

Loss of Hydroxyl Radical ($\bullet\text{OH}$) in CAD MS/MS for Identification of Oxidized Drug Metabolites

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Introduction

Recently, LC/MS has become the predominant means for drug metabolite characterization and identification. Meanwhile, formation of *N*-oxides and quaternary *N*-*O*-glucuronides in nitrogen-containing xenobiotics, as well as sulfoxides in sulfur-containing compounds, has been demonstrated to be common metabolic pathways, in addition to hydroxylated derivatives and their glucuronides. Due to the limitation of mass data, it is challenging for analytical chemists to determine the type of oxidation and to differentiate quaternary *N*-*O*-glucuronides from *C*-*O*-glucuronides by LC/MS. Here we report some observations of loss of a hydroxyl radical ($\bullet\text{OH}$) in CAD MS/MS from *N*-oxides, quaternary *N*-*O*-glucuronides, and some types of sulfoxides. We also report for the first time reduction of acetonitrile under ESI conditions to yield ethyl amine, which can attach to analytes to form ethyl amine adduct molecular ions $[\text{M}+\text{CH}_2\text{CH}_2\text{NH}_2]^+$.

Experimental

Liquid chromatography:

LC System: Waters 2690 Separations Module
Mobile Phases:
A: CH_3CN
B: 0.4% HCOOH in H_2O
C: 0.01M NH_4OAc in H_2O

Gradient: A generic gradient was used. Mobile phases A and C were used for positive ion mode and mobile phases B and C were used for negative ion mode.

Mass Spectrometry:

MS Systems I & II: Finnigan MAT TSQ-7000 Triple Quadrupole Finnigan LCGTM Mass Spectrometers.

Ion spray (IS): 4.5 kV

Capillary temperature: 170-300 °C

MS System III : AB 4000 Q TRAP LC/MS/MS System

IS: 5.5 kV

Turbo probe temp: 300-500 °C

MS System IV : AB QSTAR[®] XL MS/MS System

IS: 5.0 kV

Turbo probe temp: 300-500 °C

Results and Discussion

1 Loss of a hydroxyl radical ($\bullet\text{OH}$) was observed in CAD MS/MS from an aromatic *N*-oxide (XBL0001) in the negative ion mode. However, loss of $\bullet\text{OH}$ was not detected in a phenolic compound (XBL0002) under the same conditions (Figure 1).

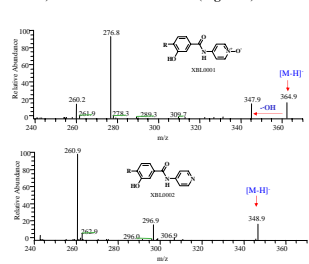


Figure 1. LC(-)ESI-MS-MS Spectra of XBL0001 and XBL0002

2 Loss of a glucuronoy moiety (176-Dalton) followed by loss of a $\bullet\text{OH}$ moiety (17-Dalton) was observed for an *N*-*O*-glucuronide (XBL0003) in the negative ion mode. Under the same conditions, loss of only a glucuronoy moiety was detected in a phenol glucuronide (XBL0004, Figure 2).

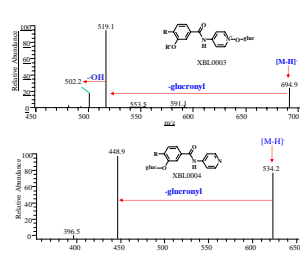


Figure 2. LC(-)ESI-MS-MS Spectra of XBL0003 and XBL0004

3 Loss of a hydroxyl radical ($\bullet\text{OH}$) was observed from a sulfoxide (XBL0005) but not from a phenolic compound (XBL0006) in the positive CAD MS/MS (Figure 3).

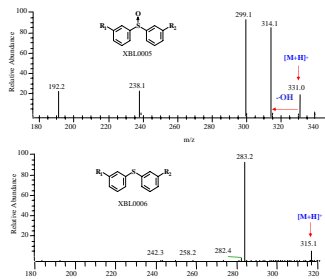


Figure 3. LC(+)ESI-MS-MS Spectra of XBL0005 and XBL0006

4 Loss of H_2O , rather than $\bullet\text{OH}$, was observed for an aromatic *N*-oxide (XBL0001) and aliphatic *N*-oxide (XBL0007) in the positive ion mode (Figure 4).

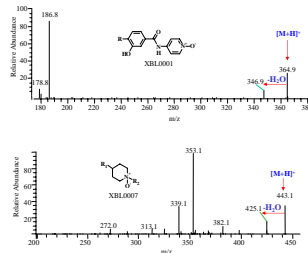


Figure 4. LC(+)ESI-MS-MS Spectra of XBL0001 and XBL0007

5 Thermal dissociation of oxidized metabolites in CAD MS/MS and typical deoxygenation¹ are summarized in the following table:

Metabolic type	CAD MS/MS (Loss of Moieties)	Thermal Dissociation
Aromatic <i>N</i> -oxide	$[\text{M}+\text{H}]^+$ (7 Da, (-), TSQ 7000 and AB 4000 Q Trap) or $[\text{M}+\text{H}]^+$ (18 Da, (+), TSQ 7000 and AB 4000 Q Trap)	$[\text{M}]^+$ (16 Da, (+) and (-), TSQ 7000 and AB 4000 Q Trap)
Aliphatic <i>N</i> -oxide	$[\text{M}+\text{H}]^+$ (18 Da, (+), TSQ 7000 and AB 4000 Q Trap)	$[\text{M}]^+$ (16 Da, (+) and (-), TSQ 7000 and AB 4000 Q Trap)
Sulfoxides (Some types)	$[\text{M}+\text{H}]^+$ (2, LCG)	NA
Aliphatic hydroxyls	$[\text{M}+\text{H}]^+$ (18 Da, (+) and (-), some)	NA
Aromatic hydroxyls	$[\text{M}+\text{H}]^+$ (18 Da, (+) and (-), weak)	NA
<i>N</i> - <i>O</i> -Glucuronides	Glucuronoyl (176 Da, (-), TSQ 7000) and $[\text{M}+\text{H}]^+$ (176 Da, (+), TSQ 7000)	NA
<i>N</i> - <i>O</i> -Glucuronide	Glucuronoyl (176 Da, (+))	NA
Phenol- <i>O</i> -Glucuronide	Glucuronoyl (176 Da, (-) and (-))	NA
Aliphatic- <i>O</i> -Glucuronide	Glucuronoyl (176 Da, (+) and (-)) and $[\text{M}+\text{H}]^+$ (18 Da, (+) and (-))	NA

6 It was found that acetonitrile could be converted to ethyl amine under ESI conditions. The formed ethyl amine was added to the molecules of some analytes, resulting in formation of a characteristic molecular ion cluster of $[\text{M}+\text{H}]^+$ and $[\text{M}+\text{CH}_2\text{CH}_2\text{NH}_2]^+$ (Figures 5 and 6).

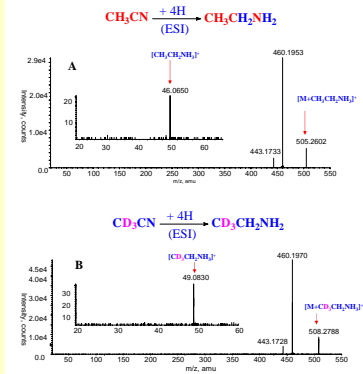
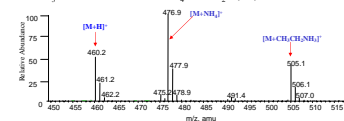
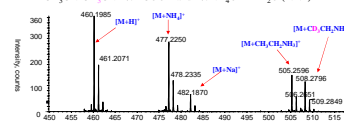


Figure 5. Product Ion Scan of m/z 505 (A) and 508 (B)

in CH_3CN -1% HCOOH and 1% NH_4OH in H_2O (1:1)



in CH_3CN - CD_3CN -1% HCOOH and 1% NH_4OH in H_2O (1:1:2)



in CD_3CN -1% HCOOH and 1% NH_4OH in H_2O (1:1)

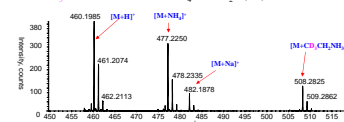


Figure 6. LC(+)ESI-MS-MS of XBL0008

Conclusions

Loss of a $\bullet\text{OH}$ (17 Da) or H_2O under CAD MS/MS appeared to be characteristic of different types of oxidized derivatives, e.g., *N*-oxides, sulfoxides, and hydroxyls. The observations, combined with some mass fragmentations proposed by other scientists,¹ are very useful for characterization of oxidized metabolites by LC/MS.

The novel adduct ion, i.e., $[\text{M}+\text{CH}_2\text{CH}_2\text{NH}_2]^+$, formed through reduction of acetonitrile from the LC mobile phase under ESI conditions, was found to be useful for the confirmation of the molecular ions.

Reference

1. Ramanthan, Su A-D, Alvarez, N, Chowdhury SK, Alton K, Patric JE, *Anal.Chem.* 200, 72, 1352.

