small control laboratories. However, although most of the published applications in the field have their origin not in the highly HPLC-oriented first world, the approach must not be considered to be only valuable and suitable for simple control laboratories with very limited assets.

Pharmaceutical analytical laboratories work under enormous economic and time pressure. Sample throughput has to be increased and lead times decreased, both without any consequence to the reliability of the results and at best without increase in personnel and operational costs. Therefore it must be recommended that TLC is considered as a possible replacement or substitute for compendial or noncompendial HPLC assays and content uniformity testing, especially for tablets.

Future Perspectives

A shift away from the traditional, semiquantitative TLC compendial purity testing of active pharmaceutical ingredients must be expected. Instead, a higher level quantitative TLC will be recommended and promoted by various national/international regulatory agencies for on-site and port-of-entry testing of pharmaceuticals. This development will be stimulated by the development of modern video imaging systems. Originally developed for documentation, modern closed-circuit device cameras are now combined with powerful software to collect the information stored on a plate in a very short time. Although not yet generally as precise and accurate as scanning densitometry, latest published results indicate that at least in the UV-region, video imaging can produce assay results that are equivalent to those derived by scanning densitometry. The lower price of video integration systems makes them the ultimate choice for rapid quantitative TLC applications.

The use of scanning densitometry will be limited to more delicate analytical tasks like stability or quality control testing procedures such as assays or content testing of finished pharmaceuticals, especially tablets, where TLC will expand because of its economical and environmental advantages, compared to HPTLC.

Highly sophisticated hyphenated techniques or combinations of TLC with spectrometric techniques such as FT-NIR, FT-IR, Raman or MS with different ionization mechanisms and MS-MS will be improved, but they will remain special technical solutions to special problems and must not be expected to become routine methods.

See also: **II/Chromatography: Thin-Layer Planar:** Densitometry and Image Analysis; Historical Development; Instrumentation; Layers; Mass Spectrometry; Modes of Development: Conventional; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal. **III/Pharmaceuticals:** Basic Drugs: Liquid Chromatography; Capillary Electrophoresis; Neutral and Acidic Drugs: Liquid Chromatography; **Thin-Layer Chromatography-Vibration Spectroscopy.**

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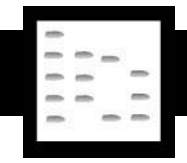
PHENOLS



Gas Chromatography

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Phenolic compounds are extensively used in the chemical industry. Some simple phenolic species are known to be products of vascular plant metabolism, and also contribute to the polymeric structures of tannins and lignin. In addition to their use as intermediates in industry and in the production of dyes, plastics and pharmaceuticals, chlorophenols have

been extensively utilized as preservative agents, pesticides, antiseptics and disinfectants. Chlorophenols can also be obtained by hydrolysis, oxidation and microbial degradation of chlorinated pesticides, and can be produced when phenol-contaminated water is chlorinated for purification. The US Environmental Protection Agency (EPA) listed 11 phenols as priority pollutants, including phenol, 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2-chlorophenol (2-CP), 2,4-dinitrophenol (2,4-DNP), 2,4-dichlorophenol (2,4-DCP), 2,4-dimethylphenol (2,4-DMP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DMP), 4-chloro-3-methylphenol (4-C-3-MP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP). Several phenolic compounds are also listed in the European Community (EC) Directive 76/464/EEC regarding dangerous substances discharged into the aquatic environment. For drinking purposes, EC Directive 75/440/EEC states that maximum levels of phenolic compounds in surface water should be within the range 1–10 ng mL^{-1}. Therefore, rapid and reliable methods must be developed to analyse these compounds.

Owing to its high sensitivity and resolving power, chromatography is the most commonly used analytical technique to determine phenols. Previous investigations have developed gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) to monitor these pollutants. Government-approved analytical methods, e.g. US EPA 604 and 625 (acid-extractable section), are based on gas chromatography using electron capture or mass spectrometry detection.

Gas chromatography is the most widely used technique in analytical chemistry. The merits of gas chromatography include its speed, precision and accuracy. Gas chromatography is appropriate for separating and analysing nonpolar volatile materials but it is limited in application to materials that exert vapour pressures of at least 10 mmHg at the column operating temperature. Also, owing to their high polarity, phenols tend to produce broad-tailed peaks, and this effect increases as the column ages. In order to obtain more favourable chromatographic peaks, less polar derivatives of phenols are often prepared. When GC is applied to determine trace amounts of phenols in water or other samples a pre-concentration or extraction step is frequently required. Proper sampling largely determines the validity of an analytical sample for trace analysis. Various pre-concentration techniques have been developed for phenol analysis. The sensitivity of GC for phenol analysis depends markedly on the detector used. Sample preparation methods, including various types of extraction techniques and derivatization procedures, are described below. The effectiveness of the techniques is demonstrated by investigations on real samples.

Sample Preparation

As many investigations have confirmed, determining phenolic compounds in water or other matrices in the range below 1 ng mL^{-1} is extremely difficult. The extraction and preconcentration of a mixture of phenols present difficulties owing to their wide range of polarities. In addition, volatilization may cause losses in preconcentration because of their relatively high vapour pressure. The conventional preconcentration technique is liquid–liquid extraction (LLE) for aqueous samples. The US EPA method for the analysis of phenols in water (EPA 604 and 625, acid-extractable section), consists of acidification of the sample to pH 2, followed by dichloromethane extraction. Merits of LLE include its simplicity and the need for inexpensive equipment. Nevertheless, despite its extensive use, LLE has many drawbacks, such as emulsion formation and different extraction efficiencies for different compounds. In addition, the method requires a large amount of solvent, and is slow and laborious. It is also hazardous to health since it uses toxic organic solvents that are also relatively expensive in terms of disposal. Solid-phase extraction (SPE) protocols are more commonly used than conventional LLE procedures, thereby reducing loss of analytes and the use of large amount toxic solvents. The complete extraction is performed in a series of stages, including washing, conditioning, eluting and drying. Many SPE materials are used in the preconcentration of phenols from aqueous samples including Empore disks, cartridges or small precolumns. Sorbents, including C_{18}, C_{10}, C_8, C_2, CH, CN and RLRP-S (styrene-divinylbenzene copolymer), have been used to investigate the extraction efficiency of phenols in water. The C_{18}-based sorbents have the property of low breakthrough volume of the more polar analytes and are therefore inappropriate for the simultaneous preconcentration of polar and nonpolar compounds. Notably, the breakthrough problem occurs for catechol, phenol and 4-NP extracted with C_{18} sorbent. According to a previous investigation, a polymeric material such as Amberlite XAD-2 or XAD-4 or PRP is the most appropriate sorbent, yielding recoveries of up to 80% for most phenolic compounds; however, the breakthrough problem for some phenols still occurs. To overcome this problem, polymeric phases with high cross-linking have been proposed, e.g. by using the packing materials based on styrene-divinylbenzene copolymer, such as Isolute ENV^+, Lichrolut EN,

Envi-chrom, PLRR-S and Porapak RPX. However, SPE can be expensive because the cartridges are normally discarded after one extraction. Moreover, the extraction still uses organic solvents that potentially threaten health and the surrounding environment.

Solid-phase microextraction (SPME) does not require the use of an organic solvent. The mechanism of SPME is based on an equilibrium of analytes between the sample and the solid phase coating on a quartz fibre. The analytes are directly determined by thermal desorption from the fibre into a GC. SPME has been extensively applied to extract trace organic compounds from aqueous samples owing to its solvent-free methodology, simplicity and rapidity. The feasibility of applying SPME to extract phenols from a complex matrix with polyacrylate (PA)- or poly(dimethylsiloxane) (PDMS)-coated fibres has been thoroughly evaluated. Extraction optimization procedures have been systematically studied, involving the pH of the sample, the desorption time and salting-out. Supercritical fluid extraction (SFE) and Soxhlet extraction techniques have been used for the solid samples. The extraction of 11 phenolic compounds from a river sediment by methanol–CO_2 mixtures under supercritical conditions and Soxhlet extraction with methylene chloride have been evaluated.

In the GC analysis of high polarity and vapour pressure phenols, a derivatization step is frequently executed to provide the mixture with better chromatographic characteristics. Until now, the derivatives used for this purpose have been based on the formation of esters, ethers and silyl derivatives. The derivatizing agents used are acetic anhydride, diazomethane, 2,4-dinitrobenzene, heptafluorobutylimidazole, pentaflurobenzoyl chloride, pentafluorobenzoyl bromide and silanizing agents. Acetylating agents have been widely used for the derivatization of phenols. Phenols can be acetylated in the aqueous organic sample or after extraction. EPA method 8041 recommends the derivatization of phenols to methylated phenols using diazomethane but diazomethane gas is extremely toxic, irritating and carcinogenic, so derivatization must be handled in a hood and with safety equipment.

Environmental Applications

Analysing phenol residues in environmental samples by GC has been the subject of extensive investigations. A novel means of determining eight chlorophenols in tap water was reported: it involved the direct acetylation of the chlorophenols with acetic anhydride in the presence of K_2CO_3. A graphitized carbon catridge was used for extraction and preconcentration. Chlorophenols were quantitatively measured by a microwave-induced plasma atomic emission detector at the sub-p.p.b. level in tap water. A methylphenylsilicone capillary column was used for separation.

The purge-and-trap technique and laboratory-made pulsed-spray techniques to extract five chlorophenols in water have been evaluated. The absorbent tubes were packed with 100 mg Tenax TA (0.2 μm). The absorbent tubes were attached to the injector port of a GC and heated to 200°C for 3 min to desorb the trapped analytes. GC separation was followed by MS detection and quantitation in the selected ion monitoring (SIM) mode. **Figure 1** depicts the gas chromatogram of spray-and-trap GC-MS of a water sample containing 100 μg L^{-1} chlorophenols and 20 μg L^{-1} internal standard (2,4,6-tribromophenol). Low ng L^{-1} levels of detection were obtained for the studied chlorophenols.

The optimization of SPME conditions for the determination of phenols was investigated. The SPME method, based on a polyacrylate-coated fibre, gave a detection limit at 32–0.01 μg L^{-1} level for GC–flame ionization detector (GC-FID) and GC–mass spectrometry (GC-MS) using saturated sodium chloride solution at pH 2. Applying the method to analyse a sewage sample indicated that the matrix significantly influenced the extraction of heavier chlorinated phenols. The feasibility of SPME-GC-MS was examined for detecting phenolic compounds in waste water and the effects of humic acid and surfactants on recovery were studied. SPME using pencil lead as a sorbent for analysis of 2-CP in water was also investigated. The detection limit for the determination of chlorophenols by GC-ECD (electron-capture detection) was 1 ng mL^{-1}. According to their results, the dissolved humic susbstances (10 mg L^{-1}) did not affect the analysis. Analysis of chlorophenols from an aqueous sample by GC-MS following SPME and reaction with diazomethane has been studied. A silica fibre coated with polyacrylate yielded good extraction efficiency. **Figure 2** displays the mass chromatogram of chlorophenols derivatized with diazomethane after SPME, with detection limits at the level of ng L^{-1}.

The direct determination phenols in water using headspace–gas chromatography (HS-GC) has been developed. A method has been proposed based on the GC headspace analysis of phenols in solids as acetate derivatives. The acetate derivatives were directly prepared in the wet solid samples by acetic anhydride in the presence of $KHCO_3$ and separated on a diisodecylphthalate capillary column with FID. The detection limit ranged between 0.03 and 0.08 μg g^{-1}. We have evaluated SPME with a polyacrylate fibre

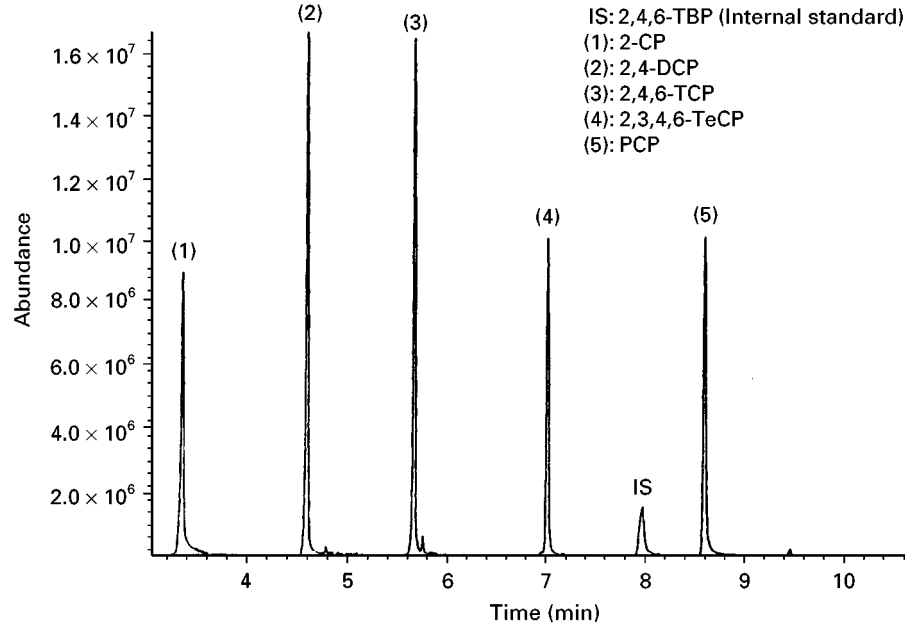

Figure 1 Chromatogram of pulsed spary-and-trap GC-MS of a water sample containing 100 μg L^{-1} chlorophenols and 20 μg L^{-1} internal standard (2,4,6-tribromophenol).

coupled with GC-MS (electron impact ionization and negative chemical ionization) to determine chlorophenols in landfill leachates and soil. The chlorophenols were analysed without any derivatization. The method is precise and can be used over a wide linear range, with detection limits of 1 ng L^{-1} levels of chlorophenols in water. The chlorophenols were determined in soil contaminated with PCP from

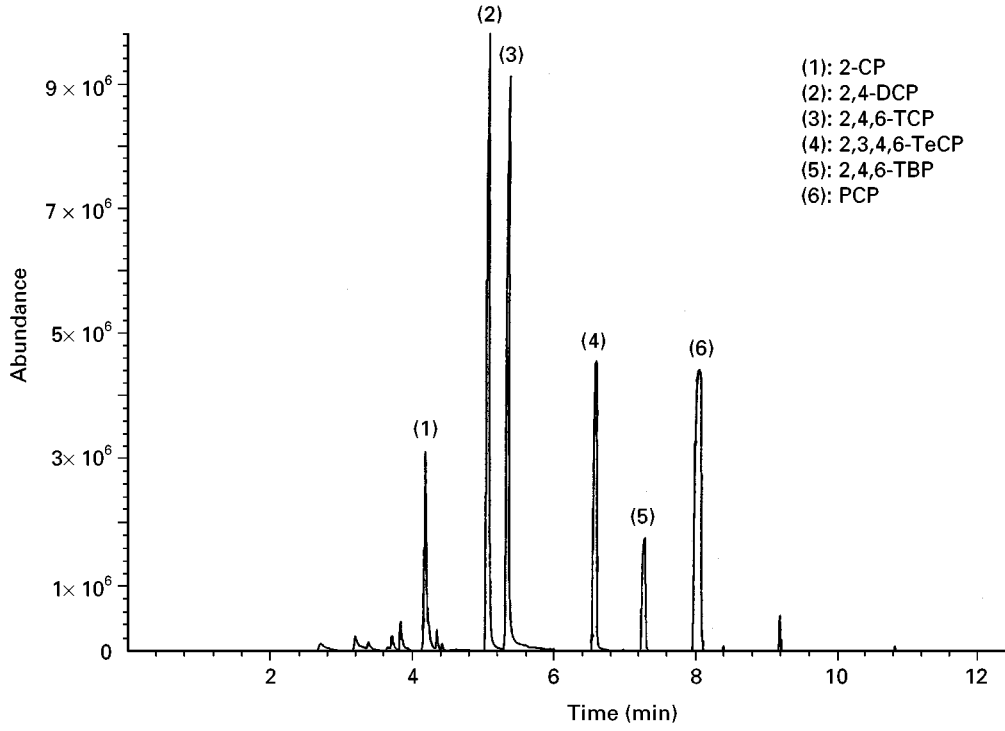

Figure 2 Chromatogram of 100 μg L^{-1} chlorophenols by post-derivatization following SPME with diazomethane produced using GC-MS (SIM).

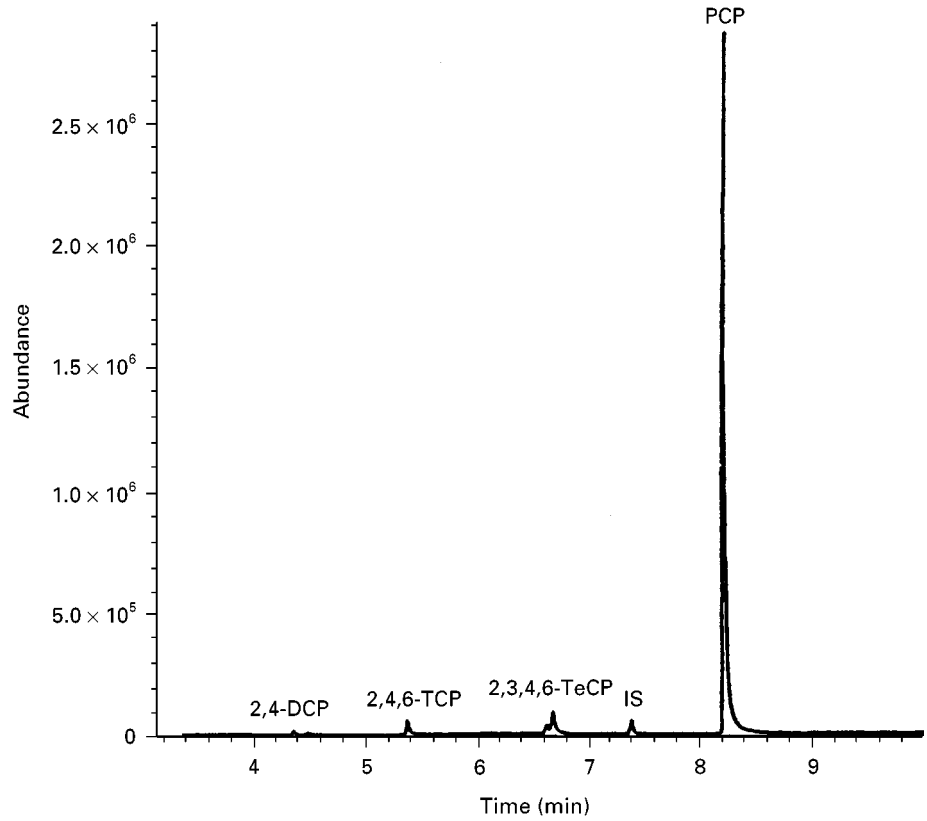

Figure 3 Mass ion chromatogram (TIC) of a real soil sample. (Reproduced from Lee MR (1998) *Journal of Chromatography A* 806: 323, with permission from Elsevier Science.)

a chemical manufacturing plant. **Figure 3** depicts the mass ion chromatogram of a real soil sample. Chlorophenols detected were 2,4-DCP, 2,4,6-TCP, 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and PCP. The internal standard was 2,4,6-tribromophenol. The PCP detected in the soil was estimated to be 534 μg g^{-1}.

Biological Fluid Analysis

The monitoring of phenols in human urine and other biological samples is used as an indication of occupational exposure or exposure to environmental contamination. The efficiency of extracting analytes from complex matrices, particularly urine samples, affects the detection level. Selecting the optimum sample preparation method is a prerequisite for trace analysis of phenols in urine. Many methods of extraction combined with chromatographic techniques have been proposed to determine phenols in urine. One method determined the urinary chlorophenols as acetyl derivatives after hot acid hydrolysis, then extracted them using LLE with toluene. The chlorophenol derivatives were then determined by GC-MS using selected ion monitoring. The method was used to monitor the urine of sawmill workers who are still occupationally exposed to chlorophenols because of a contaminated work environment. The limit of quantification was in the level of 3.6 μg g^{-1} creatinine for all the studied chlorophenols. A sensitive and selective method was developed for the quantitation of total *o*-phenylphenol (free plus conjugates) found in human urine samples. Conjugates of *o*-phenylphenol were acid-hydrolysed to free *o*-phenylphenol, extracted into toluene and derivatized to the pentafluorobenzol ester derivative. The analysis was performed via negative ion chemical ionization–GC-MS (NCI-GC-MS). In this case, the lower limit of quantification was 1 ng mL^{-1} urine.

As the result of the degradation of proteins and amino acids, the presence of some phenols, such as phenol, *p*-cresol, *p*-ethylphenol and catechol in free and conjugated forms, is commonly found in normal urine at the 5–20 p.p.m. level. The variety and amount of phenols excreted into urine are quite individual and vary with nutrition, smoking habits and intake of antibiotics. In dietary studies, it is required to monitor the relationship between nutrition and the levels of the phenols in faeces and urine samples. In occupational hygiene determination of phenol in urine after exposure to benzene or toluene is to detect

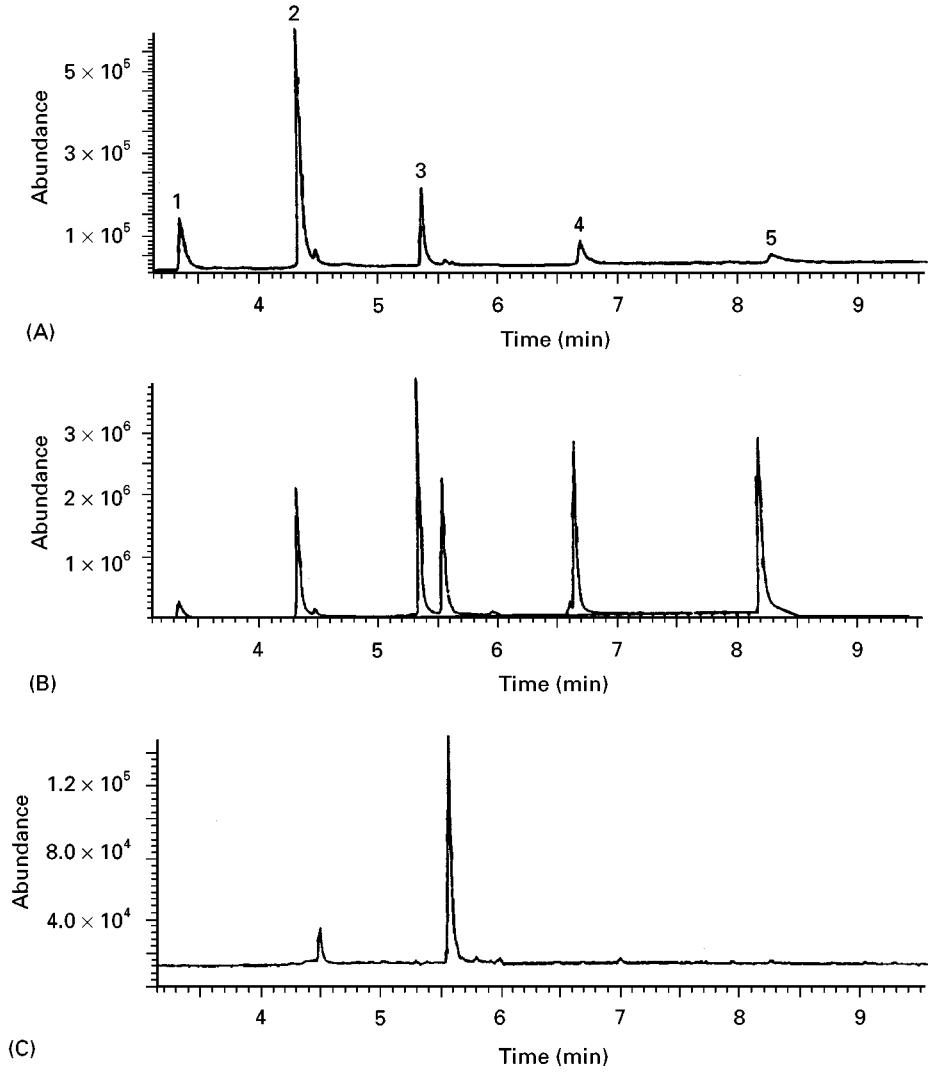

Figure 4 Mass chromatogram of (A) 25 μg L^{-1} chlorophenols in urine at pH 7, (B) at pH 1 and (C) blank urine, produced by SPME-GC-MS. Peaks are assigned as 1, 2-chlorophenol; 2, 2,4-dichlorophenol; 3, 2,4,6-trichlorophenol; 4, 2,3,4,6-tetrachlorophenol; 5, pentachlorophenol. (Reproduced from Lee MR (1998) *Journal of Chromatography B* 707: 95, with permission from Elsevier Science.)

the increased levels of phenol and *p*-cresol. Hydrolysis of urine samples with concentrated sulfuric acid during stream distillation followed by GC has been described. The condensate is buffered with H_3BO_3-NaOH and acetylated with acetic anhydride. The derivatives of all cresols and xylenols were completely separated on an SE-54 capillary column. In this case, acid hydrolysis of phenolic conjugates must be combined with steam distillation, which is not performed offline. Acid hydrolysis of phenol conjugates in urine by concentrated H_3PO_4, followed by extraction and acetylation, then GC on an OV-1 or OV-17 packed column with FID has been used to monitor normal levels of phenol and *p*-cresol or phenol and *o*-cresol after exposure to benzene or toluene vapours. The detection limit is 1 mg L^{-1}, which is adequate for the purpose. The feasibility of combining SPE on Separcol SI C_{18} with GC-ECD to determine chlorophenols and cresols in human urine after acid hydrolysis has been discussed. Before GC determination, the isolated compounds were derivatized with pentafluorobenzyl bromide. The limit of determination of phenols varied from 5 to 20 ng mL^{-1}. Using this method, 52 from occupationally and nonoccupationally exposed groups were examined for the presence of chlorophenols in urine.

The feasibility of applying SPME and GC-MS to determine chlorophenols in urine has been evaluated. The amount of an analyte extracted relies heavily on the conditions of the SPME. **Figure 4** shows that the extraction is enhanced by decreasing the pH of the urine solution from 7 to 1.

According to these results, the optimum SPME experimental procedures to extract chlorophenols in urine were with a polyacrylate-coated fibre at pH 1, extraction time 50 min and desorption into the GC injector at 290°C for 2 min. The technique offers a low ng L^{-1} sensitivity to determine trace amounts of chlorophenols in a urine sample containing high levels of interference. The method has been successfully used to analyse urine samples of workers in a sawmill where chlorophenol-containing anti-stain agents were previously used. Analysis indicated that chlorophenols were found in 9 out of 10 urine samples. The concentration of chlorophenols ranged from 0.02 μg L^{-1} (PCP) to 1.50 μg L^{-1} (2,4-DCP).

Future Prospects

Phenols, particularly chlorophenols, are toxic at concentrations of a few μg L^{-1} and are also persistent. Determining trace amounts of phenols is not easy for real samples that consist of extremely complex matrices. Methods for monitoring trace amount of phenols in real samples must be sensitive and selective, and should be rapid and simple. A mixture can be separated by GC into its individual components and, at the same time, the amount of each compound present can be determined. Furthermore, analysis can be performed with various detectors at a moderate cost. The applications presented here demonstrate the effectiveness of GC to analyse quantitatively trace levels of phenols in complex mixtures. Although various GC methods for phenol analysis have been widely used, novel techniques and sample pretreatment methods are continually being introduced. Simple retention times are not very reproducible and linking with a mass spectrometer as a detector is desirable for unambiguous identification. This approach has the merits of speed, sensitivity and selectivity. The MS-MS technique will become less expensive in the future and, eventually, the preferred means of analysing phenols when coupled to GC. Currently GC-MS-MS is more expensive than GC-MS but offers an extra separation stage to resolve the problem of analysing mixtures not amenable to GC-MS.

See also: **II/Chromatography: Gas:** Column Technology; Derivatization; Detectors: Mass Spectrometry; Detectors: Selective; **III/Phenols:** Liquid Chromatography; Solid-Phase Extraction; Thin-Layer (Planar) Chromatography.

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Liquid Chromatography

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Phenols include a considerable range of substances which possess an aromatic ring with one or more hydroxyl substituents. These compounds are present in many different types of sample, which means that the determination of phenolic compounds is of wide interest.

These compounds may be natural or synthetic. They are present in all plant tissues and are frequently the most abundant secondary metabolites in fruits, in which they sometimes reach high concentrations. Phenolic compounds may also be found in combinations to form flavones and glucosides in trees and plants.