A Rapid Gas Chromatographic Method for the Determination of Free Phenol in Blood

A rapid method for the determination of free phenol in whole blood has been developed. Phenol was extracted from blood into ethyl acetate and the extract analyzed by gas–solid chromatography using the porous polymer Chromosorb 101 as the solid adsorbent. Because of the surface nature of this adsorbent, no special treatment was needed to prevent tailing of phenols. The detectability was 0.1 mg/L in 2 mL of blood, and recoveries of greater than 90% were obtained from blood fortified with known amounts of phenol.

In recent years in Australia there has been a growing interest in the use of phenol solutions to permanently defleece discrete areas around the breech, pizzle, and jowl regions of sheep. The purpose of these treatments is to lessen the incidence of blowfly strike, wool blindness, and wool staining in sheep flocks, leading to a reduction in handling costs and stock losses (Stephenson, 1977; Pratt and Hopkins, 1978).

Officers of this department investigating the effectiveness of such treatments and possible induction of toxic or stress effects in the sheep required the determination of free phenol in a large number of blood samples. A literature search indicated that gas–liquid chromatography (GLC) was the preferred method, being specific for individual phenols and very sensitive (Wengle and Hellstrom, 1971; Robers and Anderson, 1973; Bartle and Elstub, 1977). However, the polar nature of free phenol was a major problem in GLC analysis, producing peak tailing and loss of sensitivity on most stationary phases (Bartle and Elstub, 1977; Makita et al., 1978; Bhattacharjee and Bhaumik, 1977). Derivatization of phenol with various reagents prior to GLC determination and the use of specially prepared stationary phases have been employed to overcome this problem. For example, Makita et al. (1978) separated phenols as the *o*-isobutyloxycarbonyl derivatives, Wengle and Hellstrom (1971) as the trimethylsilyl ethers, and Coutts et al. (1979) as the acetate esters, while Bartle and Elstub (1977) separated free phenols using a stationary phase consisting of Tenax GC modified with poly(*m*-phenyl) ether.

The problem of extracting phenol from a biological matrix has been approached by using a variety of techniques. Distillation was used by Wengle and Hellstrom (1971), microdiffusion by Sunshine (1969), dialysis by Pemberton (1970), and solvent extraction by Roberts and Anderson (1973) and Makita et al. (1978). These methods either gave low recoveries or produced extracts which required evaporation to concentrate the phenols prior to GLC. Not only was this a time-consuming process but also Wengle and Hellstrom (1971) and Roberts and Anderson (1973) showed that even under carefully controlled conditions phenol losses of up to 60% occurred during evaporation of phenolic solutions.

A simple procedure was developed for the rapid gas chromatographic determination of free phenol in whole blood. The method eliminated the need for time-consuming concentration and derivization steps and was particularly suited to screening large numbers of samples.

MATERIALS AND METHODS

Apparatus. A Varian Aerograph 1400 gas chromatograph equipped with a flame ionization detector and a glass column (1.8 m by 4 mm i.d.) packed with Chromosorb 101 (80–100 mesh) were used. The operating parameters were as follows: injection port temperature, 220 °C; column temperature, 210 °C, detector temperature, 220 °C; nitrogen flow rate, 35 mL/min. The chromatograph was interfaced to a Spectra-Physics Systems I computing integrator which was programmed to print out retention time, peak area, and phenol concentrations in milligrams per liter in the original blood sample.

Reagents and Materials. All reagents were analytical reagent grade. Ethyl acetate was redistilled prior to use.

Preparation of Standard Solutions. An analytical standard of phenol was prepared by dissolving 1.00 g of phenol in ethyl acetate and making up the solution to 1 L. Working standards were prepared by series dilutions with ethyl acetate. A stock solution of phenol was prepared by dissolving 0.1000 g of phenol in water and diluting it to 100 mL. Calibration standards were prepared by adding suitable aliquots of this stock solution to whole blood to give phenol concentrations in the range of 0.1–50 mg/L. An extraction solution was prepared by dissolving 0.025 g of *o*-cresol in ethyl acetate and the solution made up to 1 L.

Analysis of Blood. Two milliliters of whole blood, sample or calibration standard, was transferred to a 15 × 100 mm screw-cap culture tube and 1 mL of extraction solution added. The tube was sealed, shaken by hand for 5 min and centrifuged at 1200*g* for 15 min. Two microliters of the ethyl acetate extract was drawn off in a chromatographic syringe for injection into the gas chromatograph.

Calibration. The integrator was programmed according to the Spectra-Physics Systems I handbook (Section 6C) internal standardization program 2C, and calibrated with three 2-μL injections of the 10 mg/L calibration standard, to print out phenol concentration in milligrams per liter. The integrator was recalibrated after every 15 samples, and 5 and 20 mg/L calibration standards were analyzed periodically to check the calibration.

RESULTS AND DISCUSSION

Ethyl acetate was selected as the extracting solvent, because it had the highest partition coefficient of the solvents investigated. A single extraction of 2 mL of blood with 1 mL of ethyl acetate gave better than 95% recoveries of added phenol. So that similar recoveries could be obtained with ether, a single extraction with 4 mL was required, while with chloroform, three extractions with 4-mL volumes were required. Thus, the use of ethyl acetate as extractant achieved a concentration step, avoiding the need for solvent evaporation, saving both analysis time and losses of volatile phenols.

The internal standard used was *o*-cresol. It was chosen as it has similar chemical properties to phenol and was well resolved from it. Analysis of a number of blood samples from both phenol-treated and untreated sheep indicated that *o*-cresol was not a normal constitutent of ovine blood nor was its presence induced by phenol treatment. In addition, no interfering peaks were detected in any samples

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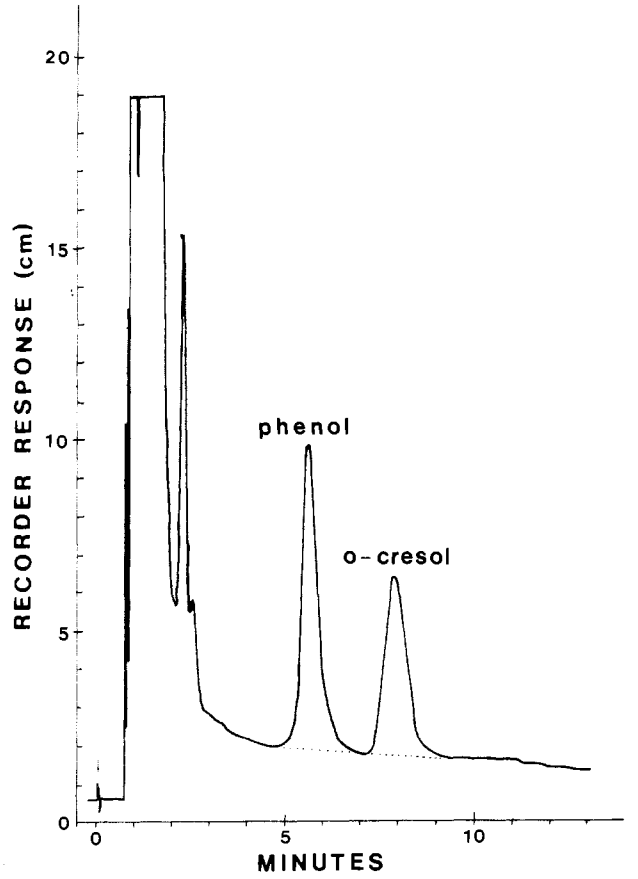

Figure 1. Chromatogram obtained after injection (denoted by arrow) of an extract solution prepared from 2 mL of ovine blood fortified with 30 mg/L phenol. The phenol peak represents 60 ng of phenol. Retention times on the Chromosorb 101 column were 5.8 min for phenol and 8.1 min for *o*-cresol.

in the region of the chromatogram where phenol and *o*-cresol peaks occurred.

A chromatogram obtained after injection of an extract prepared from ovine blood containing 30 mg/L phenol is shown in Figure 1. The retention time for phenol and *o*-cresol under the conditions described was 5.8 and 8.1 min, respectively. Because of the low affinity of Chromosorb 101 for hydroxyl groups (Johns-Manville), there is no interaction between this adsorbent and phenol or *o*-cresol, and symmetrical peaks were obtained for both compounds. A calibration plot of peak area against phenol concentration in the range 0–500 mg/L was linear and passed through the origin, indicating negligible adsorption of phenol onto chromsorb 101.

The accuracy and precision of the method were estimated by using blood samples spiked with 10 mg/L phenol. Analysis of eight separate samples, carried out on the same day, gave a mean concentration of 10.0 mg/L with a coefficient of variation of 3.4%. The recovery of phenol from spiked samples ranged from 91 to 106% with a mean of 99% (Table I). Total analysis time was 35 min/sample, and 25–30 analyses could be completed in 1 day by one operator.

With 2-μL injections and a 1:2 ratio of ethyl acetate to blood, it was possible to detect a phenol concentration of

Table I. Recovery of Phenol from Spiked Blood Samples

phenol added, mg/L	phenol found, mg/L	recovery, %
0.50	0.53	106
1.00	0.97	97
10.0	9.8	98
50.0	50.3	101
100.0	91.3	91

0.1 mg/L. As this method was intended for use in a study concerned with fairly high phenol levels, i.e., generally above 1 mg/L, no attempt was made to detect less than 0.1 mg/L. However, this could be achieved by using larger injection volumes or by altering the extraction volume ratio of ethyl acetate to blood.

Phenol may be present in blood either free or conjugated as a sulfate (Wengle and Hellstrom, 1971; Roberts and Anderson, 1973). However, as an important function of conjugation is to detoxify and excrete phenol and as this study was concerned with possible toxic and stress effects of phenol, only free phenol levels were determined. If, however, total phenol levels were required, the method could be modified to include an acid hydrolysis step, prior to solvent extraction, similar to that described by Roberts and Anderson (1973). This would add some 35 min to the analysis time.

The method has now been used in this laboratory to analyze over 100 blood samples from treated sheep, and the results of these will be reported in a forthcoming publication.

Registry No. Phenol, 108-95-2.

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