Rapid metabolite identification using ion trap mass spectrometry

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1. Introduction

Determining the sites of metabolism of pharmaceutical compounds is often a laborious and time-consuming process

The difficulties associated with this practice have been cited as a restriction with respect to high-throughput metabolite analyses[1]

Radical losses from sulfoxides have been used to accurately and definitively identify the site of oxidation in certain instances[2]

Further characteristic fragmentation behaviour could allow a reduction in interpretation times, particularly for common biotransformations *e.g. S*-oxidation, *N*-oxidation

2. Experimental

All compounds were provided by Pfizer Global Research and Development or purchased from Sigma-Aldrich (Gillingham, UK) and used without further purification

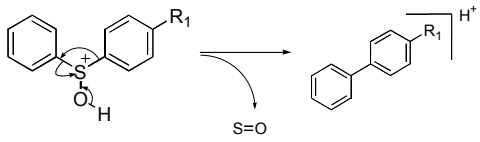
Solutions were prepared at 10 µg mL^{-1} in 0.1% analytical grade methanoic acid and LC-MS grade methanol (Fisher Scientific, Loughborough, UK)

Product ion spectra were acquired using a LCQ classic ion trap mass spectrometer (Thermo Fisher, San Jose, USA) with WideBand activation on and off

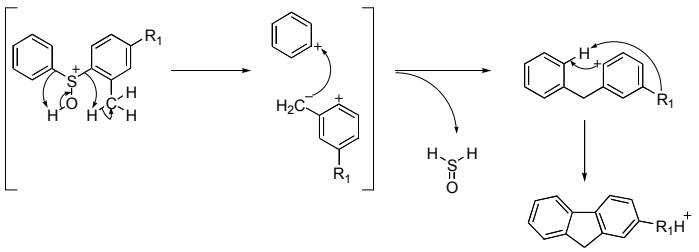
Solutions were directly infused into the electrospray ionisation source at a flow rate of 3 µL min^{-1}

3. Results and Discussion

- Product ion spectra of a series of simple sulfoxides and *N*-oxides were acquired
- Neither class of compounds produced common product ions or neutral losses which could indicate *S*- or *N*-oxidation
- Potentially useful fragmentation behaviour was identified that could indicate particular substructures in drug metabolites
- Two compounds with aromatic rings bridged by a sulfoxide group exhibited a 48 Da loss (SO)

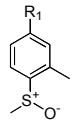


- A structurally similar compound with a methyl group *ortho* to the sulfoxide functionality showed a 50 Da loss (H_2SO)

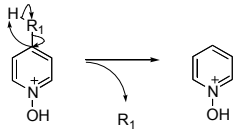


- Deuterium exchange experiments were performed
  - → 51 Da loss observed
  - → Indicated that one exchangeable proton was involved in the transition
  - → Suggests that the site of protonation is the sulfoxide oxygen and supports the hypothesis of an *ortho* effect

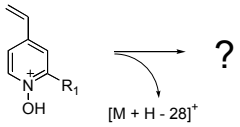
- A product ion spectrum of a compound containing both a methyl substituent and an *ortho* methyl group was acquired
  - → Loss of 15 Da observed, no 50 Da loss seen
  - → Either 50 Da loss is compound specific or functionalities form a hierarchy that dictate dissociation pathways[3]



- Four *N*-oxide compounds with various subtituents positioned *meta* and *para* to an oxidised pyridine nitrogen generated product ions at *m/z* 96
  - → Product ion at *m/z* 96 not observed for analogous pyridines
  - → Ion may be characteristic of *N*-oxidation
  - → Substituent may allow definitive assignment of the site of metabolism when multiple nitrogen atoms are present



- Three *N*-oxide compounds with either an ethanone, carbaldehyde or vinyl substituent *para* to an oxidised pyridine nitrogen displayed a 28 Da loss in their product ion spectra
  - → 28 Da loss seen for analogous pyridines with ethanone and carbaldehyde groups
  - → No 28 Da loss observed for analogous pyridine with a vinyl substituent
  - → Loss may be characteristic of *N*-oxidation at a pyridine nitrogen *para* to a vinyl group



4. Conclusions

- Loss of 48 Da → suggests oxidation at an sulfur atom bridging two aromatic rings?
- Loss of 50 Da → suggests oxidation at a sulfur atom *ortho* to a methyl group?
- Product ion at *m/z* 96 → suggests oxidation at a pyridine nitrogen with specific substituents positioned *meta* or *para*?
- Loss of 28 Da → suggests oxidation at a pyridine nitrogen with a vinyl substituent positioned *para*?
- Hierarchy possibly exists whereby certain functionalities influence dissociation pathways to a greater extent → a methyl substituent more dominant than an *ortho* methyl group on the fragmentation of sulfoxides?

5. Future work

- Determine whether dissociation pathways observed for simple molecules apply to more complex structures
- Identify further structurally dependent dissociation pathways that may allow assignment of sites of metabolism
- Investigate use of multivariate analyses *e.g.* PCA, to discriminate between spectra of drug molecules and their metabolites

References

1. M. L. Bandu, K. R. Watkins, M. L. Bretthauer, C. A. Moore and H. Desaire, *Anal. Chem.*, 2004, **76**, 1746-1753.
2. P. Wright, A. Alex, D. Gibson, R. Jones and P. Macrae, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 2005-2014.
3. K. Klagkou, F. Pullen, M. Harrison, A. Organ, A. Firth and G. J. Langley, *Rapid Commun. Mass Spectrom.*, 2003, **17**, 1163-1168.

Acknowledgements

University of Southampton
bbsrc biotechnology and biological sciences research council
Pfizer
BMSS BRITISH MASS SPECTROMETRY SOCIETY