Approaches towards rapid drug metabolite identification using ion trap mass spectrometry

Stephen W. Holman[1], Patricia Wright[2], and G. John Langley[1]

[1] *School of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK.*
[2] *Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Sandwich, CT13 9NJ, UK.*

Overview

- A 50 *m/z* unit loss from a model biaryl sulfoxide was observed and investigated.[1]
- The data was consistent with an *ortho* effect due to the methyl group.
- The fragmentation behaviour could be used to identify *S*-oxidation at a sulfur atom with an *ortho* methyl group.

Introduction

- Methodologies to reduce interpretation times of mass spectra of drug metabolites are of interest to the pharmaceutical industry.
- Structurally dependent dissociation pathways have been shown to be of use in identifying *S*-oxidation in many instances.[2]
- Another example of potentially useful fragmentation behaviour for identifying sulfoxides involving an *ortho* effect has been investigated using ion trap mass spectrometry (IT-MS), Fourier transform ion cyclotron resonance-mass spectrometry (FT-ICR MS) and molecular modelling calculations.

Methods

- 4-Benzenesulfinyl-3-methyl-phenylamine and 4-benzenesulfinyl-phenylamine were provided by Pfizer Global Research and 2-aminofluorene was purchased from Alfa Aesar.
- Solutions were prepared in analytical grade methanoic acid and LC-MS grade methanol [0.1:99.9, v/v]. For the deuterium exchange experiments a solution of 4-benzenesulfinyl-3-methyl-phenylamine was prepared in 99.5% deuterated ethanoic acid and >99.5% deuterated methanol [1:99, v/v].
- Positive ion electrospray product ion spectra were acquired using either a LCQ Classic QIT-MS with WideBand activation on and off or an Apex III FT-ICR MS.
- The gas phase proton affinity of 4-benzenesulfinyl-3-methyl-phenylamine was calculated at the density functional theory level (B3LYP) using the 6-31G** basis set. The equilibrium geometry was calculated at the ground state starting from AM1 geometry. Spartan '02 was used to perform the calculations.

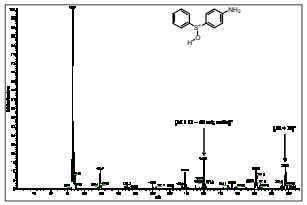

Figure 1. IT-MS first generation product ion spectrum, WideBand activation **ON**

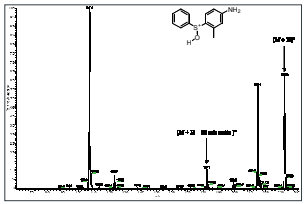

Figure 2. IT-MS first generation product ion spectrum, WideBand activation **ON**

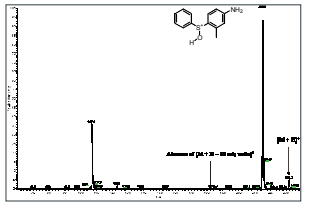

Figure 3. IT-MS first generation product ion spectrum, WideBand activation **OFF**

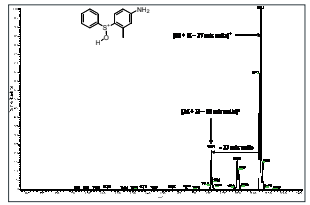

Figure 4. IT-MS second generation product ion spectrum, WideBand activation **OFF**

Results

- Protonated 4-benzenesulfinyl-phenylamine displayed a 48 *m/z* units loss (Figure 1).
- Protonated 4-benzenesulfinyl-3-methyl-phenylamine, displayed a 50 *m/z* unit loss with Wideband activation on (Figure 2) *c.f.* spectrum of protonated 4-benzenesulfinyl-phenylamine, suggesting an *ortho* effect in the fragmentation.
- Sequential losses of 17 and 33 *m/z* units demonstrated using MS/MS and MS^3 experiments with WideBand activation off (Figures 3 and 4).
- Molecular modelling showed the sulfoxide oxygen to be the most favourable site of protonation, which would be consistent with loss of a hydroxyl radical loss during the first stage of fragmentation.
- Deuterium exchange experiments proved hydroxyl radical loss during the first stage of mass analysis (Figure 5).
- Deuterium exchange experiments also showed that only one exchangeable hydrogen atom was involved in the 50 *m/z* loss (Figure 5) ⇒ support for the hypothesis of an *ortho* effect with the non-exchangeable hydrogen coming from the methyl group.
- FT-ICR MS confirmed that sequential losses of a hydroxyl and a thiol radical accounted for the 50 *m/z* unit loss (Figure 6).
- Comparison of the third generation product ion spectrum of protonated 4-benzenesulfinyl-3-methyl-phenylamine with the first generation product ion spectrum of protonated 2-aminofluorene was consistent with the latter being the structure of the ion at *m/z* 182 (Figures 7 and 8).
- Figure 9 shows a partial mechanism for the fragmentation with the planar product ion structure the likely driving force.

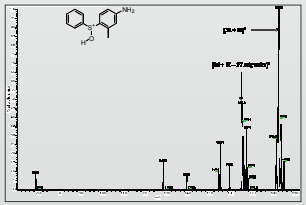

Figure 7. IT-MS third generation product ion spectrum, WideBand activation **OFF**

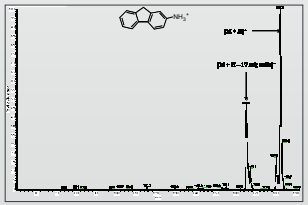

Figure 8. IT-MS first generation product ion spectrum, WideBand activation **OFF**

Conclusions

- Loss of 50 *m/z* units from protonated 4-benzenesulfinyl-3-methyl-phenylamine shown to be a two-step fragmentation process involving sequential losses of a hydroxyl and a thiol radical.
- Resultant product ion spectrum shown to be consistent with protonated 2-aminofluorene.
- Deuterium exchange can be useful in determining the site of protonation.
- The 50 *m/z* unit loss may be useful in identifying *S*-oxidation at a sulfur positioned *ortho* to a methyl group.
- Identity of neighbouring groups thought to be important in the fragmentation → loss seen for a biaryl substructure but not for aryl-alkyl systems (data not shown). Further investigations are being conducted.

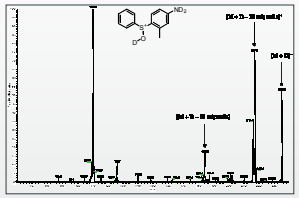

Figure 5. IT-MS first generation product ion spectrum, WideBand activation **ON**

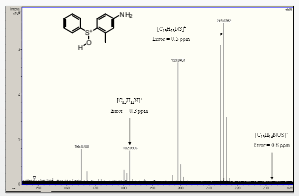

Figure 6. FT-ICR MS first generation product ion spectrum

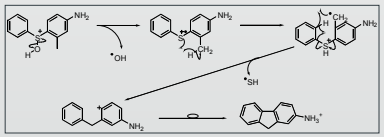

Figure 9. Proposed partial fragmentation mechanism

References

1. S. W. Holman, P. Wright and G.J. Langley, *Rapid Commun. Mass Spectrom., Accepted.*
2. P. Wright, A. Alex, D. Gibson, R. Jones and P. Macrae, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 2005-2014.

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