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Note

Detection of mefenamic acid and its metabolites in urine by thin-layer chromatography

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Mefenamic acid —N-(2,3-xylyl)anthranilic acid, Ponstan®; Parke-Davies (Pontypool, Glam, Great Britain)— is a non-narcotic analgesic used in the management of rheumatoid arthritis[1–4]. Fluorimetric[5] and colorimetric[6] assay methods for this drug have been reported but, for screening purposes, thin-layer chromatography (TLC) would be a more convenient technique. The characteristic pattern of spots obtained for mefenamic acid and its metabolites is a definite aid to detection.

EXPERIMENTAL

Plates were prepared from a slurry of silica gel G (30 g) in water (65 ml). Layers of thickness 250 μ were prepared and dried at 110° for 30 min. Reference solutions of mefenamic acid and synthetic N-(2-methyl-3-carboxyphenyl)anthranilic acid (metabolite 'A') in methanol were prepared (1 μg/μl)[5,7].

Sodium nitrite (1% in 1% sulphuric acid) was used as the location reagent.

For the extraction procedure urine (10 ml) was treated with sodium hydroxide (10 *M*, 1 μl) for 20 min at room temperature. The sample was then acidified with concentrated hydrochloric acid and extracted with petroleum ether (b.p. 40–60°; 10 ml) for 10 min on a mechanical shaker. The organic layer was separated, placed in a 10-ml conical tube, and the solvent removed.

Urine (10 ml) was boiled for 15 min with concentrated hydrochloric acid (1 ml) in an autoclave at 15 p.s.i. The sample was then extracted with petroleum ether (b.p. 40–60°; 10 ml) and, after separation, the solvent was distilled off from the organic layer. Pure mefenamic acid and synthetic metabolite 'A' were each subjected to this treatment.

In each case, the extract was reconstituted with methanol (100 μl) and a 30-μl aliquot of the solution was used for spotting, along with 10-μl aliquots of the appropriate reference solutions. The solvent systems used for the development of the chromatograms were toluene–acetic acid (9:1) and (97.5:2.5), for the non-

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autoclaved and the autoclaved extracts and reference samples, respectively. In all cases, location of the spots was effected by spraying the plates with an acidified solution of sodium nitrite (1% in 1% sulphuric acid).

N-(2-Methyl-3-carboxyphenyl)anthranilic acid was prepared by a standard procedure[8] from *o*-chlorobenzoic acid and 3-amino-2-methylbenzoic acid in the presence of copper powder and potassium carbonate[9].

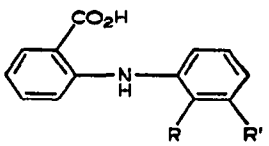
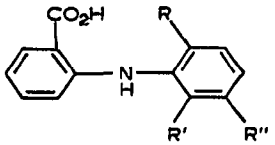
RESULTS AND DISCUSSION

The structures of mefenamic acid and its metabolites are shown in Table I (i).

Being secondary amines, the compounds form coloured N-nitroso derivatives on treatment with nitrous acid in the cold and this reaction has been used in the development of the analytical procedure described here for their selective detection. The selectivity of the proposed procedure for mefenamic acid and its derivatives has been confirmed by applying it to urine samples from hospital patients on treatment with a variety of commonly used drugs, such as barbiturates, paracetamol, aspirin, various antidepressants like amitryptyline, imipramine, etc. None of these interfered with the detection of Ponstan.

TABLE I

STRUCTURES OF FENAMIC ACIDS AND METABOLITES

Structure	*Name*	*Substituents*
(i) 	Mefenamic acid	$R=R'=CH_3$
	Metabolite 'A'	$R=CH_3$, $R'=CO_2H$
	Metabolite 'B'	$R=CH_3$, $R'=CH_2OH$
(ii) 	Flufenamic acid	$R=R'=H$, $R''=CF_3$
	Meclofenamic acid	$R=R'=Cl$, $R''=CH_3$

In the organism, mefenamic acid and its metabolites are present as the corresponding acyl glucuronides and are excreted as such in the urine. Treatment of urine with dilute alkali followed by acidification is used in order to hydrolyse the acyl glucuronides to the free compounds which are then solvent extracted and spotted on TLC plates. The R_F values and the colours of the spots due to these compounds are listed in Table II. Authentic samples of mefenamic acid and metabolite 'A' used as reference compounds in this work gave identical R_F values and colours as the spots due to the corresponding compounds extracted from urine.

An additional procedure which may be necessary in cases where a positive distinction must be made between mefenamic acid and the structurally related,

TABLE II

R_F VALUES OF MEFENAMIC ACID AND ITS METABOLITES

Compound	*R_F Values*			*Colour after treatment with nitrous acid*	
	Toluene–acetic acid (9:1)	*Toluene–acetic acid (97.5:2.5)*			
Mefenamic acid	0.75	0.57	0.99*	green	green-blue*
Metabolite 'A'	0.45	0.16	0.44*	yellow-green	green-purple
Metabolite 'B'	0.28	0.08	0.26*	yellow-green	purple*
Flufenamic acid	0.73	0.60	0.91*	dark green	blue-green*
Meclofenamic acid	0.74	0.60	0.98*	pale yellow	yellow*

* Decarboxylated.

but far less often prescribed drugs flufenamic and meclofenamic acid —see Table I (ii)— has also been developed. This differs from the method already described in that (a) the urine sample is treated with concentrated hydrochloric acid in an autoclave at 15 p.s.i. prior to extraction, and (b) the solvent system used for the development of the chromatogram is toluene–acetic acid (97.5:2.5). Autoclaving causes decarboxylation of diphenylamine-2-carboxylic acids[10] and thus TLC of the decarboxylated compounds gives a new set of R_F values and spot colours (Table II).

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