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Short Communication

A rapid method for the isolation and identification of drug metabolites from human urine using solid phase extraction and proton NMR spectroscopy

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Recently a number of applications of proton NMR spectroscopy to drug analysis in untreated biological fluids have been reported [1, 2]. The technique enables rapid multicomponent analysis to be performed on plasma and urine samples with a minimum of sample preparation. As part of an investigation on the application of this approach to the study of drug metabolism the use of solid phase extraction (a technique used previously in sample preparation for high-performance liquid chromatography) has been examined in order to simplify the spectra as an aid to interpretation.

One of the model compounds used in the work is the non-steroidal anti-inflammatory drug, naproxen (*d*-2(6-methoxy-2-napthyl)-propionic acid). Samples of urine were obtained following administration of a single oral dose of 500 mg to a female subject taking the drug as treatment for suspected osteoarthritis. Urine samples were collected as voided, and 2-ml aliquots were freeze-dried and redissolved in D_2O. These samples were then analysed by proton NMR on a Bruker WM 250 Fourier transform NMR spectrometer. The spectra were measured at ambient probe temperature (25 ± 1°C) using 16 384 data points over a 3401.36 Hz sweep width and an acquisition time of 2.4 s. The spectra obtained were the result of collecting up to 350 free induction decays using 90° pulses with a delay of 5 s between pulses. In order to suppress signals due to residual water protons a secondary irradiation field was applied at the resonance frequency of water (4.8 ppm), the decoupler being gated off during acquisition in order to minimize decoupler noise breakthrough.

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Examination of the spectrum of the sample obtained for the period 0–5.5 h after administration of the dose revealed a number of drug-related signals. Thus, signals due to the protons of the aromatic nucleus, methoxyl and side-chain methyl groups, and the characteristic doublet of the anomeric proton of glucuronic acid (indicating the presence of glucuronide conjugates), were all present (Fig. 1a).

This sample was then acidified with 10 μl ml^{-1} of formic acid (98/99% m/m) and applied to a 3-ml Bond Elut® column (Analytichem International purchased from Jones

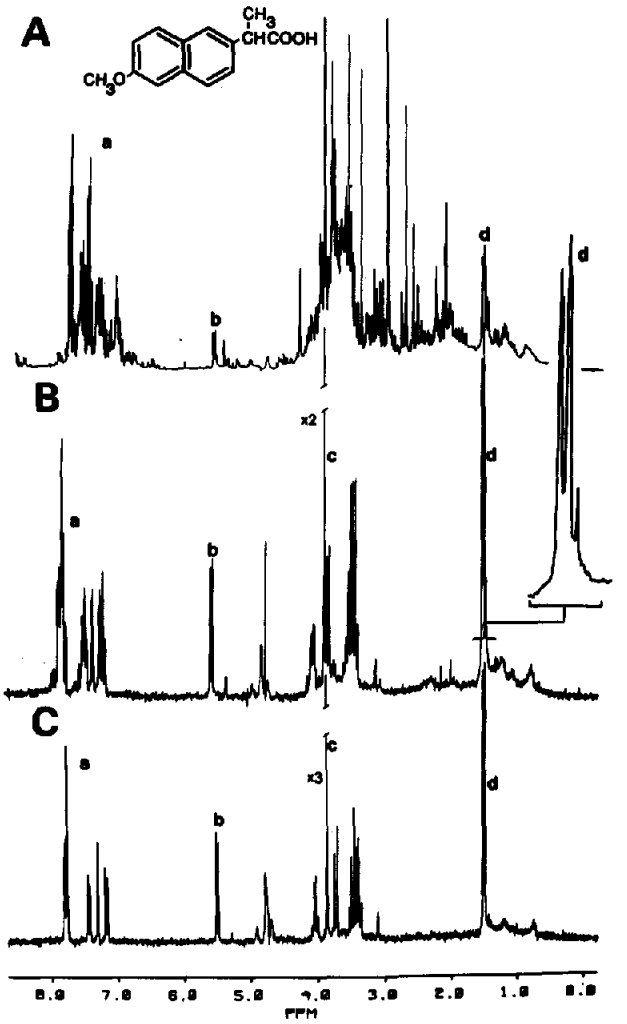

Figure 1
(A) A proton NMR spectrum of a urine sample (treated as described in the text) containing naproxen (structure top left) and its metabolites.
(B) The mixture of *O*-demethyl naproxen and naproxen, both present as glucuronides, eluting from the Bond Elut column with acidified D_2O–deuteromethanol (60:40, v/v). An expansion of the region between 1 and 2 ppm is given to show the signals due to the α-methyl group of the drug and *O*-demethyl metabolite.
(C) Naproxen glucuronide eluted with D_2O–deuteromethanol (40:60, v/v).
Key: a = aromatic protons; b = anomeric proton of D-glucuronic acid (as a glucuronide); c = *O*-methyl group; and d = α-methyl group.

Chromatography Ltd, Glamorgan, UK). The column had been activated prior to sample application by washing with 5 ml of methanol and then 5 ml of water. Once the sample had been applied, the column was washed with 2 ml of acidified D_2O followed by stepwise gradient elution with 5 ml of each of the following mixtures of acidified D_2O and deuteromethanol: 80:20 (v/v), 60:40 (v/v), and 40:60 (v/v). Each fraction was collected separately; the deuteromethanol was removed under a stream of nitrogen, and the remaining D_2O was removed by freeze-drying. The dry residues were then redissolved in 1 ml of D_2O and analysed by proton NMR.

All the endogenous contaminants were eluted in the early fractions. Drug-related material was not eluted until the deuteromethanol content of the solvent reached 40%. Analysis by NMR showed that this fraction contained two components, the parent drug and its *O*-desmethyl metabolite. Both were present as glucuronides (Fig. 2b). In the 60% deuteromethanol fraction the principal signals were due to naproxen glucuronide (Fig. 2c).

In the past the isolation of drug metabolites, particularly conjugates, from biological fluids for spectroscopic identification has been a lengthy and tedious process usually requiring several chromatographic steps. To obtain an unambiguous spectroscopic identification of metabolites using a simple and rapid procedure of the type described in the present work is noteworthy. However, preliminary experiments using this approach to study other compounds (in both plasma and urine) suggest that this result is not exceptional and that the technique may be broadly applicable to the investigation of drug metabolism.

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