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Separation and determination of phenol, α-naphthol m- and p-, o-cresols and 2,5-xylenol, and catechol in the urine after mixed exposure to phenol, naphthalene, cresols, and xylenols

GRAŻYNA BIENIEK[1] AND T WILCZOK[2]

From the Departments of General and Analytical Chemistry,[1] and Biochemistry and Biophysics,[2] Silesian Medical Academy, 41-200 Sosnowiec, Poland

The evaluation of mixed exposure to chemical compounds may be carried out by measuring the rate of excretion of each of their metabolites in the urine, separating the compounds beforehand. It has been shown that phenol may be detected after exposure to benzene[1-4] or phenol.[4-6] Paradowski *et al* have shown that after mixed exposure to phenol and cresol the presence of phenol and o- and p-cresol may be shown in the urine of exposed workers.[7] Since the metabolism of benzene[8] and phenol compounds[9] is well known one may assume that phenolic compounds are excreted into the urine as glucuronides and sulphates[10] and that α-naphthol is also eliminated.[11] The splitting of the phenols and cresols from their carriers is made possible by the use of enzymes or by simple acid hydrolysis.[12 13] When the compounds are liberated it is easy to transfer them quantitatively into ethyl ether by simple extraction.[13] There is no published information about the levels of α-naphthol when xylenols are present in the urine of people exposed simultaneously to both phenols and xylenols. We have attempted to develop a method for simultaneously determining phenol, o-, m-, and p-cresol, α-naphthol, and 2,5-xylenol in the urine of industrial workers after mixed exposure to phenol, cresols, xylenols, and naphthalene.

Material and methods

CHEMICALS

Seventy per cent perchloric acid pa (Riedel De Haën AC Seelze-Hannower), 1-naphthol, ethyl ether, rectified spirit, chloroform, methanol, acetone. All other chemicals were reagents of analytical purity produced by Poch (Poland).

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A solution of 1% fluoroborate p-nitrobenzenediazonium in acetone was obtained by dissolving 14 g of p-nitroaniline in 30 ml of concentrated HCl together with 30 ml of water cooled to 5°C and the subsequent addition of 8 g of $NaNO_2$ in 20 ml of water. When 60 ml of 40% fluoroboric acid had been mixed with the solution, the filtered precipitate was washed with fluoroboric acid, alcohol, and ethyl ether and then dried in a vacuum desiccator. The reagent is stable, but the solution has to be prepared just before use.

Hydrolysis of urine and extraction of metabolites: 25 ml of urine in a round bottom flask was acidified with 5 ml perchloric acid and refluxed in a boiling water bath for 30 minutes. After cooling, the condenser was washed with 5 ml of ethyl ether, the contents of the flask were then transferred to a separation funnel where 10 ml of ethyl ether were added and the solution shaken. The separation procedure was repeated twice with 10 ml and 8 ml ethyl ether respectively and the combined extracts evaporated to dryness. The residue was dissolved in 1 ml of ethyl ether and used for chromatography. The absorbent used was Polyamid 11 F_{254} Merck Art 5557, the developing system was chloroform-methanol (99:1 v/v) and the developing reagent was fluoroborate p-nitrobenzenediazonium acetone solution 1%. Spectrophotometric absorption measurements were carried out on substances taken from the TLC plates after reaction with fluoroborate p-nitrobenzenediazonium in ethanol using a recording Carl-Zeiss UV-Vis spectrophotometer.

Procedure

Triplicates of 25 ml samples taken from the prepared urine extract were used for TLC chromatography on plates covered with Polyamid 11 F_{254} and developed

Average values of R_f coefficients of phenol, naphthalene, cresol, and xylenol metabolites obtained by TLC on Polyamid 11 F_{254} plates in the developing system chloroform-methanol (99:1 v/v)

Determined compounds	*Values of R_f*	*Wavelength (nm)*
2,4-Xylenol	0·59	350
2,5-Xylenol	0·57	395
o-Cresol	0·51	387
3,4-Xylenol	0·46	350
3,5-Xylenol	0·46	385
m- and p-Cresol	0·41	391
Phenol	0·35	386
α-Naphthol	0·26	390
Catechol	0·1	396

in chloroform-methanol (99:1, v/v); to develop the colour, 1% p-nitrobenzenediazonium fluoroborate in acetone was applied.

The spots obtained were quantitatively removed from the plates, extracted with ethyl alcohol, and the absorbancy of the extracts was determined spectrophotometrically against ethanol at 386 nm for phenol, 387 nm for o-cresol, 391 nm for p-cresol, 390 nm for α-naphthol, and 395 nm for 2,5-xylenol.

Results

The separation of the analysed compounds obtained under the conditions described was satisfactory with the exception of the isomers of m- and p-cresols and 3,4- and 3,5-xylenols. The table presents the R_f values and the maximum absorption wavelengths of the compound analysed. Para-nitrobenzenediazonium fluoroborate gave stable complexes with phenol, cresols, and xylenols which were visible on the TLC plates as yellow orange spots. Ethanol gave good results and removed these complexes quantitatively from the Polyamid which had been used as the sorbent.

Discussion

Under industrial conditions, mixed exposure to various compounds is common and the evaluation of exposure is based on the determination of the level of metabolites found in the urine. The separation of phenol, cresols, xylenols, catechols, and other phenolic compounds was described by Dirmikis and Darbre,[13] but α-naphthol, as a metabolite of naphthalene, was not analysed for. The methods they described, however, used gas chromatography and liquid-chromatography methods which are not widely available in factory laboratories. We have described a cheap and simple TLC method that in a relatively short time enables many analyses to be performed. To achieve the complete liberation of the metabolites present in urine in the form of glucuronides and sulphates, acid or enzymatic hydrolysis has been used.[13] The data of Dirimikis and Darbre suggest that acidification gives better results than enzymatic treatment.[13] The acid hydrolysis carried out in our experiments with the use of perchloric acid splits all the complexes of phenols, cresols, xylenols, and naphthols. All the liberated compounds are readily extracted with ethanol. The difficulty noted in the separation of the isomers of m- and p-cresol are comparable with those observed when gas or liquid chromatography was used.[13] Paradowski, however, has stated that exposure to m-cresol is not important from the toxicological point of view.[7] The separation procedure described here may be used to measure levels of phenol, o-cresol, m- and p-cresols, α-naphthol, catechol, and 2,5-xylenol in the urine of workers exposed simultaneously to phenolic compounds or aromatic hydrocarbons such as benzene and naphthalene.

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