Accurate Mass Determination of Amino Alcohols by Turboion-spray/Time-of-Flight Mass Spectrometry

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Amino alcohols were studied by turboionspray/time-of-flight mass spectrometry (TIS/TOF-MS) with the aim of determining the accurate mass of their protonated molecule ions. Polyethylene glycol (PEG) was used as the internal reference. Compared with the theoretical values, all relative errors were less than 5×10^{-6}. The effects of nozzle potential, nozzle temperature, acquisition rate *etc.* on accurate mass determination were also studied.

Keywords amino alcohols, turboionspray/time-of-flight mass spectrometry, accurate mass determination

Introduction

Amino alcohols are an important class of compounds in synthetic organic reactions. Their molecule structures become unsteady when analyzed by electron impact (EI). The fragments obtained from EI are usually assigned to neutral losses such as H_2O, CH_3OH (Fig. 1). EI is not a useful method when comes to analyzing the accurate mass of amino alcohols. Compared with other desorption/ionization methods, it is evident that electrospray is a 'softer' ionization method, which operates by an 'ion evaporation' process, whereby ions are emitted from droplet into gas phase. The amount of internal energy imparted to generated ion is the lowest among all mass spectrometric ionization techniques,[1] which leads to an extremely low degree of fragmentation (Fig. 2).

Some unsteady compounds such as amino alcohol can get their $[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$, $[M + K]^+$ and further adducts easily with this 'soft' ionization technique, so that we could determine the accurate mass (or atomic compositions) by determining the accurate mass of their adducts.

Turboionspray/time-of-flight mass spectrometry (TIS/TOF-MS) was developed to determine the accurate masses of five amino alcohols A—E (Fig. 3), using polyethylene glycol (PEG) 400 as the internal reference compound.[2,3] Turboionspray is an optional electrospray ionization (ESI) that works at higher flow rates than the standard ones. Turboionspray module includes an ion spray probe that delivers sample and solvent and sprays in aid of flow of nebulizer gas and a high potential, and a heated turbo probe that directs a jet of heated gas at the sample steam in the orthogonal direction. The heated gas increases the rate of droplet evaporation, desolvation, and the intensity of ion signal. The turboionspray source provides increased ionization efficiency leading to improved sensitivity at higher flow rate than conventional electrospray, particularly for compounds with extremely high polarity and low surface activity. It is mild enough to be used with labile compounds such as peptides, proteins, and thermally labile pharmaceuticals. Samples were injected into the turboionspray module through a
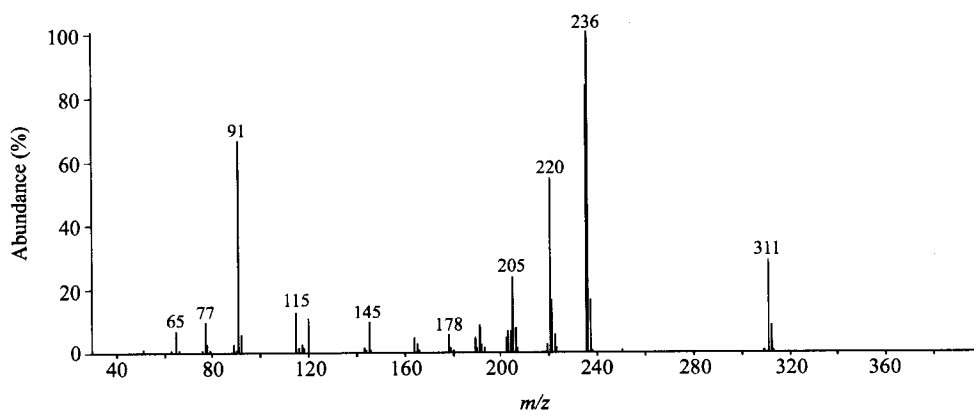
Fig. 1   EI mass spectrum of compound **D** ($M_W = 343$).

Fig. 2   ESI mass spectrum of compound **D**.

A $C_{21}H_{24}F_3NO$

B $C_{22}H_{29}NO$

C $C_{21}H_{27}NO_2$

D $C_{24}H_{25}NO$

E $C_{23}H_{29}NO$
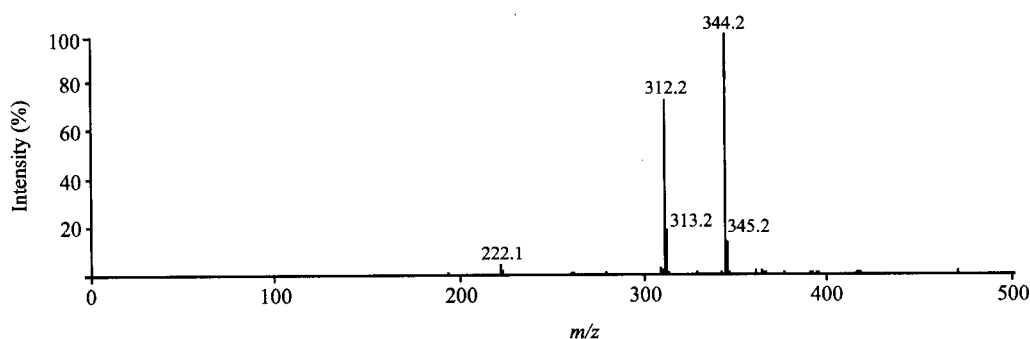
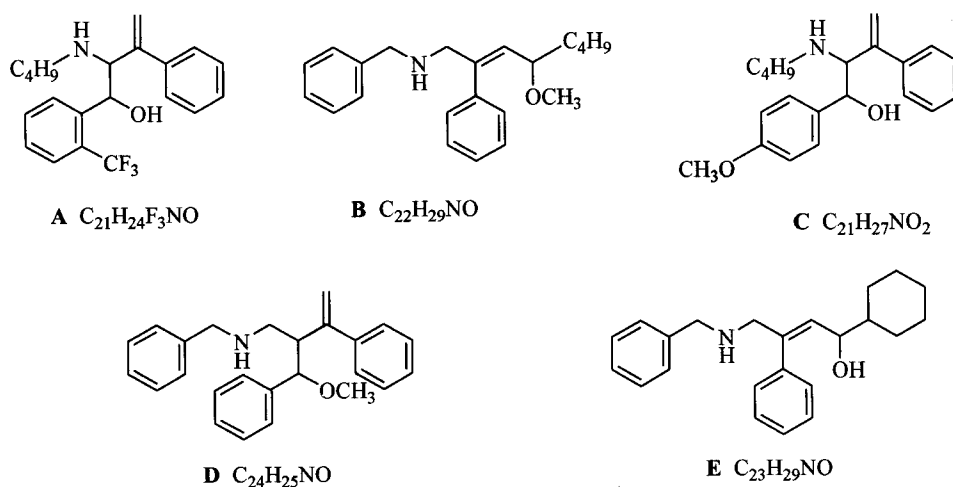**Fig. 3**   Structures of the compounds analyzed

sixway valve. Compared with a syringe pump, the method using a manual injector is faster and reduces the possibility of contamination. Compared with other accurate mass determination methods,[4] it is proved to be a simple and rapid method, and is suitable for routine determination in large-scale. This method with an autosampler can be used in high throughput determination.

It is useful for quantitation, particularly when coupled with liquid chromatography. As a result, all the absolute values of relative errors compared with theoretical values were less than 5×10^{-6}. The effects of nozzle potential, nozzle temperature, acquisition rate, etc. on accurate mass determination were studied. The difference between mass calibrations with dual reference

masses and those with multi-reference masses was also discussed.

Experimental

Chemicals

All reagents were of HPLC grade. PEG 400 was of analytical grade. All samples were synthesized by Zhao Shi-Min, and were identified by ^1H NMR, ^{13}C NMR and IR spectra.[5] The structures of tested compounds are listed in Fig. 3.

Sample preparation

The sample solutions used in the accurate mass determination were composed of PEG 400 and the compounds of interest both at the concentration of 10 $\mu L/$ mL. Mobile phase (water/methanol: 30/70) was used as the solvent.

ESL/TOF-MS *conditions*

Experiments were performed on a PE Mariner time-of-flight mass spectrometer configured with a Turboion-spray source (PE SCIEX Co. U.S.). The source was operated in the positive mode. Source conditions were as follows: spray tip potential, 5000 V; SCIEX heater temperature, 200 ℃; nozzle potential, 100 V; nozzle temperature, 140 ℃; nebulizer gas flow rate, 3.0 L/ min; curtain gas flow rate, 1.0 L/min. The detector voltage was 1850 V. Spectra were collected from m/z 100—600 with the acquisition rate of 5 seconds pre scan. Mobile phase (water/methanol: 30/70) was delivered by a Hewlett-Packard 1100 series binary pump at 0.06 mL/min, and sample solutions were injected through a Rheodyne 7725i injector with a 20 μL-sample loop. 305.15707, 349.18329, 393.20950, 371. 22756, and 415.25377 of PEG 400 were selected as reference masses for external calibration (mass score calibration).

Result and discussion

Reference masses

The masses of PEG 400, such as [HO (CH$_2$-

CH$_2$O)$_n$H + Na]$_n^+$ (n = 6—8) and [HO(CH$_2$CH$_2$O)$_n$H + H]$_n^+$ (n = 7—9), were selected as reference masses, the accurate masses of which are 305.15707, 327.20135, 349.18329, 371.22756, 393.20950 and 415.25377.

Condition of accurate mass measurement

This was the pivotal part of this experiment. The stability of the whole experiment system was inspected further. The following factors contribute to the accuracy of mass measurements: symmetrical peak shape, signal-to-noise ratio, similar signal intensity of reference and analyte peaks, two internal reference masses that bracket and close to the mass of interest.

External calibration

The instrument calibration inspects the stability of the instrument and has a great influence on its sensitivity. It also verifies whether the shapes of spectrum peaks appeared in Gaussian distribution. In this process we acquired five standard peaks that ideally bracketed the mass range of interest. In the spectrum acquisition control page, the seconds per spectrum was set to 5 to obtain better peak statistics (for example, peak shape, signal-to-noise ratio) for calibration. In this section average deviations were less than 50×10^{-6}.

Accurate masses of the five compounds were determined with two reference masses. The selected reference masses and the results are listed in Table 1. The influences of some factors on mass accuracy and the calibrations with multi-reference masses were also studied.

Effect of nozzle potential

Compound **D** was analyzed under different nozzle potentials, ranging from 70 V to 250 V. Nozzle potential affects sensitivity, signal stability, and fragmentation. The result showed that a higher nozzle potential caused higher sensitivity and signal stability, but fragmentation increased when the potential was above 100 V. The mass peaks of adduct ions were hardly observed when nozzle potential was 120 V. As a result, the nozzle potential of 100 V was selected in all other experiments.
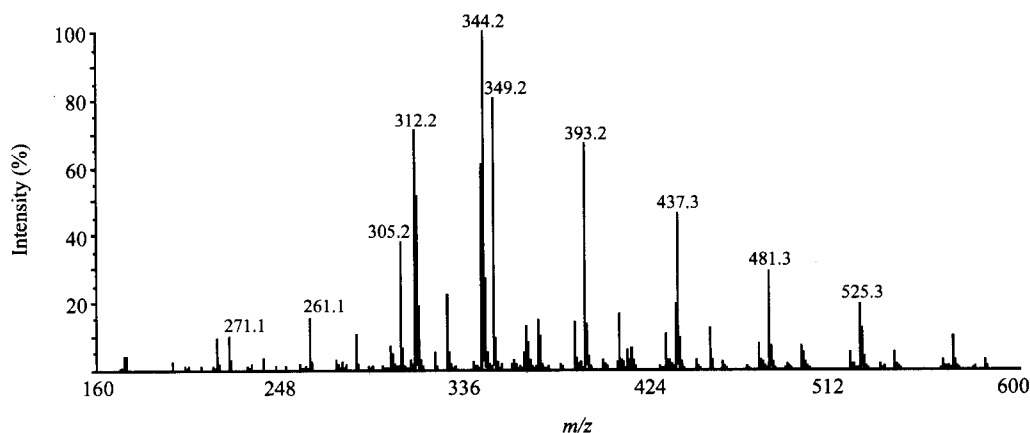
Fig. 4 ESI mass spectrum of sample with compound **D** and PEG 400.

Table 1 Accurate mass measurements of MH$^+$ for the samples with two reference masses

Compound	Theoretical mass value (amu)	Reference		Average detected mass value (amu)	Error ($\times 10^{-6}$)
		Low mass	High mass		
A	364.18828	327.20135	393.20950	364.18919	2.5
B	324.23219	305.15707	349.18329	324.23141	-2.4
C	326.21146	305.15707	349.18329	326.21263	3.6
D	344.20089	305.15707	393.20950	344.20185	2.8
E	336.23219	305.15707	393.20950	336.23111	-3.2

Effect of nozzle temperature

The study of the influence of nozzle temperature on mass accuracy showed that nozzle temperature had little effect on mass accuracy, but sensitivity decreased if it was too high. Better results were obtained when the nozzle temperature was set between 250—300 ℃. 280 ℃ was chose in the further experiments.

Influence of different acquisition rate (seconds per scan)

The influence of different acquisition rate on mass accuracy was studied. Compound **D** was tested respectively with 1, 2, 4, 5, 6, 8 and 10 seconds per scan (Fig. 4). As discussed before, acquisition rate influenced the peak statistics such as peak shape, signal-to-noise ratio, *etc*. and these factors contribute to the accuracy of mass measurements. From the experiment it could be seen that higher acquisition rate caused better mass accuracy. Because the aim of the study was to develop a rapid determination method, 5 seconds per scan was selected to test the other relevant experimental pa-

rameters.

Comparison of two calibration methods

Mass calibrations were processed with more than 3 reference masses, which were chosen from 305.15707, 327.20135, 349.18329, 371.22756, 393.20950, 415.25377 and the closest to the to-be-measured masses.

The comparison of the results obtained from two calibration methods was listed in Table 2. It can be seen that, the absolute values of relative errors from two calibration methods were all less than 5×10^{-6}. And the results obtained from method 2 had better accuracy than those from method 1. Comparing the two methods, calibration line with multi-reference masses is better than that with two reference masses, which showed that method 2 is better. Ideally, if the experimental calibration-system has a perfect linearity, either method is available.

Table 2　Comparison of two calibration methods

Compound	Error ($\times 10^{-6}$)	
	Method 1[a]	Method 2[b]
A	2.5	1.6
B	− 2.4	− 0.4
C	3.6	− 0.5
D	2.8	1.6
E	− 3.2	− 1.4

[a] Calibration with two reference masses. [b] Calibration with 4 (or 5) reference masses.

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