A NEW HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF 1-AND 2-NAPHTOLS IN URINE

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A sensitive, specific and rapid high-performance liquid chromatographic (HPLC) procedure has been developed for the detection and determination of urine levels of 1- and 2- naphtols.

Urine samples were adjusted to pH 1.5 and extracted with dichloromethane. HPLC on a Micropak CN-10 column with a fixed wavelength U.V detector (254nm) and hexane: dichloromethane: isopropyl alcohol (89:10:1) as solvent system allowed the measurement of 1-naphthol at 100ng ml-1 and 2-naphtholat 500ng ml-1 levels. The utility of this method for the detection and determination of 1-and 2-naphthols in urine of jaundiced neonates inadvertently exposed to naphthalene was demonstrated.

Naphthalene (1) is widely used as a moth-repellant in form of mothballs, flakes and deodorant cakes. 1 Naphthalene itself is not toxic but on absorption following dermal and/or inhalation exposure, its major oxidative metabolites, 1-and2-naphthols have been implicated in neonatal jaundice. 2,4. There is no report in literature for the determination of these metabolities in urine of such neonates. However, a number of gas liquid chromatographic (GLC) and HPLC methods for the determination of 1-naphthol/2 naphthol in some biological fluids and insecticide preparations have erarlier been reported. 5-9

The GLC methods 5,6 involved time consuming extraction, derivatisation and purification procedures whilst the HPLC methods 7-9 appeared unsatisfactory due to the poor resolution of the two metabolites envisaged. This paper describes a rapid, specific and sensitive method for the determination of these metabolites. The method was applied to urine of jaundiced neonates.

MATERIALS AND METHODS

1-naphthol, 2-naphthol, 2,4-dichlorophenol, isopropyl alcohol (BDH); dichloromethane, n-hexane and hydrochloric acid(Koch-Light). The solvents were analar and glass distilled before use.

The high performance liquid chromatograph (varian model 5000) was fitted with a 10ul manual loop injector, a fixed wavelength UV detector (254nm) and a prepacked CN-10 column (10um, 30cm x 4mm i.d.) The flow rate of the mobile phase was 2ml min-1. Chromatograms were recorded at 1cm min - 1 chart speed. A solution containing hexane: dichloromethane: isopropyl alcohol (89:10:1) was filtered and prepared freshly daily and used as mobile phase. Elution was performed at room temperature.

Solutions of 1-naphthol in methonal (500ug ml^{-1}) and 2-naphthol (500ug ml^{-1}) were used to spike urine samples. 2,4 Dichlorophenol dissolved in methanol (500ug ml^{-1}) was used as the internal standard.

Quantitation

1ml control urine from neonates was measured into screw-capped extraction tubes and spiked with the standard solutions to give final concentrations of 0.25, 1.0, 2.0, 3.0, 4.0 and 5.0ug ml-1 for 1-naphthol and 0.1, 0.25, 0.50, 1.0, 1.5, 2.0 and 2.5ug ml-1 for 2-naphthol. The spiked urine samples were adjusted to pH 1.5 using 2M hydrochloric acid. 50ul of the internal standard solution and 6ml of dichloromethane was added to each tube. The tubes were vortex mixed with a whilmixer for 2 minutes and centrifuged for 10 minutes at 2,000g to separate the layers.

The dichloromethane layer was transferred to tappered and evaporating tubes and evaporated to dryness under a stream of nitrogen gas at 40°C. The residue was reconstituted in 50ul of dichloromethane and 10ul of the solution was injected onto the column.

A standard curve was prepared by plotting the ratios of the peak heights (1-and 2-naphthols- Internal Standard) against concentrations expressed as ug ml^{-1}. The levels of recovery of 1- and 2-

naphthols in urine were determined by comparing peak heights of the spiked urine with those of naphthol standards of same concentration by direct injection.,

Samples Collection and assay

Urine samples of jaundiced neonates were collected within 24 hours of admission into the hospital either by attachment of uring bag or by suprapubic baldder aspiration. 1 ml of each urine sample collected was assayed using the procedure described above. The urine levels of the metabolites were determined from the calibration curve.

RESULTS AND DISCUSSION

Under the described chromatographic conditions 1-naphthol was eluted at 8.3 min from the column well separated from its isomer, 2-naphthol which appeared at 10.6 min. The retentionof the internal standard was 7 min (Fig.1). This result showed a marked improvement in resolution compared to earlier methods 7,8. The detection limit was found to be 100 and 50ng of 1 and 2 - naphthols ml -1 of urine respectively. A linear regression analysis was performed for the calibration curves prepared from naphthol spiked urine samples. It resulted in the equations $y = 0.404x + 0.041$ and $y = 0.971x + 096$ respectively for 1 - and 2 - naphthols where y is the ratio of the height of the 1- or 2-naphthol peak to the peak height of the internal standard and x is the concentration of 1-and 2-naphthols in urine.

Recoveries of 1- and 2-naphthols from urine were nearly quantitative at concentration of 1.0 - 3.0ug ml-1 for 1-naphthol and 0.5 - 1.0ug ml-1 for 2-naphthol with average recoveries ranging from 80.8 - 85.6% and coefficient of variation from 1.25 - 7.30% for both within run and betweenrun assays (Tables 1 and II).

In an assay of fifty urine samples of jaundiced neonates, twenty-five with history of exposure to naphthalene and the others without exposure, none of the latter group showed the presence of 1-and 2-naphthols. However, five of the samples of the exposed group showed peaks consistent with that of 1-naphthol, while no peak due to 2-naphthol was detected.The small number of cases with positive 1-naphthol detection may be due to possible time lag between exposure, manifestation and report to the hospitalas well as the level of exposure.That only 1-naphthol is picked up is due to the fact that naphthalene is almost exclusively oxidatively metabolised by liver microsomes to 1-naphthol.

The utility of this method has, however, been demonstrated in clinical situations and further work on the clinical implications of our findings viz-a-viz G6PD deficiency, bilirubin level, etc. are the subject of further investigations in our laboratories.

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Table 1: Recovery of 1-naphthol and 2-naphthol from urine

Sample	Concentration *ug ml-1)	Mean recovery +S.D.%	n
1-naphthol	1.0	82.8 ± 3.6	4
	3.0	82.1 ± 1.1	4
2-naphthol	0.5	80.8 ± 7.3	4
	1.5	85.6 ± 7.7	4

Table 2: Precision of the analytical method.

Sample	Concentration ug ml^{-1}	C.V. %	n
Within-Run			
1-naphthol	1.0	3.7	4
	3.0	1.3	4
2-naphthol	0.5	7.3	4
	1.5	2.5	4
Between-Run			
1-naphthol	1.0	6.7	8
	3.0	2.0	8
2-naphthol	0.5	5.8	8
	1.5	3.6	8

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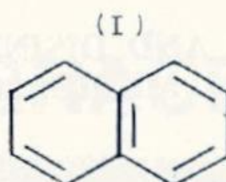
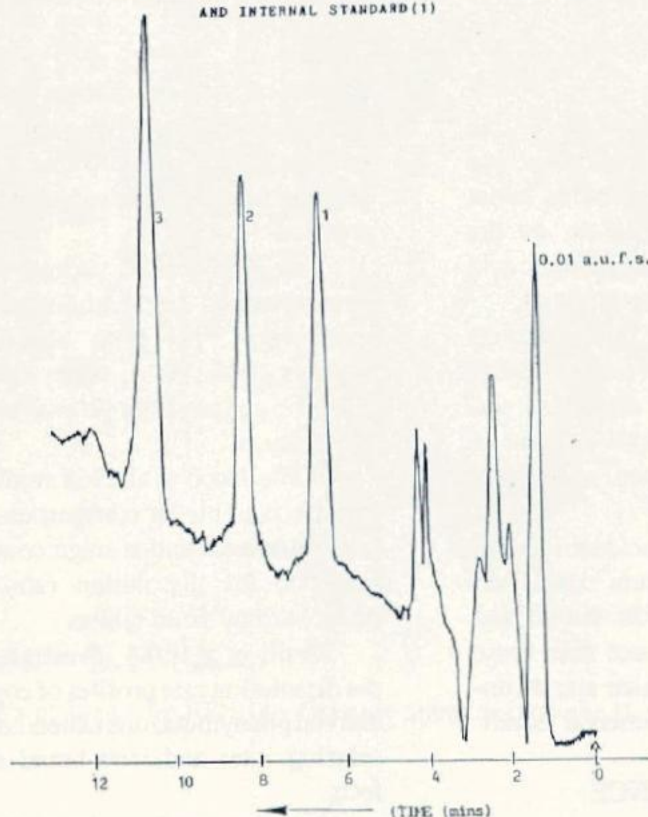


FIG. 1: HIGH-PERFORMANCE LIQUID CHROMATOGRAM OF EXTRACTED SPIKED URINE CONTAINING 1-NAPHTOL(2), 2-NAPHTOL(3), AND INTERNAL STANDARD(1)



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