Comprehensive Investigation of Injection Time and Auto Gain Control on Ion Trap Instruments for LC/MS Application of Metabolite Identification

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Overview

➢Space charge effect in an ion trap instrument was investigated by monitoring Auto Gain Control (AGC) and injection time.

➢Mass accuracy and spectral accuracy of the ions measured at different AGC and injection time were obtained by self calibrated instrument line shape isotope profile search (sCLIPS).

➢Best data acquisition conditions for accurate mass measurements and formula ID are recommended and remained challenges are discussed.

Introduction

Ion trap mass spectrometers are widely used in drug metabolism study, proteomics, product impurity and degradant identification, and other areas of qualitative analysis due to their great sensitivity, high order of MS/MS capability, fast scan speed, and high mass resolution. However, this type of instrument suffers from space charge effect and could not be used for accurate mass measurements to determine elemental composition, which quite often is a valuable and necessary additional confirmation for structural elucidation. In this study, we focus on space charge effect on both mass accuracy and isotope profile variations. The goal is to discover the optimal instrument conditions to achieve necessary mass accuracy and isotope distribution for identification of metabolites by LC/MS analysis.

Methods

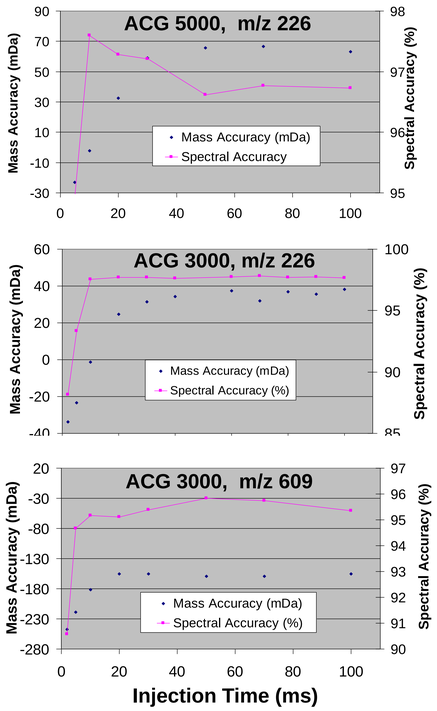
All experiments are performed on a Thermo LTQ linear ion trap instrument. The injection time and auto gain control (AGC) value as two important instrument parameters related to space charge effect are investigated. Zoom scan mass spectra of Terbutaline and Reserpine by infusion under different injection time and AGC values. The injection time varies from 5 to 100 ms, while different AGC values including 1000, 2000, 3000, and 5000 are selected. The acquired zoom scan spectra are averaged over up to 1 minute and used for accurate mass measurements and isotope pattern comparison with theoretical one from both Terbutaline and Reserpine. The zoom scan spectra of Terbutaline were also averaged with 3 seconds to evaluate their accurate mass and isotope profile search performance in a LC/MS time scale. Both mass and spectra accuracy are calculated and formula search is performed by MassWorks.

Results and Discussion

Space charge effect in an ion trap instrument is simply due to too many ions confined in a limited space. Their interactions result in mass shift and isotope distortion during mass analysis. In order to minimize the space charge effect and maintain sufficient sensitivity, the number of ions injected into the trap were optimized by adjusting injection time and AGC values. These experiments were performed with data acquisition of zoom scan of Terbutaline by infusion. Under the conditions of AGC at 5000 and injection times at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ms, the mass accuracy for Terbutaline varies from -2.2 to 66.7 mDa while spectral accuracy, i.e. the isotope pattern match between experimental and theoretical spectra, was observed from 94.9 to 97.6% (100% is the best possible match). The best mass accuracy of -2.2 mDa was found with injection time of 10 ms. With mass tolerance of 10 mDa, elemental composition determination for this measurement shows the Terbutaline was ranked number 3 based on mass accuracy and ranked number 2 based on spectral accuracy.

Results and Discussion

Mass and Spectral Accuracy vs. Injection Time

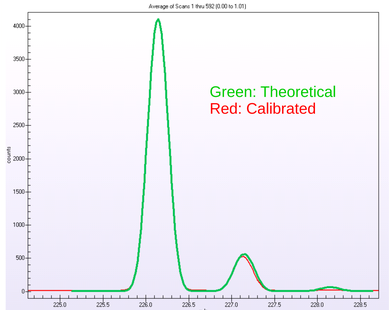


sCLIPS Search Results for m/z 226

Accurate Mass	226.1421			Element	Min	Max
Charge	1			C	0	34
Mass Tolerance (mDa)	10			H	0	411
Electron State	Even			N	0	29
DBE (min.)	-1			O	0	25
DBE (max.)	50					
Profile Mass Start (Da)	-1					
Profile Mass End (Da)	1.5					

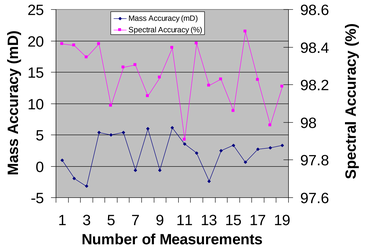
Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
C8H16N7O	226.1416	-0.4668	-2.0643	98.5942	17	4.5
C12H20NO3	226.1443	2.2185	9.8103	98.2181	21	3.5
C7H20N3O5	226.1403	-1.8042	-7.9783	96.8165	38	-0.5
C6H20N5O4	226.1515	9.4292	41.6957	96.501	42	-0.5
C14H16N3	226.1344	-7.6775	-33.9498	96.0101	48	8.5
C3H16N9O3	226.1376	-4.4896	-19.8529	94.8954	61	0.5
C2H16N11O2	226.1488	6.7438	29.8211	94.5626	65	0.5

Theoretical and Calibrated Mass Spectra of Terbutaline



What is interesting is that the observed isotope pattern of Terbutaline have normal distribution for monoisotope (M) and 13C peaks (M+1), but a large portion of (M+2) peak is missing. In this case, sCLIPS search for m/z 226 achieved high spectral accuracy of 98.2% using isotope peaks of M and M+1 only.

Mass and Spectral Accuracy for Zoom Scan Spectra at LC Time Scale



The zoom scan spectra of Terbutaline averaged for 3 seconds have mass accuracy better than 10 mDa and spectral accuracy better than 98% for all the 19 measurements. These results indicate the zoom scans can be used for LC/MS data acquisition for the purpose of accurate mass measurements and formula identification.

Recommendations and Future Work

Preliminary results show the best injection time is about 10 to 20 ms. The injection time longer than 20 ms will resulted in mass errors higher than 10 mDa. The injection time less than 10 ms also posed a problem with poor spectral accuracy.

Zoom scan spectra provide high resolution MS data and can be directly analyzed by sCLIPS for accurate mass measurements and formula ID with no need of any calibration. However, the distortion of isotope distribution of the spectra with M+2 peak nearly missing was observed throughout this study. Further investigation is necessary to understand this behavior and to find optimized instrument conditions to get ideal isotope pattern for compound ID.

Under the same conditions of AGC and injections time, mass errors appear to be compound dependent. Terbutaline has about 2 mDa with injection time of 10 ms and AGC of 3000 while reserpine has mass errors about
-180 mDa.

Conclusions

➢ Space charge effect in an ion trap instrument can be managed by selecting ion injection time to achieve good mass and spectral accuracy for formula ID. Current study indicates 10 to 20 ms are the best injection time for acquiring zoom scan LC/MS data in a LTQ type ion trap.

➢ With 10 mDa mass accuracy and normal isotope pattern, it is possible to identify unknowns with zoom scan spectra by sCLIPS.

➢ The compound dependent mass shift and the distortion of isotope pattern in the ion trap instrument observed in this study are complicated and warrant further investigation.