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GAS–LIQUID CHROMATOGRAPHY OF N-ACETYL-*p*-AMINOPHENOL (PARACETAMOL) IN PLASMA AND URINE

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SUMMARY

A procedure is described for determining unconjugated paracetamol in plasma and urine. The method, based on gas chromatography of the unchanged drug, is more rapid than a spectrophotometric assay and has a limit of detection of 2 μg/ml.

Plasma and urine levels are reported after therapeutic and overdose ingestion.

INTRODUCTION

The methods already described[1–5] for the determination of paracetamol in body fluids normally require hydrolysis of the drug to *p*-aminophenol, followed by a coupling reaction to form an azo dye which is then estimated spectrophotometrically. The reaction time for the hydrolysis in all of these procedures is usually about 1 h, so that when extraction and colour development are also included the analysis becomes lengthy and time consuming. This is a disadvantage for the clinical biochemist or toxicologist investigating cases of therapeutic and overdose ingestion.

ROUTH *et al.*[1] have recently compared a hydrolysis assay of this type with an assay employing the combination of the free radical dipicrylhydrazyl with paracetamol, and also with differential absorbance measurements of paracetamol at 266 nm. The dipicrylhydrazyl reaction with paracetamol also requires 1 h for its completion and was considered by the authors to be the least accurate of the techniques. The differential absorbance method in our hands gave higher blanks than those obtained by the authors and, more important, the molar extinction of paracetamol was not large enough for the measurement of some therapeutic samples.

A method has been developed therefore, using gas chromatography (GC) which is more rapid and sensitive than the procedures previously reported.

MATERIALS AND METHODS

Paracetamol was given to laboratory staff in the form of two 500 mg BP tablets per person or two Panasorb tablets (Sterling: Winthrop, Research and Development Division, Newcastle-upon-Tyne).

All other chemicals were obtained from Hopkin & Williams Ltd., Chadwell

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Heath, Essex. Diethyl ether (analar grade) was redistilled and the ammonium sulphate purified by washing with ether.

Diphenyl phthalate was used as the internal standard for the GC measurements. Two separate methanolic solutions were made up to contain: (i) 0.4 mg per 100 ml and (ii) 4 mg per 100 ml, respectively; the former being added when measuring therapeutic doses in the range 0.2–2 mg of paracetamol per 100 ml and the latter for overdoses in the range 2–20 mg of paracetamol per 100 ml.

Extraction of paracetamol from plasma

1 ml of plasma, derived from venous blood, was saturated with 3 g of solid ammonium sulphate and extracted twice with 15 ml of ether. The sample was centrifuged after each extraction and the ether layers pooled. 1 ml of the internal standard solution (i) or (ii) was added to the organic layer. The extract was transferred in small portions to a 10 ml conical tube (BC/C14T Quickfit & Quartz, Stone, Staffordshire) and evaporated to dryness under a stream of air. The residue was then carefully reconstituted in 0.1 ml of ether and 5 μl injected into the gas chromatograph.

Extraction of paracetamol from urine

Interfering compounds in the urine can be removed by the use of a Florisil column. A BTL chromatographic column, Type 2A, I.D. 1 cm (Baird & Tatlock, Chadwell Heath, Essex) was filled to a height of 8 cm with a slurry of Florisil which was then washed with 10 ml of distilled water and the excess allowed to drain. 30 ml of urine were then passed through this column, the first 10 ml were rejected and the remainder was collected. 1–5 ml of eluate was then saturated with solid ammonium sulphate and extracted twice with 15 ml of ether. Subsequent analysis was carried out by adding 1 ml of the internal standard solution and proceeding as described for plasma.

Gas–liquid chromatography

A Pye 104 gas–liquid chromatograph, equipped with a flame ionisation detector, was used for the analysis. The column was a 7 ft. glass coil previously silanised for 24 h with 5 % dimethyldichlorosilane in benzene, and packed with 2 % FFAP on Aeropak 30, mesh size 70–80 (Varian Aerograph, Fife, Great Britain). This was conditioned at 100° for 48 h without the carrier gas flowing. A further 48-h conditioning at 250° was made with nitrogen flowing at 60 ml/min. The instrument settings were: injection temperature, 280°; column temperature, 240°; detector temperature, 280°; gas flow rates: air, 400 ml/min; hydrogen, 45 ml/min; nitrogen, 60 ml/min; sensitivity 2×10^{-10} A.

A gradual deterioration of the column response and sensitivity occurred after three months continual use. Reconditioning of the column was necessary when this happened.

Measurements

Stock solutions were prepared so as to contain 20 mg of diphenyl phthalate in 100 ml of ether and 100 mg of paracetamol in 100 ml of ethanol–ether (5:95). These were diluted with ether so as to prepare a range of standard solutions containing 40 μg/ml of diphenyl phthalate and from 20–200 μg/ml of paracetamol, at intervals of 20 μg/ml. To construct the calibration curve, 2–5 μl of each of these samples were injected

on to the column. The retention times of paracetamol and diphenyl phthalate were 6.5 min and 11 min, respectively. The ratio of the peak heights of paracetamol to diphenyl phthalate was linear with respect to the concentration of paracetamol (Fig. 1). This line did not pass exactly through the origin, probably due to the slight tailing of the paracetamol peak. However, the calculation of paracetamol concentration from peak height ratio was used for the sake of simplicity, rather than the more precise peak area ratio which passed through the origin.

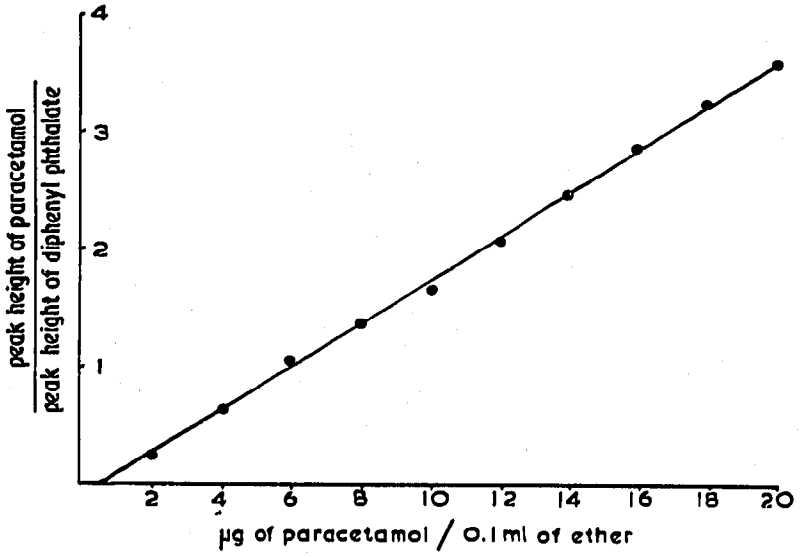

Fig. 1. The standard calibration graph of the ratio of the peak heights of paracetamol and diphenyl phthalate against the number of µg of paracetamol in the extract.

The concentration of the samples was calculated from this calibration curve by measuring the ratio of the peak heights of paracetamol to diphenyl phthalate in the sample and adjusting the figure obtained to account for the amount of internal standard employed. Thus no adjustment was necessary if the 0.4 mg/100 ml diphenyl phthalate solution was used. However, if the larger 4 mg/100 ml diphenyl phthalate solution was employed, the result must be multiplied by a factor of 10.

Interferences

There is the possibility that this assay may be applied to samples where phenacetin has been ingested and, therefore, its behaviour was investigated on the FFAP column. Phenacetin eluted after 1.5 min, while *p*-aminophenol (another metabolite of phenacetin that might be extracted) did not elute. This was expected since the hydroxy group renders it more polar than paracetamol.

Common drugs manufactured in tablet form in combination with paracetamol are aspirin, codeine and orphenadrine. Orphenadrine did not chromatograph, aspirin appeared in the solvent peak and codeine had a retention time of 7.5 min.

RESULTS

This method has been used for the analysis of therapeutic and overdose samples. Tables I and II show the plasma levels obtained when six normal adults had taken 1 g

of paracetamol BP or the equivalent amount of paracetamol in the form of a Panasorb (a mixture of paracetamol and sorbitol in the proportion 5:1). Free paracetamol plasma levels were generally lower than those obtained by spectrophotometric methods. However, GWILT *et al.*[6] have demonstrated that the absorption characteristics of paracetamol tablets differ with the source of the tablet, thus valid comparisons between our findings and those of other authors cannot be made. The difference between the plasma levels of paracetamol BP and Panasorb in the two tables shows that, usually, Panasorb is more rapidly absorbed to give higher levels than paracetamol B.P. Individual variations in the maximum plasma levels attained was quite

TABLE I

PLASMA PARACETAMOL LEVELS IN μg/ml

Volunteer				*Hours after ingestion of 1 g paracetamol B.P.*								
	Sex	*Age (yr)*	*Weight (kg)*	¼	½	1	1½	2	3	4	5	6
J.G.	M	34	73	—	7.1	10.1	9.2	8.2	4.3	3.2	1.7	0.9
D.B.	M	25	68	—	6.4	7.8	6.1	4.7	2.9	1.3	0.4	0.4
G.W.	M	24	73	—	0.4	0.3	4.2	5.7	3.9	3.1	2.9	1.9
L.C.	F	21	51	0	0	0	0.3	8.0	9.9	2.9	—	0.9
F.H.	F	22	70	0	3.4	13.3	9.2	7.0	4.1	2.7	—	0.7
B.H.	F	19	57	0	0	0	2.1	6.2	2.2	0.4	—	0

TABLE II

PLASMA PARACETAMOL LEVELS IN μg/ml

Volunteer				*Hours after ingestion of 1 g paracetamol in the form of Panasorb*								
	Sex	*Age (yr)*	*Weight (kg)*	¼	½	1	1½	2	3	4	5	6
J.G.	M	34	73	4.2	5.0	5.1	4.2	3.2	1.7	0.7	—	0
D.B.	M	25	68	8.9	14.8	10.3	8.4	6.3	3.1	2.1	—	0
G.W.	M	24	73	1.8	5.5	3.9	2.9	2.4	0.5	0.4	—	0
L.C.	F	21	51	3.6	4.1	6.8	8.5	14.3	11.1	5.4	—	1.6
F.H.	F	22	70	1.2	11.9	11.9	8.9	5.7	4.7	2.7	—	0.8
B.H.	F	19	57	0	0.8	3.2	7.3	16.8	6.4	6.7	—	3.0

TABLE III

TOTAL FREE PARACETAMOL VOIDED IN 24 h AFTER INGESTION OF PARACETAMOL AS PERCENTAGE DOSE

Volunteer		*Paracetamol*	*Panasorb*
J.G.	M	2.2	5.6
D.B.	M	4.4	18.9
G.W.	M	2.0	8.0
L.C.	F	3.5	16.7
F.H.	F	2.1	14.7
B.H.	F	3.3	1.3

marked, falling between 5.7 and 13.3 μg/ml for paracetamol BP and 5.1 and 16.8 μg/ml for Panasorb.

Table III shows the free paracetamol found in the urine voided in the first 24 h after ingestion. Volunteers had considerably more free paracetamol in their urine after taking Panasorb.

Table IV gives some results obtained using the method described when following the progress of some cases of paracetamol overdose.

TABLE IV

PATIENTS INGESTING A PARACETAMOL OVERDOSE

Patient	*Plasma levels (mg %)*	*Urine levels (mg %)*	*Remarks*
Female (43 years) took 50 tablets	On admission 27.8 6 h later 18.8 36 h later 1.1	1st day 195	Peritoneal dialysis containing albumen. 20 l cleared approximately 0.75 g paracetamol.
Female (23 years) took 40 tablets	12 hours after admission 5.3	1st day 20.9	
Male (28 years) access to 97 tablets	On admission 13.6 1 h later 15.4 2 h later 11.4 4 h later 8.7 8 h later 1.6		Stomach washout with 10 l of warm tap water. 5 l of 1 *N* saline and 5 % dextrose infusion over 14 h.
Female (25 years) access to 200 tablets	5 days after admission 0.8		Plasma level taken before cross circulation with husband for 10 h. None found in husband after circulation. Died 10 days after admission.

DISCUSSION

The ability of the stationary phase FFAP to separate polar compounds, such as fatty acids, indicated that paracetamol might be similarly analysed by GC. This has been substantiated and, although some tailing of the paracetamol peak occurs it is by no means unacceptable when so much time and accuracy is gained. The ratio of blood paracetamol/plasma paracetamol concentration has been established by GWILT *et al.*[3] as being 1.1. Therefore, since no major difference in paracetamol concentration occurs between plasma and red cells, plasma was taken for analysis, ether extracts of whole blood giving unsuitable gas chromatograms.

Recoveries of paracetamol from water at the 5 mg/100 ml level were quantitative when saturated with either ammonium sulphate or sodium chloride. However, when plasma was saturated with ammonium sulphate slightly better recoveries were obtained than with sodium chloride. An average recovery of 95 % was obtained from plasma, in triplicate determinations, at levels of 0.5, 1.0, 1.5, 2.0 and 20.0 mg of paracetamol per 100 ml.

Fig. 2 shows the chromatogram obtained from a blank plasma extract without the addition of the internal standard, diphenyl phthalate. Fig. 3 shows an extract from the plasma of a volunteer taking paracetamol. The unidentified peak at 13 min

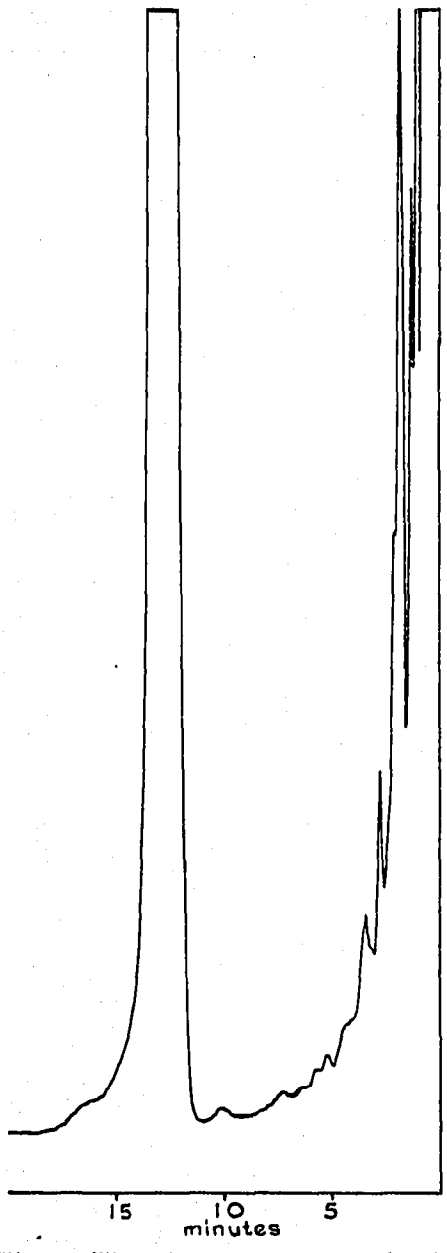

Fig. 2. The chromatogram obtained from 1 ml of a normal blank plasma without addition of the internal standard diphenyl phthalate.

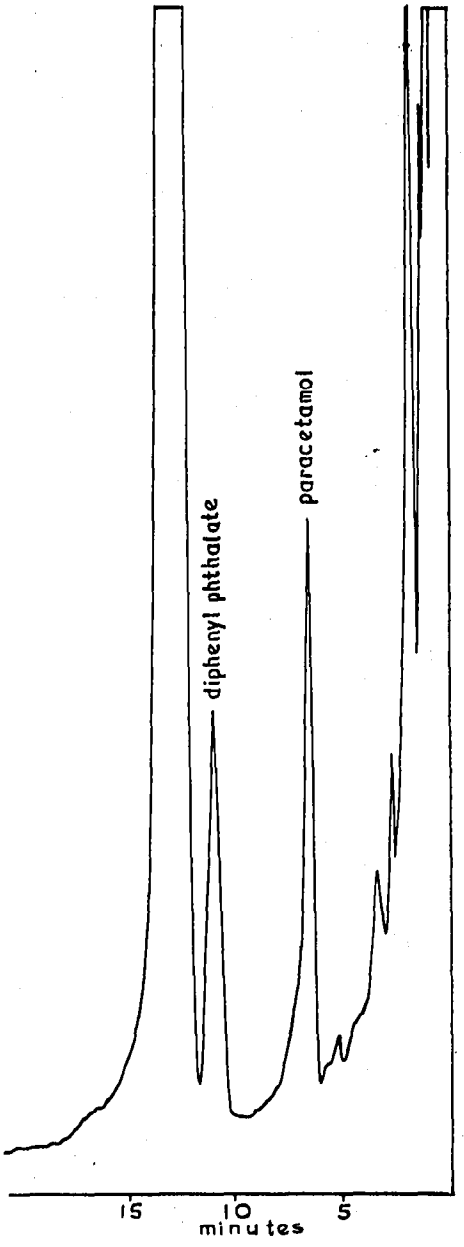

Fig. 3. The chromatogram obtained from 1 ml of plasma from a volunteer taking paracetamol.

(found in all plasma extracts), although close to the internal standard peak, can be removed by protein precipitation prior to extraction. However, the addition of another step to the analysis and the lowering of the recovery did not offer any major advantage.

The passage of urine through Florisil columns as a means of purification prior to analysis has already been noted[7,8]. When paracetamol was added to urine and passed through a Florisil column and subsequently analysed, quantitative recoveries demonstrated that the drug was not absorbed on the column. In cases of overdose, however,

when the free paracetamol level is high and, quite often, after the therapeutic doses when the urine is only lightly pigmented, this step may be safely omitted.

The ingestion of an overdose of paracetamol can be extremely dangerous because of the associated liver damage. The present method may be useful in correlating plasma levels with the prognosis of cases of self poisoning and may be applied to the study of regimes designed to eliminate paracetamol from the body.

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