22
Ethers	5	7.09
Acetates	10	3.52
Sulfides	1	1.27
Diols	2	0.94
Diones	1	4.92
Nitriles	3	0.81

* Each mean coefficient of variation value represents 4–9 duplicate determinations per compound.

wide variety of sample types. The only requirement is that aqueous suspended solids be removed by filtration or centrifugation and the analyst must be aware of possible problems with compound ghosting[4,5] and peak tailing[2], which could cause errors if unnoticed. The method is fast, precise, accurate and requires little sample preparation. These advantages, together with the applicability to a wide variety of compounds as demonstrated in this paper, make this method useful for the analysis of many organic compounds in water.

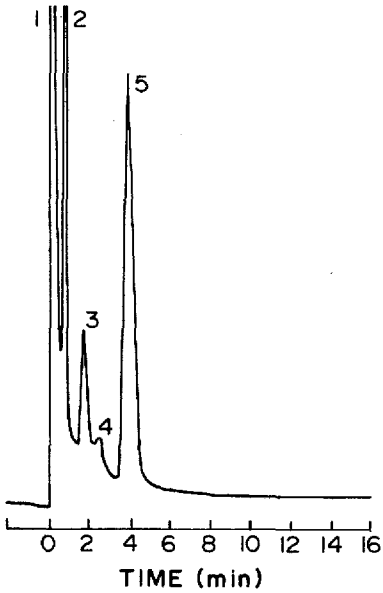

Fig. 1. Typical chromatogram of a multi-component standard analyzed isothermally at 180°C on a 91 cm × 2 mm I.D. Tenax GC column. Peaks: 1 = solvent (methanol) (4%, v/v); 2 = hexanol (45 μg/ml); 3 = 2-octanone (14 μg/ml); 4 = 1-octanol (5.1 μg/ml); 5 = acetophenone (65 μg/ml).

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