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Short Communication

Thin-layer chromatography for detection of aromatic acids in urine after occupational exposure to aromatic compounds*

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Introduction

The estimation of the burden by substituted aromatics is very important in occupational health as well as in environmental medicine for the recognition of possible health risks, at locations such as gas-filling stations, congested roads and chemical, printing and foodstuff factories. Indoor and outdoor measurements only provide information about the available amounts of hazardous agents but nothing about their absorption in the body. Additionally, toxicokinetic and toxicodynamic factors have to be considered. These factors depend, for example, on the physical activity and genetic profiles of the individuals concerned. The assessment of absorbed harmful agents is normally made by determination of their metabolites in urine. The problem of their specific determination is the large number of endogenous aromatic compounds in urine coupled with the need for rapid analysis of a large number of samples. The published photometric [1], GC [2–4], LC [5, 6], isotachophoretic [5] and TLC procedures [7, 8] generally employ complicated extractions and derivatizations that are impractical for screening applications that may also require quantification. For these purposes, TLC should be a viable, economic and selective method [9] for which the selectivity can also be modified by post-chromatographic derivatization [10].

Methods

TLC of hippuric acids

Hippuric acids are the major metabolites of the methylbenzenes [11]. Samples were diluted with water, and 5 μl spotted directly onto silicagel thin layers containing soluble starch as a binder (Silufol™, CSSR or Empore™, UK). The plates were then developed as follows: for hippuric acid (HA) (the main metabolite of toluene) with a 10-cm run in chloroform–acetone–glacial acetic acid (40:10:5, v/v) (Fig. 1); for methylhippuric acids (metabolites of xylenes) with toluene–methanol–nitromethane–glacial acetic acid (5:8:2:2, v/v); and for chlorohippuric acids (metabolites of chlorotoluenes) with nitrobenzene–ethylacetate–glacial acetic acid (45:8:5, v/v). After drying the chromatograms at 130°C the hippuric acids were detected with a solution of 6.25% *p*-dimethyl-aminobenzaldehyde (pDAB) in ethylacetate containing 25% acetic anhydride, heated at 130°C again for 10 min for semiquantitative evaluation. It was also possible to quantify the spots either by densitometry at 495 nm or by photometry at 468 nm after elution with ethylacetate.

TLC of mandelic and phenylglycilic acid

Mandelic acid (MA) and phenylglyoxilic acid (PGA) are the main metabolites of styrene and ethylbenzene, respectively. The preparation of

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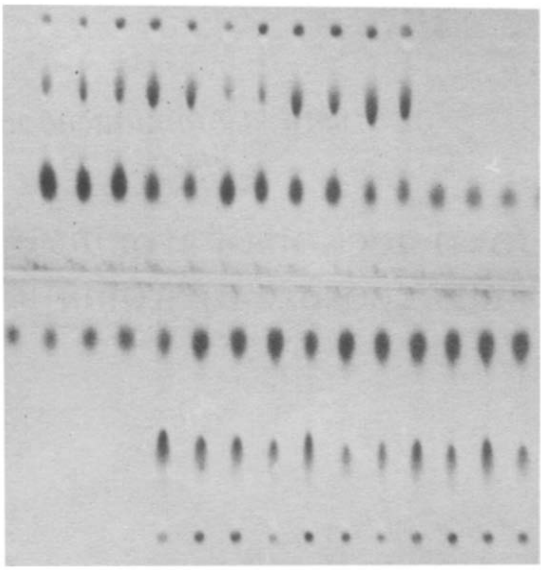

Figure 1
TLC of hippuric acid (HA) in urine on Silufol™, CSSR (10-cm run, spotted on two sides of a 20 × 20 cm plate). Spots after the origin 1, urea; 2, hippuric acid. From the left: lanes 1–4; HA-standard; lanes 5–15, urine samples.

the urine samples ensued as described before and 5 µl were spotted directly onto Silufol™ (CSSR) and developed with toluene–acetonitrile–glacial acetic acid–acetone (30:8:4:1, v/v) (Fig. 2).

These metabolites could also be separated on Merck–Kieselgel 60-UV 254™ (FRG) with *n*-hexane–butanone–ethylacetate–glacial acetic acid (10:30:1:1, v/v). However, that separation was inadequate for UV detection and MA and PGA were detected with a selective reagent such as Tetrazolium Blue, TB).

UV-detection. MA, HA and PGA were determined either densitometrically (225–270 nm) or photometrically after elution with 0.2 M HCl. Quantitative measurements of HA and PGA were made at 235 and 255 nm, respectively. For the determination of MA derivative spectroscopy in the range of 210–230 nm (second derivative) was more useful because it accentuates the absorption of the CH(OH)—COOH-chromophore which appears as a shoulder at 217 nm [12]. Figure 3 shows the incomplete resolution of MA on Merck–Kieselgel 60-UV 254™-foil.

Visible detection. Screening for MA, PGA and HA was also possible by spraying the chromatogram with a 0.5% TB solution in methanol–conc. ammonia (1:1, v/v) and exposure to light (366 nm) for 75 min (Fig. 4). After semi-quantitative evaluation it was then possible to quantify either by densitometry (475–540 nm) or by elution with methanol and further TLC separation with chloroform–ether–*n*-butanol (90:10:5, v/v) to remove un-

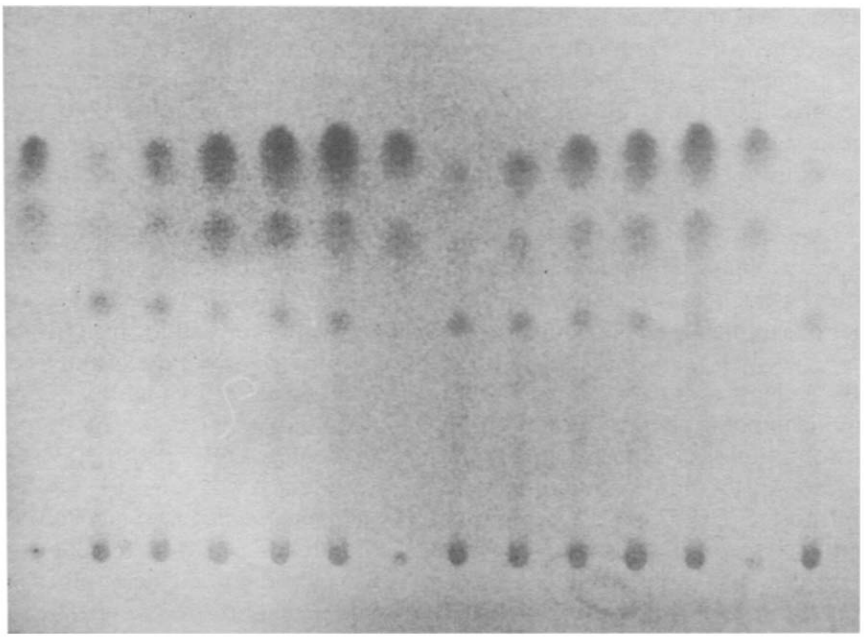

Figure 2
TLC of hippuric- (HA), mandelic- (MA) and phenylglyoxilic acid (PGA) on Silufol™ (CSSR) after UV 254 nm detection: Spots after the origin: 1, HA; 2, MA; 3, PGA from left: lanes 1 and 13 MA/PGA-standard; lane 14 control urine, other lanes spiked urine.

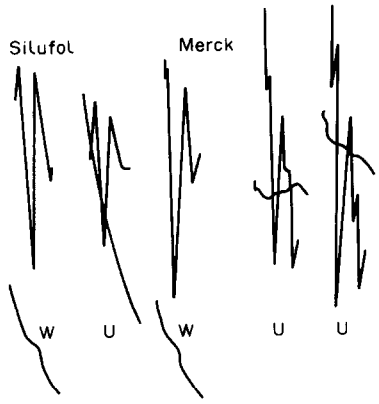

Figure 3
Derivative spectroscopically curve of mandelic acid (MA) after separation on Silufol™ (CSSR) and Merck Kieselgel 60 UV 254 (Specord M 40, VEB Carl Zeiss, Jena, second derivative). From left after separation on Silufol™ in water (lane 1) and urine (lane 2). On Merck–Kieselgel 60 UV 254™ in water, lane 3; and urine, lanes 4 and 5.

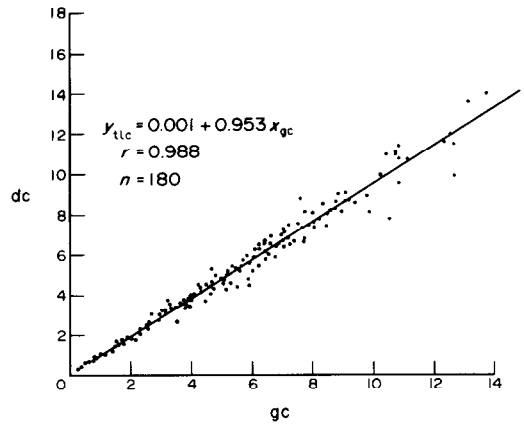

Figure 5
Comparison of the determination of hippuric acid in urine by GC and TLC by linear regression.

reacted TB. PGA and the more important MA were measured photometrically at 450 and 515 nm, respectively.

Results and Discussion

Figure 5 is a linear regression analysis of the results obtained by TLC with those obtained by GC for the analyses of some 180 urine samples. The results (Fig. 5) indicate that the two methods gave equivalent quantitative results. The RSD for the TLC method was 2.85% which compares with a value of 1.2% for analysis by GC. The limit of detection for the TLC analysis of HA was 15 μg ml^{-1} of urine. The advantage of the TLC method is that it allows the rapid simultaneous screening of many samples on the same plate. More than 200 samples of urine can be screened in a single working day. It has been the experience of this

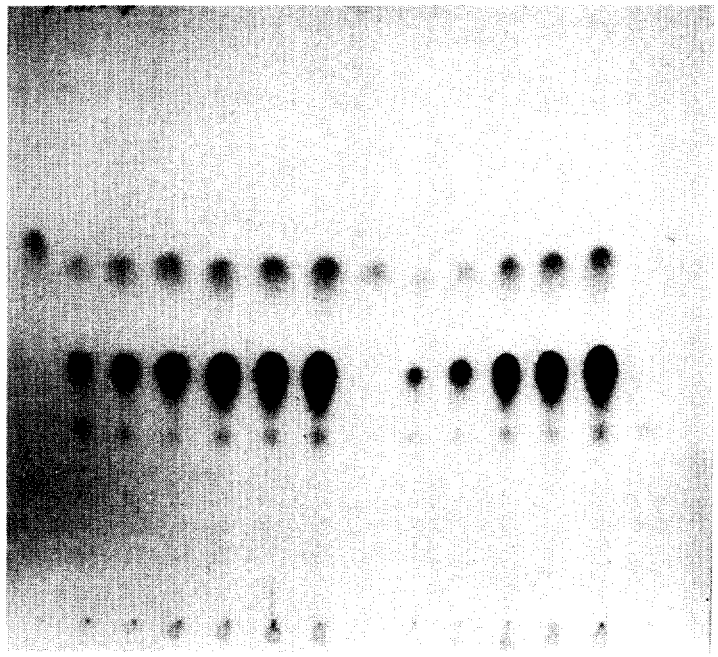

Figure 4
TLC of hippuric- (HA), mandelic- (MA) and phenylglyoxilic acid (PGA) on Silufol™ (CSSR) after Tetrazolium Blue detection (visible) Spots (see Fig. 2) from left: lanes 1 and 8 PGA-standard, lane 9 MA-standard, lanes 2–7, and 9–13 urine samples, lane 14 control urine.

group that proper collection of urine samples is important if an accurate measure of the exposure to potentially harmful aromatic compounds is to be obtained. Collection of the urine passed during the last 4 h of a workshift appears to give the best indication of exposure to potentially harmful substances absorbed from the atmosphere in the workplace. It was also found important to correct for differences in urine volume. This was done in a semi-quantitative fashion by dilution of the samples and determination of the relative concentrations of xenobiotics compared with an endogenous marker. Salicyluric acid did not interfere with the determination of HA.

The metabolites of styrene and ethylbenzene were well separated on Silufol UV 254™ (CSSR) with detection by densitometry or UV photometry. MA, the major metabolite of styrene, can be determined with high sensitivity by derivative spectrometry or, with lower sensitivity, by measurement at three wavelengths (214, 217 and 221 nm), making use of the relationship (equation 1)

$$\Delta E_{217} = E_{217} - \frac{E_{214} + E_{221}}{2} . \qquad (1)$$

In contrast with UV detection, selective detection of MA, PGA and HA for screening purposes was possible after reaction with a spray reagent containing Tetrazolium Blue (TB). The limit of detection of MA by TLC was 50 $\mu g\ ml^{-1}$ of urine.

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