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LIQUID CHROMATOGRAPHY-HIGH RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY SYSTEM (LC-HRTOF)

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Recent instrumental developments have enabled TOFMS to provide accurate mass measurement. especially with the aid of internal calibrants. This breakthrough offers the capability of operation routine LC/MS analyses in the high-resolution mode. We have evaluated major commercial instruments for this objective. Two test samples were employed in this evaluation, one for confirmation of known compounds and the other for identification of trace components. The first sample consisted of several known components, ranging in concentration from approximately 1% to 20%. The second sample consisted of several trace components at the 100 ppm level which needed plausible molecular formulas. The HPLC conditions included a conventional reversed chase column, a gradient mobile phase (water/methanol or water/acetonitrile) and an auxiliary UV detection system. The HPLC run time was 40-60 minutes.

All instruments showed similar strengths and weaknesses, satisfactory exact mass measurement capability but inadequate software systems for routine LC-HRMS operation. It was found that with external calibration, the mass accuracy was approximately 50 ppm. This is sufficient for confirmation of known compounds but ineffective for identification of unknown compounds. Infusion of calibrant(s) into the mobile phase provided reference peaks for internal calibration. The mass accuracy was improved to approximately 10 ppm or better.

Table 1 Exact Mass Measurements with Extemal and internal Calibrants Peak

Peak	Theoretical	External(dev.ppm)	Internal(dev.ppm)
A	227.1255	227.1310(+24.2)	227.1233(-9.7)
B	285.1310	285.1206(-36.5)	285.1300(-3.5)
C	343.1354	343.1383(+5.2)	343.1334(-5.8)
D	327.1779	327.1556(-68.2)	327.1781(+0.6)
E	385.1834	385.1786(-14.0)	385.1792(-10.9)
F	427.2303	427.2364(+14.3)	427.2335(+7.5)

Table 1 shows the results for six major components in Sample 1 with external calibration and internal calibration. With the accuracy of internal calibration, a plausible molecular formula can be confidently selected as shown in Table 2, which shows the identification of six trace components in sample 2.

Table 2 Identification of 1 00 ppm Level Trace Components (using internal calibration)

Peak	MH measured	Proposed Formula	Dey. ppm
A	340.2007	$C_{20}H_{26}N_3O_2$(340.2025)	-5.3
B	340.2035	$C_{20}H_{26}N_3O_2$(340.2025)	+2.9
C	300.1360	$C_{16}H_{28}N_3O_3$(300.1348)	+3.9
D	461.2155	$C_{26}H_{29}N_4O_4$(471.2189)	-7.4
E	477.2128	$C_{26}H_{29}N_4O_5$(477.2138)	-2.1
F	448.2252	$C_{26}H_{30}N_3O_4$(448.2236)	+3.6

The mass accuracy deteriorated when the mass difference between the sample and the calibrant exceeded 20%. The effect of the intensity difference between the sample and the calibrant was less pronounced.

A major weakness of most current commercial TOFMS is that the software systems are not yet capable of handling LC-MS operation routinely in the high-resolution mode. Most noticeably, in high resolution mode, each LC peak is treated separately as an individual analysis rather than as part of an LC/MS experiment. Many common features in magnetic sector instruments were still unavailable at the time of our evaluation. Current TOFMS instruments do not have the ability to calibrate the entire mass range, lack universal calibrants, do not automatically convert exact masses during the LC/MS analysis, do not remove reference peaks from the acquired mass spectra and have difficulty handling specific heteroatoms in molecular formula searches, just to name a few.

In summary, most current HR-TOFMS instruments routinely perform exact mass measurements if each chromatographically separated peak is treated independently. However, LC-HRMS analysis is a continuous operation. Handling the entire chromatogram as a single analysis is still at the developing stage. The interfacing device and especially the software system are still not fully satisfactory. There is no doubt that we will see rapid improvements for these subjects in the near future.