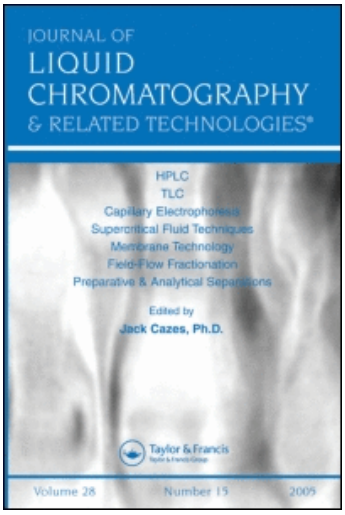

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Plasma Phenol Determination by HPLC

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PLASMA PHENOL DETERMINATION BY HPLC

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ABSTRACT

After acetonitrile deproteinization, plasma phenol was determined on a CN column with 30 % acetonitrile elution. It was detected by both an ultraviolet and an electrochemical detector. The latter detector was about 30 times more sensitive than the first one and also gave cleaner chromatograms. The method is simple and rapid.

INTRODUCTION

Phenol is used occasionally as a sympathetic blockade and as a treatment for chronic peripheral pain disease (1,2). Serum phenol level has been determined by gas chromatography (1-3) after solvent extraction and derivatization. Here we demonstrate that phenol can be determined more conveniently by HPLC on a CN with simple acetonitrile deproteinization.

MATERIALS AND METHODS

Patients: Volunteer patients for an approved special study on pain control were administered (prevertebral space) 20-40 mg/Kg of phenol. Blood samples were collected in EDTA-oxalate tubes at 0, 5, 15, 30, 60, 120, 180, 240, 300 and 1440 min after drug administration and were centrifuged to remove the red blood cells from the plasma.

Procedure: Plasma samples (100 uL) were deproteinized with 200 uL of acetonitrile and centrifuged for 30 S at 15,000 rpm in a centrifuge, (Microfuge, Beckman Instruments, Fullerton, CA). An aliquot of 50 uL of the supernatant was injected on the column for the ultraviolet detector. However, when the electrochemical detector was used, the deproteinized samples were diluted 30-fold with the mobile phase before injection on the column.

Equipment: A Model 110 A pump (Beckman Instruments) was used to deliver the solvent 30 % acetonitrile in 10 mmol/L phosphate buffer , pH 6.7 through a 100 X 8 mm (i.d.) CN (10 um) cartridge column in the RCMM module (Waters Associates, Milford, Ma) at a flow rate of 1.2 ml / min. The detection was at 280 nm by a fixed wavelength detector, Model 153 (Beckman Instruments), set at 10 mA full scale and also with an in-house-built electrochemical detector set at 1.1 V.

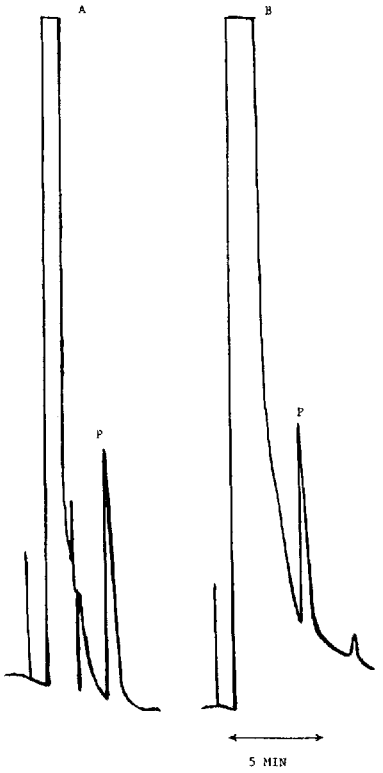

Fig 1. Representative chromatogram of: A- Standard 10 mg/L and B-Patient receiving phenol. Detection at 280 nm (P= Phenol).

RESULTS AND DISCUSSIONS

Phenol has a good absorptivity in the ultraviolet region of the spectrum with maxima at 270 and 210 nm. The absorptivity at 270 nm is about 5 times less than that at 210 nm but is more suitable for injecting deproteinized plasma samples. Phenol is also an electroactive compound. A representative chromatogram of a

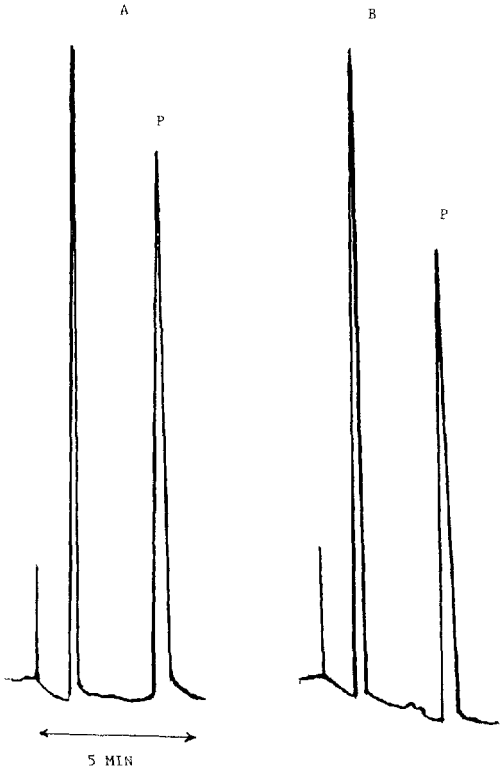

Fig 2. Representative chromatogram of: A- Standard 10 mg/L and B-Patient receiving phenol. Electrochemical Detection at 1.1 V (P= Phenol).

patient sample, 30 min after receiving phenol, with the ultraviolet detection is illustrated in Fig 1. The same sample was also detected by the electrochemical detector as illustrated in Fig 2. The chromatograms with electrochemical detection are so much cleaner and show increased sensitivity such that the samples were diluted 30-fold before injection on the column. However, from a

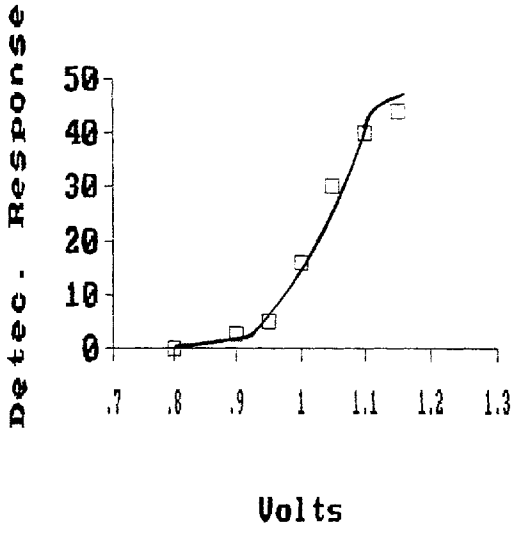

Fig 3. Relationship between peak height and electrode voltage.

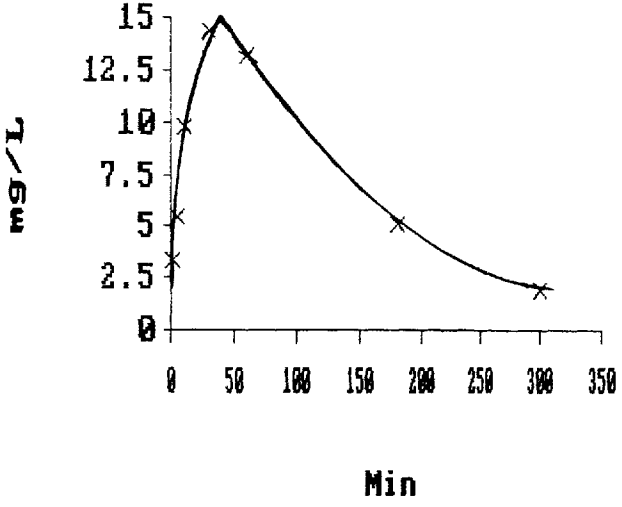

Fig 4. Kinetics of of plasma phenol after the phenol dose.

practical view point the ultraviolet detector had adequate sensitivity to detect the phenol at the doses used in this study. Thus either detector can be utilized for this assay depending on their availability in the laboratory. The relationship between the voltage and peak height for the electrochemical detection is illustrated in Fig 3. The recovery of 19 mg/L added to a pool of plasma samples was 99.4 % (n=3). Since there is no extraction or complicated steps involved in this procedure the need for an internal standard is not critical.

The plasma peak for this drug occurred about 30-60 min after the dose, Fig 4. The drug becomes undetectable about 5 hours after the dose. The mean level for 5 patients was 14.4 +- 6.7 mg/L. These results are similar to what has been reported earlier (1). Since this method avoids extraction and concentration it is much faster (about 6 min) and simpler than the gas chromatographic methods (1-3).

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