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Note

Simultaneous gas chromatographic determination of the cumene metabolites 2-phenylpropanol-1 and 2-phenylpropanol-2 in urine

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Cumene is a common constituent of many petroleum distillates. It is widely used as a thinner for paints and enamels and has become a commercial source of phenol and acetone. The acute toxicity of cumene is greater than that of benzene and toluene: rats exposed to cumene developed hyperemia and congestion in the lungs, liver and kidneys[1]. Experiments on rabbits showed that an oral dose of cumene is metabolized in the liver: 40% is converted into 2-phenylpropanol-2 (2-P-P-2), 25% into 2-phenylpropanol-1 (2-P-P-1) and 25% into 2-phenylpropionic acid. These metabolites are excreted in the urine as glucuronides[2].

After exposure to cumene vapour for 8 h, man converts 35% of the absorbed cumene into 2-P-P-2 within 48 h. The urinary metabolite was determined by gas chromatography[3].

This paper describes the simultaneous gas chromatographic determination of 2-P-P-2 and 2-P-P-1 in urine.

EXPERIMENTAL

Extraction procedure

A 100-ml volume of a 24-h urine is acidified (pH = 1; HCl) and hydrolysed in a boiling water-bath for 15min. Then the urine is rendered alkaline (pH = 9; NaOH) and extracted twice with methyl chloride. The combined organic phases are dried over sodium sulphate, filtered and taken to dryness on a rotary evaporator below 40°. The residue is taken up in 1 ml of acetone and 0.5 μl of an internal standard solution (1 ml of *n*-undecane dissolved in 10 ml of acetone) is added. A 1-μl volume of the final solution is injected into the gas chromatograph.

Gas chromatography

A Pye 104 gas chromatograph was fitted with two 9 ft. × 2 mm I.D. glass columns packed with 5% OV-17 on 100–120-mesh Chromosorb WHP. A heated dual flame-ionization detector was used. The flow-rates were: hydrogen 28, air 700 and nitrogen 20 ml/min. The injection heater was kept at 200° and the detector at 350°. After injection, the temperature in the oven was maintained at 70° for 4 min. Then

the programme was started at the rate of 4°/min until 250° was reached, and this temperature was maintained for a further 10 min. Peak areas were calculated by an electronic integrator (Vidar Model 6300). The attenuation was $4 \cdot 10^{-10}$ A f.s.d.

Calibration

Volumes of 100, 200, 300, 400 and 500 μl of an acetone solution containing 95 mg of 2-P-P-2 and 94 mg of 2-P-P-1 per 100 ml were added to 100 ml of urine. The samples were extracted and analysed as described above. The integrator counts of the individual substances were divided by the integrator counts for *n*-undecane.

The calibration line $y = mx + b$ was evaluated by using a regression analysis, where the dependent variable (y) represents the relative integrator counts and the independent variable (x) is given by the amount of substance in 1 ml of solution.

RESULTS AND DISCUSSION

Fig. 1 shows the gas chromatogram of the blank urine extract (A) and of 2-P-P-2 and 2-P-P-1 extracted from urine (B) (0.38 mg-%). In Fig. 1A it can be seen

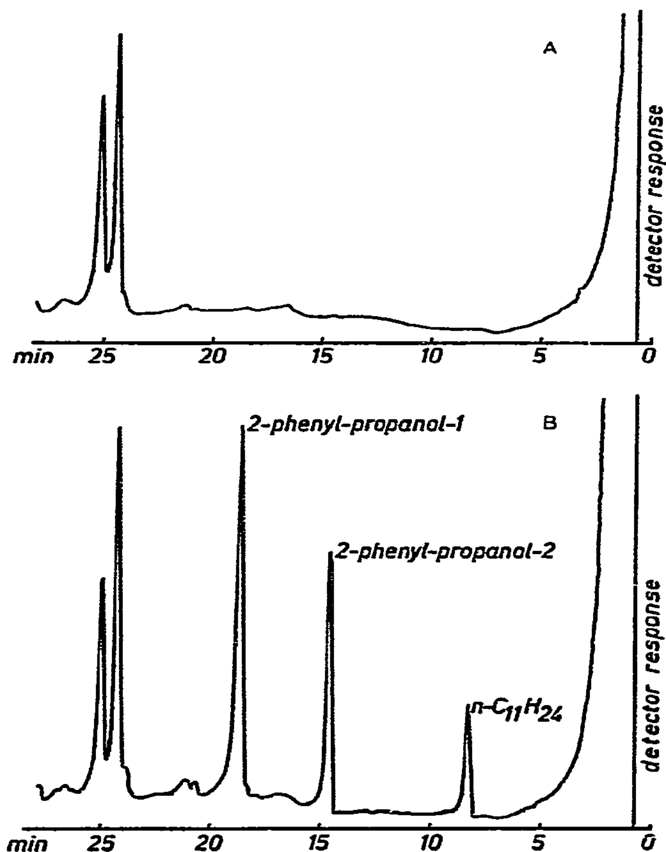

Fig. 1. Chromatogram of (A) blank urine extract and (B) 2-P-P-2 and 2-P-P-1 extracted from urine (0.38 mg-%).

that there are virtually no substances in the urine extract that interfere with either the 2-P-P-2 or 2-P-P-1 signals.

The recoveries of the individual alcohols, the constants *m* and *b* of the calibration graphs and the correlation coefficients *r* are given in Table I.

TABLE I

RECOVERY OF 2-P-P-2 and 2-P-P-1, THE CONSTANTS *m* AND *b* OF THE CALIBRATION GRAPHS AND THE CORRELATION COEFFICIENTS *r*

Substance	*Recovery (%)*	*m*	*b*	*r*
2-P-P-2	79.3	7.59	0.22	0.972
2-P-P-1	90.6	10.55	0.14	0.966

The reproducibility of the complete urine analysis was determined on ten repeated examinations: 0.3 mg each of 2-P-P-2 and 2-P-P-1 were added to 100 ml of urine, which was then analysed. The resulting coefficients of variation were 6.9% for 2-P-P-2 and 4.2% for 2-P-P-1.

The method described seems to be suitable for determining 2-P-P-2 and 2-P-P-1 simultaneously in the urine of subjects who are exposed to cumene vapour: according to Senczuk and Litewka[3], subjects who were exposed during an 8-h period to a cumene concentration near the maximal allowed concentration [245 mg/m^3 (ref. 4)] excrete about 50 mg of 2-P-P-2 per 24 h. If a 24-h urine volume between 1 and 2 l is considered, the concentration of 2-P-P-2 lies between 50 and 25 mg per litre of urine. This concentration is between 16 and 8 times higher than the concentration that can be determined with an acceptable reproducibility. Similar considerations apply to 2-P-P-1.

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