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The application of Ultrafast LCMS to optimizing detection in the analysis of tramadol and its metabolites

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Introduction

The detection of drug residues in samples such as urine is often limited by the extent of metabolism of the parent drug, the structural relationships between parent and metabolite and the availability of standards of the metabolites. Method optimization in such cases is limited by the need to extract the residues from urine and carry out repeated analyses to determine the best fragmentation conditions or alternatively base detection on the fragmentation conditions derived for the parent compound. We describe an Ultrafast LCMS approach to the optimising of CID energies based on the analysis of urine specimens containing metabolites of tramadol.

Method

Urine samples were obtained from a 80 kg human male that had received Tramal™ (Tramadol hydrochloride 500 mg, Grunenthal-CSL, Melbourne, Australia) for acute pain relief 2, 5 and 8 hours after administration. An equine urine sample previously found to contain tramadol metabolites during routine analysis was also included for study.

Extraction and Analysis

Methanol was HPLC grade and water purified using a Millipore synergy system. Portions of urine (3 mL) were diluted with 0.5 M ammonium acetate buffer (pH 5.5, 4.5 mL) and the pH adjusted to 5.5-6. The samples were subject to protease and beta-glucuronidase treatment, centrifuged to remove sediment and extracted (Fig 2A) on mixed-mode C8-SCX columns (Bond Elut-Certify, 130 mg, 3 mL, Agilent, CA, USA) previously conditioned with methanol (2 mL) and water (2 mL). Each sample was passed through a column. The column was washed with water (4 mL) then 1 M acetic acid (2 mL) for pH adjustment and dried with nitrogen at 200 mL/sec for 6 minutes. The sorbent was washed with methanol (2 mL) and again dried with nitrogen for 6 minutes. The base fraction was eluted with ethyl acetate /dichloromethane /2-propanol (5:4:1v/v) containing 2% concentrated aqueous ammonia (2 mL).

LC-UFMSMS analysis

Analysis was completed on a Nexera LC-30 - LCMS-8040 (Shimadzu, Kyoto, Japan) equipped with a Shimpack XR-ODS III (75 mm × 2.1, 1.7 µm particle) column. The sample was injected with a Sil-30AC autosampler and an injection volume of 0.1 µL (equivalent to 30 µL of urine). Analysis was at a temperature of 40°C, flow rate of 0.4 mL/min. The mobile phase comprised 10 mM aqueous ammonium acetate (solvent A) and 10 mM ammonium acetate in methanol (solvent B).

The initial solvent composition was 5% B held from 0 to 0.5 min then ramped to 95% solvent B at 6 min, held from 6 – 9 min then returned to the starting composition. Precursor Ion scanning for *m/z* 44 and *m/z* 58 and Product Ion Scanning for MH^+ 264 (tramadol), 310, 296, 282, 280, 268, 266, 252, 250 and 236 was conducted at 15000 µ/sec from *m/z* 40-310 for each analyte at collision energies of -15, -25 and -35 V. All channels were acquired from 0-6 min for a total loop time of 0.95 sec. Alternatively specific retention time windows were applied to individual analytes to allow further collision energies to be applied. The drying line was 280°C, N2 nebulising gas 3 L/min, heater block 400°C, the drying gas at 15 L/min and the CID gas was argon at 230 kPa.

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Discussion

Tramadol is extensively metabolised in various species by aryl and alkyl hydroxylation and by N- and O-demethylation (Fig. 1). As targets for screening, both tramadol and its more abundant metabolites are of interest and we use them as a proof of principle in this study. We have been concerned with the difficulty in determining optimum collision energies for metabolites identified in controlled administration urine but for which there are no authenticated standards.

By using LC-UFMS in combination with a CID process which has demonstrably low crosstalk, we have initially scouted for the presence of metabolites without regard to retention time (Fig. 2) and in a second analytical run identified retention time windows and optimised the collision energy by rapidly acquiring 36 CID spectra at different collision energies with a total cycle time of less than 1 second (Fig. 3).

The array of PIS spectra may be viewed as a function of CID voltage and detection criteria optimised on the basis of base-peak intensity, the presence of qualifying ions or by weighting the structural importance of fragment ions. We applied this method successfully on-the-fly to extracts of post-administration urine samples. In the case of tramadol and its major metabolites, fragmentation was dominated by the formation of the alkylamine ions (Fig. 3) and the method therefore showed high sensitivity and was ideally suited to a MRM based technique. The use of even higher collision energies did not result in substantial changes to the distribution of fragment ions and resulted in a loss of intensity in the base peak. We believe that the informing power of the analysis may be increased by analysing derivatised urine extracts. In continuing work, we are repeating the analysis using acetylated analogues to investigate the characteristic losses of acetyl from O-acetyl and N-acetyl moieties. As tramadol itself also has a free hydroxyl group, the opportunity exists to generate qualifying ions for the parent compound.

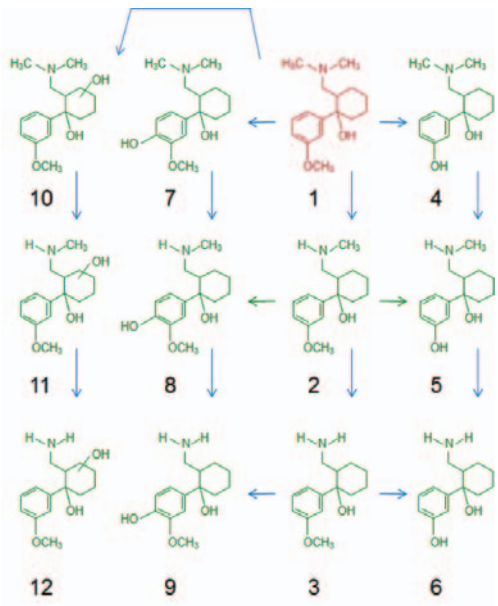

Fig. 1 (above) The metabolism of tramadol in different species.

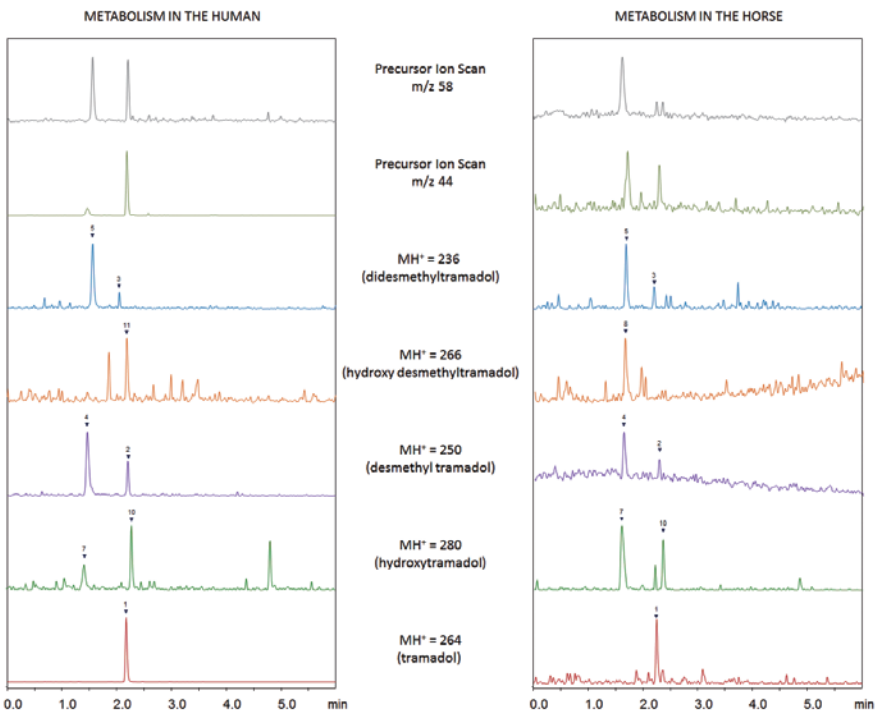

Fig. 2 (right) UFMS applied to detecting predicted metabolites using PIS across the entire chromatographic range.

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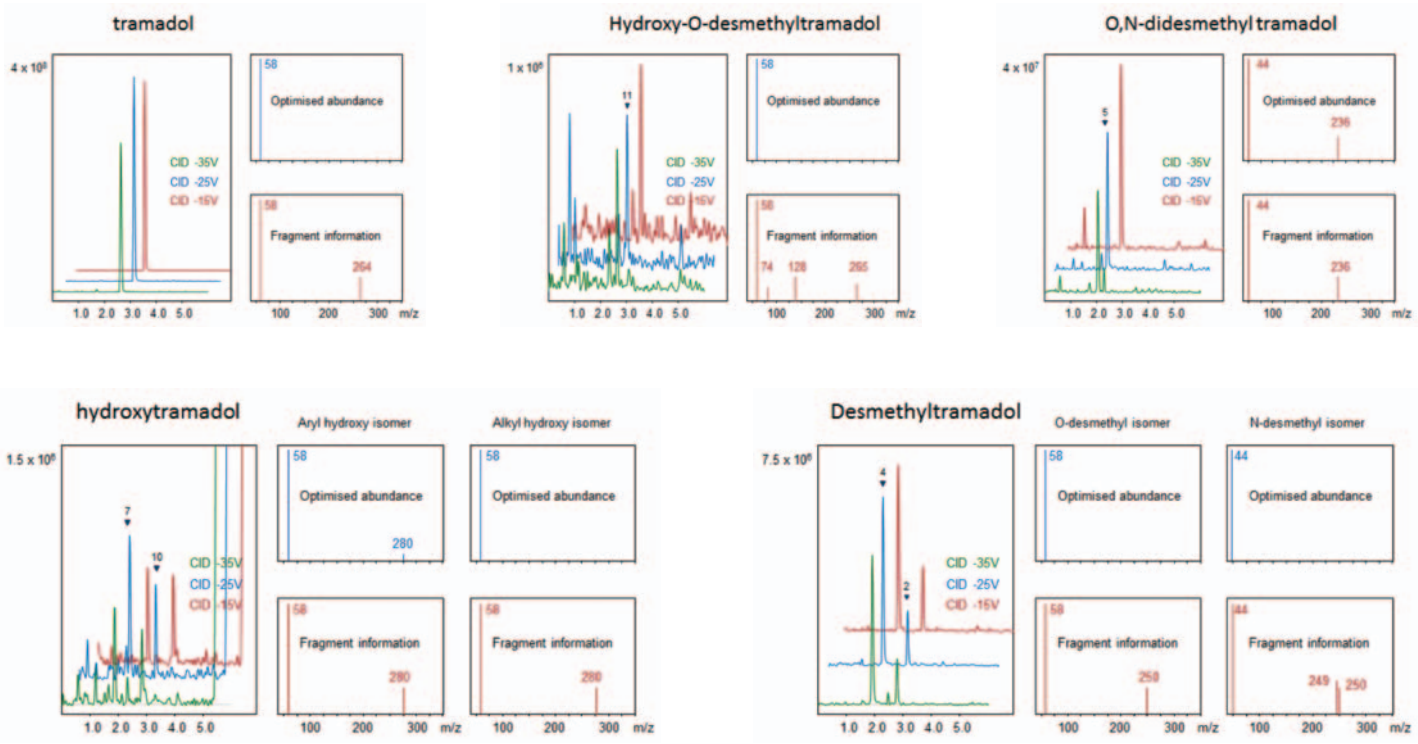

Fig. 3 On-the-fly optimisation of CID energy. The Product Ion Scan for each analyte was recorded at three different collision energies (-15V, -25V and -35V) in a cycle that also included 33 dummy transitions and a total cycle time of less than one second. Scan speed was 15000 µ/sec. The dummy transitions demonstrate the capacity for creating a PIS data array for each analyte from a single injection and so allow the optimisation of the collision process in favour of either base peak abundance or the generation of other fragment ions.

Tramadol metabolites show a strong loss of the alkylamine moiety and this product ion (m/z 58 for $(CH_3)_2NCH_2^+$ or 44 for $CH_3NHCH_2^+$) dominates the CID spectra for most analytes.

Conclusion

UFMS was used for metabolite scouting using product ion scanning without loss of peak fidelity. Targeted precursor ions were predicted for modification including hydroxylation and dealkylation. Ultrahigh scan speeds and low cross talk CID were also used to generate collision spectra at multiple CID voltages across analytical peaks to allow optimisation of detection conditions. The collision energy may be translated directly to MRM experiments and is useful for developing screening protocols for metabolites for which there are no authenticated standards. The method enables detection but does not remove the regulatory need for external standards that are used for the unequivocal confirmation of structure.

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