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Determination of short-chain aliphatic, oxo- and hydroxy-acids in drinking water at low microgram per liter concentrations

Sigrid Peldszus*, Peter M. Huck, Susan A. Andrews

Department of Civil Engineering, University of Waterloo, Waterloo, Ont. N2L 3G1, Canada

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Abstract

A fast and reliable ion chromatography method has been developed and applied to study the formation and consumption of organic acid ozonation by-products in a drinking water treatment plant. Water samples are injected directly into the ion chromatograph using a large sample loop (740 μl) without any sample preparation step other than possibly filtration. Organic and inorganic anions are determined by separation on a high-capacity anion-exchange column followed by conductivity detection. The average recovery for the organic acids investigated (β-hydroxybutyric, acetic, glycolic, butyric, formic, α-ketobutyric and pyruvic acid) ranged from 96 to 105%, and their method detection limits ranged from 1 to 5 μg/l. When applied to samples taken from a drinking water treatment plant, the method proved to be reliable.

Keywords: Water analysis; Organic acids; β-hydroxybutyric acid; Acetic acid; Glycolic acid; Butyric acid; Formic acid; α-Ketobutyric acid; Pyruvic acid

1. Introduction

Discussions on the health risks of chlorination by-products in drinking water have led to an increased interest in alternative disinfectants such as ozone. During ozonation, organic matter present in the source water is oxidized to form a variety of compounds including alcohols, aldehydes, ketones and carboxylic acids [1–3]. Some of these substances serve as nutrients for those microorganisms which escape disinfection, thus leading to bacterial regrowth in drinking water distribution systems. The investigation arose from another in which the objective was to identify and then quantify selected ozonation by-products so that they could be compared to parameters such as ozone dose and water quality. This research focused on short-chain organic acids, which are present at low μg/l concentrations.

Methods for the analysis of organic acids which are currently available involve either gas chromatography (GC) or ion chromatography (IC). Although many GC methods possess the desired sensitivity (low μg/l detection limits), they involve rather time-consuming sample prep-

* Corresponding author.

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aration steps such as extraction and derivatization. Some of these methods are also able to include hydroxy- and/or oxo- substituted organic acids [4–9]; however, they usually involve additional, even more time-consuming derivatization steps.

Ion-exclusion chromatography is the most commonly used IC technique to determine organic acids [10–13]. The majority of the methods using this technique have detection limits of approximately 1 mg/l, which makes them unsuitable for the present research. Nonetheless, Haddad and Jackson [14] successfully combined an anion-exchange concentrator column with an ion-exclusion analytical column achieving detection limits ranging from 5.6 to 9.2 μg/l for formic, acetic, propionic and butyric acid. Unfortunately, these authors reported that it would not be possible to preconcentrate these organic acids in the presence of even moderately high concentrations of inorganic anions as would be found in drinking water samples. Furthermore, ion-exclusion chromatography is only well suited for weak acids, those with pK_a values greater than approximately 3. Anions of acids with low pK_a values, such as pyruvic acid, are likely to elute very early in the chromatogram together with anions of strong mineral acids such as Cl^-, SO_4^{2-} and NO_3^-.

For the purpose of the present investigation, a fast and sensitive method was required. Due to the limitations of the techniques currently in use, a different method was developed which utilizes anion-exchange chromatography. To increase the sensitivity, a large sample loop (740 μl) was used for sample injection. Due to the relatively high resin capacity of the analytical anion-exchange column, the initially broad sample band entering the chromatographic system was effectively focused at the front of the column, resulting in acceptable peak widths in the final chromatogram. Seven different carboxylic acids including hydroxy- or oxo- substituted acids (β-hydroxybutyric, acetic, glycolic, butyric, formic, α-ketobutyric and pyruvic acid) could be determined at low μg/l concentrations without any sample preparation step other than filtration, and the filtration step was normally easily accommodated as part of an autosampler routine.

2. Experimental

2.1. Reagents

Deionized water (Ultrapure water system: Milli Q-UV plus, Millipore, Bedford, MA, USA) was used for the preparation of eluents and stock solutions. Sodium hydroxide eluents (8 m*M* and 150 m*M*) were prepared using a 50% (w/w) sodium hydroxide solution with low carbonate content (Na_2CO_3 0.09%) obtained from Fisher Scientific (Ottawa, Ont., Canada). Organic acids (D,L-ß-sodium hydroxybutyrate, sodium acetate, glycolic acid, sodium butyrate, sodium formate, α-ketobutyric acid, sodium pyruvate, lactic acid, hemicalcium glycerate, propionic acid and oxalacetic acid) were purchased from Sigma (St. Louis, MO, USA) with purities of at least >98%, and used as received.

2.2. Stock solutions

Individual 1000 mg/l stock solutions were prepared for each acid in ultrapure water. An amount of 10 ml of each stock solution was pipetted into 100 ml deionized water resulting in a 100 mg/l acid mixture. Weekly measurements of 20 μl of this mixture indicated that the solution was stable for at least twelve weeks. Stock solutions as well as the 100 mg/l mixture were prepared fresh every three months.

2.3. Working standard solutions

In order to account for sample matrix effects, working standards were prepared using solutions which were as similar as possible to the sample matrix. These solutions were prepared by adding salts of the most common anions to deionized water in approximately the same concentrations as expected for the water samples (CO_3^{2-}: 100 mg/l; Cl^-, SO_4^{2-}: 50 mg/l; F^-, Br^-, NO_2^-, PO_4^{3-}: 0.1 mg/l). They were then used to prepare organic acid standards with concentrations between 5 and 1000 μg/l by serial dilution of the 100 mg/l mixture.

A 1 mg/l standard was determined to be stable for a week, whereas standards with lower concentrations were only stable for approximately

three days. Hence, the 1 mg/l standard was prepared every week and standards with lower concentrations were prepared every day.

2.4. IC system

All equipment was purchased from Dionex (Sunnyvale, CA, USA). The IC was equipped with an autosampler (ASM), an advanced gradient pump (AGP), a high-pressure, three-way injection valve (BF-2) and a large sample loop (740 μl = 370 cm PEEK tubing with 0.51 mm I.D.). The anion-exchange column (AS 10, 250 × 4 mm I.D.) was composed of 8.5 μm ethylvinylbenzene–divinylbenzene substrate agglomerated with completely aminated anion-exchange latex. The latex particles had a diameter of approximately 65 nm and carried the ion-exchange sites. The capacity of the column was 170 μeq. To protect the analytical column, a guard column (AG 10, 50 × 4 mm I.D.) containing the same resin as the AS 10 was installed. Preliminary experiments also involved a concentrator column (AC 10, 50 × 4 mm I.D.) and a different anion-exchange column (AS 11, 250 × 4 mm I.D.). The detection system consisted of an anion self-generating suppressor (ASRS I, 4 mm) and a conductivity detector (CDM-2). Due to the high sodium hydroxide concentration at the end of the elution gradient, the suppressor had to be used in the external water mode (flow 1.8 ml/min). When run in the autosuppression mode, the suppressor would become overloaded and initiate a shut down procedure. The current at the suppressor control unit was set to 3, which is equivalent to approximately 300 mA. Eluents were degassed with helium, and were further purified by an anion trap (ATC-1, 24 × 9 mm I.D.) placed between the pump and the injection valve.

2.5. Determination of the acids

In general it was not necessary to manually filter the samples because filter units were incorporated into the caps of the autosampler vials. However, water samples containing visible solids, for example certain raw or settled waters, required an additional filtration through a 0.45-μm glass fiber filter prior to injection. This prevented solids from entering the chromatographic system causing pressure increases. Using the autosampler, water samples or standards were injected through a sample loop into the chromatographic system. The organic anions under investigation were separated from the inorganic anions utilizing a gradient with a flow of 1 ml/min. The gradient started with an eluent concentration of 8 m*M* NaOH, which was held for 10 min. Over the next 13 min the eluent concentration was increased at a constant rate to 125 m*M* NaOH (17% NaOH 8 m*M*, 83% NaOH 150 m*M*). This concentration was held for 12 min and then brought back to the initial conditions, where it was held for 10 min to equilibrate for the next injection.

2.6. Sample preservation

Chloroform ($CHCl_3$) was tested as a preservative for several water types. Raw, settled, post-ozone and post-filter water samples were spiked with the organic acid mixture adding 50 μg/l to the acids already present in these samples. Each sample was divided into an unpreserved and a preserved portion (0.1%, v/v, $CHCl_3$ addition) and stored at 4°C. The organic acid content in these samples was monitored for several days, and also remeasured after four weeks.

2.7. Drinking water sampling

A full-scale water treatment plant, which uses ozone as the primary disinfectant, provided samples for the method evaluation. Initially, water samples from different stages of the treatment process were analyzed the same day they were obtained. Later, 0.1% $CHCl_3$ (50 μl into 50-ml sample) was added to each sample. These samples were stored at 4°C for up to a maximum of one week before analysis.

3. Results and discussion

The original objective of this research was to develop a suitable method for the analysis of drinking water for short-chain (C_1 to C_4) mono-

and dicarboxylic acids, preferably including hydroxy- and keto-substituted species. Preliminary experiments showed that acids which elute after chloride had either nonreproducible retention times or were not sufficiently separated from inorganic anions. As a consequence acids such as malonate, oxalate or ketomalonate were not included in this investigation.

Fig. 1 shows typical standard chromatograms. Inorganic anions were added to these standards at concentrations similar to those known for drinking water samples to account for competitive effects of these anions in this type of sample. Due to the large injection volume, the peaks from chloride and sulfate (50 mg/l each) were very large and broad, and dominated the chromatogram (Fig. 1a). However, the organic acids still displayed acceptable peak shapes and sensitivities even for concentrations as low as 25 μg/l (Fig. 1b).

Some of the compounds of interest were later found to coelute with other organic acids as shown in Fig. 1b. However, these other acids have not been reported to be present in drinking water, and are therefore expected to be only very minor constituents of drinking water. In addition, since the ozonation investigations described herein use the same type of water for each series of experiments, it can be expected that in general the products which are formed vary mainly in concentration and not in structure. If necessary, this assumption may be proved by comparing results obtained with the IC method to those obtained using a confirmation method, perhaps one involving gas chromatography [15]. This confirmation step, however, was considered to be unnecessary in the present study.

3.1. Preconcentration vs. large injection volume

In order to optimize the method sensitivity in a simple way, use of an anion-exchange concentrator column seemed to be an appropriate choice for initial investigations. However, concentrator columns have a limited resin capacity and since inorganic anions are usually retained more strongly on anion-exchange resins, organic anions can be made to elute when the capacity of the concentrator column is exceeded. This means that high concentrations of inorganic anions in a sample could displace the compounds of interest, the organic acids. When use of a preconcentration column (AC 10) was attempted, the result was that the concentrations of chloride and sulfate (50 mg/l each) were high enough to displace the organic acids from the column with as little as 2.3 ml of sample.

Attempts were then made to increase the sensitivity without the use of a concentrator column by simply installing a 740-μl sample loop instead of a standard 20-μl loop. Since the AS 11 column (45 μeq/column) was especially designed for organic acid analysis it was used in preliminary experiments. Injections of organic acid standards containing inorganic anions in mg/l concentrations resulted in chromatograms with com-

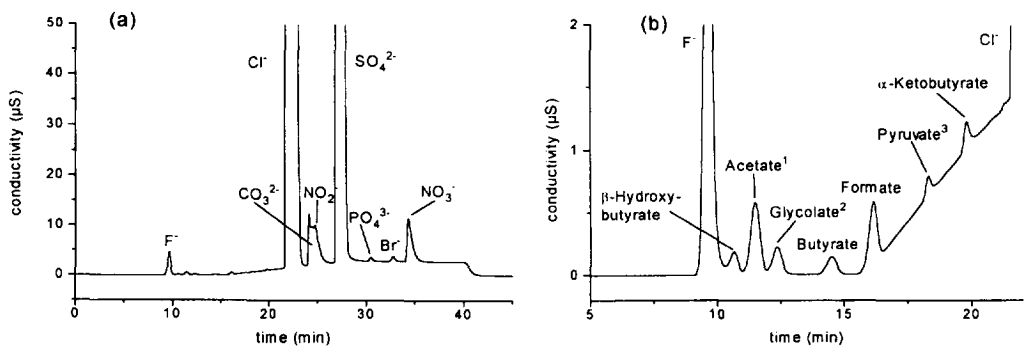

Fig. 1. Typical standard chromatogram with a concentration of 25 μg/l for each acid: (a) complete chromatogram showing the inorganic anions; (b) enlarged section showing the organic acids (annotations indicate coelution: 1, with lactate and glycerate; 2, with propionate; and 3, with oxalacetate).

pletely distorted peaks. Therefore, in order to cope with the relatively high amounts of inorganic anions entering the chromatographic system, the analytical column had to have a relatively high resin capacity. Hence, the AS 10 column with its resin capacity of 170 μeq/column was chosen. This column effectively enabled a large sample to be injected onto the column such that a relatively narrow band of analytes could be established at the front of the column. Thus, the large sample loop (370 cm length) did not cause excessively large peak widths in the final chromatogram. This behaviour, sometimes called the "relaunch effect", has been described previously [16–19].

3.2. Method calibration and detection limits

To evaluate the described method calibration curves, recoveries and detection limits were determined.

The calibration curves were linear over the investigated concentration range (5 to 200 μg/l), which is the normally expected range for ozonation by-product concentrations. The equations for the calibration curves and their correlation coefficients can be found in Table 1. The high correlation coefficients (>0.99) indicated a good fit of the actual data points with the calculated calibration curves.

Analyte recoveries were determined at six concentrations ranging from 5 to 200 μg/l with at least six but mostly eight replicates for each concentration. The average overall recoveries were generally very good. They were close to 100% with standard deviations from 4 to 12% (Table 2). For the different concentrations evaluated the average recoveries ranged from 80 to 117%. As expected, the recoveries for the lower concentrations showed greater deviations from 100% than the ones for the higher concentrations.

The method detection limits (MDL) were determined according to standard protocols (Standard Methods for the Examination of Water and Wastewater [20]) and are evident from Table 3. The MDL is defined as the lowest concentration of a compound detected with 99% confidence. For the investigated compounds (c = 5 μg/l, $n = 7$), the MDLs ranged from 1 to 5 μg/l, indicating that the desired sensitivity was achieved. Acetate had the highest MDL with 5 μg/l, even though the response for acetate was higher than for most of the other compounds. This was possibly caused by a background contamination problem with acetate, which may have led to a significantly higher standard deviation for repeated injections of acetate at the 5 μg/l level, the concentration used to determine the MDL.

3.3. Sample stability and preservation

Ideally, samples were analyzed the same day they were taken. However, in order to accommodate requirements for sample shipping or to save

Table 1
Calibration curves[a]

Compound	Calibration curve equation[b]	Correlation coefficient	Standard error
β-Hydroxybutyrate	$y = 1.34 \cdot 10^5 x - 3.57 \cdot 10^5$	$r^2 = 0.9998$	$2.38 \cdot 10^5$
Acetate	$y = 2.17 \cdot 10^5 x + 5.60 \cdot 10^6$	$r^2 = 0.9916$	$1.49 \cdot 10^6$
Glycolate	$y = 2.02 \cdot 10^5 x - 2.55 \cdot 10^5$	$r^2 = 0.9996$	$2.49 \cdot 10^5$
Butyrate	$y = 1.42 \cdot 10^5 x + 2.59 \cdot 10^4$	$r^2 = 0.9997$	$6.03 \cdot 10^5$
Formate	$y = 3.81 \cdot 10^5 x + 1.01 \cdot 10^6$	$r^2 = 0.9991$	$3.46 \cdot 10^5$
Pyruvate	$y = 6.63 \cdot 10^4 x - 3.32 \cdot 10^5$	$r^2 = 0.9969$	$2.40 \cdot 10^5$
α-Ketobutyrate	$y = 1.18 \cdot 10^5 x - 7.89 \cdot 10^5$	$r^2 = 0.9978$	$3.49 \cdot 10^5$

[a] Using the following concentrations: 5, 10, 25, 50, 75, 100, 150 and 200 μg/l.
[b] y = Peak area (area counts); x = concentration (μg/l).

Table 2
Recoveries of carboxylic acids from drinking water (%)

Compound	Concentration (μg/l)						Average recovery ± S.D.
	5	10	25	50	100	200	
β-Hydroxybutyrate	152	112	85	91	96	96	96 ± 10[a]
Acetate	80	110	106	98	104	107	101 ± 11
Glycolate	90	104	104	101	103	102	101 ± 5
Butyrate	93	98	109	112	112	108	105 ± 8
Formate	80	117	104	101	103	107	102 ± 12
Pyruvate	111	95	104	101	104	105	103 ± 5
α-Ketobutyrate	109	97	101	101	103	102	102 ± 4

[a] Calculated for concentrations above 5 μg/l.

Table 3
Typical concentrations (μg/l) of organic acids in drinking water treatment plant samples

Compound	Raw	Settled	Post-ozone	Post-filter
β-Hydroxybutyrate	<2	<2	<2	<2
Acetate	≤5	≤5	140	55
Glycolate	<2	<2	55	8
Butyrate	<1	<1	<1	<1
Formate	<2	<2	277	33
Pyruvate	<3	<3	49	<3
α-Ketobutyrate	<2	<2	<2	<2

< = Compound not detected.
≤ = Compound detected, but below method detection limit.

time, it would be of advantage if the samples could be stored. To evaluate the maximum storage time possible, raw, settled, post ozone and filtered water samples were spiked with organic acids, and their concentrations were monitored over a period of one week and remeasured after four weeks.

Fig. 2a shows the results for a raw water sample (surface water). All compounds decreased in their concentration over time, with

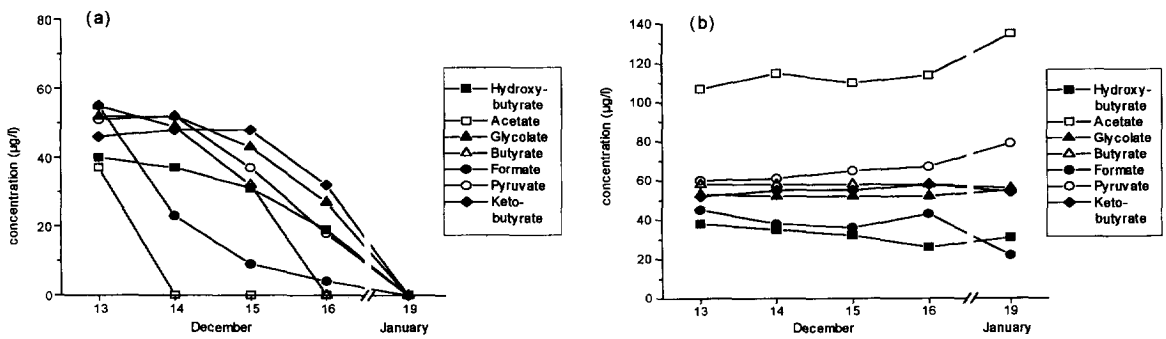

Fig. 2. Stability of organic acids in raw water during storage at 4°C: (a) unpreserved; (b) preserved with $CHCl_3$ (0.1% v/v).

acetate and formate disappearing more quickly than the others. After four weeks none of the compounds was detectable. The other water samples displayed similar trends. The rate of decay was dependent on the source of the sample with raw water showing the most rapid and post-ozone water the slowest decline in organic acids. Presumably microbiological activity is one of the major factors responsible for the organic acid consumption.

Use of a preservative was, therefore, considered. Traditional preservation methods such as acidification or metal salt addition were unsuitable since they would interfere with the chromatography by introducing excessively high concentrations of inorganic anions. However, because the analytical column was solvent compatible, the addition of 0.1% (v/v) chloroform to the samples as described by Lawrence and Koutrakis [10] would not cause any problems. Organic acids in preserved, spiked water samples were monitored together with unpreserved samples. Fig. 2b indicates clearly that preserved raw water samples can be stored at least for a few days without organic acid degradation. The same is true for settled, post-ozone and filtered waters, when these are preserved with $CHCl_3$.

3.4. Drinking water samples

A series of experiments at a full-scale water treatment plant provided an opportunity to use the method to measure the production and removal of short-chain organic acids in drinking water [21]. Only some typical results will be presented.

Fig. 3a shows an enlarged section of a chromatogram of a typical raw water sample, which was the influent to the water treatment process. Besides an unidentified compound (indicated by X) none of the organic acids under investigation could be detected.

After several other treatment steps, ozone was applied as the main or primary disinfectant. The chromatogram of the ozonated water sample (Fig. 3b) indicates that several organic acids were detected, as expected. Acetate and especially formate were formed in higher concentrations than the other acids, glycolate and pyruvate (Table 3). The unidentified compound X was still present after the ozonation step, now accompanied by another unidentified peak Y.

The ozonation step was followed by a granular media filtration step. In the filter effluent the organic acid concentrations were significantly smaller than in the post-ozone water thus indicating that the majority of the detected organic acids was removed (Table 3). It has been postulated that microbiological activity on such filters is a likely removal mechanism [9].

4. Conclusions

With the proposed IC method, seven organic acids were determined at low μg/l concentrations in drinking water. A large sample loop (740 μl = 370 cm) was used to inject the water sample

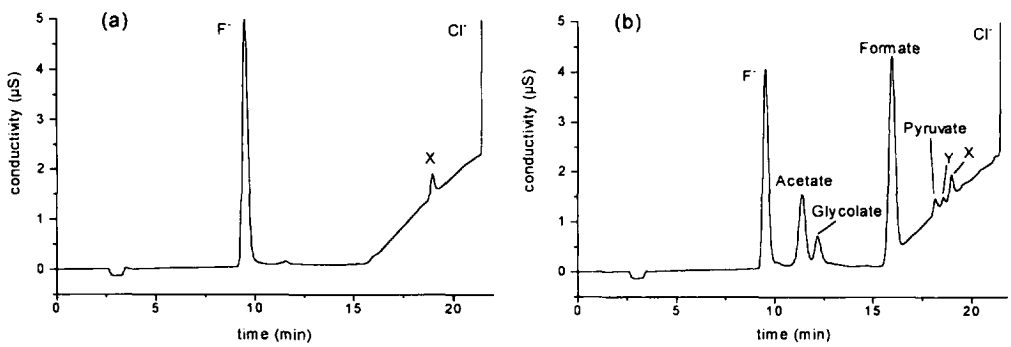

Fig. 3. Chromatograms of (a) a raw water sample and (b) a post-ozone water sample.

directly onto an anion-exchange column (AS 10), where it could be focused into a relatively narrow band due to the high resin capacity of the analytical column. The compounds were separated on the anion-exchange column and detected with a conductivity detector. Depending on the component, the average analyte recoveries ranged from 96 to 105% and the method detection limits ranged from 1 to 5 μg/l.

An additional study with different types of water showed that samples could be stored for a few days if a preservative (0.1%, v/v, $CHCl_3$) was added. Measurements made on samples from a full-scale water treatment plant proved this method to be applicable to drinking water samples, and showed that ozonation during drinking water treatment can result in the formation of organic acids. Removal of acids thus formed was shown to occur in subsequent filtration steps, possibly because of microbiological activity in the filters.

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References

[1] W.H. Glaze, Environ. Health Perspect., 69 (1986) 151.
[2] W.E. Coleman, J.W. Munch, H.P. Ringhand, W.H. Kaylor and D.E. Mitchell, Ozone Sci. Eng., 14 (1992) 51.
[3] H. Matsuda, Y. Ose, T. Sato, H. Nagase, H. Kito and K. Sumida, Sci. Total Environ., 117/118 (1992) 521.
[4] I. Molnar-Perl, V. Fabian-Vonsik and M. Pinter-Szakacs, Chromatographia, 18 (11) (1984) 637.
[5] M.I. Daneshvar and J.B. Brooks, J. Chromatogr., 433 (1988) 248.
[6] H.M. Liebich and A. Dotzauer, J. High Resolut. Chromatogr., 12 (3) (1989) 160.
[7] J.T.F. Wong, G.B. Baker and R.T. Coutts, J. Chromatogr., 428 (1988) 140–146.
[8] K. Kawamura, K. Steinberg and I.R. Kaplan, Int. J. Environ. Anal. Chem., 19 (1985) 175.
[9] Y. Xie and D.A. Reckhow, Ozone Sci. Eng., 14 (1992) 269.
[10] J.E. Lawrence and P. Koutrakis, Environ. Sci. Technol., 28 (1994) 957.
[11] R. Widiastuti, P.R. Haddad and P.E. Jackson, J. Chromatogr., 602 (1992) 43.
[12] K. Tanaka and J.S. Fritz, J. Chromatogr., 361 (1986) 151.
[13] J.S. Fritz, J. Chromatogr., 546 (1991) 111.
[14] P.R. Haddad and P.E. Jackson, J. Chromatogr., 447 (1988) 155.
[15] S.A. Andrews, PhD thesis, University of Alberta, Dept. of Civil Engineering, 1993.
[16] A.E. Buchholz, C.I. Verplough and J.L. Smith, J. Chromatogr. Sci., 20 (1982) 499.
[17] A.L. Heckenberg and P.R. Haddad, J. Chromatogr., 229 (1984) 301.
[18] M. Legrand, M. De Angelis and R.J. Delmas, Anal. Chim. Acta, 156 (1984) 181.
[19] T. Okada and T. Kuwamoto, J. Chromatogr., 350 (1985) 317.
[20] APHA–AWWA–WEF, Standard Methods for the Examination of Water and Wastewater, 18th ed. and Suppl., Washington, DC, 1100 pp.
[21] D. Mutti, M.Sc Thesis, University of Waterloo, Dept. of Civil Engineering, 1995.