AN ECD-GLC TECHNIQUE FOR THE DETERMINATION OF PARACETAMOL IN HUMAN BIOFLUIDS

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A quantitative, rapid, selective and sensitive method for the determination of paracetamol in biofluids is essential for investigations such as poisoning due to acute overdose, research into factors affecting drug absorption in the G.I.tract when the drug is used as a marker, and studies on pharmacokinetics and bioavailability of this drug in various innovative dosage forms. The generally accepted GLC methods (Prescott, 1971) suffer from interference peaks which often mask analytical peaks or prolong analysis time (Thomas & Coldwell, 1972; Kalra, Mamer & others, 1977).

We have developed an ECD-GLC technique (using 10% Apiezon L on Diatomite 80-100 mesh in a 5ft., $\frac{1}{4}$in. o.d. glass column at 240°C, nitrogen carrier at 60ml/min. and a Pye104 gas chromatograph fitted with an ECD) which is rapid and specific and can measure therapeutic concentration of paracetamol in a 0.1ml plasma or urine sample. The method involves an ethereal extraction of the drug and internal marker (N-butyryl-p-aminophenol) from the biofluid, followed by pentafluorobenzylation using pentafluorobenzyl bromide and potassium carbonate as the base catalyst (Scheme 1).

This method has several advantages over those previously reported. Only a small sample of biofluid (0.1ml) is required for analysis and this enables blood-sampling from finger tip punctures. The high sensitivity of the assay allows a more thorough study of the fate of paracetamol in the body. The selectivity of ECD gives a cleaner chromatogram and enables analysis of up to 10 samples per hour (Fig.1). A disadvantage is that all organic solvents used should be distilled twice. Using this technique a pharmacokinetic study of an oral dose of Panadol (1G) in a fasting subject was performed. The results obtained (an elimination $t_{\frac{1}{2}}$ of 2.69 hr. and a 24 hr. urinary recovery of 5.57% as unchanged drug) compare favourably with previously reported data and indicate that the technique is reliable. This method has also been used successfully for the determination of indomethacine in human biofluids (Sibeon, Chan & others, 1978).

SCHEME 1. Analysis of paracetamol in biological fluids

STEP 1: Plasma or urine (0.1ml), water (0.9ml), 30µg internal marker, 20µl 5NNaOH, extracted with 8ml of diethyl ether	Discard ethyl layer to remove basic, and lipid-soluble substances
STEP 2: Aqueous layer, 20µl 5NHCl, 1ml phosphate buffer (pH 7.4), 1G $(NH_4)_2SO_4$, extracted with 2x6ml diethyl ether	Evaporate organic extract to dryness by nitrogen stream
STEP 3: Add K_2CO_3 (25mg), 0.1% pentafluorobenzyl bromide in acetone (0.5ml) to the dry residue; incubate at 50°C for 30min. Cool to room temperature	Evaporate organic extract to dryness by nitrogen stream
STEP 4: Add water (0.5ml) to dissolve K_2CO_3; extracted with n-hexane (1ml)	Inject 1µl to GLC fitted with ECD

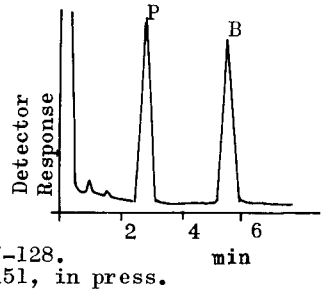

Fig.1 Gas chromatograms of pentafluorobenzylated paracetamol (P) and N-butyryl-p-aminophenol (B) from a plasma extract treated with pentafluorobenzyl bromide.

Prescott, L.F. (1971). J.Pharm.Pharmac. 23, 807-808.
Thomas, B.H. & Coldwell, B.B. (1972). Ibid., 24, 243.
Kalra, J., Mamer, O.A. & others (1977). Ibid. 29, 127-128.
Sibeon, R., Chan, K. & others (1978). J.Chromatogr. 151, in press.