Accurate Mass Measurement of High Concentration Samples by LCMS-IT-TOF

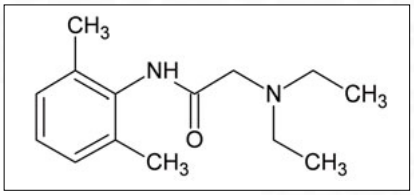
Technical Report vol.27

1. Introduction

Time of Flight (TOF) type mass spectrometers are used because they are capable of providing accurate high-resolution mass information. Mass accuracy for TOF analyzers can deteriorate under certain conditions when analyzing high concentration samples. A small error in the measurement values used to predict composition formulas can lead to mistaken conclusions. When the upper limit of the detector is exceeded by an especially large electrical signal, the mass accuracy is adversely affected, in comparison with the accuracy displayed with typical signal intensities. To avoid this problem, which is common with any mass spectrometer, it is usually necessary to dilute the sample and adjust the injection volume, and then re-analyze. However, this approach can lead to difficulty in determining the appropriate dilution, especially if the sample consists of a mixture of components at varying concentrations. When a sample contains components with different concentrations, a lower concentration substance might become too dilute due to dilution to provide sufficient signal for accurate mass determination. To avoid this situation, the LCMS-IT-TOF instrument incorporates a feature referred to as ASC (Automatic Sensitivity Control). This function automatically adjusts the ion accumulation time in the octapole according to the signal intensity, thereby averting detector saturation when especially high concentration components are introduced. ASC allows stable mass accuracy to be obtained even when measuring samples of differing concentration levels or samples containing multiple constituents, and eliminates the bother of performing dilution and re-analysis during the process, while also eliminating mobile phase waste.
To illustrate the benefits of the ASC function, lidocaine solutions were prepared at concentrations ranging from 100 ng/mL to 1 mg/mL, and analyzed using the flow injection method. At each concentration, the mass spectral peak shape and mass accuracy were checked to determine the benefit of enabling the ASC function.

2. Method

A 1 mg/mL solution of lidocaine in acetonitrile was diluted stepwise to prepare solutions at concentrations of 100 µg/mL, 10 µg/mL, 1 µg/mL, and 100 ng/mL, respectively.



Lidocaine
$C_{14}H_{22}N_2O$
Exact Mass: 234.1732
$[M+H]^+$: 235.1805

Fig. 1 Structural Formula of Lidocaine

Using a mobile phase of acetonitrile/water (90/10), flow injection analysis was conducted at a flow rate of 0.2 mL/min. The injection volume at each concentration was 2 µL. Ionization was conducted in the ESI positive mode, and scanning analysis was carried out from m/z 150 - 1000. The ASC settings are indicated on the following page.

3. ASC (Automatic Sensitivity Control) Settings

Fig. 2 shows the ion path of the instrument. In the LCMS-IT-TOF, ions are injected into the ion trap in pulses that are produced through the action of a combination of the skimmer, octapole and lens optics (compressed ion injection). In creating these pulses, the ions are first accumulated in the octapole for a fixed period of time, with more ions accumulated at longer accumulation times for higher signal intensity where needed. The value for the ion accumulation time can be freely set in the method file, but when the ASC feature is activated, the ion accumulation time in the octapole is adjusted automatically and instantaneously during the analysis to effectively avoid saturation of the detector.

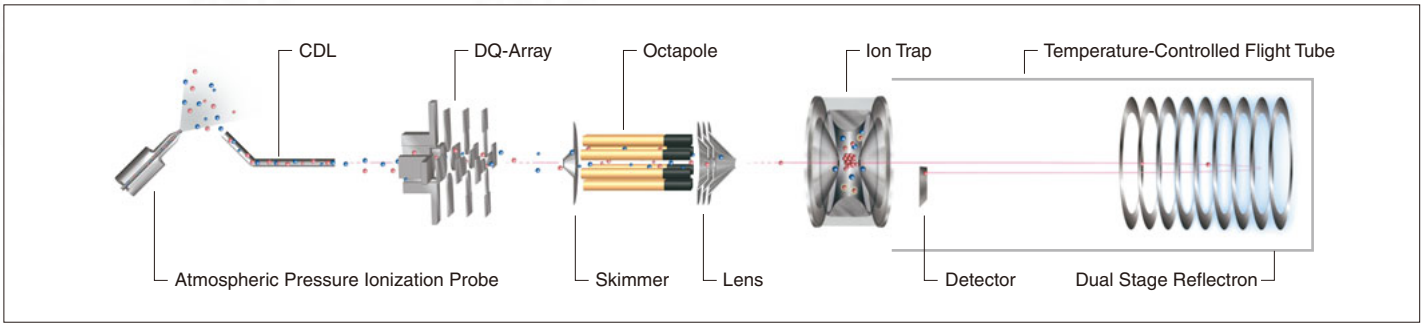


Fig. 2 LCMS-IT-TOF Ion Path

Fig. 3 shows the method file screen that was used. The portion enclosed in the red frame is used for setting the ion accumulation time. When ASC is not used, a fixed ion accumulation time is used for every scan (in Fig. 3, 30 msec). To use ASC, select the [ASC] checkbox and set a target ion quantity. The target ion quantity is set as the threshold at which the ASC begins to function, so when the signal intensity of the base peak exceeds 50% (as set in Fig. 3) of the full scale of the detector signal, the ASC starts working by automatically shortening the ion accumulation time according to the intensity (it is never adjusted to be longer than the entered time). The amount of decrease in the ion accumulation time during ASC operation is also considered, and the intensity corrected with respect to the actual signal intensity during data processing is displayed.

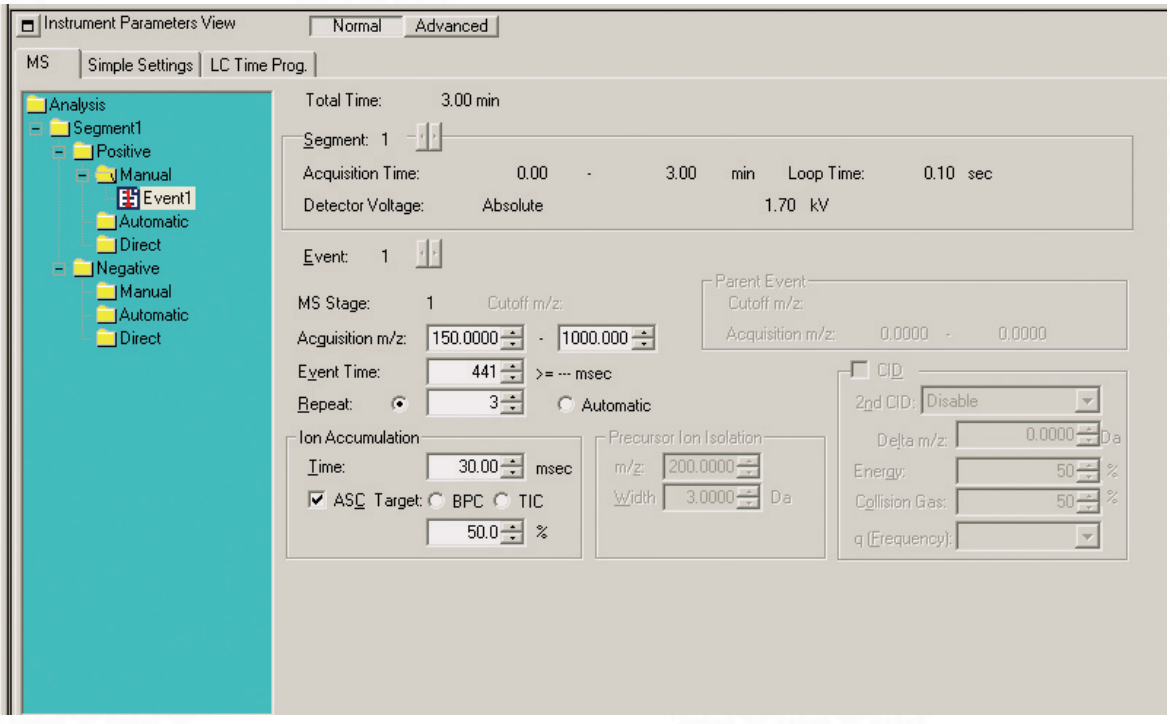


Fig. 3 Method Setting Example for ASC (used for this analysis)

4. Results

Fig. 4 shows the mass spectra enlarged in the vicinity of the peak for each concentration of lidocaine solution. The data acquisition results on the left pertain to ASC (OFF), and on the right to ASC (ON).

At 100 ng/mL, a fairly low level, a good peak shape is obtained without saturation in both ON and OFF conditions. At concentrations from 1 µg/mL to 100 µg/mL, saturation is seen when ASC is not used (saturated signal is indicated with red-colored spectrum). As the concentrations increase, the peak shapes deteriorate further, but there are no obvious changes in mass accuracy. At 1 mg/mL, a very high concentration, the peak apex is flattened, and the mass accuracy is also affected. On the other hand, when ASC was used, the same good spectral peak shape as at 100 ng/mL is shown at all the other concentrations, and the mass accuracy is stable.

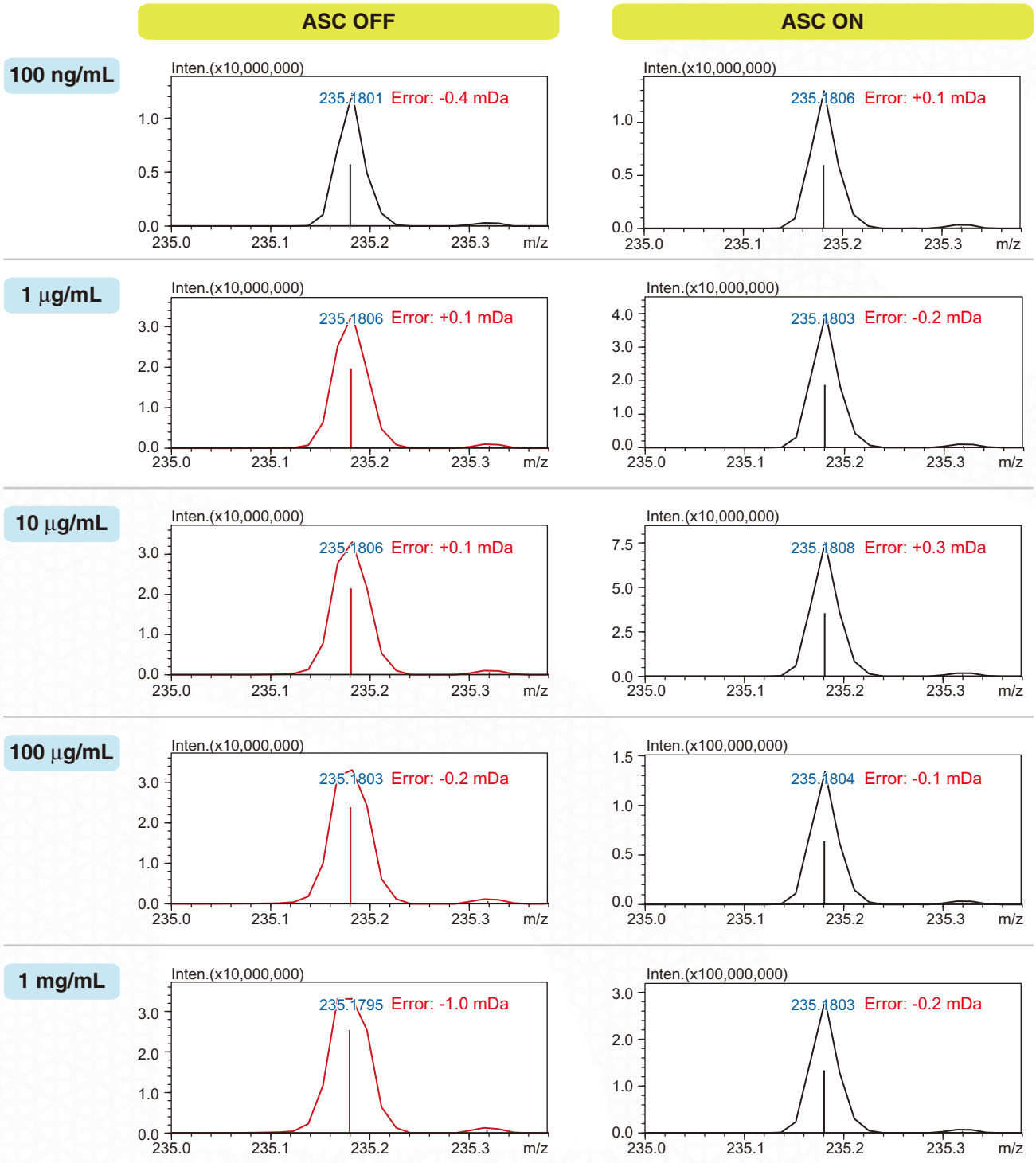


Fig. 4 Enlarged Mass Spectra of Lidocaine (100 ng/mL to 1 mg/mL)

It is clear from the results shown in Fig. 4 that spectral peak shape and mass accuracy deteriorate as the detector gets closer to saturation, but that this problem is avoided with the use of ASC. However, it can also be seen in Fig. 4 that extreme saturation does not occur, and that deterioration of mass accuracy is limited. To see the effectiveness of ASC under conditions of even greater severity, a measurement of a 1 mg/mL solution was conducted using a detector voltage increased to 0.1 kV (4 times greater, Fig. 5). Saturation became intense and the spectral peak was completely distorted into a trapezoid when ASC was not used. The error increased to a margin greater than 10 mDa, precluding any possibility of using the data for composition formula prediction or structural analysis. On the other hand, when ASC was used, excellent peak shape and mass accuracy were well maintained even under these adverse conditions.

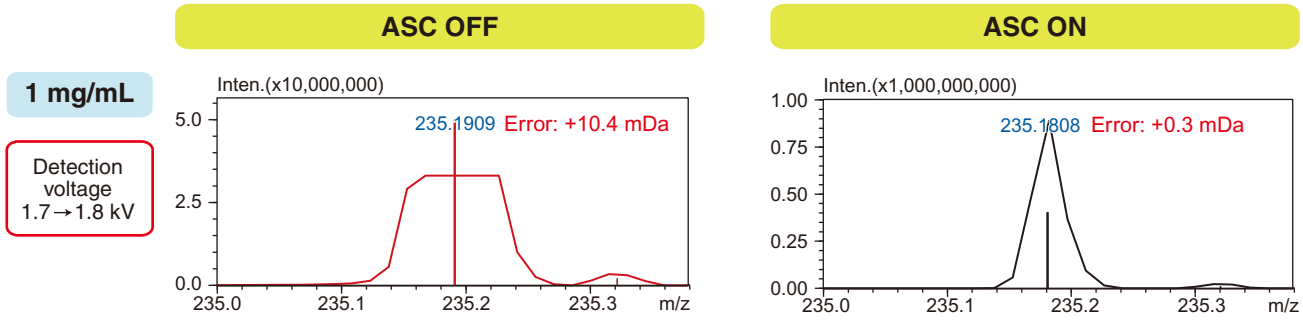


Fig. 5 Enlarged Mass Spectra of Lidocaine (1 mg/mL, detector voltage 1.8 kV)

5. Summary

This investigation shows that ASC function is an effective tool for preventing loss of mass accuracy due to saturation of the detector. Using ASC, highly accurate measurement results can be obtained in a single analysis in cases where the concentration is unknown or when the sample contains a mixture of components at varying concentrations. This ability to maintain good mass spectral peak shape regardless of the concentration not only plays a role in preserving mass accuracy, but also influences isotopic ratios, making it an important factor in controlling the quality of composition prediction results. ASC is a highly useful feature built into the LCMS-IT-TOF which not only reduces the costs associated with re-preparation and re-analysis of samples in order to maintain mass accuracy, it also plays a role in reducing the environmental impact of analysis.

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SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
URL http://www.shimadzu.com

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