TECHNICAL NOTE

Amy P. Hart,[1] *M.D. and Amitava Dasgupta,*[1] *Ph.D.*

A Novel Derivatization of Phenol after Extraction from Human Serum Using Perfluorooctanoyl Chloride for Gas Chromatography-Mass Spectrometric Confirmation and Quantification

REFERENCE: Hart AP, Dasgupta A. A novel derivatization of phenol after extraction from human serum using perfluorooctanoyl chloride for gas chromatography-mass spectrometric confirmation and quantification. J Forensic Sci 1997;42(4):693–696.

ABSTRACT: Phenol (carbolic acid) is widely used as a disinfectant as well as in the chemical industry as an intermediate in the synthesis of a variety of chemicals. Phenol is also the major metabolite of benzene which is used in many commercial solvents. Phenol is toxic and caustic and may cause death even from dermal absorption. Therefore, measurement of phenol in postmortem blood is essential. The concentration of phenol in blood can be measured by gas chromatography with flame ionization or mass spectrometry. Phenol can also be analyzed by high performance liquid chromatography. However, in forensic toxicology, unambiguous confirmation of phenol by mass spectrometry is as important as quantification in blood. Here we describe a novel derivatization of phenol after extraction with chloroform from human serum using perfluorooctanoyl chloride. The perfluorooctanoyl derivative of phenol showed a strong molecular ion at m/z 490 (relative abundance: 23%) whereas the base peak was observed at m/z 77. The derivative of the internal standard 3,4-dimethylphenol showed a very strong molecular ion at m/z 518 (relative abundance: 56%) and the base peak was observed as m/z 121. The derivative of p-cresol, a chemically related phenolic compound, showed a strong molecular ion at 504 m/z (relative abundance: 54%) and a base peak at m/z 107. We observed baseline separation between derivatized phenol (retention time: 6.1 min), p-cresol (retention time: 7.8 min), and the internal standard (retention time: 9.4 min). We observed no interferences in our assay from grossly hemolyzed serum. Within and between run precision was studied using a serum standard containing 25 mg/L of phenol. The within run precision was 6.6% ($\bar{X}$ = 24.3, SD = 1.6 mg/L, n = 8) whereas the between run precision was 8.6% ($\bar{X}$ = 25.5, SD = 2.2 mg/L, n = 8). The assay was linear for serum phenol concentrations of 10–200 mg/L. The detection limit was 1 mg/L of serum phenol concentration. The average recoveries were 92.1% to 94.0% for various serum phenol concentrations.

KEYWORDS: forensic science, forensic toxicology, phenol, perfluorooctanoyl derivative, gas chromatography/mass spectrometry

[1]Resident in Pathology and director of clinical chemistry and toxicology and associate professor of Pathology, University of New Mexico Health Sciences Center, Albuquerque, NM.

Received 12 Aug. 1996; and in revised form 11 Oct. 1996; accepted 14 Oct. 1996.

Phenol (carbolic acid) was originally introduced as an antiseptic and was used preoperatively as a skin disinfectant. Phenol is still used today commercially as a disinfectant and also as an intermediate in chemical synthesis. Phenol is also used in outpatient surgery by podiatrists. The over the counter Campho-Phenique contains 2–5% phenol (1). Cresol and soap solution is part of the British Pharmacopoeia and is known as lysol. Some American brand name "Lysol Disinfect" also contains phenol and cresol. Phenol is used in the manufacture of phenol-formaldehyde resins and plastics and also in photographic developers. Phenol denatures proteins and is considered a general protoplasmic poison. It is readily absorbed from all parts of the body and is caustic, causing severe irritation and corrosion of the skin and other tissue.

Ingestion or dermal absorption of the germicidal/fungicidal agents or the medicinal 89% phenol preparation can be life threatening. A workman fell into a vat full of phenol solution. Although he was immediately removed and washed, he died within 30 min and his postmortem blood showed a phenol concentration of 90 mg/L (2). In another fatal case of dermal exposure, the postmortem blood phenol level was 56 mg/L (3). A man who ingested lysol in a suicide attempt died shortly and showed a postmortem blood phenol level of 46 mg/L (4).

Phenol concentrations in blood have been measured by gas chromatography (GC) with flame ionization detection (5,6) or mass spectrometry (7). However, the polarity and volatility of phenol may cause a reproducibility problem in the GC methods (8,9). Moreover, in the GC method, the identification of phenol is solely based on the retention time, and other compounds like p-cresol, may interfere. Determination of phenol using high performance liquid chromatography also suffers from similar problems (10).

In forensic toxicology, unambiguous confirmation of phenol by mass spectrometry is equally important as the blood level of phenol. Phenol is a small molecule that is not ideal for mass spectrometric confirmation. A new less volatile derivative of phenol which could produce strong peaks at a much higher mass range could be more useful for unambiguous identification and confirmation by mass spectrometry. In this paper we report a novel derivatization of

phenol using perfluorooctanoyl chloride which produced a strong molecular ion at m/z 490 (molecular weight of underivatized phenol is 94) in the electron ionization mass spectrometry. Quantitation of phenol can be easily achieved by using 3,4-dimethylphenol as an internal standard which was also derivatized using our protocol.

Materials and Method

Phenol, p-cresol, and the internal standard 3,4-dimethyphenol were obtained from Aldrich Chemical Company (Milwaukee, WI). The derivatizing agent perfluorooctanoyl chloride was obtained from PCR Chemicals (Gainsville, FL). The gas chromatography-mass spectrometric analysis was performed by using a Model 5890 series II gas chromatograph coupled with a 5972 series mass selective detector (Hewlett Packard, Palo Alto, CA). A 25 m by 0.2 mm ID Ultra one column (Hewlett Packard) with a film thickness of 0.33 micron was used. The initial column oven temperature was 120°C. After 5.5 min, the oven temperature was increased at a rate of 15°C/min to 170°C. Then it was increased at a rate of 25°C/min to 290°C. The mass spectrometer was operated using electron ionization with a scan range of 50 to 550 m/z.

Plasma and whole blood were obtained from a local blood bank. The whole blood was hemolyzed to investigate whether gross hemolysis which is often present in postmortem blood, has any effect on our assay for phenol. We prepared stock solutions of phenol (10 mg/mL) in deionized water and in chloroform (for recovery studies). We prepared a stock solution of 3,4-dimethylphenol, the internal standard in chloroform (10 mg/mL). Plasma or grossly hemolyzed plasma was supplemented with various concentrations of phenol using the aqueous stock solution.

A 2-mL aliquot of specimen was supplemented with 10 μL of the internal standard to achieve a final concentration of 50 mg/L. Phenol along with the internal standard was extracted from plasma using 10 mL of chloroform (vortexed for 1 min followed by mixing for an additional 10 min). The upper aqueous layer along with precipitated protein was aspirated off. The bottom organic layer was concentrated at room temperature to an approximate volume of 50–100 μL. After addition of 50 μL of perfluorooctanoyl chloride, the derivatizing agent, the specimen was heated at 80°C for 25 min. Then, the specimen was again concentrated to approximately a volume of 50 μL. After reconstitution with 50 μL of ethyl acetate, 1–2 μL was injected into the GC/MS.

Results and Discussion

Mass Spectral Characteristics of Derivatized Phenol

We observed baseline separation with excellent peak shapes for derivatized phenol (retention time: 6.1 min), p-cresol (retention time: 7.8 min), and the internal standard, 3,4-dimethylphenol (retention time: 9.4 min) (Fig. 1). Because these derivatized phenols elute at much higher temperature than the underivatized phenols, our analysis is free from interferences from volatile components of the plasma matrix.

The perfluorooctanoyl derivative of phenol showed a strong molecular ion at m/z 490 (relative abundance: 23%), whereas the base peak was observed at m/z 77. We also observed another strong characteristic peak at m/z 93 (Fig. 2). The derivative of the internal standard, 3,4-dimenthylphenol showed a very strong molecular ion at m/z 518 (relative abundance: 56%) and the base peak was observed at m/z 121 (Fig. 3). The derivative of p-cresol also showed a strong molecular ion at m/z 504 (relative abundance: 54%) and a base peak at m/z 107 (Fig. 4).

Precision, Linearity and Detection Limit

The within and between run precision of the assay was determined using a serum control containing 25 mg/L of phenol. The within run precision showed a CV of 6.6% ($\bar{X}$ = 24.3, SD = 1.6 mg/L, n = 8), whereas the between run precision showed a CV of 8.6% ($\bar{X}$ = 25.5, SD = 2.2 mg/L, n = 8). The assay was linear for serum phenol concentrations between 10 and 200 mg/L. Using the target concentration as the x-axis and the observed concentration as the y-axis in the linearity study, we observed the following regression equation:

$$y = 1.11 \times -4.66 \ (r = 0.99)$$

The detection limit of the assay was 1 mg/L of serum phenol

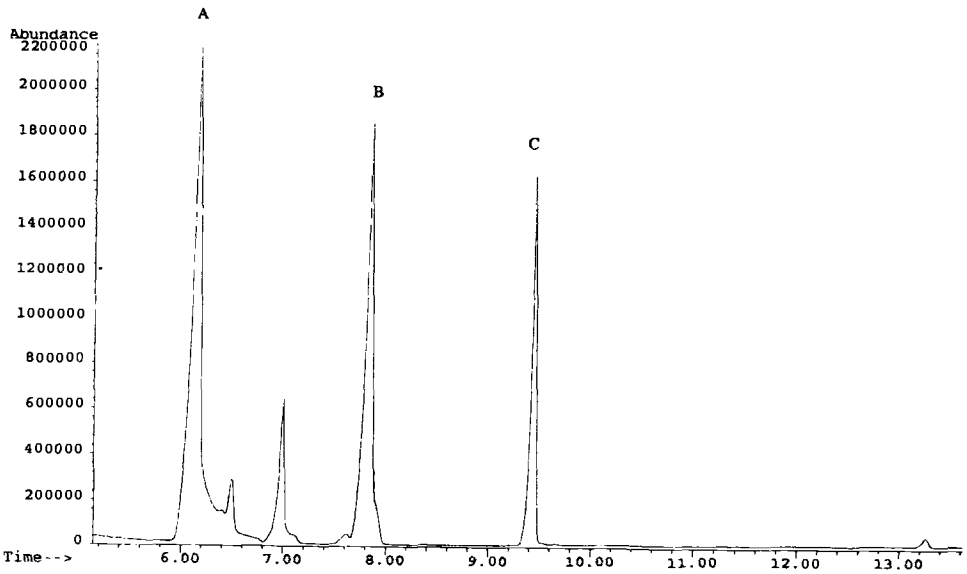

FIG. 1—*Total ion chromatogram showing separation between derivatized phenol (A), p-cresol (B), and the internal standard (C) isolated from a highly hemolyzed serum.*

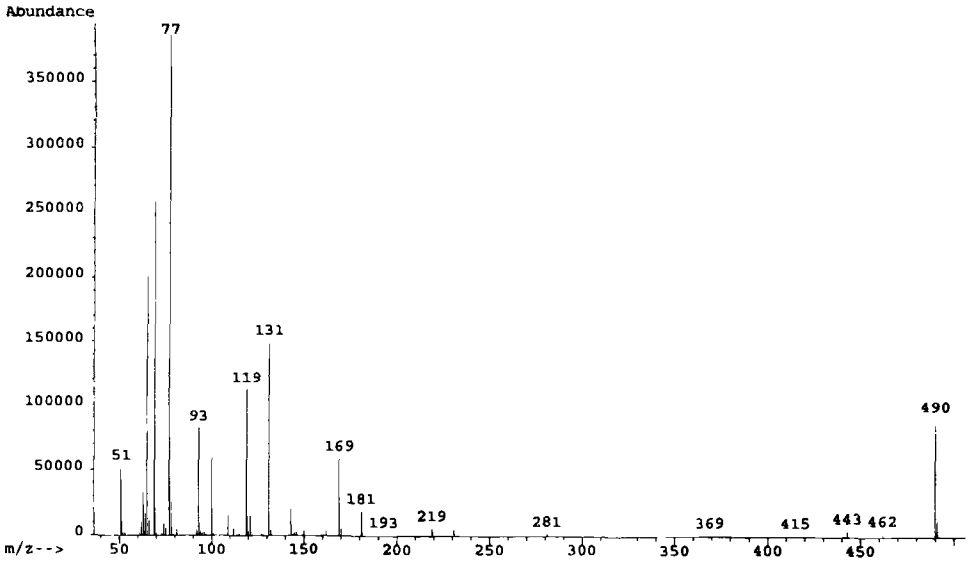

FIG. 2—*Electron ionization mass spectrum of perfluorooctanoyl derivative of phenol.*

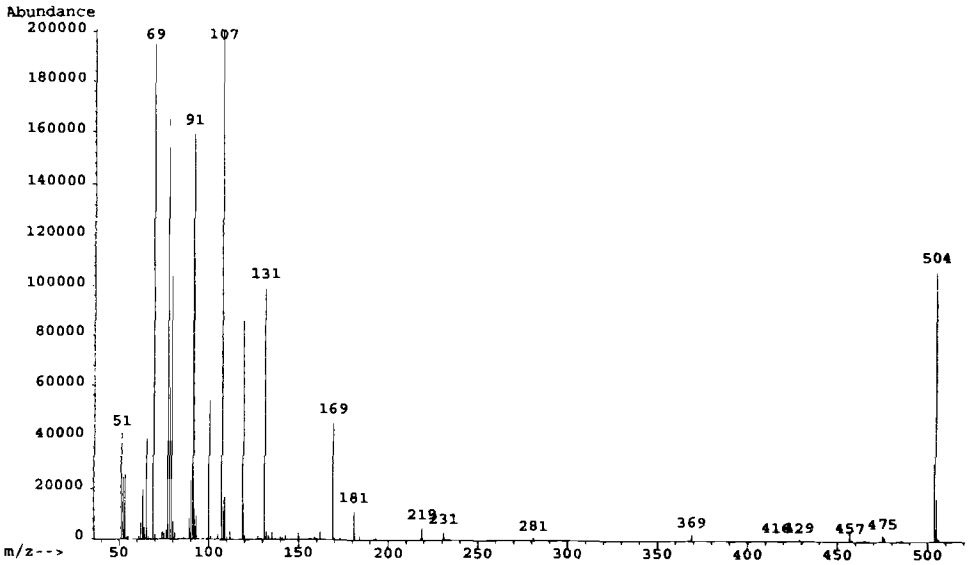

FIG. 3—*Electron ionization mass spectrum of perfluorooctanoyl derivative of p-cresol.*

concentration. The detection limit can be further lowered if selected ion monitoring was used instead of full scan mass spectrometric analysis.

Recovery

We studied the recovery of phenol using various serum standards. Serum standards were made by supplementation with a stock solution of phenol prepared in deionized water, whereas we used a stock solution prepared in chloroform for derivatization without extraction from serum. The average recovery of phenol was 92.1% at a serum phenol concentration of 25 mg/L, 94.0% at a serum phenol concentration of 50 mg/L and 93.2% at a serum phenol concentration of 200 mg/L.

Effect of Hemolysis

Because many postmortem blood specimens are highly hemolyzed, we studied the effect of highly hemolyzed serum on our assay. Whole blood was hemolyzed using a syringe and the serum was separated by centrifugation at 1500 g for 10 min. We achieved grade three to grade four hemolysis. The hemolyzed serum was supplemented with phenol and analyzed after extraction and derivatization by GC/MS. Our extraction protocol did not extract hemoglobin and the organic phase was colorless as observed with normal serum. Moreover, the observed phenol concentrations were similar to target concentrations in hemolyzed specimens indicating that gross hemolysis has no effect on recovery of phenol.

Application of the Assay

Phenol intoxication may lead to death, and unambiguous identification and quantitation of phenol in postmortem blood is essential for such death investigation. Phenol is also the major metabolite of benzene and exposure of benzene vapor is a serious health hazard in industry which may even cause death (11). Phenol is the major metabolite of benzene and the exposure to benzene can

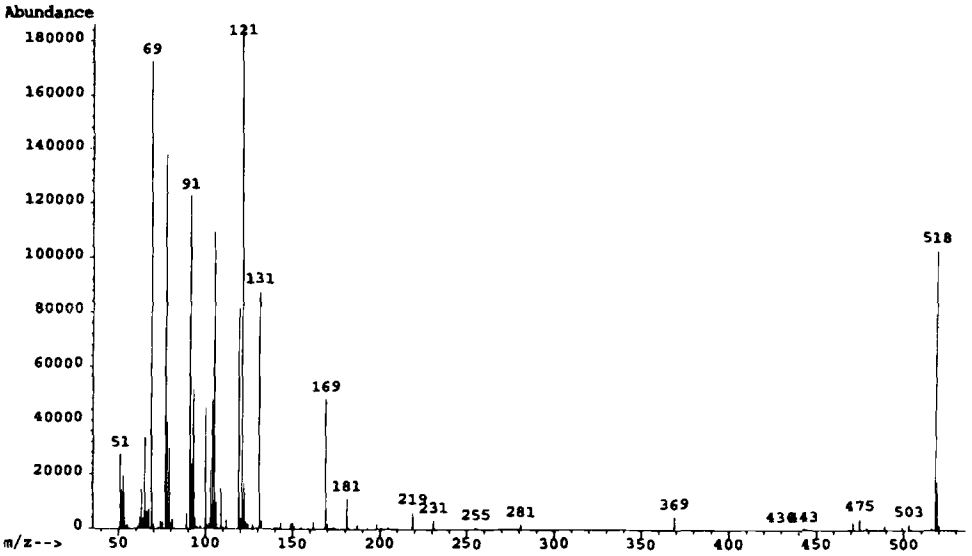

FIG. 4—*Electron ionization mass spectrum of perfluorooctanoyl derivative of 3,4-dimethylphenol, the internal standard.*

be determined by measuring concentration of phenol in blood or urine. Cresols are used as disinfectants (lysol), and acute toxicity from cresol can cause death. A man who ingested at least 25 g of cresol died and showed a postmortem blood cresol level of 90 mg/L (12). A one-year-old boy who died within 5 h of accidental dermal application of cresol antiseptic fluid demonstrated a postmortem blood cresol level of 120 mg/L (13). A blood cresol concentration of 190 mg/L has been reported for a woman who ingested a cresol disinfectant (14). Usually the protocol applied for determination of phenol can be used to determine cresol concentrations in blood.

The currently used GC techniques for detection of phenol without derivatization use only retention time to identify phenol. Moreover, the peaks elute at a relatively lower temperature due to the high volatility of phenol. In our protocol, derivatized phenol elutes at a much higher temperature and our assay is free from interferences by the volatile components of serum. In addition, due to the presence of a strong molecular ion at a much higher mass, unambiguous confirmation of phenol can be easily accomplished. We also observed baseline separation between derivatized phenol, cresol, and the internal standard. The perfluorooctanoyl derivative of cresol demonstrated a very strong molecular ion at a relatively high mass range, aiding in unambiguous confirmation of cresol. Our extraction protocol also successfully extracted cresol from serum as well as hemolyzed serum. Therefore, our protocol for the determination of phenol in serum after derivatization with perfluorooctanoyl chloride can also be adapted easily for determination of cresol in blood if needed.

References

1. Haddad LM. Phenol in "Clinical management of poisoning and drug overdose." Haddar LM, Winchester JF, editors, W.B. Saunders Company, Philadelphia, 1983:810–2.
2. Baselt R, Cravey R. Disposition of toxic drugs and chemicals in man. Chemical Toxicology Institute, Foster City, CA 1995: 614–6.
3. Soares RJ, Jift JP. Phenol poisoning: Three fatal cases. J Forensic Sci 1982;27:729–31.
4. Briglia R. Presented at the Quarterly Meeting of the California Association of Toxicologists, Millbrae, CA, 7 Feb. 1981.
5. Hadson PD, Hanrahan P. A rapid gas chromatographic method for the determination of free phenol in blood. J Agr Food Chem 1983;31:447–8.
6. Nomoto Y, Fujita T, Kitani Y. Serum and urine levels of phenol following phenol blocks. Can J Anaesth 1987;34:307–10.
7. Harrison LM, Morrison JE, Fennessey PV. Microtechnique for quantifying phenol in plasma by gas chromatography-mass spectrometry. Clin Chem 1991;37:1739–42.
8. Dimikis SM, Darbre A. Gas-liquid chromatography of simple phenols for urinalysis. J Chromatogr 1974;94:169–87.
9. Ahmad N, Hale K. A capillary micro assay for urinary phenols using capillary gas chromatography and optimized enzymatic hydrolysis. Clin Chem Acta 1994;230:201–8.
10. Murray KE, Adams RF. Determination of simple phenols in faeces and urine by high performance liquid chromatography. J Chromatogr 1988;431:143–9.
11. Avis SP, Hutton CJ. Acute benzene poisoning: A report of three fatalities. J Forensic Sci 1993;38:599–602.
12. Arthurs GJ, Wise CC, Coles GA. Poisoning by cresol. Anaesthesia 1977;32:642–3.
13. Green MA. A household remedy misused-fatal cresol poisoning following cutaneous absorption (a case report). Med Sci Law 1975;15:65–6.
14. Bruce AM, Smith H, Watson AA. Cresol poisoning. Med Sci Law 1976;16:171–6.

Additional information and reprint requests:
Dr. Amitava Dasgupta
Pathology Services
University of New Mexico Hospital
2211 Lomas Blvd N.E.
Albuquerque, NM 87106