Expect the unexpected: An example of how unanticipated fragmentation behaviour could preclude correct assignment of sites of metabolism

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Introduction

- Identification of pharmaceutical drug metabolites is performed in drug discovery projects primarily using mass spectrometry.
- Understanding fragmentation under collision-induced dissociation (CID) conditions is critical to any structural elucidation.
- Unexpected rearrangements under CID conditions have been reported.[1,2]
- Herein, an unexpected rearrangement is discussed showing that incorrect assignments of sites of metabolism are possible if a rigorous analytical approach is not applied.

Methods

- 3-dimethylaminomethyl-4-(4-methylsulfanylphenoxy)-benzenesulfonamide (**1**), 3-dimethylaminomethyl-4-(4-methanesulfinyl-3-methyl-phenoxy)-benzenesulfonamide and (**2**) and 3-dimethyl-$^2H_6$-aminomethyl-4-(4-methanesulfinyl-3-methyl-phenoxy)-benzenesulfonamide (**3**) were synthesised by Pfizer Global R & D.
- Solutions were prepared in analytical grade HCOOH and LC-MS grade MeOH [0.1:99.9, v/v] or 99.5% deuterated CH_3COOD and >99.5% deuterated MeOH [1:99, v/v].
- Positive ion electrospray product ion spectra were acquired using either a LCQ Classic QIT-MS with WideBand activation on or an Apex III FT-ICR MS *via* direct infusion at 3 μL min^{-1}.

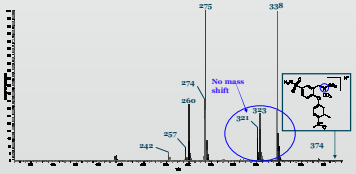

Figure 5. First generation product ion spectrum of protonated **3**

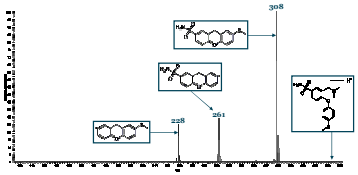

Figure 1. First generation product ion spectrum of protonated **1**

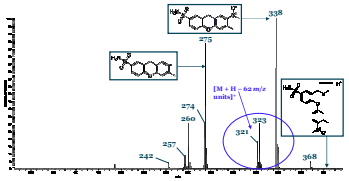

Figure 2. First generation product ion spectrum of protonated **2**

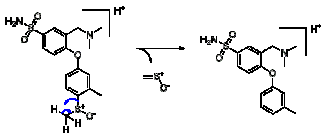

Figure 3. Proposed mechanism for the loss of 62 *m/z* units

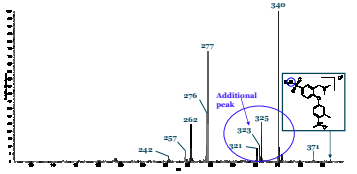

Figure 4. First generation product ion spectrum of fully exchanged, deuterated **2**

Results

- Protonated **1**, the parent compound, produced three major product ions under CID conditions through losses of the three side chains, or combinations thereof (Figure 1).
- Protonated **2**, a model *S*-oxidised metabolite, produced a more complex product ion spectrum (Figure 2).
- A loss of 62 *m/z* units was only observed for the model metabolite. The dissociation was assumed to be due to the oxidation and involve the loss of methanethial, *S*-oxide, facilitated by a 1,3-proton shift, *via* a four-centred rearrangement (Figure 3).[3]
- Deuterium-exchange experiments showed losses of the labels, disproving the initial hypothesis. The appearance of an additional peak indicated that, in protonated solvents, two losses formed nominally isobaric ions. The loss due to all three labels suggested loss of the primary amine group in the dissociation (Figure 4).
- Dissociation of a deuterium labelled analogue, **3**, indicated that both dissociations lost the tertiary amine group due to a single peak at *m/z* 321 being observed (Figure 5).

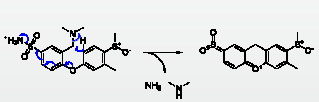

Figure 7. Proposed mechanism for loss of $C_2H_{10}N_2$ for compound **2**

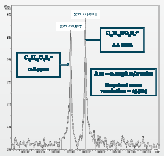

Figure 6. First generation product ion spectrum of protonated **2** using FT-ICR-MS

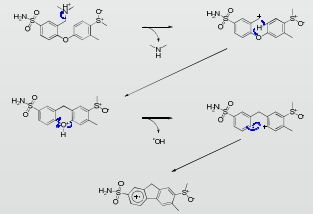

Figure 8. Proposed mechanism for loss of $C_2H_8NO^{\cdot}$ for compound **2**

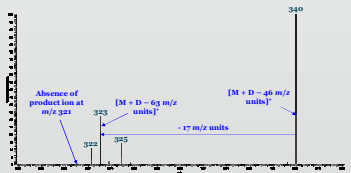

Figure 9. Second generation product ion spectrum of fully exchanged, deuterated **2**

- High-resolution FT-ICR-MS showed the existence of two ions at a nominal *m/z* of 321 and allowed assignment of the losses as $C_2H_{10}N_2$ and $C_2H_8NO^{\cdot}$ (Figure 6). Mechanisms for these losses have been proposed (Figures 7 and 8).
- Both dissociations involve the loss of the tertiary amine group, one facilitated by the ionising proton and the other by the shift of a non-exchangeable hydrogen atom.
- The loss of the tertiary amine group is also observed in the first generation product ion spectrum *via* protonation at that site for compound **2**. Supportive evidence for the mechanisms was provided through acquisition of the second generation product ion spectrum *via* dissociation of the ion at *m/z* 338 (experiment performed in deuterated solvents to allow mass resolution on the QIT-MS; thus ion observed at *m/z* 340).
- The shift of the first generation product ion to *m/z* 340 indicated protonation at the tertiary amine. The second generation loss of 17 *m/z* units supported the mechanism for the loss of $C_2H_8NO^{\cdot}$ as proceeding *via* protonation at the tertiary amine followed by the loss of dimethylamine and a hydroxyl radical, the latter facilitated by the shift of a non-exchangeable hydrogen atom (Figure 9).
- The absence of the ion at *m/z* 321 supported the mechanism for the loss of $C_2H_{10}N_2$ where protonation is proposed at the primary amine and involves loss the tertiary amine group through the shift of a non-exchangeable hydrogen atom (Figure 9).

Conclusions

- *S*-oxidation can significantly change the dissociation of a compound.
- Dissociation under CID conditions can be difficult to predict and is poorly characterised.
- Extensive experimentation required to fully understand dissociations.
- Can not assign sites of metabolism confidently without a rigorous analytical approach.
- Deuterium-exchange experiments useful for determining sites of protonation and elucidating different dissociation pathways.

References

1. P. R. Tiller, C. Raab and C. E. C. A. Hop, *J. Mass Spectrom.*, 2001, **36**, 344-345.
2. A. G. Craig and S. W. Taylor, *J. Am. Soc. Mass Spectrom.*, 2001, **12**, 470-474.
3. C. E. Hudson and D. J. McAdoo, *J. Am. Soc. Mass Spectrom.*, 2004, **15**, 972-981.

Acknowledgements