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Review

Chromatographic determination of volatile solvents and their metabolites in urine for monitoring occupational exposure

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ABSTRACT

The determination of volatile solvents and their metabolites in biological materials such as expired air, blood or urine allows the estimation of the degree of exposure of these chemicals. Chromatographic methods are now universally employed for this purpose and numerous analytical procedures are available for the determination of the most commonly used volatile solvents and their metabolites in urine. GC methods appear well adapted to the determination of the parent volatile solvents in blood and urine and may be used for the determination of their urinary metabolites, but these methods often require several prechromatographic steps. However, HPLC is becoming a powerful tool for the accurate and easy determination of urinary metabolites of volatile solvents, considering its decisive advantages for routine monitoring. Further, recent developments in HPLC could widen the usefulness of this method for most complex analytical problems that could be encountered during this measurement. However, despite the relative neglect of planar chromatography in this area of concern and considering the great interest in methods that could permit the simultaneous assay of numerous samples often required by routine monitoring, new approach using improved methods such as overpressured TLC could be very fruitful in the future.

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1. INTRODUCTION

Organic solvents and their vapours are widely distributed in the modern environment, both in the home and at work. Although the occupational exposure to these solvents is generally well monitored in developed countries, the domestic exposure may be greatly underestimated (dry-cleaning fluids, paint diluents, nail-polish removers, etc.). Moreover, the problem of solvent abuse ("glue sniffing") among teenagers has become a subject of growing concern for health practitioners and toxicologists [1]. Most of these solvents are very toxic.

Vapours of volatile or volatilizable liquids are

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often readily absorbed by the lungs but the skin may be also an important route of absorption. Since the vapour in the alveoli equilibrates almost instantaneously with blood passing through the pulmonary capillary bed, the rate of absorption is variable and strongly dependent on the toxicant's blood/gas solubility. If the vapour has a low blood solubility, the rate of transfer is blood-flow dependent (perfusion limited), whereas for vapours with higher solubility it is strongly dependent of the respiratory process (ventilation limited) [2].

Many of the early toxic effects of inhaled solvents after an acute exposure are observed on the central nervour system. In general, these effects are directly related to the unchanged substance and resolved well after the cessation of exposure. However, during exposure, most of these solvents follow metabolization pathways that can generate highly toxic short-lived intermediates such as free radicals or arene epoxides (Fig. 1). As volatile solvents are generally highly hydrophobic, the metabolization allows initially conversion into a more hydrophilic compound by a phase-I reaction such as hydroxylation of an aromatic ring to phenol. This reaction is then followed by a phase-II conjugation process (acetylation, glucuroconjugation, glycinoconjugation, etc.) leading to very soluble conjugated derivatives that are easily excreted by the kidneys.

2. MONITORING OF VOLATILE SOLVENT EXPOSURE —THE ANALYTCAL PROBLEM

The determination of volatile solvents and their metabolites in biological materials such as expired

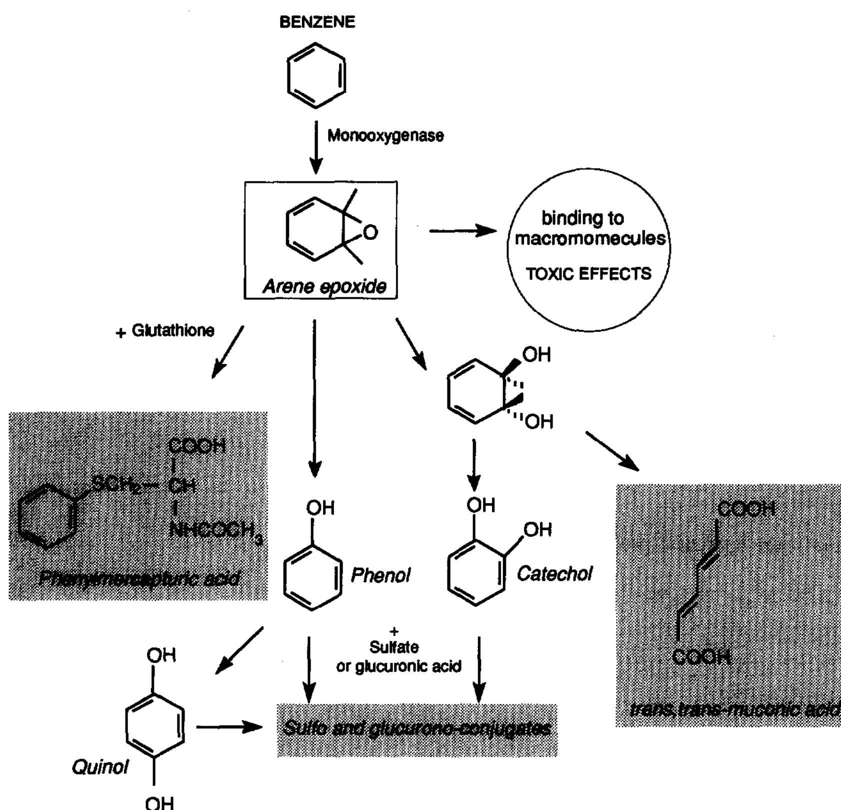

Fig. 1. Metabolization profile of benzene. Blood benzene is oxidized by liver or bone-marrow mixed-function oxidase systems, generating the highly reactive short-lived intermediate benzene epoxide. This toxic metabolite is able to bind to macromolecules such as proteins or nucleic acids leading to deleterious effects (benzene-induced haematological abnormalities). Various detoxification pathways lead to several derivatives, which are subsequently conjugated and excreted in urine. Shaded areas show the major urinary metabolites that are currently determined for monitoring purposes.

air, blood or urine allows the estimation of the degree of exposure of these chemicals [3–5]. The definition of permissible levels of exposure to chemical agents can be expressed in terms of allowable atmospheric concentrations [5] or permissible biological levels for these chemicals or their metabolites expressed as the biological "threshold limit values" (TLV), representing a biological marker of the ambient conditions under which it is considered that nearly all workers may be exposed for 8 h a day without significant adverse effects [6].

In occupational laboratory medicine, if the problem in question is the routine monitoring of workers exposed to ambient chemicals, the analytical performance of a suitable method has to answer the following question: had the workers been exposed to higher levels of chemicals than those accepted on the basis of their biological TLVs? As the biological TLVs of the studied chemicals are relatively high, the sensitivity is not a real problem. However, an acceptable specificity, a low cost and a rapid and possibly automated method are required. Indeed, high specificity is less critical for routine monitoring than for pharmacokinetic or forensic studies, as a more detailed and specific analysis can be performed if a higher metabolite concentration is found than its biological TLV.

The determination of metabolites in urine offers important and decisive advantages over other monitoring methods: (1) it takes into account absorption by all the possible routes (skin, oral ingestion, lungs), (2) it can consider indvidual variations in the toxicokinetics and biotransformations of the solvent, (3) it may also reflect the total exposure (amounts excreted during a given period) and (4) it is easily performed for routine purposes.

However, the nature of the metabolite to be determined can be critical and may influence the choice of the analytical method. For example, urinary phenol determinations have classically been used to monitor high levels of occupational benzene exposure. However, the same technique cannot be used to monitor low levels of exposure (*e.g.*, environmental exposure) because of the possible high excretion of phenol resulting from the metabolism of alimentary and endogenous aromatic amino acids. Thus, alternative biological indices for exposure, such as urinary muconic acid, may be useful [7]. As phenol may be present at high levels in urine, relatively simple analytical methods can be sufficient. However, for muconic acid determination, a more sophisticated method (GC–MS) is required. Similarly, hippuric acid is the glycine conjugate of benzoic acid, the oxidative metabolite of the toxic solvent toluene, but benzoic acid may also be present as an additive in foods and beverages.

Moreover, urinary excretion of unchanged solvents may be also closely related to the degree of exposure. For example, a close relationship between benzene exposure level and urinary concentrations was found in a group of workers exposed to low environmental benzene concentrations (mean value 1.2 mg/m^3) [7]. Consequently, the choice of an appropriate analytical method must be an acceptable compromise between several conflicting requirements.

In toxicological analysis, chromatographic methods such as GC, HPLC and planar chromatographic techniques such as TLC are now extensively used. Thus, for the urinary determination of volatile solvents and their metabolites, these methods are now almost universally employed. For this purpose, elution chromatographic methods exhibit decisive advantages over global methods such as spectrophotometry because they can often separate a toxic compound and its metabolites from the biological matrix materials. However, the level of specificity, governing the choice of an analytical method, is an important (but not always resolved) problem in practice.

In a clinical situation (*e.g.*, acute poisoning) a simple answer ("yes/no") may be sufficient because other clinical or biochemical data must be also considered in order to confirm the possible diagnosis. However, in a medicolegal situation or for monitoring exposed workers when legal and socioeconomic consequences may be important, more precise identification and determination are essential. Consequently, other physical parameters, such as IR or mass spectra, may be required. These considerations may explain the growing use of coupled techniques such as GC–MS in this area.

For the collection and handling of specimens containing solvents, special care is required. Lipophilic organic solvents may be readily absorbed into plastic and therefore blood samples should be collected in glass tubes. Moreover, contamination during sampling (venepuncture swabs containing

alcohols) or during handling (laboratory environment) should be guarded against.

For the determination of the urinary metabolites of solvents, untimed or random specimens are generally unsuitable because of the wide circadian variations of urine volume and dilution. Thus, urine specimens for quantitative analysis must be collected over an interval of time, such as 24 h, providing an "integrated" picture of the metabolite production (*e.g.*, amount excreted per day) which is more clinically relevant than its concentration. However, the urine concentration of metabolites is currently corrected for dilution using the expression grams of excreted metabolite per gram of urinary creatinine.

3. METHODS

3.1. Gas chromatography

The advantages of GC for the determination of volatile substances in complex mixtures such as biological materials are well known. First, the separation of volatile solvents from the biological matrix can easily be achieved by the headspace method [8,9], exploiting their favourable gas/liquid distribution ratio. Second, temperature programming leads to a good chromatographic pattern of high-boiling-point solvents such as glycols or branched-chain aromatic hydrocarbons. Third, the use of sensitive detectors permits the determination of minor components such as various isomers in the presence of important amounts of the major metabolite and a precise determination of trace concentrations of the parent solvent. Among these, flame-ionization detector is a widely used device that is an excellent general-purpose detector, particularly with capillary columns. This detector is extensively used to the determination of aliphatic or aromatic solvents in biological materials [10–12]. For submicrogram amounts of halogenated solvents or carboxylic metabolites, the electron-capture detector is very useful. However, GC–MS appears very suitable for the identification and determination of most volatile solvents and their metabolites [13–16] and a growing use of this coupled method can be observed [7,17]. In addition, the use of stable isotope tracer techniques permits more precise metabolic studies of solvents in humans and animals.

Unfortunately, not all compounds of interest are amenable to GC study because of poor thermal stability or volatility. Thus, polar compounds are often analysed by GC after a derivatization process such as methylation or silylation. Consequently, GC remains the method of choice for the determination of volatile solvents in biological samples but appears less adapted for the routine determination of their polar urinary metabolites.

GC has been used for the blood determination of almost all volatile solvents, including aliphatic and halogenated hydrocarbons, ketones, aromatic hydrocarbons, nitro and amino derivatives, carbon disulphide, nitriles and complex mixtures of these compounds. In particular, headspace GC appears very suitable for the simultaneous blood determination of several solvents [8,9,18]. The analytical diagnosis of solvents abuse has been reviewed by Oliver [1,19], who gave useful procedures for the extraction of solvents from biological materials.

GC has been less used for urinary than for blood determinations but appears to be the method of choice for the determination of the unchanged form of some volatile toxic compounds, such as ketones, aliphatic and halogenated hydrocarbons and the non-conjugated hydroxylated metabolites of several solvents [11,20].

In general, published methods use a classical headspace procedure to isolate volatile compounds from the normal components of urine [21,22], but direct injection of the biological material may be sometimes employed, especially when Porapak or polyethylene glycol (PEG) columns are used [23–26]. Alternatively, microdiffusion techniques are often used [27]. Various sample-handling procedures and chromatographic conditions have been proposed by Duboswski [12] for the determination of 52 volatile substances in biological materials including urine. For headspace analysis of a wide range of volatile substances in biological materials, a Carbopak B column coated with 5% Carbowax 20M permits an acceptable separation [22]. However, a Tenax GC column appears well adapted for the analysis of substances involved in solvent abuse.

For the detection of low levels of volatile solvents in blood samples, a precise and rigorous degassing procedure using a cold trap has been described by Anderson *et al.* [28]. In addition, the sensitivity of the simple headspace procedure can be improved by

degassing the blood using helium in a double-syringe arrangement [1].

Recently, high-precision sampling of trace gas-borne volatiles by a dynamic solvent effect was described [29]. In addition, a new method was described by Fiorentino *et al.* [30] for determining low concentrations of benzene in urine samples by means of a dynamic headspace method. The urine (50 ml in a 120-ml vial) is saturated with anhydrous sodium sulphate and the volatile substances are extracted and concentrated on an adsorbent substrate (Carbotrap 100) by means of a suction pump with simultaneous entry of charcoal-filtered air. Benzene is then thermally desorbed and injected into a capillary column. A detection limit of 50 ng/l (R.S.D. 4.7%) was claimed. This method could be very useful for the trace determination of several other solvents in urine samples. Moreover, the blood/urine and urine/air partition coefficients for 43 commonly employed industrial substances have been determined by GC using a multiple equilibration method [31]. Although packed columns are still used, a growing use of capillary column GC in toxicology, especially for trace-level determinations, can be observed [1]. Moreover, for volatile solvents and their metabolites, the use of capillary columns combined with MS is becoming popular.

The biological monitoring of occupational exposure to methyl ethyl ketone (MEK) by means of urinary determination of MEK itself and its metabolites (2-butanone, 2-butanol and 3-hydroxy-2-butanone) has been described using a capillary headspace GC method [11,32–34]. Acetone can also be easily determined in urine after exposure by using GC [11,34]. Capillary GC has also been extensively used for the determination of the neurotoxic 2,5-hexanedione, the main urinary metabolite of hexane [11,15,35–41]. Similarly, cyclohexanol, the urinary metabolite of cyclohexane, and several urinary metabolites of *n*-heptane, including 2,5-heptanedione, have been determined by GC and GC–MS [14,42].

Halogenated solvents are mainly excreted in urine as hydroxylated and acidic metabolites such as trichloroethanol and trichloroacetic acid. Thus, several GC methods have been used for the accurate determination of urinary metabolites of trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane and propylene dichloride [11,20].

Simple aliphatic alcohols, such as methanol or ethanol, are sparingly determined in urine because of the lack of correlation between urinary levels and toxicity but, if required, these determinations can be easily performed by simple injection on to packed columns. However, the determination of urinary metabolites of 2-ethoxy- or 2-methoxyethanol, methoxyacetic and ethoxyacetic acid, has been performed by capillary GC [43] and with electron-capture detection [44].

For the urinary determination of low-volatility conjugated metabolites, GC has been lesser widely employed than other chromatographic methods such as HPLC or TLC, perhaps because of the necessity for a derivatization step. Several organic acids have been isolated and determined for routine metabolic screening by GC–MS [45]. Hippuric and methylhippuric acid, metabolic derivatives of toluene and xylene, have been determined in urine by capillary GC after derivatization with diazomethane [46–49]. Styrene and its metabolites (mandelic acid, phenylglyoxylic acid, phenylethylene glycol and phenylethanol) have been determined by capillary GC [50–53] and a comparison between HPLC and GC has been performed. Diazomethane derivatization has been used by some workers for the simultaneous determination of mandelic and phenylglyoxylic acids [54].

Separation of the isopropyl esters and isopropylisopropylurethane esters of the diastereoisomers of mandelic acid has been described by Korn *et al.* [55] using GC capillary columns coated with Chirasil-Val.

Phenols are important urinary metabolites of aromatic solvents such as benzene, toluene, xylene and halo, nitro and amino aromatic compounds. Phenolic compounds are highly sulpho- and glucurono-conjugated and require acid [56] or enzymatic [57,58] hydrolysis of urine samples before chromatography. The relative volatility of simple phenols such as phenol and cresols has permitted the development of several GC methods after simple steam distillation and without derivatization [10,59,60]. However, derivatization of phenols is often required, using diazomethane, heptafluorobutyryl anhydride [61] or hexamethyldisilazane [62]. The simultaneous determination of phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and 2,3,5,6-tetrachlorophenol in urine of exposed workers has been reported [63].

In conclusion, GC methods appear well adapted to the determination of the parent volatile solvents in blood and urine and may be used for the determination of their urinary metabolites. However, these methods often require several prechromatographic steps.

3.2. HPLC

The introduction of HPLC into occupational medicine represents a valuable tool for the routine monitoring of workers exposed to volatile solvents. As previously indicated, a wide range of lipophilic volatile solvents are metabolized and then excreted by the kidneys in more hydrophilic forms such as phenols or carboxylic acids, in the free or conjugated form. The well known versatility of HPLC methods increased the range of compounds that can be measured, complementing GC and also proving more suitable for the determination of low-volatility or thermally labile substances. Thus, for the urinary determination of solvent metabolites, especially for routine monitoring, HPLC is becoming a method of choice. Indeed, this method offers decisive advantages in this area.

First, the wide range of separation mechanisms now permits the determination of almost all urinary solvent metabolites, regardless of their polarity, molecular mass and thermal stability. However, reversed-phase chromatography is probably the most widely used mode of chromatography. Second, HPLC permits the simultaneous determination of several metabolites in an isocratic mode or by gradient analysis if needed, leading a complete metabolic profile, and can be easily automated. Moreover, separation of substances of interest from structurally similar compounds or isomers is feasible. Third, urine samples do not usually require extraction or derivatization steps and pretreatment is generally limited to sample filtration and acidic or enzymatic hydrolysis of conjugates.

The major inconvenience of HPLC for the routine urinary determination of solvent metabolites is a lower sensitivity than GC. However, as previously indicated, the urinary TLVs of many common volatile solvents are high, causing no real difficulties despite the direct injection of unprocessed urine samples (except for a simple filtration step) used in many "improved" methods. The detector most widely used in this context depends on UV absorption as many solvent metabolites, especially from aromatic solvents, absorb strongly in the UV–visible region. For non-UV-absorbing metabolites, derivatization with chromophore-adding reactants could be performed using a pre- or postcolumn mode and has been widely used in toxicology [64]. However, this approach had not been sufficiently explored for low-UV-absorbing solvents and their metabolites except for the HPLC determination of ethylene glycol [65]. Similarly, the use of a more sensitive detector such as a spectrofluorimeter with or without derivatization has not been investigated. Indeed, this approach could be an attractive alternative to capillary GC for the determination of trace levels of hydroxylated or acidic metabolites from haloalkanes or phenolic derivatives from polyhalogenated phenyls.

The HPLC determination of acidic and phenolic urinary metabolites of aromatic solvents (*e.g.*, benzene, toluene, xylene, ethylbenzene, styrene) has been extensively studied. Several papers were focused on the HPLC determination of metabolites from a specific solvent such as catechol and quinol from benzene [66], hippuric acid and cresols from toluene [59,67–69] or mandelic acid and phenylglyoxylic acid from ethylbenzene or styrene [70,71]. However, the simultaneous determination of acidic and phenolic metabolites from these aromatic solvents for screening purpose has been widely performed [72–83]. A simple reversed-phase method, using a mixture of acetonitrile or methanol and water at acidic pH, was generally sufficient for separating the acidic metabolites, but some workers preferred an ion-pair method [79]. Conjugated phenolic compounds were hydrolysed by both acidic or enzymatic methods and the free phenols (phenol, *o*-cresol) were then separated using a specific mobile phase.

However, the simultaneous determination of phenol and four urinary acidic metabolites (hippuric, methylhippuric, mandelic and phenylglyoxylic acid) has been achieved using acetonitrile–1% phosphoric acid (10:90) in an isocratic mode [83] and by an automated method [78]. Further, urinary metabolites of halo or nitro aromatic solvents such as 4-chlorocatechol and chlorophenols from chlorobenzene [84], bromophenols from bromobenzene [85] and 4-nitrophenol from nitrobenzene [83] have

also been determined using HPLC.

HPLC has been also employed for a more sophisticated approach to volatile solvent exposure than the simple monitoring. The simultaneous determination of styrene and acetaminophen metabolites was described by Colin *et al.* [86], as acetaminophen, which is a widely used drug, and its metabolites can interfere during routine monitoring, allowing to a false interpretation of results. HPLC also permits the accurate determination of cresol isomers, as toluene exposure markers [87,88]. An improvement in the HPLC of *trans,trans*-muconic acid, a substitute for phenol in the biological monitoring of benzene exposure, was recently described by Ducos *et al.* [89]. As HPLC is a simpler and cheaper approach than the GC–MS method used by others [7,16,87,90], this method appears to be a powerful tool for a more sophisticated approach to the routine monitoring of solvent exposure, separation of isomers, trace-level determination or toxicokinetics [91–93]. The determination of *p*-chloronitrobenzene and its complex metabolic by-products in urine has been studied by both GC–MS and HPLC by Yoshida and co-workers [94–96] in rats treated by this aromatic compound and in patients suffering from acute poisoning. Evaluation of occupational exposure to carbon disulphide in biological materials including urine has been performed by an HPLC method [97]. S-Phenyl-N-acetylcysteine, as a marker of benzene exposure, has also been determined in urine by Jongeneelen *et al.* [98]. Moreover, some papers indicate that HPLC and GC give similar results [72].

In conclusion, it now appears evident that HPLC is becoming a powerful tool for the accurate and easy determination of urinary metabolites of volatile solvents.

3.3. Planar chromatography

In the past, classical planar chromatography (*i.e.*, TLC) has been widely used for the biological monitoring of solvent exposure. Compared with GC and HPLC, this method presents clear advantages in terms of simplicity, rapidity, low cost and the possibility of the simultaneous determination of several samples. Urinary metabolites of aromatic solvents have mostly been measured by this method [57,99,100]. TLC has also permitted the determination of *p*-aminophenol after mixed exposure to aniline and toluene [101] and a simple method for detecting ethylene glycol in urine by TLC has been proposed [102]. In these papers, TLC was used mostly for mass screening and semi-quantitative determination of urinary levels.

A major drawback to TLC remains the difficulty of the quantitative assessment of the spots but the use of suitable densitometers can allow a more accurate determination. However, for routine monitoring or detection of intoxication, classical TLC could be sufficient, as urinary levels will be high in cases of massive exposure such as solvent abuse or solvent handling without protective devices.

HPTLC could be an attractive improvement of classical TLC [103], but this method has not been employed in the biological monitoring of solvent exposure. Similarly, overpressured thin-layer chromatography (OTLC) could be a promising and powerful tool in this area [104–106]. As one-line detection can be coupled to OTLC, this method could combine the major advantage of OTLC (simultaneous assay of 50–100 samples) and the specific and quantitative determination furnished by appropriate detectors such as a UV spectrophotometer.

4. CONCLUSIONS

The biological monitoring of solvent exposure is a growing field of interest in toxicological and occupational laboratories. As always in biomedical analysis, the choice of the most appropriate analytical method remains a major problem. As an ideal method does not exist, the best method will be a compromise between several conflicting requirements.

For research purposes or for an accurate diagnosis of a rare and complicated intoxication, sophisticated methods such as GC–MS and a specialized laboratory will be required. Indeed, in these situations, cost, length, complexity and workload problems generated by the analytical method are not necessarily major concerns. In contrast, for the routine monitoring of several hundred individuals exposed to volatile solvents in an industrial environment, the cost per determination, simplicity and automation will be important considerations.

HPLC appears to be the primary choice for the

determination of urinary metabolites. The apparatus is relatively economical, automatable and sufficiently versatile to be used for other determinations in related areas such as drug monitoring or clinical toxicology that are currently of interest in the same laboratory. As previously indicated, the analyst now has a choice between several methods, well adapted to routine and simultaneous determinations of the urinary metabolites of some common volatiles solvents that require biological monitoring. Moreover, HPLC is also becoming an attractive tool in the growing field of more elaborate approaches with new urinary markers such as specific conjugates or isomers.

Whereas capillary GC and its coupled methods remain currently of major interest for the determination of parent volatile solvents in blood and urine [107], HPLC appears to be the best choice for the measurement of their urinary metabolites. Further, recent developments in HPLC such as high-efficiency columns, chiral phases, diode-array or electrochemical detectors and HPLC–MS, which are seldom used in this domain, could widen the usefulness of this method for most complex analytical problems that may be encountered during this measurement. However, despite the relative neglect of planar chromatography in this area and considering the great interest in methods that could permit the simultaneous assay of numerous samples, as often required in routine monitoring, a new approach using improved methods such as OTLC is urgently required.

REFERENCES

1 J. S. Oliver, in A. S. Curry (Editor), *Analytical Methods in Human Toxicology*, Vol. 1, Macmillan, London, 1984, pp. 89–100.
2 C. D. Klaassen, in J. Doull, C. D. Klaassen and M. O. Amdur (Editors), *Casarett and Doull's Toxicology*, Macmillan, New York, 2nd ed., 1980, pp. 33–35.
3 R. R. Lauwerys, in J. Doull, C. D. Klaassen and M. O. Amdur (Editors), *Casarett and Doull's Toxicology*, Macmillan, New York, 2nd ed., 1980, p. 699.
4 E. T. O'Brien, W. B. Yeoman and J. A. Hobby, *Br. Med. J.*, 3 (1971) 29.
5 R. R. Lauwerys, *Industrial Chemical Exposure: Guidelines for Biological Monitoring*, Biomedical Publications, Davis, CA, 1983.
6 H. H. Cornish, in J. Doull, C. D. Klaassen and M. O. Amdur (Editors), *Casarett and Doull's Toxicology*, Macmillan, New York, 2nd ed., 1980, p. 468.
7 W. E. Bechtold, G. Lucier, L. S. Birnbaum, S. N. Yin, G. L. Li and R. F. Henderson, *Am. Ind. Hyg. Assoc. J.*, 52 (1991) 473.
8 J. F. Brien and D. J. Hoover, *Pharmacol. Methods*, 4 (1980) 51.
9 A. Prebel-Cabic, A. Cailleux and P. Allain, *Clin. Chim. Acta*, 56 (1974) 5.
10 P. B. Van Roosmalen, J. Purdham and I. Drummond, *Int. Arch. Occup. Environ. Health*, 48 (1981) 159.
11 S. Ghittori, M. L. Fiorentino and M. Imbriani, *G. Ital. Med. Lav.*, 9 (1987) 21.
12 K. M. Dubowski, in I. Sunshine (Editor), *Methodology for Analytical Toxicology*, Vol. 1, CRC Press, Cleveland, OH, 1975, pp. 407–411.
13 M. A. Mori, Y. Naruse and H. Kozuka, *Chem. Pharm. Bull.*, 29 (1981) 1147.
14 L. Perbellini, F. Brugnone, V. Cocheo, E. De Rosa and G. B. Bartolucci, *Arch. Toxicol.*, 58 (1986) 229.
15 N. Fedtke and H. M. Bolt, *Int. Arch. Occup. Environ. Health*, 57 (1986) 149.
16 J. Sparling, D. Fung and S. Safe, *Biomed. Mass. Spectrom.*, 7 (1980) 13.
17 L. D. Gruenke, J. C. Craig, R. C. Webster and H. I. Maibach, *J. Anal. Toxicol.*, 10 (1986) 225.
18 J. D. Ramsey and R. J. Flanagan, *J. Chromatogr.*, 240 (1981) 423.
19 J. S. Oliver, *Hum. Toxicol.*, 1 (1982) 293.
20 S. Ghittori, M. Imbriani, G. Pezzagno and E. Capodaglio, *Am. Ind. Med. Assoc. J.*, 48 (1987) 786.
21 H. Hackenberg and A. P. Schmidt, *Gas Chromatography Headspace Analysis*, Heyden, Philadelphia, 1977.
22 C. Suitheimer, R. Bost and I. Sunshine, in I. Sunshine (Editor), *Methodology for Analytical Toxicology*, Vol. 2, CRC Press, Cleveland, OH, 1982, pp. 1–9.
23 A. S. Curry, G. W. Walkern and G. S. Simpson, *Analyst*, 91 (1966) 742.
24 D. J. Brown, N. C. Jain, R. B. Forney, F. W. Hughes and A. B. Richards, *J. Forensic Sci.*, 13 (1968) 537.
25 A. Davis, A. Roaldi and L. E. Tufts, *J. Gas Chromatogr.*, 2 (1964) 306.
26 K. M. Dubowski, in I. Sunshine (Editor), *Methodology for Analytical Toxicology*, Vol. 1, CRC Press, Cleveland, OH, 1975, pp. 149–154.
27 M. Feldstein, in C. P. Stewart and A. Stolman (Editors), *Toxicology*, Vol. 1, Academic Press, New York, 1960, p. 639.
28 H. R. Anderson, I. Thomson and W. A. Harland, *Fire Mater.*, 32 (1979) 91.
29 P. J. Apps, *J. Chromatogr.*, 504 (1990) 21.
30 M. L. Fiorentino, S. Ghittori and G. Pezzagno, *Med. Lav.*, 81 (1990) 107.
31 M. Imbriani, S. Ghittori, G. Pezzagno and E. Capodaglio, *G. Ital. Med. Lav.*, 7 (1985) 133.
32 S. Kezic and A. C. Monster, *J. Chromatogr.*, 428 (1988) 275.
33 M. Miyasaka, M. Kumai, A. Koizumi, T. Watanabe, K. Kurasako, K. Sato and M. Ikeda *Int. Arch. Occup. Environ. Health*, 50 (1982) 131.
34 M. Sakata, J. Kikuchi, M. Haga, N. Ishiyama, T. Maeda, T. Ise and N. Hikita *J. Toxicol. Clin.*, 27 (1989) 67.

35 T. Kawai, T. Yasugi, K. Mizunuma, S. I. Horiguchi, Y. Uchida, O. Iwami, H. Iguchi and M. Ideda, *Int. Arch. Occup. Environ. Health*, 63 (1991) 213.
36 L. Perbellini, D. Marhuenda Amoros, A. Cardona Llorens, C. Giuliari and J. Brugnone, *Br. J. Ind. Med.*, 47 (1990) 421.
37 I. Ahonen and R. W. Schimberg, *Br. J. Ind. Med.*, 45 (1988) 133.
38 M. Imbriani, S. Ghittori, G. Pezzagno and E. Capodaglio, *Int. Arch. Occup. Environ. Health*, 55 (1984) 33.
39 L. Perbellini, F. Brugnone, R. Silvestri and E. Gaffuri, *Int. Arch. Occup. Environ. Health*, 48 (1981) 99.
40 L. Perbellini, F. Brugnone and G. Faggionato, *Br. J. Ind. Med.*, 38 (1981) 20.
41 M. Suguria, Y. Kosada, Y. Kawano, S. Maruyama, T. Gemba, K. Katsu, M. Hatano and M. Ishihara, *Meikai Daigaku Shigaku Zasshi*, 19 (1990) 63.
42 C. N. Ong, G. L. Sia, S. E. Chia, W. H. Phoon and K. T. Tan, *J. Anal. Toxicol.*, 15 (1991) 13.
43 D. Groeseneken, E. Van Vlem, H. Veulemans and R. Masschelein, *Br. J. Ind. Med.*, 43 (1986) 62.
44 J. Begerow and J. Angerer, *Fresenius' Z. Anal. Chem.*, 336 (1990) 42.
45 J. Greter and C. E. Jacobson, *Clin. Chem.*, 33 (1987) 473.
46 H. Kataoka, K. Manabe, S. Nakase and M. Makita *J. Pharm. Biomed. Anal.*, 9 (1991) 699.
47 D. De Carvalho, V. L. Lanchote, P. S. Bonato, R. Hc. Queiroz, A. C. Santos and S. A. C. Dreossi, *Int. Arch. Occup. Environ. Health*, 63 (1991) 33.
48 J. R. Caperas and F. G. Fernadez, *Br. J. Ind. Med.*, 34 (1977) 229.
49 M. Ikeda and H. Ohtsuji, *Br. J. Ind. Med.*, 26 (1969) 162.
50 R. L. Dills, R. L. Wu, H. Checkoway and D. A. Kalman, *Int. Arch. Occup. Environ. Health*, 62 (1991) 603.
51 M. Korn, R. Wodarz, K. Drysch, W. Schoknecht and F. W. Schmahl, *Arch. Toxicol.*, 58 (1985) 110.
52 V. J. Elia, L. A. Anderson, T. J. Macdonald, A. Carson, C. R. Buncher and S. M. Brooks, *Am. Ind. Hyg. Assoc. J.*, 41 (1980) 922.
53 M. Guillemin and D. Bauer, *Int. Arch. Occup. Environ. Health*, 44 (1979) 249.
54 J. Flek and V. Sedivec, *Int. Arch. Occup. Environ. Health*, 45 (1980) 181.
55 M. Korn, R. Wodarz, W. Schoknecht, H. Weichardt and E. Bayer, *Arch. Toxicol.*, 55 (1984) 59.
56 C. N. Ong, B. L. Lee, H. Y. Ong and L. E. Heng, *J. Anal. Toxicol.*, 12 (1988) 159.
57 A. Dorantes and S. Stavchansky, *Anal. Lett.*, 24 (1991) 39.
58 W. M. Pierce and D. E. Nerland, *J. Anal. Toxicol.*, 12 (1988) 344.
59 W. Woiwode and K. Drysch, *Br. J. Ind. Med.*, 38 (1981) 194.
60 S. Dirmilius and A. Darbre, *J. Chromatogr.*, 94 (1974) 169.
61 K. W. Kirby, J. E. Keiser, J. Groene and E. F. Slach, *J. Agric. Food Chem.*, 27 (1979) 757.
62 M. F. Crammer and A. Peoples, *Bull. Environ. Contam. Toxicol.*, 5 (1969) 329.
63 P. B. Van Roosmalen, A. L. Klein and I. Drummond, *Int. Arch. Occup. Environ. Health*, 45 (1980) 57.
64 P. S. B. Minty, in A. S. Curry (Editor), *Analytical Methods in Human Toxicology*, Vol. 2, Macmillan, London, 1984, pp. 203–205.
65 R. N. Gupta, F. Eng and M. L. Gupta, *Clin. Chem.*, 28 (1982) 32.
66 O. Inoue, K. Seiji, M. Kasahara, H. Nakatsuka, T. Watanabe, S. G. Yin, G. L. Li, S. X. Cai and M. Ikeda, *Br. J. Ind. Med.*, 45 (1988) 487.
67 H. Matsui, M. Kasao and S. Imanura, *J. Chromatogr.*, 145 (1978) 231.
68 A. Astier and A. M. Deutsch, *J. Chromatogr.*, 182 (1980) 88.
69 S. H. Hansen and M. Dossing, *J. Chromatogr.*, 229 (1981) 141.
70 R. Andersson, A. Carlsson, M. Byfaelt-Nordquist and J. Sollenberg, *Int. Arch. Occup. Environ. Health*, 53 (1983) 101.
71 J. P. Gromiec and J. K. Piotrowski, *Int. Arch. Occup. Environ. Health*, 55 (1984) 61.
72 W. Will, W. Zschiesche, K. Gossler, K. H. Schaller and H. Valentin, *Fresenius' Z. Anal. Chem.*, 303 (1980) 401.
73 O. Inoue, K. Seiji, T. Suzuki, T. Watanabe, H. Nakatsuka, H. Satoh and M. Ikeda, *Bull. Environ. Contam. Toxicol.*, 47 (1991) 204.
74 G. Truchon, J. Brodeur and M. Gerin, *J. Anal. Toxicol.*, 14 (1990) 227.
75 T. Sakai, Y. Takeuchi, Y. Ikeya, T. Araki and K. Ushio, *Jpn. J. Ind. Health*, 31 (1989) 9.
76 A. Premel-Cabic, A. Cailleux and P. Allain, *Ann. Biol. Clin.*, 46 (1988) 683.
77 M. Ogata and T. Taguchi, *Int. Arch. Occup. Environ. Health*, 61 (1988) 131.
78 M. Ogata and T. Taguchi, *Int. Arch. Occup. Environ. Health*, 59 (1987) 263.
79 M. Ogata and T. Taguchi, *Int. Arch. Occup. Environ. Health*, 58 (1986) 121.
80 G. Bienek and T. Wilczok, *Br. J. Ind. Med.*, 38 (1981) 304.
81 K. Gossler, W. Will, K. H. Schaller and W. Zscheische, *Arbeitsmed. Sozialmed. Präventivmed.*, 14 (1979) 236.
82 M. Buratti, O. Pellegrino, G. Caravelli, D. Xaiz, C. Valla and A. Colombi, *Med. Lav.*, 80 (1989) 254.
83 A. Astier, *J. Chromatogr.*, 573 (1992) 318.
84 M. Ogata, T. Taguchi, N. Hirota, Y. Shimada and S. Sakae, *Int. Arch. Occup. Environ. Health*, 63 (1991) 121.
85 G. Truchon, J. Brodeur and M. Gerin, *J. Appl. Toxicol.*, 9 (1989) 123.
86 P. Colin, G. Sirois and S. Chakrabarti, *J. Chromatogr.*, 377 (1986) 243.
87 M. Korn, R. Wodarz, K. Drysch and F. W. Schmahl, *Arch. Toxicol.*, 60 (1987) 86.
88 M. Yoshikawa, Y. Taguchi, K. Arashidani and Y. Komada, *J. Chromatogr.*, 362 (1986) 425.
89 P. Ducos, R. Gaudin, A. Robert, J. M. Francin and C. Maire, *Int. Arch. Occup. Environ. Health*, 62 (1990) 529.
90 W. M. Pierce, Jr. and D. E. Nerland, *J. Anal. Toxicol.*, 12 (1988) 344.
91 W. Woiwode, R. Wodarz, K. Drysch and H. Weichard, *Arch. Toxicol.*, 43 (1979) 93.
92 M. Shoji, M. Mori, K. Moto-o, H. Kozuka and T. Honda, *Chem. Pharm. Bull.*, 33 (1985) 1687.
93 A. Lof, S. H. Hansen, P. Naslund, E. Steiner, M. Wallen and E. W. Hjelm, *Clin. Pharmacol. Ther.*, 47 (1990) 412.
94 T. Yoshida, K. Andoh and T. Tabuchi, *Arch. Toxicol.*, 65 (1991) 52.

95 T. Yoshida, T. Tabuchi and K. Andoh, *Xenobiotica*, in press.
96 T. Yoshida, M. Hirata, T. Tabuchi and K. Miyajima, *Jpn. J. Ind. Health*, 34 (1992) 3.
97 L. Campbell, A. H. Jones and H. K. Wilson, *Am. J. Ind. Med.*, 8 (1985) 143.
98 F. J. Jongeneelen, H. A. A. M. Dirven and C. M. Leijdekkers, *J. Anal. Toxicol.*, 11 (1987) 100.
99 J. Gartzke and H. J. Weigmann, *J. Chromatogr.*, 162 (1979) 234.
100 T. Wilczok and G. Bieniek, *Br. J. Ind. Med.*, 35 (1978) 330.
101 G. Bieniek, K. Karmanska and T. Wilczok, *Br. J. Ind. Med.*, 41 (1984) 272.
102 G. A. Maylin, *Cornell Vet.*, 70 (1980) 202.
103 A. Zlatkis and R. E. Kaiser (Editors), *High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977.
104 E. Tyihak, E. Mincsovics and J. Szekely, *J. Chromatogr.*, 471 (1989) 375.
105 E. Tyihak, E. Mincsovics and H. Kalasz, *J. Chromatogr.*, 174 (1979) 75.
106 E. Tyihak, E. Mincsovics, H. Kalasz and J. Nagy, *J. Chromatogr.*, 211 (1981) 45.
107 B. S. Thomas and E. Bailey, in A. S. Curry (Editor), *Analytical Methods in Human Toxicology*, Vol. 1, Macmillan, London, 1984, pp. 217–256.