Accurate Mass Measurements of Product Ions for Metabolite Identification on Unit Mass Resolution Mass Spectrometers

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Overview

- Accurate Mass measurements of product ions made available on unit mass resolution mass spectrometers
- Novel algorithms to calibrate mass spectrometers for both mass and peak shape
- Demonstrated data including infusion of drug standards and LC/MS of rat liver microsomal incubations of verapamil

Introduction

Accurate mass (AM) measurements are routinely performed on high resolution mass spectrometers for metabolite identification. However, these instruments are very expensive and often require dedicated and skillful operators. We have developed MSIntegrity™ technologies that allow AM analysis to be conducted on unit mass resolution mass spectrometers[1]. Although AM analysis of verapamil metabolites has already been demonstrated previously in full MS mode, further elucidation of the structures of these metabolites requires the recognition of their corresponding fragment ions from collision-induced dissociation experiments. Accurate measurements of these ions are desirable to pinpoint their structures[2]. As an extension to the MSIntegrity technologies, we report here the AM measurements of fragment ions to facilitate metabolite identification on unit mass resolution mass spectrometers.

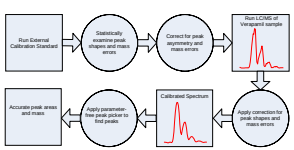
Methods

The product ion spectra of a set of small pharmaceuticals were acquired from an ABI/Sciex 4000 Q TRAP by infusion, while LC/MS/MS measurements of rat liver microsomal incubation of verapamil were performed on an ABI/Sciex 3200 Q TRAP.

Methods

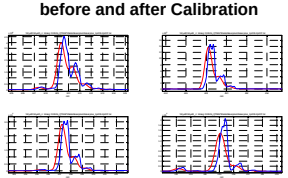
All mass spectral data were collected in profile mode and processed using the MSIntegrity technologies implemented in the MassWorks™ software. A few or a single known fragment ion(s) from the MS/MS spectra by infusion was selected as calibration standard(s) to internally calibrate the spectra. Some of the ions in the same spectra were tested for mass accuracy. LC/MS/MS fragment ions were generated on-the-fly by information dependent scans. Accurate mass measurements on demethylated verapamil were achieved by applying an external calibration made by the fragments generated from verapamil.

Calibration Procedures



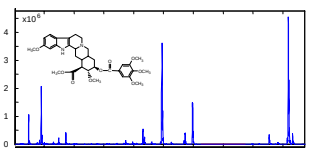
Results and Discussion

The MS/MS Peak Shape before and after Calibration



Results and Discussion

MS/MS Spectrum of Reserpine



AM of Product Ions of Reserpine

(Internal calibration)

Four point calibration

Name of Ions	Exact Mass	Formulam of Ions	Accurate Mass	Delta Mass (mDa)
Reserpine	609.2812	C33H41N2O9	Cali Ion	
Frag1	448.1971	C23H30NO8	448.1936	-3.5
Frag2	397.2127	C23H29N2O4	Cali Ion	
Frag3	195.0657	C10H11O4	Cali Ion	
Frag4	174.0919	C11H12ON	Cali Ion	

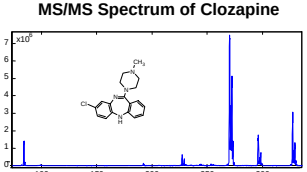
Two point calibration

Name of Ions	Exact Mass	Formula of Ions	Accurate Mass	Delta Mass (mDa)
Frag2	397.2127	C23H29N2O4	Cali Ion	
Frag3	195.0657	C10H11O4	195.0630	-2.7
Frag4	174.0919	C11H12ON	Cali Ion	

One point calibration

Name of Ions	Exact Mass	Formula of Ions	Accurate Mass	Delta Mass (mDa)
Frag3	195.0657	C10H11O4	Cali ion	
Frag4	174.0919	C11H12ON	174.0924	-0.5

MS/MS Spectrum of Clozapine



AM of Product Ions of Clozapine

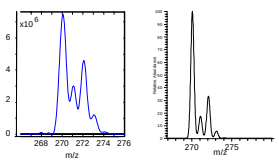
(Internal Calibration)

One point calibration

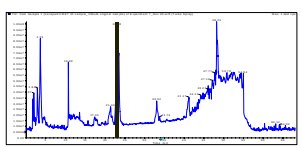
Name of Ions	Exact Mass	Formula of Ions	Accurate Mass	DeltaMass (mDa)
Clozapine	327.1376	C18H20ClN4	327.1379	0.3
Frag1	296.0955	C17H15ClN3	Cali Ion	
Frag2	270.0798	C15H13ClN3	270.0438	-36

Note: Large mass errors of m/z of 270 were due to saturation.

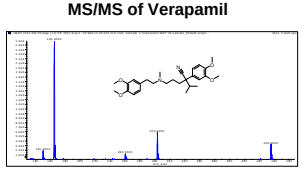
Saturated and Theoretical Isotope Distribution of C15H13ClN3



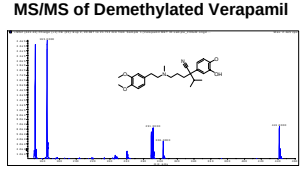
TIC of Verapamil Metabolites



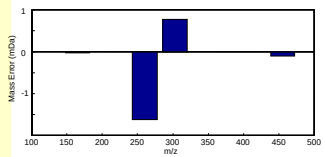
MS/MS of Verapamil



MS/MS of Demethylated Verapamil



Mass Errors of Four Calibration Ions



AM of MSMS of Demethylated Verapamil

(External Calibration)

Name of Ions	Exact Mass	Formula of Ions	Accurate Mass	Delta Mass (mDa)
Demethylated Verapamil	441.2753	C26H37N2O4	441.5840	-0.6
Frag1	303.2073	C18H27N2O2	303.4199	5.5
Frag2	289.1916	C17H25N2O2	289.3933	-5.6
Frag3	291.2073	C17H27N2O2	291.4092	-6

Conclusions

- Accurate mass measurements of product ions with 5 ppm can be achieved on unit mass resolution instruments through MassWorks
- Mass spectrometry calibration including both mass and peak shape is critical for high mass accuracy.
- Internal calibration can help achieve highest possible mass accuracy.
- Peak saturation is a key factor causing poor mass accuracy in a quadrupole system.

References

1) Gu, M. et al *RCMS*, **2006**, 20, 764.

2) Zhang, M. et al *JMS*, **2005**, 40, 1017.