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Review

Analytical isotachophoresis in biological monitoring of exposure to industrial chemicals

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ABSTRACT

Isotachophoretic methods for the determination of compounds of interest in biological monitoring are reviewed. The analytes are charged biotransformation products such as acids or amines. Comparisons are made between isotachophoretic methods and other techniques regarding sensitivity, need for preseparation or derivatization and similar technical aspects.

CONTENTS

1. INTRODUCTION

Biological monitoring of exposure to industrial chemicals requires evaluation of the uptake (internal exposure or internal dose) of such chemicals in the body by analysis of biological samples from the exposed subject. Mostly urine, blood and expired air are used for the direct measurement of the chemical or a biotransformation product. Indirect measurement of biochemical or physiological changes that occur in response to the exposure are also included. Many compounds are biotransformed to more water-soluble and ionized products and for the analysis of these, isotachophoresis (ITP) can be an attractive technique.

This paper reviews ITP methods used for analysis of compounds of interest in biological monitoring.

TABLE 1

ELECTROLYTE SYSTEMS USED IN THE CITED ISOTACHOPHORETIC METHODS

Exposure = the industrial chemical to which the subject is exposed; analyte = the product to be analysed in biological monitoring; specimen = biological specimen for analysis. Abbreviations: HA = hippuric acid; MHA = methylhippuric acid; MA = mandelic acid; PGA = phenylglyoxylic acid; HPMC = hydroxypropyl-methylcellulose; TCA = trichloroacetic acid.

Exposure	Analyte	Specimen	Leading electrolyte	Terminating electrolyte	Ref.
Anionic systems					
Toluene, xylene, styrene	HA, MHA, MA, PGA	Urine	5 mM HCl–20 mM β-alanine; pH 3.75; 0.4% HPMC	5 mM caproic acid	1
Ethylbenzene	MA, PGA	Urine	5 mM TCA + β-alanine to pH 3.35; 0.4% HPMC	5 mM caproic acid	5
Halothane	Trifluoroacetic acid	Urine, blood	10 mM HCl + β-alanine to pH 3.6–3.9; 1% Triton X-100	10 mM caproic acid	13
Methanol	Formate	Blood, plasma	5 mM HCl; 0.1% Triton X-100 or 0.4% HPMC	10 mM acetic acid	16
Vinyl chloride	Thiodiacetic acid	Urine	10 mM HCl + β-alanine to pH 3.4 for preseparation capillary and to pH 4.3 for analytical capillary; 0.2% HPMC	10 mM acetic acid	18
Ethylene glycol	Glycolic, glyoxylic oxalic and formic acids	Blood, plasma	3 mM HCl–2 mM NaCl, pH 2.5; 0.2–0.4% HPMC	10 mM acetic acid	19
Cationic systems					
–	Creatinine	Urine	10 mM potassiumacetate + acetic acid to pH 5.3; 0.2% HPMC	5 mM HCl	10
Triethanolamine	Triethanolamine	Urine	5 mM ethanolamine + acetic acid to pH 5.3; 0.4% HPMC	4 mM histidine + acetic acid to pH 5.3	9

2. STYRENE, ETHYLBENZENE, TOLUENE, XYLENE

In 1977, Sollenberg and Baldesten [1] published a method for the determination of mandelic, phenylglyoxylic, hippuric and methylhippuric acids in urine. These acids are biotransformation products of styrene (the first two acids), toluene and xylene, respectively, and their excretion in urine is used for biological monitoring of the corresponding solvents. When the investigation started, spectrophotometric and thin-layer chromatographic methods were used for routine determination of these acids. Existing gas chromatographic (GC) methods [2,3] were laborious and required derivatization of the acids with diazomethane or a silyl reagent before analysis. With the described ITP method these acids could be determined in the same run with no pretreatment other than extraction from the urine sample with diethyl ether. Table 1 gives the compositions of the electrolytes used. In a contemporary paper the first high-performance liquid chromatographic (HPLC) method for the determination of hippuric and methylhippuric acids in urine was described [4].

In connection with studies on the biotransformation of ethylbenzene in the rat, an HPLC procedure was developed for the analysis of the products; 59 rat urine samples were analysed for mandelic and phenylglyoxylic acids, which are the main products, and these samples were also analysed simultaneously by the ITP method (with minor modifications, Table 1) [5]. There was no significant difference between the results obtained by the two methods. However, the limit of determination was 0.01 mmol/l for both mandelic and phenylglyoxylic acids by HPLC whereas it was 0.04 and 0.02 mmol/l, respectively, by ITP.

Another comparison between the ITP method and an HPLC method was made when methylhippuric acid was determined in human urine [6]. The two methods gave essentially identical data for urine samples with added methylhippuric acid. However, the limit of determination was 0.2 mmol/l for both methods. In this study the pH of the leading electrolyte was 4.15.

The cited ITP method was used for routine work but also for some investigations, e.g., on xylene exposure [7] and on relationships between occupational styrene exposure and excretion of mandelic and phenylglyoxylic acids in urine [8]. From these relationships conceivable biological exposure limits were calculated. Such values can be expressed as the concentration of the excreted product in the urine sample divided by the concentration of creatinine in the same sample. This is usually done to adjust for variations in urine flow as a substitute for excretion rate when the time between voidings and the urine volume is not known. The excretion of creatinine is assumed to be constant. Mostly, creatinine in urine samples is determined spectrophotometrically in automated analysers.

However, in connection with this study, ten samples were analysed for creatinine by ITP [9] using an electrolyte system as described by Mikkers and Everaerts [10] (Table 1). The analysis is very simple and no pretreatment of the urine sample was applied before injection into the ITP instrument. The urine samples were simultaneously analysed using a Merckotest-Creatinin kit and the results were compared by linear regression analysis. The equation was $y = 0.39 + 0.96x$; $r = 0.998$, where y is the ITP result and x the Merckotest result. The concentration range of creatinine was 6–20 mmol/l.

Another slight modification of the ITP method was used for the determination

of hippuric acid in urine in a study on toluene exposure [11]. The extraction of the urine samples step was omitted and only a 10-fold dilution with water was made before analysis. The results were compared with those obtained for the same urine samples made using an HPLC method and were found to be in accordance. It was found that a metabolite of salicylic acid, salicyluric acid (o-hydroxyhippuric acid), interfered with hippuric acid in the ITP analysis. By changing the pH of the leading electrolyte from 3.75 tot 3.15, separation was achieved but the time of analysis was prolonged.

Zschiesche et al. [12] compared thin-layer chromatographic (TLC), HPLC, GC, spectrophotometric and ITP [1] methods for the determination of mandelic, phenyl-glyoxylic and hippuric acids in urine. Phthalic acid was used as an internal standard in the ITP analyses. When hippuric acid was determined, the correlations between ITP and HPLC and between ITP and GC were similar. However, GC had the lowest detection limit (0.2 μmol/l) followed by HPLC and then ITP (50 μmol/l). In contrast to the HPLC and ITP methods, the GC procedure required derivatization of the hippuric acid with diazomethane.

3. HALOTHANE

The main biotransformation product of the anaesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) that is excreted in urine is trifluoroacetic acid. Morio et al. [13] described the ITP determination of trifluoroacetic acid in urine and blood from patients who had had halothane anaesthesia. They claimed that this ITP method is advantageous compared with paper chromatographic, TLC, ion-exchange chromatographic, GC and HPLC methods for the determination of trifluoroacetic acid in urine. No preparation of the samples is required, the sample volume is small and the analysis time is short. Down to 2 nmol can be measured in a volume between 5 and 200 μl. The daily urinary excretion of trifluoroacetic acid from seven patients who had had halothane anaesthesia was measured for 14 days. The highest elimination rate was on the second day and it has ceased by the thirteenth day. However, the dose of halothane was not measured.

After occupational exposure to 5 ppm of halothane for a working week, it has been estimated that the concentration of trifluoroacetic acid in the urine at the end of the week will be about 50 μmol/l [14]. Consequently, the sensitivity of the ITP method is sufficient also for biological monitoring of halothane exposure of operating personnel.

The electrolyte system used was hydrochloric acid–β-alanine in the leading electrolyte and caproic acid in the terminating electrolyte (Table 1). In another paper [15] the composition of electrolytes for the optimum separation of trifluoroacetic acid from urinary acids is discussed. Experimentally determined conditions were assessed by computer simulation and it was shown that separation was achieved in the pH range 3.5–3.7.

4. METHANOL

Øvrebø et al. [16] developed a method for the determination of formic acid in plasma from methanol–poisoned patients. No pretreatment of the sample was neces-

sary, as opposed to other methods described for this type of analysis. However, this method was not used for occupational exposure measurements but for cases of acute poisoning where the concentration of formate is certainly higher. A plasma sample of 1–5 μl was used for the analysis and down to 0.2 mmol/l could be measured. The ITP instrument was equipped with a single capillary of 0.5 mm I.D. In plasma from ten poisoned patients the formate concentration ranged from 0.4 to 17.1 mmol/l.

In persons not exposed to methanol the concentration of formate in the blood ranged from 0 to 0.4 mmol/l and in a group of workers exposed to about 100 ppm of methanol the mean concentration of formic acid in the blood increased from 0.07 to 0.17 mmol/l [17]. The described ITP method is therefore not sensitive enough for measurements of occupational exposure without modifications. Hydrochloric acid was used as the leading and acetic acid as the terminating electrolytes (Table 1).

5. VINYL CHLORIDE

Thiodiacetic acid is a metabolite of vinyl chloride. Krivánková et al. [18] developed a sensitive ITP method for the determination of this acid in urine from vinyl chloride-exposed persons. No pretreatment of the urine was necessary, but an ITP instrument equipped with a column-coupling system was required. The leading electrolytes in the preseparation column and in the analytical capillary were of the same composition but had different pH values 3.4 and 4.3, respectively (Table 1).

Urine samples were collected from persons exposed to vinyl chloride in PVC production. In these samples the thiodiacetic acid concentration varied between 0.075 and 0.15 mmol/l, compared with a range of 0.025–0.067 mmol/l in urine from non-exposed persons. No measurements of the vinyl chloride exposure were reported, but the increase in the concentration of thiodiacetic acid in urine from exposed persons was obvious. By using the column-coupling technique the method was sensitive and the detection limit was $6 \cdot 10^{-6}$ mol/l.

6. ETHYLENE GLYCOL

Ethylene glycol is biotransformed into several products, including glycolic, glyoxylic, oxalic and formic acids. The determination of these acids by ITP has been described by Øvrebø et al. [19], who developed a method for their determination in blood from ethylene glycol-poisoned humans. The leading ion was chloride at pH 2.5 and the terminator was acetate (Table 1). Preparation of plasma by using oxalate, citrate or EDTA should be avoided as they interfere in the separation of the analytes. Furthermore, oxalic acid is one of the biotransformation products. Heparin was recommended for plasma preparation. In a plasma sample from an ethylene glycol-intoxicated patient glycolic acid was found whereas the other biotransformation products were below the detection limit of 0.2 mmol/l.

7. TRIETHANOLAMINE

Occupational exposure to triethanolamine can occur by inhalation of aerosols (the vapour pressure of the amine is very low). Sollenberg [9] developed an ITP method for the determination of triethanolamine in urine from workers exposed to

synthetic cutting fluids. Such fluids contain triethanolamine as an anti-corrosive agent. Triethanolamine taken up in the body is excreted to a large extent untransformed in the urine. A urine sample was applied to a cation-exchange column which was eluted with dilute ammonia solution, thus eluting only a fraction of the cationic content of the sample. The eluate was analysed by ITP using an instrument equipped with a column-coupling system. By using ethanolamine as the leading ion having a mobility slightly higher than that of triethanolamine and histidine as the terminator with a slightly lower mobilitiy, the analyte could be conveniently separated (Table 1). The detection limit was $2 \cdot 10^{-6}$ mol/l. Preliminary results indicated that triethanolamine can be found in urine from persons exposed to aerosols from synthetic cutting fluids. The method can therefore be useful for biological monitoring of exposure to triethanolamine.

REFERENCES

1 J. Sollenberg and A. Baldesten, *J. Chromatogr.*, 132 (1977) 469.
2 J.-P. Buchet, R. Lauwerys and H. Roels, *Arch. Mal. Prof. Med. Trav. Secur. Soc.*, 35 (1974) 511.
3 K. Engström and J. Rantanen, *Int. Arch. Arbeitsmed.*, 33 (1974) 163.
4 M. Ogata, R. Sugihara and S. Kira, *Int. Arch. Occup. Environ. Health*, 39 (1977) 199.
5 J. Sollenberg, A. W. Smallwood and L. K. Lowry, *J. Chromatogr.*, 343 (1985) 175.
6 J. Sollenberg, F. C. Phipps, B. Stringer and L. K. Lowry, *J. Chromatogr.*, 343 (1985) 419.
7 L. K. Lowry, T. W. Thoburn, F. C. Phipps, B. J. Gunter and J. Sollenberg, in M. H. Ho and H. K. Dillon (Editors), *Biological Monitoring of Exposure to Chemicals. Organic Compounds*, Wiley, New York, 1987, p. 143.
8 J. Sollenberg, R. Bjurström, K. Wrangskog and O. Vesterberg, *Int. Arch. Occup. Environ. Health*, 60 (1988) 365.
9 J. Sollenberg, unpublished results.
10 F. Mikkers and F. Everaerts, in F. M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 1.
11 R. Andersson, A. Carlsson, M. Byfält Nordqvist and J. Sollenberg, *Int. Arch. Occup. Environ. Health*, 53 (1983) 101.
12 W. Zschiesche, K. Gossler, K. H. Schaller, G.Triebig, D. Weltle and J. Angerer, *Berufskrankheiten in der keramischen und Glas-Industrie, Heft 28*, Berufsgenossenschaft der keramischen und Glas-Industrie, Würzburg, 1978–79.
13 M. Morio, K. Fujii, R. Takiyama, F. Chikasue, H. Kikuchi and L. Ribaric, *Anesthesiology*, 53 (1980) 56.
14 E. Dallmeier and D. Henschler, *Dtsch. Med. Wochenschr.*, 106 (1981) 324.
15 T. Hirokawa, H. Takemi, Y. Kiso, R. Takiyama, M. Morio, K. Fujii and H. Kikuchi, *J. Chromatogr.*, 305 (1984) 429.
16 S. Øvrebø, D. Jacobsen and O. M. Sejersted, in C. J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter de Gruyter, Berlin, 1984, p. 261.
17 K. Baumann and J. Angerer, *Int. Arch. Occup. Environ. Health*, 42 (1979) 241.
18 L. Krivánková, E. Samcová and P. Boček, *Electrophoresis*, 5 (1984) 226.
19 S. Øvrebø, D. Jacobsen and O. M. Sejersted, *J. Chromatogr.*, 416 (1987) 111.