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Thermospray liquid chromatography–mass spectrometry for the characterisation of sulphate ester conjugates

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ABSTRACT

Formation of polar conjugates is a well documented metabolic pathway for xenobiotics containing phenolic hydroxyl groups. This paper describes the analysis of two sulphate ester conjugates by fast atom bombardment mass spectrometry and thermospray liquid chromatography–mass spectrometry. Thermospray liquid chromatography–mass spectrometry proved the more succesful technique for obtaining the molecular weight of the intact conjugate, but only by removal of the buffer from the high-performance liquid chromatography eluent.

INTRODUCTION

Sulphate conjugation of phenolic compounds is a well documented metabolic pathway [1]. Characterisation of sulphate conjugates has often involved acid or specific enzyme hydrolysis and subsequent analysis of the hydrolysed product. More recently both fast atom bombardment mass spectromy (FAB-MS) and thermospray liquid chromatography–mass spectrometry (LC–MS) have been used for sulphate ester analysis. At present FAB is the MS technique of choice, provided that the sample is sufficiently pure [2].

Thermospray LC–MS of these conjugates isolated from biological fluids have been reported [3,4], however, sensitivity, which is often an important factor in metabolism studies, has been limited. Evidence of the intact molecular species is an essential part of metabolite structure elucidation. Unfortunately, when ammonium acetate buffer is used in the mobile phase, the mass spectral data often shows little, if any, indication of this species. However, published data by Watson *et al.* [5] have shown the analysis of intact steroid conjugates by thermospray LC–MS with the absence of buffer in the mobile phase.

A series of metabolic studies were performed for two drugs, salbutamol (Fig. 1) a β_2-adrenoceptor agonist used in the treatment of asthma and ondansetron (Fig. 2) a $5HT_3$ receptor antagonist used as an anti-emetic in cancer chemotherapy. Following oral administration of salbutamol and ondansetron, suspected sulphate ester conjugates were isolated from marmoset urine and dog bile, respectively. Here we report

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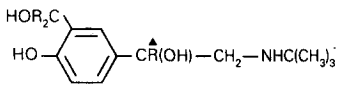


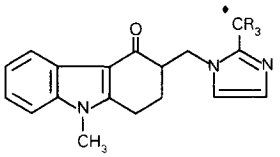


Fig. 1. The structure of salbutamol. R = H: Salbutamol; R = ^2H: [$^2H_3$]salbutamol; ▲ = Position of the tritium label.

Fig. 2. The structure of ondansetron. R = H: Ondansetron; R = ^2H: [$^2H_3$]ondansetron; ◆ = Position of the carbon-14 label.

the analysis of these intact sulphate conjugates by thermospray LC–MS following unsuccessful attempts by FAB-MS.

EXPERIMENTAL

Animal experiments

A male common marmoset received a single oral dose, 5 mg/kg, of a 1:1 mixture of salbutamol and its deuterated analogue with a trace amount of [^{3}H]salbutamol (40 μCi, 1.46 MBq), (Fig. 1). Urine was collected over the period 0–24 h and the major metabolite isolated by solid-phase extraction and semi-preparative high-performance liquid chromatography (HPLC) (Fig. 3).

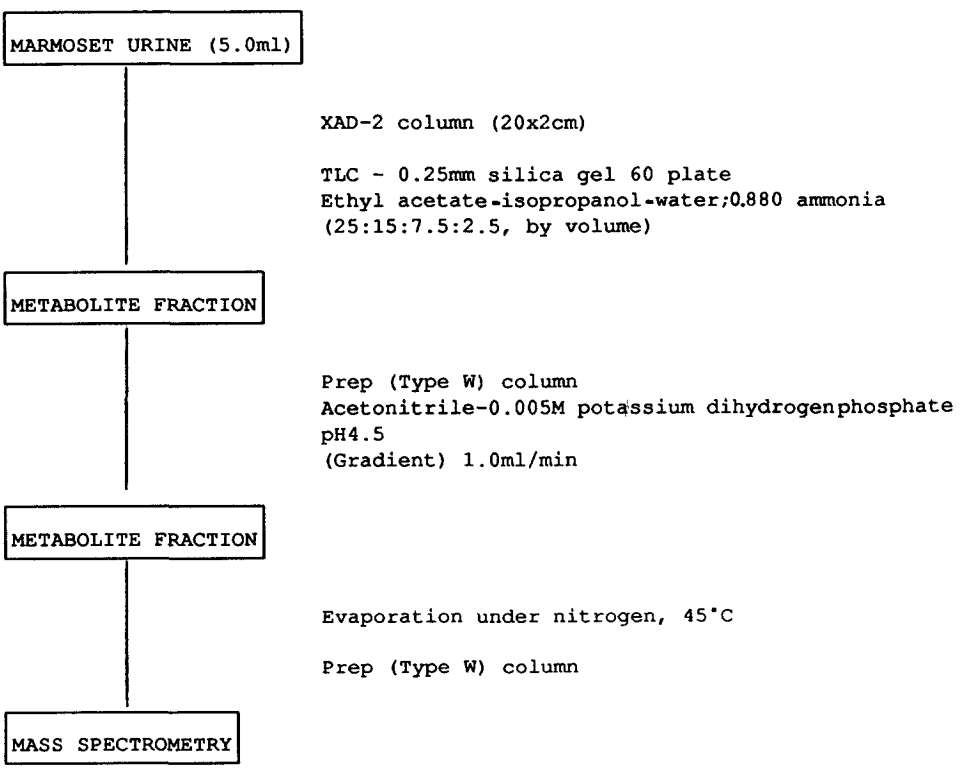


Fig. 3. Isolation of the salbutamol metabolite.

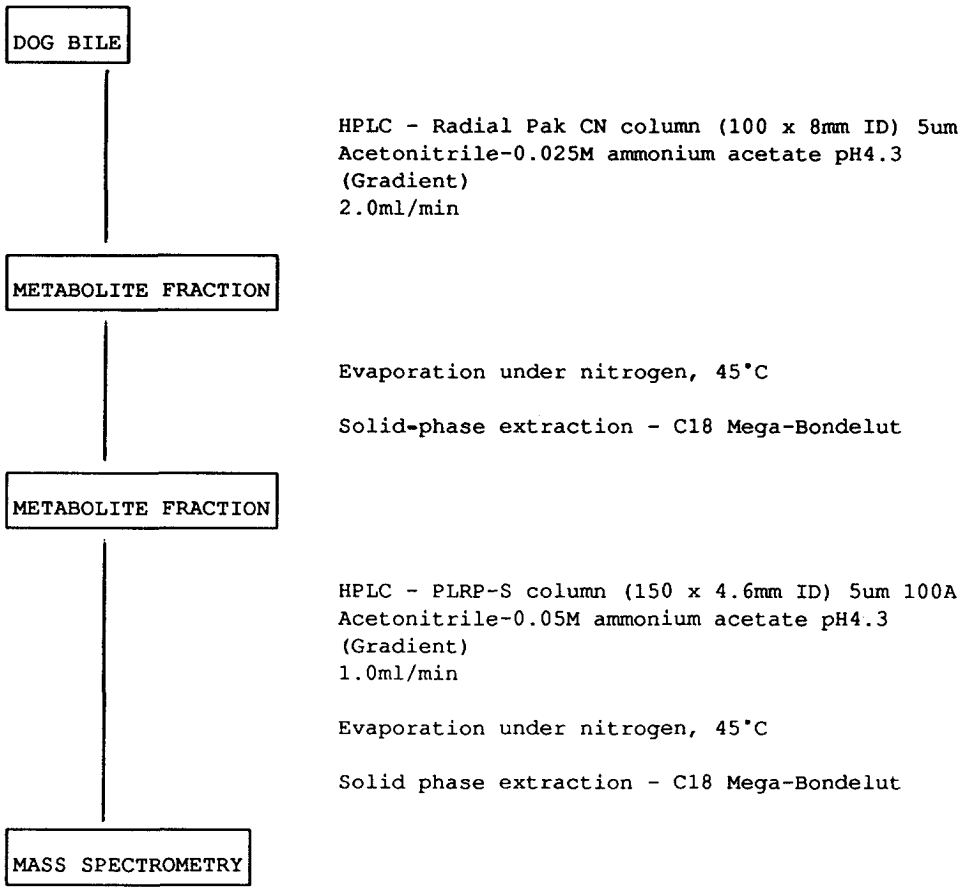


Fig. 4. Isolation of the ondansetron metabolite.

In the second experiment a male dog received a single oral dose, 1 mg/kg, of a mixture of ondansetron–[^{14}C]ondansetron–[$^{2}H_3$]ondansetron (0.85:0.15:1.0), (Fig. 2). Bile was collected over the period 0–6 h and a major metabolite was isolated by solid phase extraction and semi-preparative HPLC (Fig. 4).

HPLC

A Hewlett-Packard HP1090A liquid chromatograph (Hewlett Packard, Winnersh, UK) equipped with an auto-injector was used. The system was run at a flow-rate of 1.0 ml/min. The column used for the analysis of α-napthyl sulphate (Sigma, Dorset, UK) and the salbutamol metabolite was a Spherisorb ODS2 (100 × 4.6 mm I.D., 5 μm) (Phase Separations, Cheshire, UK) and was operated at room temperature. The ondansetron metabolite was introduced via a loop injection. The isocratic solvent system used for thermospray LC–MS analysis of α-napthyl sulphate was acetonitrile–water or acetonitrile–0.05 *M* ammonium acetate (2:98). For the analysis of the salbutanol and ondansetron metabolites the mobile phase comprised acetonitrile–water (8:92 or 50:50, respectively).

MS

FAB-MS was performed on a VG 7070E mass spectrometer (VG Analytical, Manchester, UK) equipped with a VG 11/250J data system. The instrument was operated at 6 kV accelerating voltage and a resolution of 1000. The saddle field ion gun (Ion Tech, Middlesex, UK) was operated at 8 keV and 100 μA current using xenon gas. An aliquot of the sample was added in methanol to the sample probe to which glycerol had been previously applied and spectra were recorded in negative ion mode.

Thermospray LC–MS was performed on a Hewlett-Packard HP5987A mass spectrometer equipped with an RTE-6 data system (Hewlett-Packard, Palo Alto, CA, USA) and a TSP interface (Vestec, Cheshire, UK). The TSP source block temperature was maintained at 250°C for all experiments and the vapouriser temperature in the range 209–211°C. All spectra were recorded in the negative ion mode. The TSP source was operated in the filament and discharge off mode. Approximately 10 μg of the salbutamol metabolite and 1.2 μg of the ondanestron metabolite were used for the analyses.

RESULTS AND DISCUSSION

FAB-MS analysis of the salbutamol metabolite produced a spectrum containing two weak ions at m/z 318 and 321 which could be assigned as $[M-H]^-$ for salbutamol and its deuterated analogue. However, these data proved inconclusive due to the amount of endogenous material present in the sample (Fig. 5). For similar reasons this technique also proved unsuccessful for analysis of the ondansetron metabolite. In this instance the presence of bile acids, including taurocholic acid, $[M-H]^-$ at m/z 514, dominated the spectrum (Fig. 6).

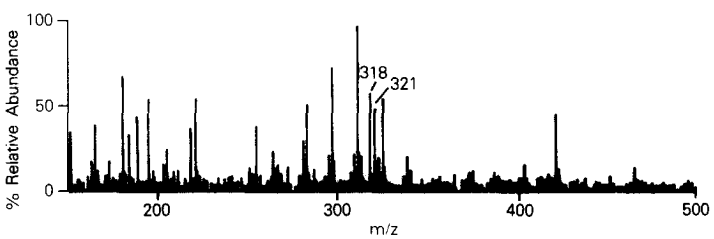


Fig. 5. FAB mass spectrum of the salbutamol metabolite.

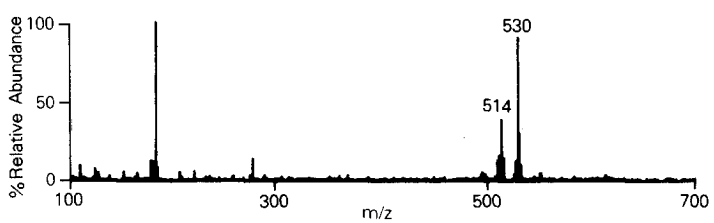


Fig. 6. FAB mass spectrum of the ondansetron metabolite.

Thermospray LC–MS was investigated an an alternative technique. However, because no authentic standards were available, conditions were optimised initially using α-napthyl sulphate. In the absence of ammonium acetate the molecular anion, *m/z* 223, is the base peak in the spectrum (Fig. 7). However, in the presence of buffer the base peak at *m/z* 203 is derived from [α-napthol + acetate]$^-$. The α-napthol is probably formed by buffer catalysed hydrolysis of the α-napthyl sulphate, similar processes have been reported previously for the thermospray of purine-6-sulfonate [6]. Thus, in the absence of buffer, an ion evaporation process predominates, whereas chemical degradation occurs in its presence.

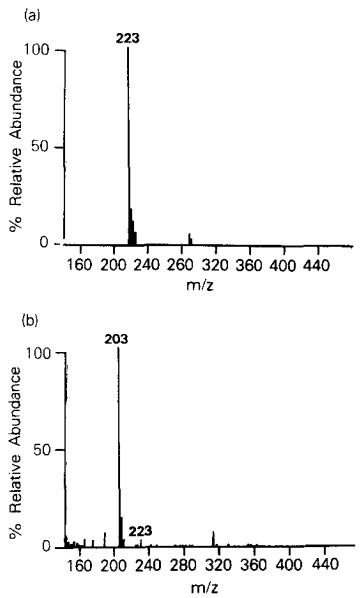
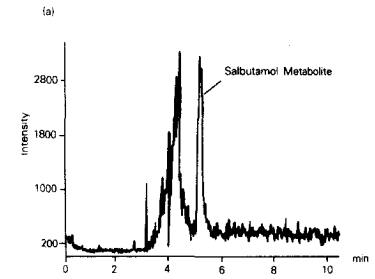
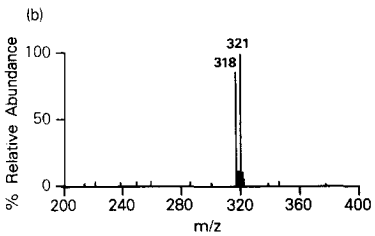


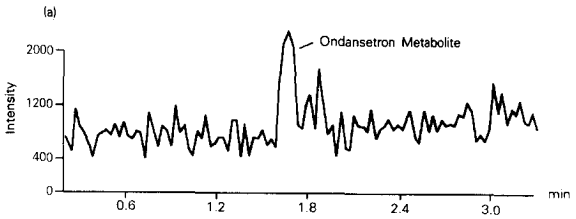
Fig. 7. Thermospray mass spectra of α-napthyl sulphate (a) in the absence and (b) in the presence of ammonium acetate buffer.

To minimuse hydrolysis and obtain important molecular weight information, thermospray LC–MS analysis of the metabolites of salbutamol and ondansetron were performed in the absence of buffer. Fig. 8 shows the total ion current (TIC) chromatogram and mass spectrum obtained for thermospray LC–MS analysis of the salbutamol metabolite. The ions at *m/z* 318 and 321 are assigned as [M − H]$^-$ for the salbutamol metabolite and its deuterated analogue. Confirmation of the position of sulphate conjugation has not been established for this metabolite, although evidence obtained from human studies have previously indicated phenolic conjugation [7].

Fig. 9 shows the TIC chromatogram and thermospray mass spectrum of the ondansetron metabolite. The ions at *m/z* 388 and *m/z* 391 are assigned as [M − H]$^-$ for the ondansetron metabolite and its deuterated analogue. The determined molecular weight of the metabolite, 389, is consistent with a sulphate ester conjugate of the mono-hydroxylated drug. Analysis by NMR has confirmed the structure of the metabolite as the 8-hydroxy sulphate ester of ondansetron.







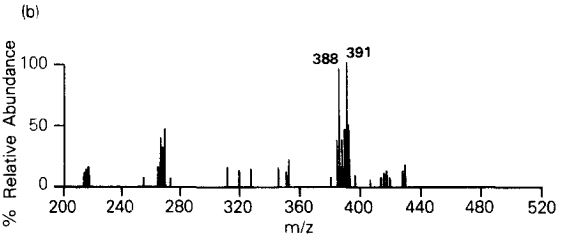


Fig. 8. Thermospray (a) TIC chromatogram and (b) mass spectrum from analysis of the salbutamol metabolite.

Fig. 9. Thermospray (a) TIC chromatogram and (b) mass spectrum from analysis of the ondansetron metabolite.

CONCLUSION

The metabolites of two drugs, ondansetron and salbutamol have been characterised by thermospray LC–MS. The results of our experiments have demonstrated that in the absence of ammonium acetate, ion evaporation of the molecular anions occurs. Use of this technique has allowed unambiguous determination of the molecular weights of intact conjugates which would not have been obtained from FAB-MS.

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