COMPARISON OF ANALYTICAL METHODS FOR STUDYING PARACETAMOL METABOLISM AFTER OVERDOSE

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In recent years there has been increasing interest in the use of high field NMR spectroscopy for the analysis of endogenous and exogenous substances in body fluids. Bales et al (1984) showed that paracetamol and its major metabolites can be detected and quantified in urine samples following administration of a normal therapeutic dose. In the present study, the urine of patients who had taken paracetamol overdose and been treated with methionine or Parvolex (acetylcysteine) was examined by NMR. The results were compared with conventional HPLC and spectroscopic analysis of the same samples.

Samples for NMR were prepared by addition of 10% D_2O as 'lock' and valine (2 mg/ml) as internal standard. 400 MHz 32K ^1H spectra were obtained with a Jeol GX400 spectrometer using a 45° pulse, a pulse delay of 5s and 128 scans. The signal due to water was suppressed by homo-gated decoupling. Resolution enhancement was achieved by applying a Gaussian function to the FID. The spectra were referenced on the higher field doublet of valine (0.99ppm) which does not shift in the pH range of urine. Metabolites were quantified by integrating peaks arising from the acetyl methyl signals which occur in a region of the spectrum clear of signals in normal urine.

HPLC analysis utilising UV detection was performed on the samples taken for NMR using a modification of the method of Adriaenssens and Prescott (1978). Quantification of the individual paracetamol metabolites (paracetamol glucuronide and sulphate) was as for paracetamol but following specific enzymic degradation of the conjugates.

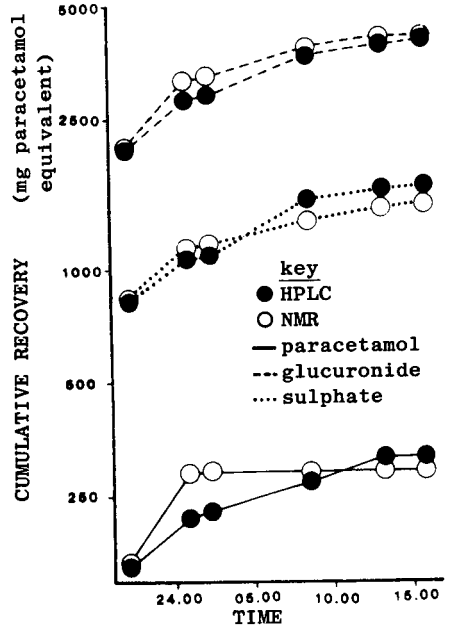

Fig 1. Urinary excretion of paracetamol after overdose.

Colorimetric analysis was performed as a comparison to the above methods since this is the routine method of analysis in hospital laboratories. The method used in this study was that of Chafetz et al (1971). Again, the individual metabolites were analysed following enzymic hydrolysis of the conjugates. The results obtained by NMR and HPLC analyses for a representative patient are presented in Fig. 1. The results obtained by visible spectroscopy are not presented but were shown to give artificially high values for paracetamol and its metabolites since selective extraction of the individual compounds is not possible by this method. The NMR and HPLC results were comparable. Some of the variation could be accounted for by the differences in sample preparation: NMR required no pre-treatment whereas the HPLC method required enzymic hydrolysis. There is no doubt that a method such as NMR where individual components may be quantified simultaneously with no need for prior extraction/hydrolysis is of benefit in the study of drug metabolism.

Adriaenssens, P. I., Prescott, L. F.(1978) Br. J. Clin. Pharmac. 6: 87.
Bales, J. R. et al (1984) Clin. Chem. 30: 1631.
Chafetz, L. et al (1971) J. Pharm. Sci. 60: 463.