Thin layer chromatography of p-aminophenol in urine after mixed exposure to aniline and toluene

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ABSTRACT A simple method of evaluating p-aminophenol in the urine of people exposed simultaneously to aniline and toluene relies on separating p-aminophenol from hippuric acid and other physiological components of the urine by thin layer chromatography. The adsorbents and developing system have been thus fixed to make possible the separation of p-aminophenol from hippuric acid, urea, and creatinine and their quantitative determination. This method also makes possible the determination of p-aminophenol in urine in the presence of hippuric acid. Hippuric acid is a physiological component of urine and also the metabolite of toluene, so the determination of p-aminophenol is possible also after simultaneous exposure to both compounds: aniline and toluene. At the same time the concentrations of urea and creatinine as additional factors may be determined. The limit of detection of the method is: 5 µg/ml for p-aminophenol, 9 µg/ml for hippuric acid, 8 µg/ml for urea, and 6 µg/ml for creatinine.

The evaluation of occupational exposure to aniline in the presence of toluene can be carried out by analysing the concentrations of p-aminophenol in urine.¹⁻⁴ Because p-aminophenol is excreted in the urine partially in the free form and partially in the form of glucuronides and sulphates, the quantitative determination of p-aminophenol is possible only after enzymatic hydrolysis using β -glucuronidase or after acid hydrolysis.⁵ Methods for the colorimetric determination of p-aminophenol have been described.^{6,7}

Material and methods

After enzymatic or acid hydrolysis⁵ had been carried out the released p-aminophenol was extracted by ethyl acetate and the concentrated extract was used for thin layer chromatography.

Adsorbents used—Plates with adsorbents: MN Kieselgel G (Macherey Nagel + CO 516 Düren), Kieselgel HF₂₅₄ (Macherey Nagel + CO 516 Düren), Kieselgel GF₂₅₄ (type 60) Art 7730 E Merck Darmstadt, Kieselgel HF₂₅₄ + 366 Mach Stahl (E Merck AG Darmstadt) Art 7741.

Developing system—Butanol saturated with water. Butanol is shaken with water, and the butanol layer used after the layers have separated.

Developing reagents—Reagent A—for localisation

of p-aminophenol and urea. To 12 g of butanol saturated with water, 0.15 g of dimethylaminobenzaldehyde and 0.6 ml of concentrated hydrochloric acid at $d = 1$, 18 g/ml are added. Reagent B—for localisation of creatinine an acetone solution of fluoroborate p-nitrobenzenediazonium is prepared after dissolving 14 g of p-nitroaniline in 30 ml of concentrated HCl with 30 ml of water cooled to 5°C and 8 g of NaNO₂ in 20 ml of water added. Then 60 ml of 40% fluoroboric acid was mixed with the sample. The filtered precipitate was washed with fluoroboric acid, alcohol, and ether, and then dried in a vacuum desiccator. Reagent B is stable, but the solution has to be prepared just before using. Reagent C—for localisation of hippuric acid; 2 g of p-dimethylaminobenzaldehyde⁷ is dissolved in 100 ml of acetic anhydride. The development of colour spots is achieved after heating at about 105°C for 3–5 minutes.

ELUTION

Spots of creatinine and hippuric acid were eluted by using 95% ethyl alcohol. Spots of p-aminophenol and urea were eluted by chloroform or acetone. The absorption spectra of colour compounds obtained as the result of the reaction between these compounds and the developing reagents in specially fixed solvents were recorded spectrophotometrically.

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Table 1 Recommended developing reagents, elution systems, and wavelengths for *p*-aminophenol, urea, creatinine, and hippuric acid determination

Compound	Developing reagent	Colour of compound	Eluting solution	Wavelength
<i>p</i> -Aminophenol	A	Yellow orange	Chloroform	446
			Acetone	445
Urea	A	Yellow	Chloroform	435
			Acetone	427
Creatinine	B	Carmine yellow	Ethanol 95%	423
Hippuric acid	C	Orange	Ethanol 95%	465

Table 2 Average R_f values obtained for creatinine, hippuric acid, urea, and *p*-aminophenol using the developing system: butanol saturated with water in the presence of given adsorbents

Compound	Adsorbent			
	MN Kieselgel G	Kieselgel HF ₂₅₄	Kieselgel HF ₂₅₄₊₃₆₆	Kieselgel GF ₂₅₄
Creatinine	0.18	0.24	0.16	0.25
Hippuric acid	0.41	0.39	0.24	0.30
Urea	0.52	0.52	0.37	0.38
<i>p</i> -Aminophenol	0.71	0.81	0.73	0.58

Procedure

Into 5 ml of urine 5 ml of water was added and acidified with 1.5 ml of concentrated H_2SO_4 ($d = 1.84 \text{ g/ml}$). The solution was heated for one hour in boiling water. The product of hydrolysis was extracted twice with 10 ml of ethyl acetate.

The extract was concentrated to the volume of 1 ml, and 25 μl put on to activated chromatographic plates. The chromatogram was developed in butanol saturated with water. When the chromatographic plates dried out, they were sprayed with the developing reagent as quickly as possible. Next, the elution and measurement of absorbance were carried out at the wavelengths characteristic for each of the compounds (table 1).

Results

Satisfactory separation of *p*-aminophenol from other components of the urine (hippuric acid, urea, and creatinine) was achieved as shown in table 2.

Quantitative determination of the compounds can be carried out after the development of the chromatograms by using the developing reagents that produce various colour compounds with hippuric acid, urea, creatinine, and *p*-aminophenol separated from each other. After the plate was sprayed with reagent A, the orange yellow spot of *p*-aminophenol and the yellow spot of urea were obtained and eluted by chloroform or acetone. Creatinine and hippuric acid gave no colour reaction. After the plate was developed by reagent B the carmine yellow spot of creatinine was obtained and this was eluted by ethyl alcohol. Urea,

p-aminophenol, and hippuric acid gave no colour reaction under this condition.

After the plate was sprayed with reagent C, the pale yellow unstable spot of urea, the orange spot of hippuric acid, and the faint pale yellow spot of creatinine were obtained. Reagent C may be used for the simultaneous determination of hippuric acid⁸ after mixed exposure to toluene and xylene.

Table 1 gives recommendations for the developing reagents for the quantitative determinations of *p*-aminophenol, creatinine, urea, and hippuric acid, eluting solutions, colour compounds, and characteristic wavelengths of absorptions.

Discussion

The developing system best able to separate *p*-aminophenol, hippuric acid, urea and creatinine on silica gels was butanol saturated with water.

Table 2 shows R_f values of *p*-aminophenol, hippuric acid, and creatinine. The developing reagent that located *p*-aminophenol best on chromatograms was acidified butanol (*p*-dimethylaminobenzoic aldehyde solution).

Reagent A also reacts with urea, but the developing system (butanol saturated with water) makes the separation of *p*-aminophenol and urea on silica gels possible.

Creatinine can be localised on chromatograms by the application of acetone solution (*p*-nitrobenzenediazonium fluoroborate). When the quantitative determination of hippuric acid in urine without analysis of *p*-aminophenol is to be carried out a more simple method of determination has been proposed. This relies on direct extraction by

using chloroform and separation by the use of thin layer chromatography⁹ and colorimetric determination.

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