
CLIN. CHEM. 37/10, 1739–1742 (1991)

Microtechnique for Quantifying Phenol in Plasma by Gas Chromatography–Mass Spectrometry
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Methods for detection and quantification of phenol have been developed primarily for use in environmental and industrial monitoring, given the widespread use of phenol as a disinfectant and antiseptic. Little information is available regarding concentrations of phenol in the blood of patients treated with phenol in regional nerve blocks (e.g., intrathecal) for temporary relief of pain or spasticity. We report a specific and sensitive method for quantifying phenol in plasma, using chemical derivatization and high-resolution capillary column gas chromatography in conjunction with mass spectrometry. The assay we describe was developed to monitor plasma concentrations of phenol in children given motor point nerve blocks with dilute phenol.

Additional Keyphrases: pediatric chemistry · nerve blocks

Phenol is of limited use in clinical treatment because of its toxic and caustic properties (1). However, very weak solutions (10–30 mL/L) can be found in topical preparations used to control microbial growth. Nonetheless, even with topical preparations, toxicity and death in humans have resulted when large areas of the body surface were exposed to phenol (2). Recently, phenol has been used in strong (~500 mg/L) solutions for “face peels” by plastic surgeons. A phenol face peel restores a clean, youthful appearance by removing the fine wrinkles of the epidermis and enhances the appearance of photoaged facial skin (3). Studies of the side effects of these face peels indicate that some form of cardiac arrhythmia was seen in 39% of the cases in which phenol solution was applied to the whole face over a short time (4, 5).

Phenol is also used in the management of spasticity (6), where neuromuscular junctions are damaged or disrupted (neurolysis), by the injection of a 50 mg/L phenol solution. Given the side effects reported for phenol, we wanted to determine concentrations of phenol in plasma during these motor point block procedures and measure concurrently cardiac irregularities (if any). In reviewing the literature, we were unable to find a

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Received April 8, 1991; accepted August 9, 1991.
simple, specific technique for quantifying phenol in human plasma (7-10). Methods for the analysis of more complex phenols were unacceptable because of the amount of background contamination in the areas of the chromatogram where phenol was eluted. We report here a specific and sensitive method for the quantification of phenol in plasma.

Materials and Methods

Materials

All reagents used were HPLC grade. Before use, glassware was oven-baked and silanized with dimethyl dichlorosilane, 100 mL, in toluene. The derivatizing agent, heptfluorobutyric anhydride (HEPTA; Pierce Chemical Co., Rockford, IL), was used as supplied by the manufacturer. Before use, triethylamine (Aldrich Chemical Co., Milwaukee, WI) was freshly distilled in the presence of 2 g of NaOH pellets. The sodium sulfate (Aldrich) was 99.99% pure, ACS-reagent grade. We identified and quantified phenol with a Model 5790A gas chromatograph (Hewlett-Packard, Allentown, PA) equipped with a 15-m DB-1 capillary column (J & W Scientific, Inc., Folsom, CA) connected to an HP Series 5970 mass-selective detector (Hewlett-Packard). Programming and data acquisition were accomplished with a Hewlett-Packard 9000 series computer. Samples were introduced into the gas chromatograph with a HP Model 7672A autosampler (Hewlett-Packard). We used helium as the carrier gas for all analyses.

Extraction

We obtained blood samples from subjects undergoing motor point block procedures. Informed consent was obtained from each subject and the procedure was approved for human study by the Internal Review Board of The Children’s Hospital. We obtained a control sample before the procedure was started and a series of samples at the end of each procedure. Samples of blood were collected in heparin, and frozen at -20 °C within minutes after acquisition until needed for analysis.

We thawed the frozen whole blood and centrifuged it to separate the plasma. A 500-μL aliquot of the plasma was added to 500 μL of distilled water supplemented with 50 μL of benzene containing a total of 5 μg of p-cresol as the internal standard. (Because of hazardous material considerations, toluene may be substituted for benzene, here and in the derivatization procedure described below.) The mixture was vortex-mixed gently for 15 s. We added 3 mL of iso-octane/diethyl ether (80/20 by vol) and gently mixed the contents for 1 h in an orbital shaker. Two milliliters of the organic solvent layer was removed and placed in a clean test tube.

Derivatization

We derivatized samples by a modification of the method of Cline et al. (7). A 100-μL aliquot of 1 mol/L triethylamine in benzene was added to the extracted phenol as a catalyst for the derivatization procedure. To this mixture we added 20 μL of HEPTA, vortex-mixed for 15 s, and incubated at room temperature for 30 min. The mixture was washed with 2 mL of a 1 mol/L phosphate buffer, pH 6.0, and the organic solvent was placed in a tube containing ~200 mg of anhydrous sodium sulfate to remove any residual water. A 500-μL aliquot of the solvent containing derivatized phenol was transferred to crimped-top vials that were compatible with the HP autosampler.

Standards

We added the internal standard to a 500-μL aliquot of human pooled plasma not containing phenol. Phenol diluted in iso-octane/diethyl ether (80/20 by vol) was added to test tubes containing the plasma and internal standard to create a standard curve containing 1, 2.5, 5, 10, and 25 μg of phenol per milliliter of plasma. The supplemented samples used for the standard curve were extracted in the same fashion as the patients’ samples.

Gas Chromatography

The gas chromatograph had an oven starting temperature of 50 °C; the injection port and transfer line were set at 250 °C. The injected sample was equilibrated for 2 min, then ramped to 300 °C at 50 °C/min and held at 300 °C for 3 min. We “baked out” each sample at 300 °C to remove residual contamination before the next injection. We made three 2-μL injections for every sample, with acetonitrile washes between injections.

Mass Spectrometry

Before analyzing the unknown samples, we obtained the complete mass spectra of derivatized phenol and p-cresol. The base peak (most intense) in these mass spectra was m/z 69, which is derived from the heptfluorobutyric moiety. A unique, abundant, high-mass ion was identified in the mass spectra of each compound. The ions that gave the strongest and most consistent signal were the molecular ions (M⁺) of the derivatized phenol (m/z 290) and p-cresol (m/z 304). A single-ion monitoring program for each of these selected ions was used in the quantitative analysis of the standard curve and the patients’ samples. Therefore, the relative abundance of phenol in the samples could be compared with the known amount of internal standard (p-cresol) added to each sample.

Results

Peak areas and height were sorted automatically by retention time for each sample. The peak-area ratio for phenol/p-cresol for each standard was plotted against the known concentration of phenol to construct a calibration curve (Figure 1). Phenol concentrations for the patients’ samples were calculated from the area ratio for phenol/p-cresol by fitting the unknown points to the calibration regression curve.

Blank plasma samples were run with every set of experimental samples to ensure the absence of background interference. The interassay coefficient of variation (CV) between different sets of samples was determined by including blood samples in each assay batch from a “quality-control pool” supplemented with either
5 or 25 mg of phenol per liter. The interassay CV averaged 5.5% for the 5 mg/L quality-control pool and 9.7% for the 25 mg/L pool. The intra-assay CV was calculated from the degree of precision between injections. The intra-assay CV for all patients' samples averaged <2%.

An example of phenol concentrations in the blood of one patient, over a typical sampling time course, is shown in Figure 2. Motor point blocks involving aqueous phenol, 50 mg/L, were administered to the patient over a 25-min interval. The total dose of phenol given during this study was 350 mg. Blood samples were taken at 5, 15, 30, 60, and 120 min after administration of the drug. The mean concentration of phenol in controls was 0.4 mg/L (SD 0.2).

Discussion

Low-molecular-mass compounds such as phenol (94 Da) present a challenging analytical problem when isolated from a complex biological matrix containing many other volatile compounds. In addition, isolation of phenol from blood and its analysis are made more difficult when organic solvents are used for the extraction or derivatization steps. Furthermore, trace contaminations in the extraction solvents or natural products in the biological matrix can interfere with the analysis. When attempting to correlate concentration data with clinical manifestations of overdose, the specificity provided by the combination of gas chromatography and mass spectrometry is required.

The method described here is rapid, precise, and sensitive. We detected 2 ng of phenol (the amount actually injected on column for the 1 mg/L standard) easily (signal-to-noise ratio >100). The sensitivity of the current analysis was 0.1 mg/L, sufficient for the measurement of plasma phenol, and adequate for the current study. However, with this procedure we could have attained low-picogram sensitivity ranges without great difficulty. Alternatively, the combination of high sensitivity and low CV suggests that phenol could be extracted and measured from 100 or 200 μL of blood with good reproducibility. Applications for which additional sensitivity is desired may require the use of a [13C]phenol in place of the p-cresol internal standard used in this study. Samples with phenol concentrations >25 mg/L (the concentration of the highest standard) were analyzed after dilution to place them within the range of the standard curve.

Occasionally peaks containing ions m/z 290 and 304 could be found in the control or analysis samples. However, the signal-to-noise ratio of these spurious ions was always low compared with that of the experimental samples (2:1 or 3:1 vs >100:1). Furthermore, by using a combination of the retention times of the authentic standards and the specific ions for phenol and p-cresol (m/z 290 and 304, respectively), we maintained specificity for phenol in the biological samples. If we had any concerns about the identity of the peak being integrated for quantification, the entire mass range could be scanned to provide full mass spectra for confirmation of structure. In this manner, we confirmed that some of our study subjects had phenol (0.4 mg/L) in their plasma before the start of the study (11). These values are in general agreement with the literature (12). Although the gas chromatography–mass spectrometry system we used for this analysis is more expensive and complicated than a simple gas chromatograph, it has numerous advantages. Its specificity is greater because both the retention times and the specific, characteristic ions are monitored simultaneously. Full scan capabilities not only enhance the capability to confirm the identity of a peak, but also help identify and eliminate consideration of the contaminants or drugs often found in the clinical samples. Lastly, the use of an autosampler greatly improves the productivity and turnaround time of the analysis, eliminating the laborious procedure of injecting the sample manually and permitting round-the-clock acquisition of data. The resulting raw data can be easily converted to phenol concentration by using a calculator or computer-driven spreadsheet such as Lotus 1-2-3.

The use of phenol in any type of clinical setting should
be accompanied by the determination of phenol concentrations in the patient's plasma. The growing acceptance of high concentrations of phenol in clinical practice for various dermatological and nerve block applications, as well as the known risk to the patient, mandates cardiac monitoring in association with rapid and accurate measurements of phenol in blood.

We thank Drs. Dennis Matthews, Department of Rehabilitation Medicine, and Reginald Washington, Department of Pediatric Cardiology, The Children's Hospital, Denver, CO, for collection and funding for the analyses of blood samples. This work was supported by NIH grant no. RR01152.

References

CLIN. CHEM. 37/10, 1742-1745 (1991)

Quantification of Serum Amyloid P by Enzyme-Linked Immunosorbent Assay
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This enzyme-linked immunosorbent assay procedure for quantifying serum amyloid P (SAP) in human plasma makes use of affinity-purified polyclonal antibodies to SAP in a "sandwich"-type format. The procedure is sensitive, reproducible, simple, and easily automatable. Results correlate well with those by a rocket immunoelectrophoresis method performed with the same antibodies. Sera from apparently normal individuals had a mean SAP content of 44.17 mg/L and increased with age.

Additional Keyphrases: amyloidosis • sex- and age-related effects

The generic name amyloidosis is applied to a family of diseases characterized by organ function disturbances attributed to amyloid deposits that replace and destroy normal tissues (1, 2). P-component has been found associated with amyloid fibrils in all forms of amyloid disease, with the possible exception of the intracerebral amyloid plaques associated with Alzheimer disease (3, 4). P-component isolated from both plasma (SAP) and amyloid deposits has the property of calcium-dependent binding to particular specific ligands (5-7). Human serum contains two known pentraxins, C-reactive protein and SAP (8). Unlike C-reactive protein, however, SAP is not an acute-phase protein (9), is glycosylated (10), and is present in serum as a complex of two pentameric discs instead of one (5, 11). SAP is synthesized in the liver and can be reproduced by hepatocytes in tissue culture (12). The short half-life of SAP, 7.8-8.5 h, suggests rapid, continuous production to maintain a stable and substantial concentration in serum, 40-50 mg/L (13-15). The function of SAP, especially its pathological role in amyloid formation or deposition, is unknown. For a better understanding of SAP, a simple assay for its determination is needed. We report here an enzyme-linked immunosorbent assay (ELISA) for the quantification of human SAP.

Materials and Methods

Serum samples. We studied sera from 150 apparently healthy subjects (100 males, 50 females) selected at the Center for Preventive Medicine (Institut Pasteur, Lille). Isolation of SAP. SAP was purified as described by De Beer and Pepys (16). The SAP preparations were analyzed without pretreatment in 4-30% gradient polyacrylamide gel electrophoresis (Pharmacia, Bromma, Sweden) precisely according to the manufacturer's instructions.

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Received March 15, 1991; accepted July 24, 1991.