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EVALUATION OF A RF-ONLY STACKED RING BASED COLLISION CELL WITH AXIAL FIELD FOR THE LC-MS-MS ANALYSIS OF THE METABOLITES OF RABEPRAZOLE

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Presented at ASMS 2002, Orlando, Florida, USA, 3rd - 6th June 2002

OVERVIEW

- Comparison of the performance of a stacked ring based collision cell with axial field and a hexapole collision cell.
- Full scan LC-MS/MS-MS using precursor, product and neutral loss modes of operation used to confirm the presence of Rabeprazole Metabolites.
- The results show the stacked ring collision cell performance to be better than the hexapole based cell for fast precursor ion and neutral loss scanning MS-MS.

INTRODUCTION

The relatively slow transit of ions through RF only collision or reaction cells as a result of thermalisation by multiple collisions can compromise mass spectrometer performance especially when rapid acquisitions are required. To this end a novel collision cell based on a stacked ring electrode design with an axial voltage gradient has been built to allow the investigation of precursor ion and neutral loss scanning modes of operation on a tandem Quadrupole mass spectrometer. The performance of the stacked ring cell has been compared with a hexapole based collision cell (without axial gradient) by studying the metabolites of Rabeprazole formed in human liver microsomal incubations. The results of the study show that the stacked ring collision cell significantly improves precursor and neutral loss sensitivity and precursor mass resolution aiding the MS-MS confirmation of metabolites.

METHODS

Sample Preparation

A microsomal sample containing 1mg/mL liver microsomal proteins was incubated with 50uM Rabeprazole, in 0.1M Tris Buffer pH7.4 at 37°C. The reaction was initialised by the addition of NADPH and stopped after 60 minutes by freezing. The microsomal incubation sample was centrifuged and diluted 1 to 1 in water prior to analysis.

This sample was chosen because a number of major metabolites could be predicted based on previously reported information[1]. These metabolites could then be used to illustrate features of the stacked ring collision cell.

LC Conditions

Column - Waters Symmetry, C18, 3.5µM, 2.1 x 100mm
Injection volume = 10µL

Phase A - 10 mM aqueous ammoinum acetate, pH 4.5 (acetic acid)
Phase B - acetonitrile
LC gradient: %B - 5% 0-2 mins, 95% 15-20 mins, 5% 21-30 mins

Flow rate - 300µL/min (no post column split)
Flow diverted to waste for first 2.5mins of the run.

MS Conditions

Mass Spectrometer - Micromass Quattro Ultima
Ionisation mode - Positive ion electrospray

Full scan MS analysis - m/z 100 to m/z 700 in 0.5seconds
Precursor ion analysis - m/z 100 to m/z 700 in 0.5seconds
Product ion analysis - m/z 100 to m/z 700 in 1.2 seconds
Neutral loss analysis - m/z 200 to m/z 800 in 1.2 seconds

MICROMASS MS TECHNOLOGIES

RF-Only Stacked Ring Based Collision Cell

In many tandem mass spectrometry systems ions are fragmented as required in a low pressure gas collision cell. The ions in such collision cells are typically confined using RF fields produced by a multi-pole system of rods. Adjacent rods have an RF voltage phase difference of 180 degrees and a DC offset is applied to axially accelerate ions for fragmentation purposes. The typical pressure regime in such devices is of the order 10^{-3} to 10^{-2} mbar, with typical collision gasses being nitrogen or argon. Once ions enter the cell and have undergone a few collisions their axial kinetic energy is reduced. In the absence of an axial field it is considered that it is mainly the ingress of additional ions, gas flow and diffusion are the means by which the ions exit the cell. The use of an axial field to reduce ion transit times in gas filled RF devices has been considered by several groups [2,3,4].

A novel device based on a RF only stacked ring technology has been built with an axial field for use as a collision cell. The new cell is a reasonably gas tight box with entrance and exit apertures each having a separate voltage supply. In the cell there are 15 isolated segments with the same RF but different DC voltages. A linearly increasing voltage is applied to each segment via a resistor ladder and two computer programmable voltage supplies. The resistor and capacitor network are mounted on two PCB's connected to the side of the cell. A schematic diagram showing the collision cell and its connections is shown in **figure 1**. A RF voltage supply providing phase (RF+) and anti-phase (RF-) voltages at a frequency of 1.75 MHz is coupled to the ring electrodes using the capacitors. Four inductors are used in the DC supply rails to reduce any RF feedback onto the DC supplies.

Each segment has two isolated sections with four electrodes with a hole in the centre shown in **figure 1**. The two sections interlock giving 8 electrodes per segment with a 1mm inter-electrode spacing.

Once interleaved adjacent electrodes in a segment have the same DC but the RF has a phase shift of 180°. Two ports are provided to allow the introduction of the collision gas into the cell and a pirani gauge to be connected. A photograph of the stacked ring based collision cell can be seen in **figure 2**.

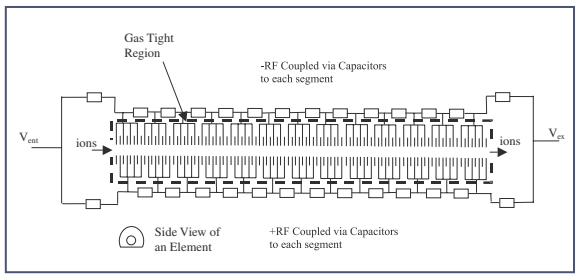

Figure 1. Schematic diagram of the collision cell showing the electrical connections

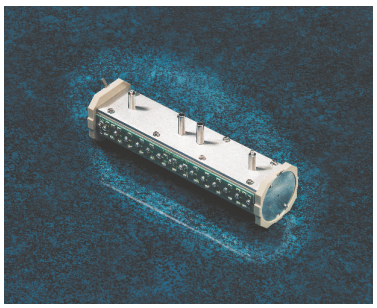

Figure 2. Photograph of the Collision Cell showing the PCB containing the resistor and capacitor network entrance an exit lenses and the gas inlet and pirani gauge port

The voltage difference applied across the gas cell varied depending upon the scan mode used. For MS scans it was 0.5V (25mV/cm), product ion scans it was 3V (150mV/cm) and neutral loss and precursor ion scans it was 7V (370mV/cm).

RESULTS

The chromatogram obtained in full scan MS mode (**figure 3**) shows the complexity of the sample used in this study.

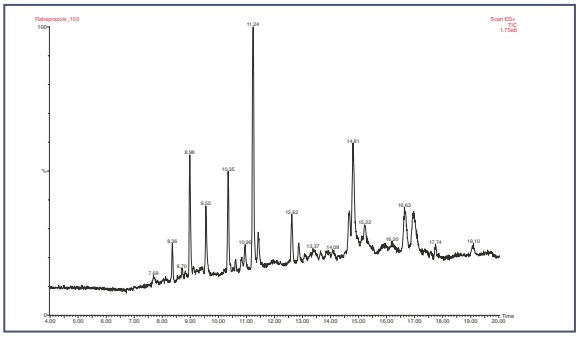

Figure 3. TIC for 60 minute incubation sample

The presence of expected major metabolites[1] was initially confirmed by generation of extracted mass chromatograms (**figure 4**).

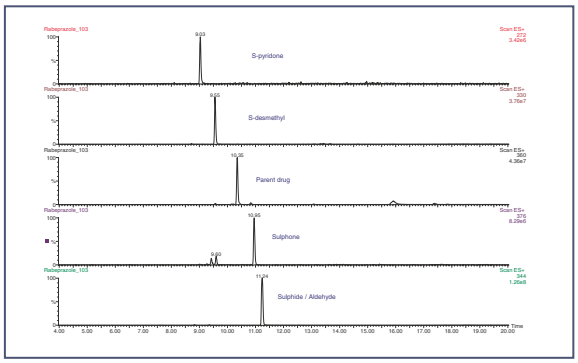

Figure 4. Extracted mass chromatograms for expected metabolites of rabeprazole

Product ion analysis revealed a fragment at m/z 119 to be common in both the parent drug and a number of the metabolites. Product ion analysis also showed a neutral loss of 118 Da to occur for both the parent drug and a number of the metabolites. This is illustrated in the example given in **figure 5**.

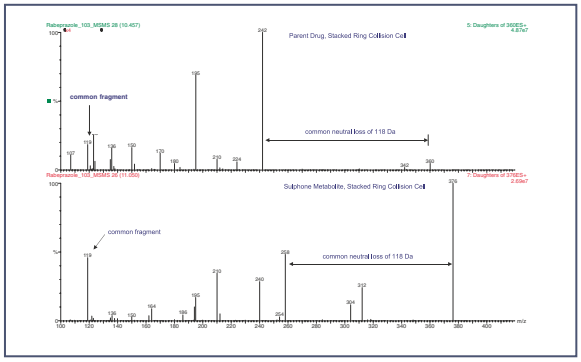

Figure 5. Comparison of product ion spectra obtained for rabeprazole and its sulphone metabolite

Fast precursor ion scanning (500 Da/sec) of the common product ion at m/z 119 was performed. Example chromatograms obtained for the parent drug and the sulphone metabolite are presented together with equivalent data obtained using the standard hexapole cell in **figure 6**.

Extracted ion chromatogram intensity was observed to increase by greater than a factor of 10 with the stacked ring collision cell. The precursor ion resolution achieved using the new collision cell was unit mass at the base compared with over 25 Da for the hexapole collision cell (**figure 7**). Due to the increased precursor ion resolution (**figure 7**) the extracted ion chromatograms obtained using the stacked ring collision cell were free of interference peaks (**figure 6**) significantly aiding confirmation of metabolite peaks.

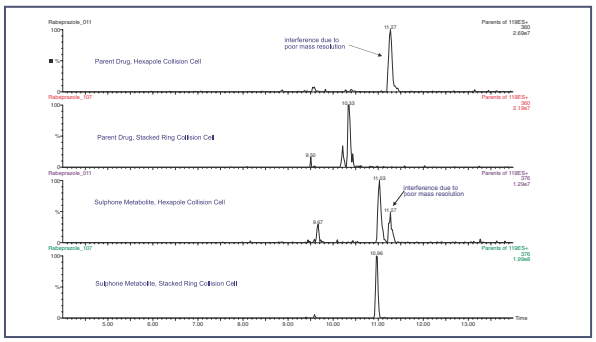

Figure 6. Precusor ion chromatograms obtained using Hexapole and stacked ring collision cells

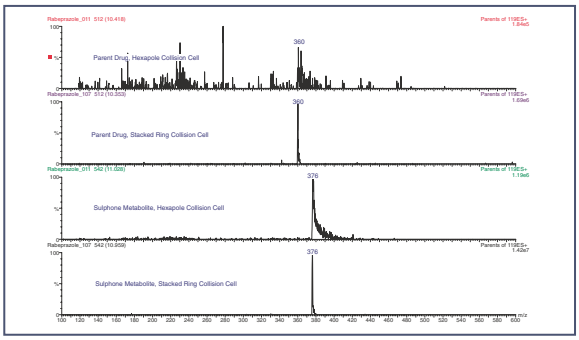

Figure 7. Precusor ion spectra obtained using Hexapole and stacked ring collision cells

Extracted mass chromatograms and corresponding spectra for the neutral loss of 118 Da obtained with the two collision cells are presented in **figures 8 and 9** for the parent drug and its sulphone metabolite. The data obtained using the new collision cell shows an increase of a factor of 10 in intensity for both spectral and chromatographic data.

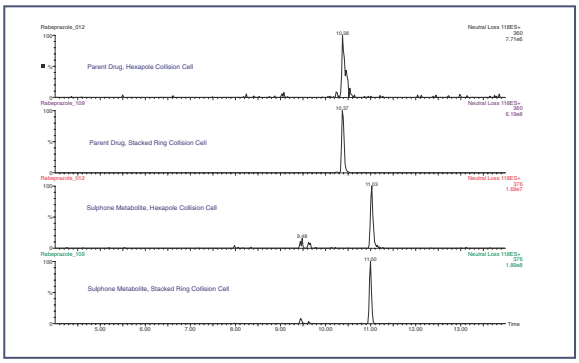

Figure 8. Neutral loss chromatograms obtained using Hexapole and stacked ring collision cells

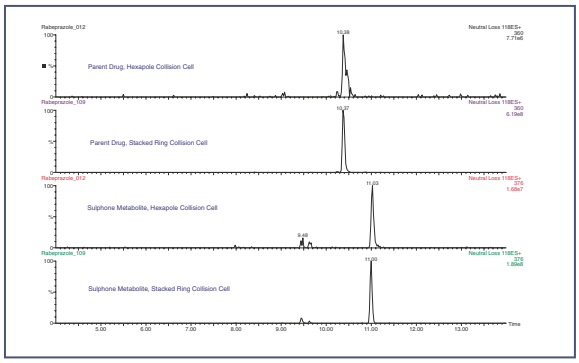

Figure 9. Neutral loss spectra obtained using Hexapole and stacked ring collision cells

CONCLUSION

In the MS and product ion scanning modes the performance of the rf-only stacked ring collision cell with an axial field was equivalent to the standard hexapole collision cell. However in the product ion scanning mode the mass resolution was maintained even at relatively high scan speeds. This was not the case for the hexapole cell. As a result of the increased resolution (at 500 Da/s) the precursor ion spectral intensities were significantly better for the stacked ring collision cell. The increased resolution also resulted in extracted ion chromatograms free of interference peaks, ensuring unambiguous confirmation of metabolite peaks.

The neutral loss data obtained using the stacked ring collision cell showed an increase of a factor of 10 in intensity for both spectral and chromatographic data as compared to the hexapole collision cell when operated at a scan speed of 500 Da/s.

The neutral loss and precursor ion experiments were repeated at scan speeds of 1000 Da/s using the stacked ring collision cell. This collision cell showed no deterioration in performance when compared to the data obtained at 500 Da/s.

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ACKNOWLEDGEMENTS

Thompson, C. Jolliffe and R. Javahery
Lars Weidorlf, AstraZeneca R&D, Sweden, is gratefully acknowledged for providing the sample.

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