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LC-MS-MS ANALYSIS OF THE METABOLITES OF RABEPRAZOLE USING HUMAN LIVER MICROSOMES

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SUMMARY

The aim of this study was to demonstrate how a tandem quadrupole mass spectrometer could be used to confirm the presence of previously reported metabolites in human liver microsomal incubation samples. The results presented were for a 60-minute incubation sample. LC-MS and LC-MS-MS data was processed in an automated fashion to confirm the presence of a number of significant metabolites. The mass spectrometer was equipped with a novel stacked ring based collision cell having an axial field. The results illustrate benefits of this design to both parent and neutral loss analysis.

INTRODUCTION

Rabeprazole is a relatively new drug, which belongs to a class of medicines called proton pump inhibitors. It is used to treat people diagnosed as having active duodenal and stomach ulcers and gastro-oesophageal reflux disease. Samples were obtained from in-vitro metabolism studies using human liver microsomes[1].

LC-MS data was processed in an automated fashion to confirm the presence of a number of significant metabolites. Daughter ion analysis was automatically performed by the system based on the LC-MS results obtained to allow confirmation of structural similarities between parent drug and potential metabolites. This daughter ion analysis subsequently demonstrated a common fragment (m/z=119). This was used in neutral loss and parent ion analysis to confirm published metabolic pathways.

The relatively slow transit of ions through collision cells as a result of 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 developed.

The sample analysis was performed initially with a mass spectrometer equipped with a standard hexapole based collision cell and then repeated using an instrument equipped with the new stacked ring based collision cell. The results demonstrated that the new collision cell can significantly improve precursor and neutral loss sensitivity and precursor mass resolution, aiding the MS-MS confirmation of metabolites.

METHODS

Sample Preparation

The sample used in this study was obtained from AstraZeneca, Sweden. This sample was orginally part of an investigation jointly performed by Astra Zeneca and Micromass UK LTD into the use of tandem time of flight mass spectrometry for metabolite confirmation[1]. This sample was chosen because a number of major metabolites could be predicted based on previously reported information[1].

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.

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.

MICROMASS MS TECHNOLOGIES

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

RF-Only Stacked Ring Based Collision Cell

In many tandem mass spectroscopy 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 having an RF voltage phase difference of 180 degrees. A DC offset is applied to enhance the fragmentation process. The typical pressure regime in such devices is of the order 10^{-3} to 10^{-2} mbar, with typical collision gases being nitrogen or argon.

A novel device based on a RF only stacked ring collision cell has been designed. In the cell there are 15 isolated segments each consisting of a number of ring electrodes. The same RF is applied to all ring electrodes in the cell. A linearly increasing DC voltage is applied to each segment. The voltage gradient applied across the gas cell is varied depending upon the MS scan mode used. For MS scan it was 0.5V, product ion scans it was 3V and neutral loss and precursor ion scans it was 7V.

RESULTS

The chromatogram obtained in full scan MS mode (**figure 1**) shows the complexity of the sample used in this study.

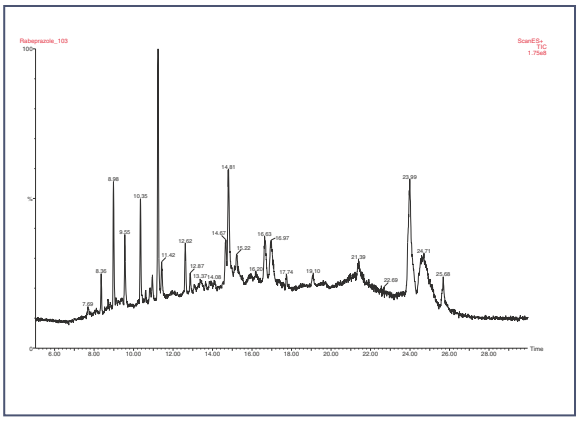

Figure 1. TIC for 60-minute incubation sample.

The LC-MS data was processed using an automated metabolite search algorithm (MetaboLynx). This search was performed against an extensive list of expected metabolites. A report was automatically generated in the form of an interactive browser (**figure 2**) highlighting which metabolites are present in the microsomal incubation sample. A summary of the extracted mass chromatograms representing the metabolites found in the MetaboLynx report are shown in **figure 3**.

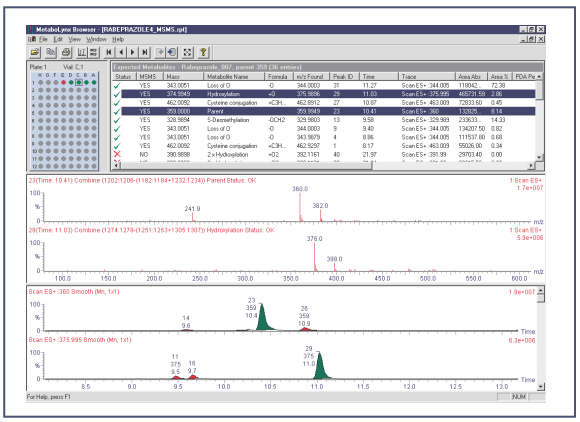

Figure 2. LC-MS metabolite browser report.

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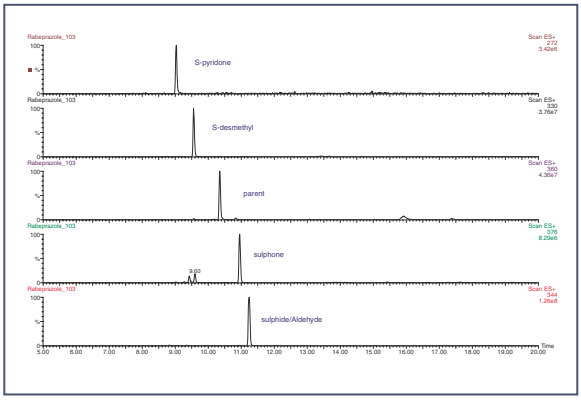

Figure 3. Metabolites of rabeprazole confirmed by LC-MS to be present in the sample.

Using the confirmed metabolites in the browser report the MetaboLynx program automatically performs product ion analysis of the sample (**figure 4 and 5**). The browser report is updated with the product ion data and comparisons are automatically made between metabolite spectra and that of the parent drug to identify common fragments and common losses. In this automated way basic structural similarities of the parent drug and its metabolites can be confirmed. 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 6**.

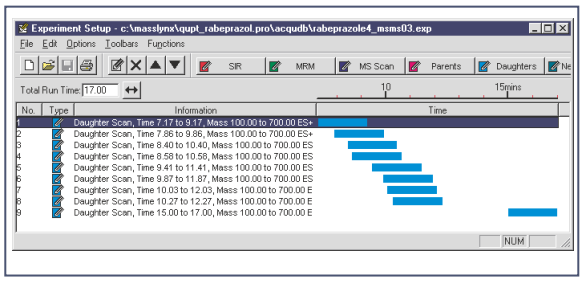

Figure 4.Product ion scan experiment automatically generated by MetaboLynx.

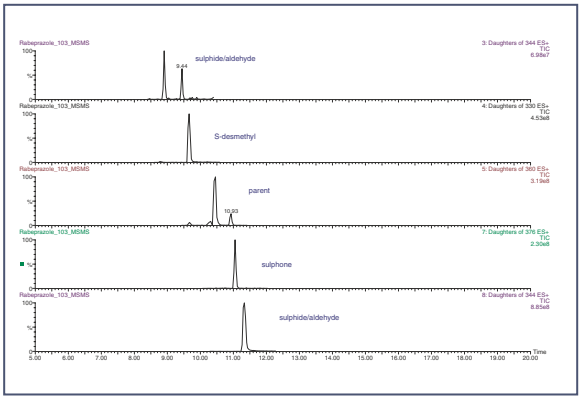

Figure 5. Product ion chromatograms used to confirm LC-MS findings.

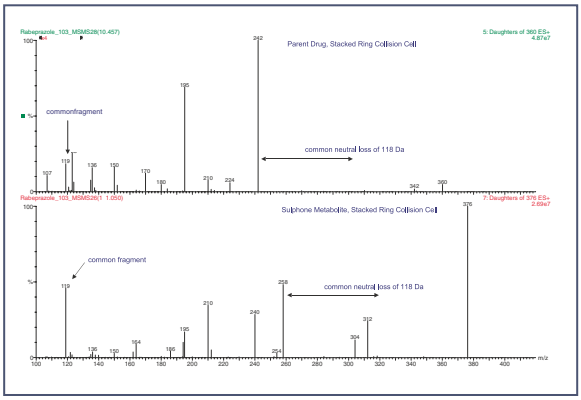

Figure 6. Example comparison of product ion spectra obtained for rabeprazole and one of its metabolites.

Fast precursor ion scanning (500 Da/sec) of the common product ion at m/z 119 was performed to confirm a common structural feature of rabeprazole and a number of its major metabolites (**figure 7**).

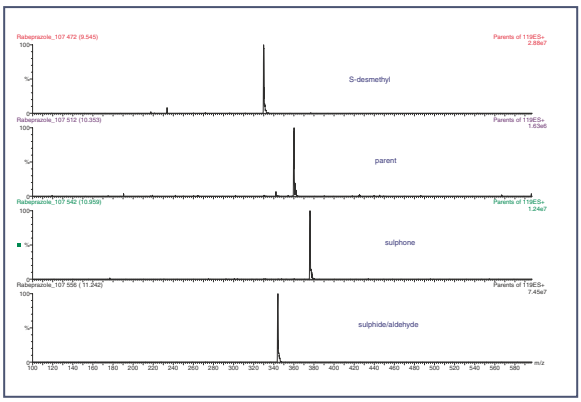

Figure 7. Precursor ion analysis results

Precursor ion analysis was repeated using a mass spectrometer equipped with a standard hexaople based collision cell. Extracted ion chromatogram intensity was observed to be 10 times greater with the stacked ring collision cell (**figure 8**). Due to the increased precursor ion resolution the extracted ion chromatograms obtained using the stacked ring collision cell were free of interference peaks significantly aiding confirmation of metabolite. 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 9**).

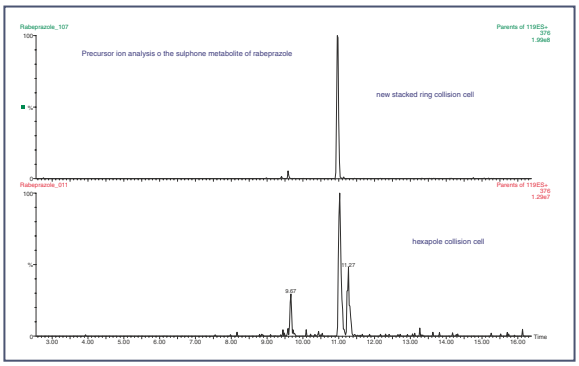

Figure 8. Comparison of two designs of collision cell for precursor ion analysis.

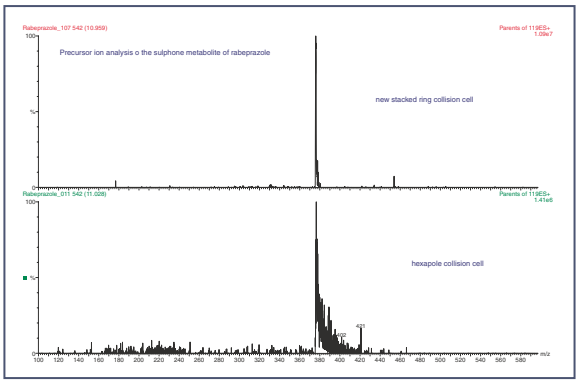

Figure 9. The effect of collision cell design on precursor ion resolution

A comparison was also made between the two designs of collision cell for neutral loss analysis. Using the neutral loss of 118Da a number of metabolites could be located in the sample (**figure 10**). The new collision cell showed an increase of a factor of 10 in intensity for both spectral and chromatographic data (**figure10 A and B**).

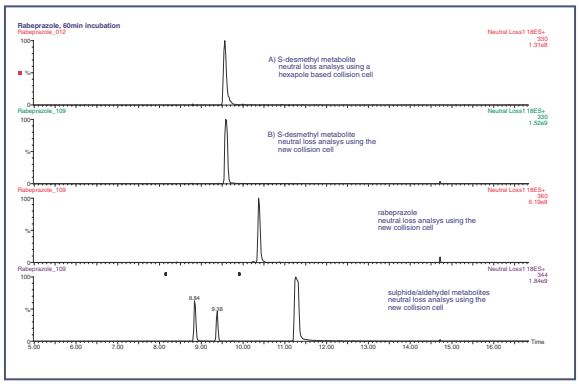

Figure 10. Neutral loss analysis.

CONCLUSIONS

The data presented shows how a tandem quadrupole mass spectrometer can be used to detect and confirm the presence of previously reported metabolites of rabeprazole in a microsamal incubation sample. The detection of metabolites in LC-MS data files can be automated. LC-MS-MS data is shown to be useful for confirmation of the LC-MS results.

The new collision cell was shown to have an advantage over the standard hexapole based collision cell when performing precursor and neutral loss analysis. In the precursor ion mode the mass resolution was maintained even at relatively high scan speeds. As a result of the increased resolution (at 500 Da/s) the precursor ion spectral intensities was significantly better for the new 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 new collision cell also 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.

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REFERENCES

1. High throughput automated metabolite search using LC-MS-MS. Jose. Castro-Perez and Steve Preece. Poster presented at PITTCON 2001.

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