ANALYSIS OF HUMAN URINARY METABOLITES OF NAPROXEN USING A Q-TOF PREMIER™ WITH APCI AND LOCKSPRAY™

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INTRODUCTION

The use of exact mass measurements provides a greater confidence in metabolite confirmation through the ability to determine actual elemental compositions. LockSpray™ has been established as a routine and rugged hardware configuration of choice with the Q-Tof Premier™. This configuration provides a single reference peak against which any subsequently acquired mass spectra are accurately mass measured. Historically it was common working practice on magnetic sector and orthogonal – acceleration time of flight (oa-Tof) mass spectrometers to introduce a reference solution into the eluent stream prior to the ionisation source, in order to allow exact mass measurement of LC peaks. This approach was also utilised when APCI was performed. The associated inherent problems of teeing in the reference compound can result in a variation in the signal intensity when LC gradients are applied, mass interference with analytes having the same nominal mass and suppression of the reference response with high concentration analyte response. In these instances mass measurement errors can occur. Enhancement of the APCI performance of the Q-Tof Premier has been made. LockSpray functionality has been extended to APCI.

The configuration of APCI LockSpray is shown in Figure 1. New hardware and power supply design enable the analyte to be acquired using APCI and a reference sample to still be acquired using electrospray ionisation. To switch from ES to APCI on the Q-Tof Premier, it is only necessary to switch the ES probe for the APCI probe and add the corona pin. No instrument recalibration or tuning is required, those utilised for ES can be used routinely, and the enhanced instrument stability enables accurate mass measurement below 3ppm to be obtained over extended periods of time.

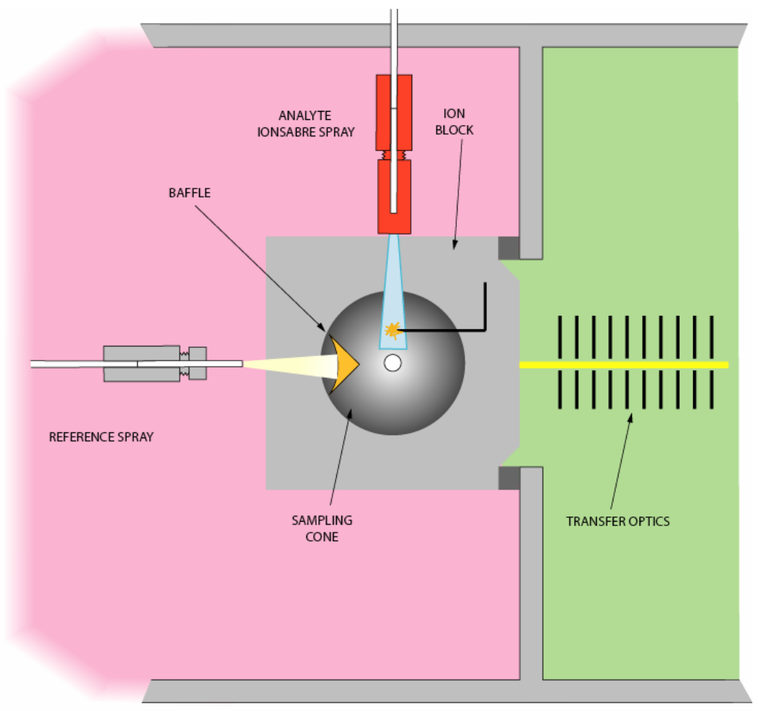

Figure 1. Schematic representation of Q-ToF Premier™ APCI LockSpray™.

Naproxen is a member of the aryl acetic acid group of non steroidal anti inflammatory drugs and is used for treatment of conditions such as tendonitis, rheumatoid arthritis, gout and other inflammatory conditions. Analysis of urine has been performed to determine and identify the metabolites of Naproxen, where the patients' prescribed daily dose was 2 x 500 mg (Naprosyn). Using the Q-Tof Premier in APCI mode with accurate mass measurement, phase I and phase II metabolites of Naproxen have been rapidly identified using the application manager MetaboLynx™. Neutral loss experiments were also performed to further confirm the presence of the acyl-migrated isomers of β-1-O-acyl glucuronides. These metabolites have been implicated in drug toxicity because they can bind to proteins. The process of metabolite identification was improved by being able to consecutively acquire full spectrum data with collision induced fragmentation, this enabling structural information to be acquired and further confirm the metabolite identification shown within the MetaboLynx browser.

METHODS

UPLC Conditions

System: Waters ACQUITY UPLC™
Column: Waters ACQUITY UPLC™ BEH C18 (100 mm x 2.1 mm, 1.7 mm particle size)
Column temperature: 45°C
Flow Rate: 0.6 mL/min
Mobile phase: MeCN (B) : H2O (0.1% HCOOH) (A)
Gradient: 0 min 2% B, 14.0 min 40% B, 16.0 min 95% B, 16.1 min 2% B,
Run time 18 mins
Injection volume= 10µL

Sample Analysis
A patient was prescribed a daily dose of 2 x 500 mg Naprosyn (Naproxen). A urine sample was taken for analysis at time point 4 days and4.5 hrs and analysed directly.

Data Processing
Mass measurement errors were determined with automatic processing with MetaboLynx™ application manager

MS Conditions

Mass Spectrometer: Waters® Micromass Q-Tof Premier™
Ionization Mode: Analyte; APCI- at 5 µAmps, Reference ESI- at 3kV
Sample cone voltage: 20V
Reference mass: Sulphadimethoxine, [M-H]- =309.0658
Acquisition Parameters: 100-1000 m/z;
0.15 second/spectrum; 0.02 second inter acquisition delay
Resolution = 15500 FWHM (W mode)
Low Collision Energy = 4 eV
High Collision Energy = 20 eV
Neutral Loss MS/MS Collision Energy = 12 eV

RESULTS AND DISCUSSION

The determination of urinary metabolites of Naproxen using a Q-Tof Premier in APCI mode with LockSpray enabled has been performed. The enhanced source configuration has allowed the inherent interference, suppression problems and reduced flexibility produced when teeing in a reference compound into the eluent flow when conducting APCI analysis.

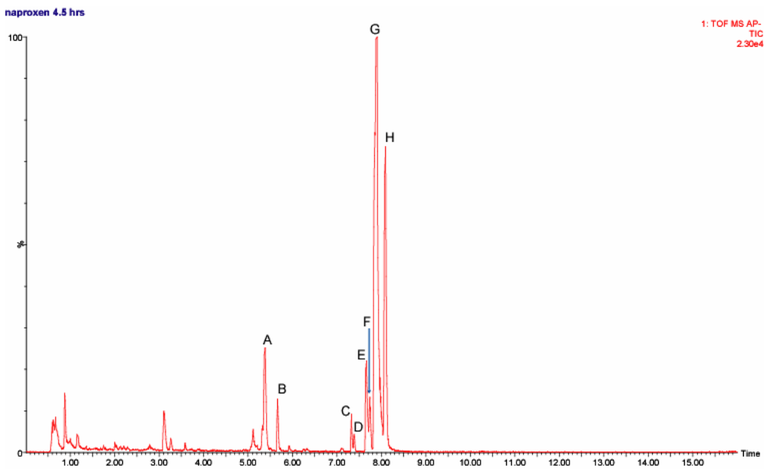

Figure 2. Total ion chromatogram acquired with negative ion APCI for the determination of urinary metabolites of Naproxen in human at 1000 mg/day dosing level.

It can be seen in Figure 2, eight major urinary metabolites have been identified using negative mode APCI. Using MetaboLynx™ a comparison of the control and the analytes sample was performed. Metabolites A and B were identified as demethylated glucuronides. The six remaining metabolites C to H were identified as glucuronides.

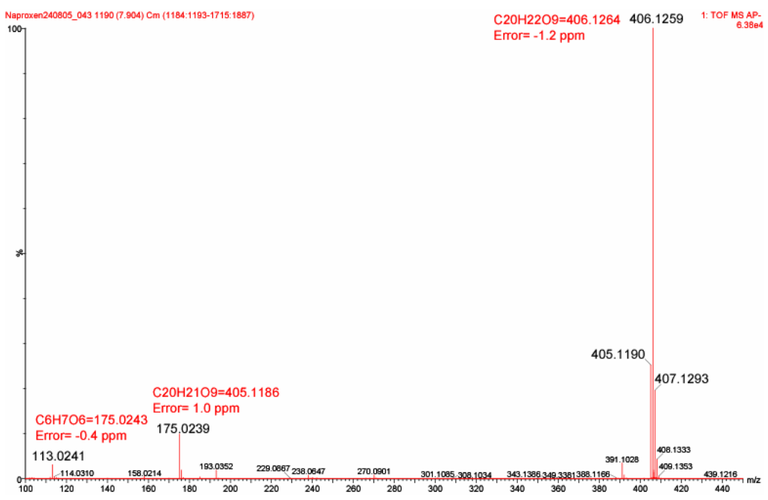

Figure 3. Exact mass spectrum acquired major naproxen glucuronide metabolite G.

The exact mass spectrum, elemental composition along with a mass measurement error of only 1.2ppm for the glucuronide metabolite G (m/z 406, radical anion) has been shown in Figure 3, the deprotonated molecular ion is also present (m/z 405). In addition evidence of the loss of the glucuronide is observed at m/z 175 (-0.4ppm). Prior to analysis the identification of two glucuronide metabolites of Naproxen was expected. However using UPLC/Tof, the presence of six glucuronides was determined. Using high/low collision energy switching, it was possible to obtained full spectra acquisition data as well as fragmentation spectra, and produce structural information in order to confirm in one analysis that the six metabolites believed to be glucuronide metabolites were correctly identified. In each case evidence of the loss of m/z 175 was found.

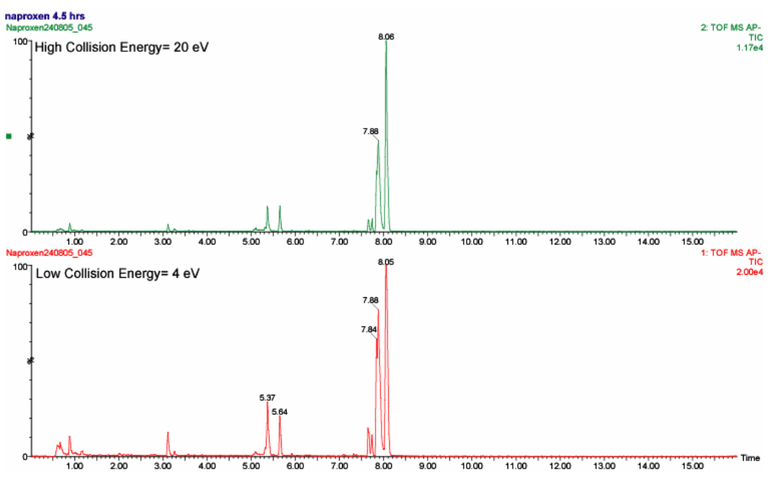

Figure 4. Total ion chromatograms acquired with negative ion APCI for the determination of urinary metabolites of Naproxen in human at 1000 mg/day dosing level using collision energy switching.

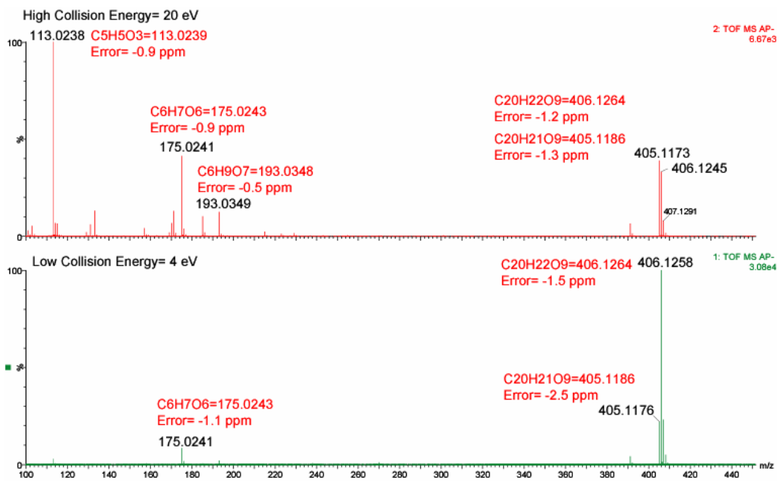

Figure 5. Consecutively acquired low collision energy spectrum and high collision energy fragmentation spectrum for glucuronide metabolite G.

As can be seen in Figures 4 and 5 where the high/low energy TICs and spectra are shown respectively, both full MS and CID spectra can be acquired with exact mass measurement routinely. In the data presented mass measurement errors of less than 3ppm have been obtained routinely. Further confirmation of that the glucuronide metabolites were identified correctly using the neutral loss functionality of the Q-Tof Premier. In the case shown, the experiment was set up to perform high/low energy switching, and where a loss of m/z 175 was observed MS/MS would be performed, to produce specific structural information.

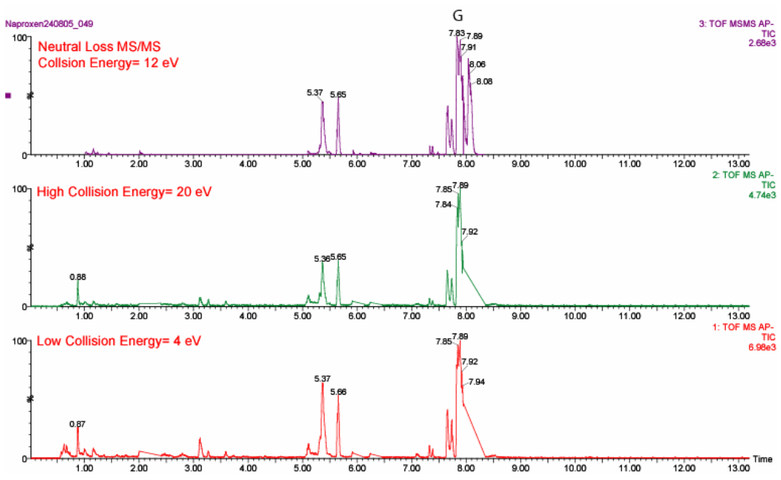

Figure 6. Low energy, high energy and MS/MS total ion chromatograms acquired in negative ion APCI neutral loss mode for the determination of urinary glucuronide metabolites of Naproxen.

In Figure 6, the TIC obtained for the three functions of the neutral loss experiment are shown, where the standard operating collision energy of 4eV was increased to 20eV to induce full spectra CID fragmentation and a collision energy of 12eV was utilised to perform MS/MS where a neutral loss of m/z 175 was observed.

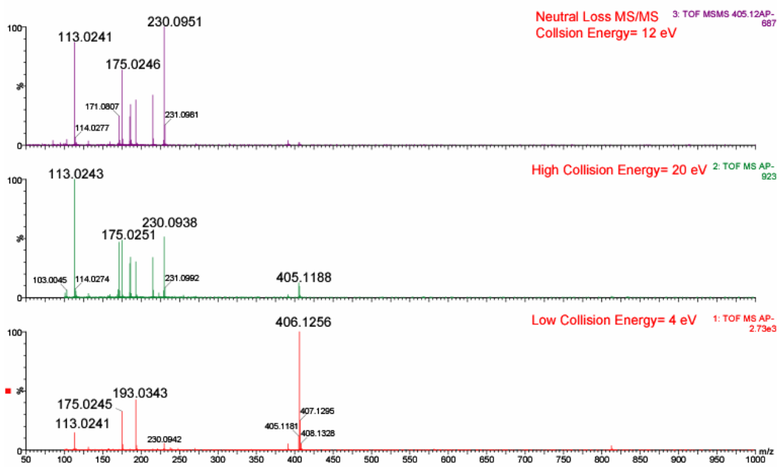

Figure 7. Low energy, high energy and neutral loss MS/MS total ion mass spectra acquired using negative ion APCI for the naproxen glucuronide (metabolite G).

For metabolite G, the exact mass spectrum produced in normal low energy operation mode is shown with the high energy CID exact mass spectrum (20eV) and the neutral loss triggered MS/MS spectrum are shown in Figure 7. The exact mass neutral loss data acquired further confirmed that six glucuronide metabolites had been determined to be present, and the additional unexpected glucuronides metabolites of Naproxen were in fact produced via acyl migration[1].

CONCLUSIONS

- The enhancement of APCI to incorporate LockSpray functionality has enabled exact mass measurement errors of less than 3ppm to be obtained routinely.
- Utilising UPLC/TOF/MS eight metabolites of Naproxen were separated and identified.
- High/low collision energy switching is routinely used to maximise information from one sample analysis, where both full spectra and CID fragmentation spectra are produced.
- Confirmation of the identification of six human urinary Naproxen glucuronide metabolites was performed using neutral loss MS/MS.

REFERENCES

1. Ramus W Mortensen et al. S-Naproxen-β-1-O-Acyl Glucuronide Degradation Kinetic Studies By Stopped-Flow High-Performance Liquid Chromatography-1H NMR and High Performance Liquid Chromatography-UV. Drug Metabolism and Disposition 29:375-378, 2001.

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