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アプリケーションノート

LCT Premier Mass Spectrometer: APCI Sensitivity, Exact Mass Measurement, and Dynamic Range Performance

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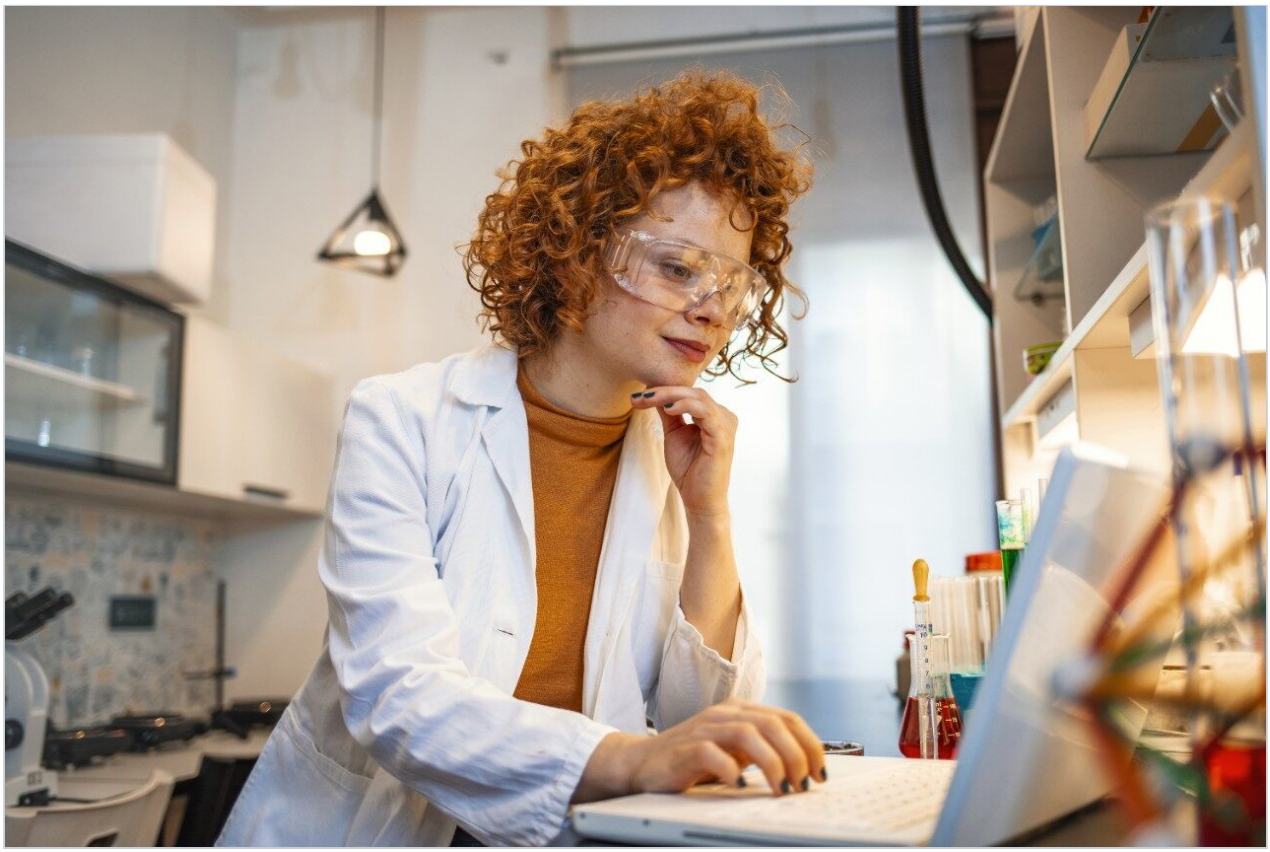

Abstract

This study evaluates the sensitivity and mass measurement of the Waters Micromass LCT Premier Mass Spectrometer when configured with the IonSABRE APCI probe. Using positive ion mode APCI, excellent mass accuracy is achieved routinely with continuum and real-time centroid data acquisition. The system used is comprised of a Waters Alliance HT 2795 Separations Module, Symmetry C_{18} Column and LCT Premier Mass Spectrometer.

Introduction

Although the vast majority of LC-MS applications are per formed with electrospray ionization, atmospheric pressure chemical ionization (APCI) is often chosen when compounds under analysis require extension of the polarity range (typically more nonpolar).

In this study, APCI is coupled with the newly developed LCT Premier orthogonal acceleration time of flight (oa-TOF) mass spectrometer (Figure 1). This benchtop mass spectrometer incorporates new hardware and software control technology to meet the analytical demands of pharmaceutical, environmental and clinical applications.

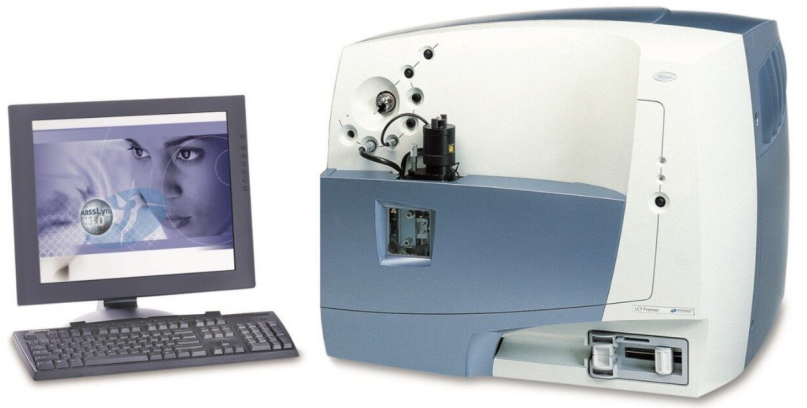

Figure 1. LCT Premier oa-TOF Mass Spectrometer.

This study uses a set of standard compounds to evaluate LCT Premier performance with APCI using both continuum and real-time exact mass centroid data acquisition. The high duty cycle of the TOF is utilized for qualitative studies, generating full spectra at high mass accuracy (<3 ppm RMS). This mass measurement accuracy provides an extra degree of information that aids in the interpretation of data.

Experimental

HPLC Conditions

HPLC system:	Waters Alliance HT 2795 Separations Module
Column:	Waters Symmetry C_{18}, 4.6 mm x 100 mm, 3.5 µm
Flow rate:	1 mL/min
Mobile phase:	A: H_2O, B: MeCN
Isocratic:	70% A: 30% B

MS Conditions

Mass spectrometer:	Waters Micromass LCT Premier oa-Tof
Ionization mode:	APCI Corona +ve = 10 µA
Sample cone voltage:	100V
Reference mass:	Propranolol , $[M+H]^+$ = 260.1650, post-column tee-in
Acquisition parameters:	100 - 1000 *m/z* 1 spectrum/second 0.1 second inter scan delay 5500 FWHM (V-mode) 10000 FWHM (W-mode)

Sample Preparation

Standards were prepared in 50/50 acetonitrile/water. The compounds used in this study are presented with the elemental formula in Table 1.

Compound	Elemental Formula	$[M+H]^+$
4-acetamidophenol	$C_8H_9NO_2$	152.0711
Sulphadimethoxine	$C_{12}H_{14}N_4O_4S$	311.0814
17α Hydroxyprogesterone	$C_{21}H_{30}O_3$	331.2773
Terfenadine	$C_{32}H_{41}NO_2$	472.3215
Reserpine	$C_{33}H_{40}N_2O_9$	609.2812

Table 1. Standards used to determine the LCT Premier APCI mode performance.

Results and Discussion

Calibration

Presented in Figure 2 is an example of a typical APCI calibration using sodium formate acquired using the LCT Premier auto-calibration procedure.

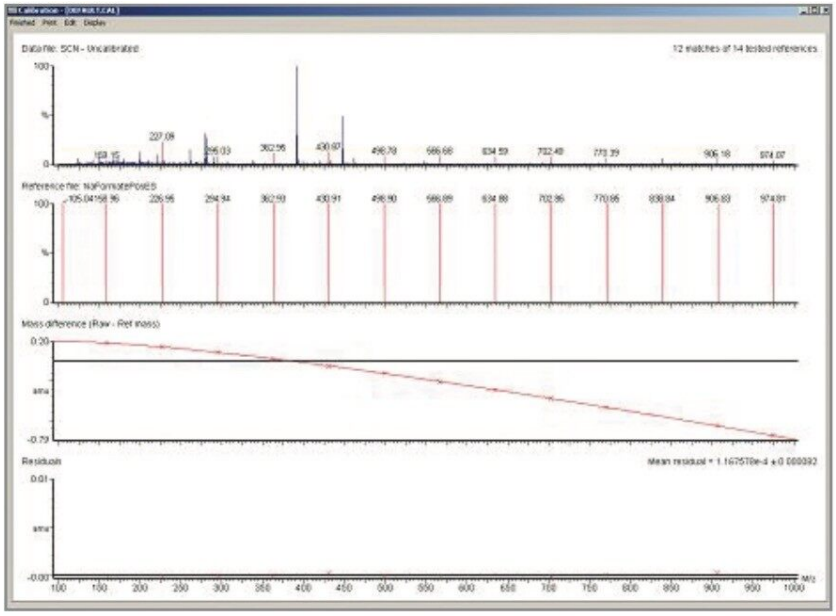

Figure 2. APCI calibration m/z 100–1000 with resolution > 10000 FWHM. Residuals are <0.08 mDa.

Mass Resolution

Table 1 lists the standard compounds used to test the LCT Premier APCI performance. The standards were chosen in order to illustrate the mass resolution and accuracy that can be obtained for small molecule applications. The data acquisition mass range utilized was *m/z* 100-1000. The standards used range from *m/z* 152 to *m/z* 609. The resolution achieved for progesterone (*m/z* 331) at >5000 FWHM (V-mode) and >10000 FWHM (W-mode) is presented in Figure 3.

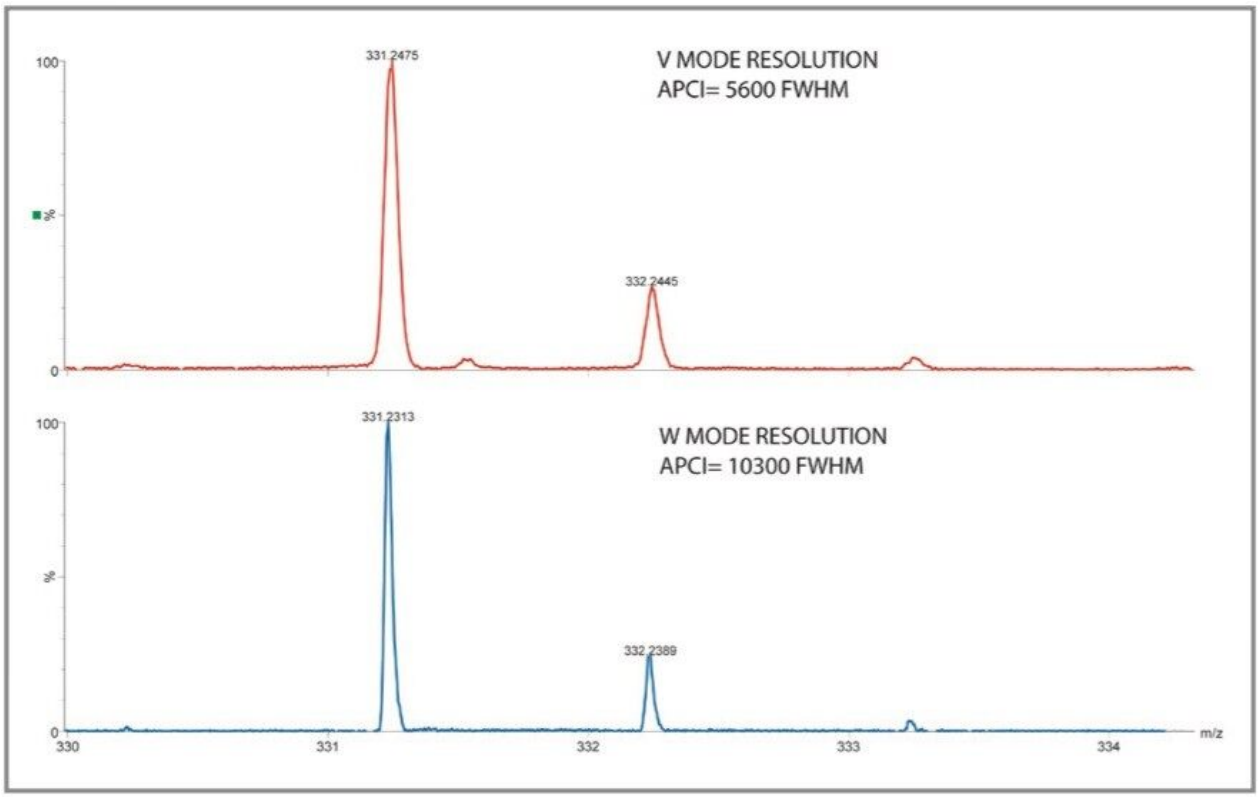

Figure 3. APCI positive mode resolution for hydroxyprogesterone (V > 5000 FWHM and W > 10000 FWHM).

Sensitivity

To deter mine the sensitivity of the LCT Premier in positive APCI mode, consecutive 10 µL loop injections of 17α hydroxyprogesterone (1ng/µL) were made and the signal to noise determined. Over six injections, an average S/N= 452:1 was achieved, with a coefficient of variation of 3.6% as shown in Figure 4. The sensitivity in negative APCI mode was determined with consecutive 10 µL loop injections of sulphadimethoxine (1ng/µL).The signal to noise determined over six injections gave an average S/N= 2992:1, with a coefficient of variation of 3.6% as shown in Figure 5.

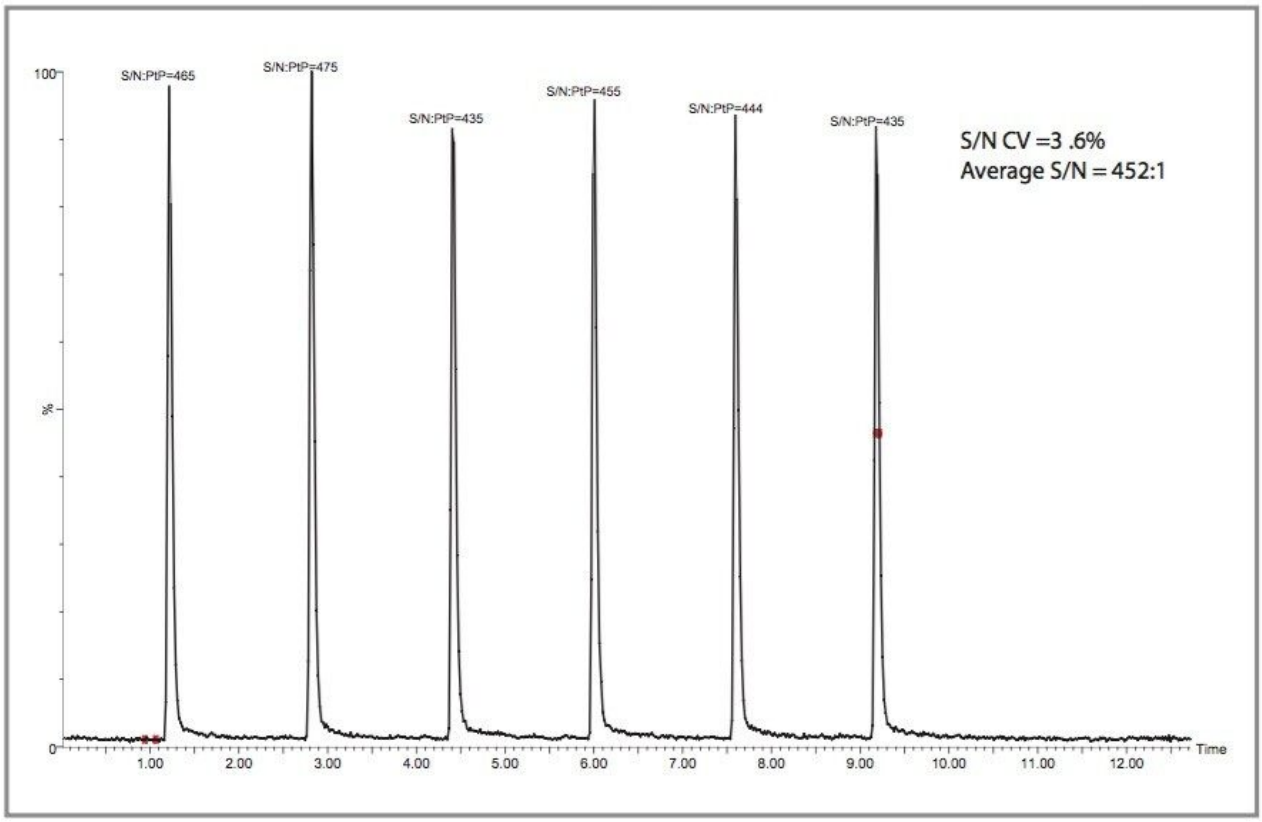

Figure 4. S/N for 10 µL injection of 17α hydroxyprogesterone (1 ng/µL) using positive mode APCI.

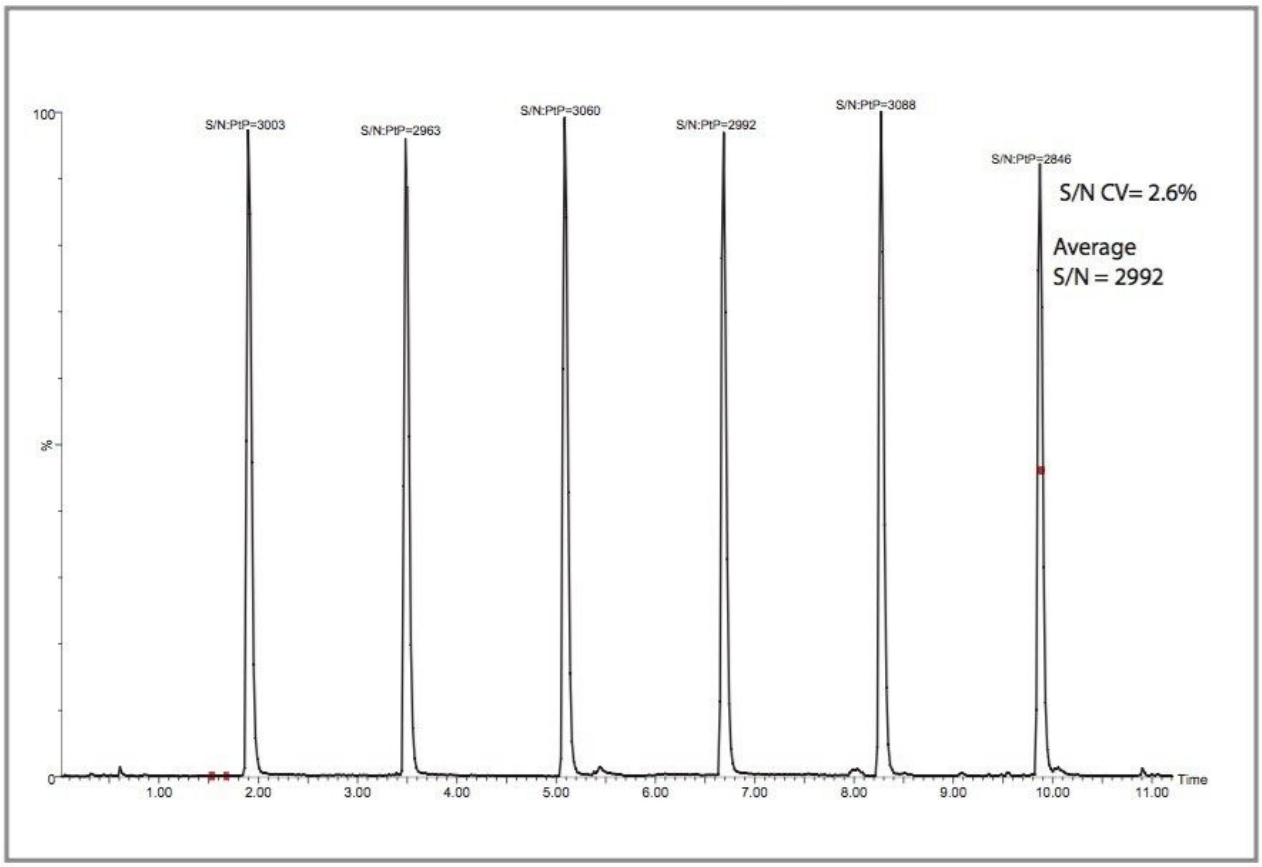

Figure 5. S/N for 10 µL injection of sulphadimethoxine (1 ng/µL) using negative mode APCI.

Mass Measurement Accuracy in Continuum Mode

Using loop injections of a mixture of five standards, six consecutive injections were performed to determine the reproducibility of the response and the exact mass measurement achieved. Initially, continuum data was acquired and centered. This is illustrated in Figure 6. The five masses of interest are labelled 1 to 5. It can be seen in Figure 7 that excellent exact mass measurement has been obtained. The calculated masses are shown alongside the mass measured. The error varied from –2.58 ppm to 2.11 ppm, giving an RMS error of just 1.61 ppm over the mass range acquired.

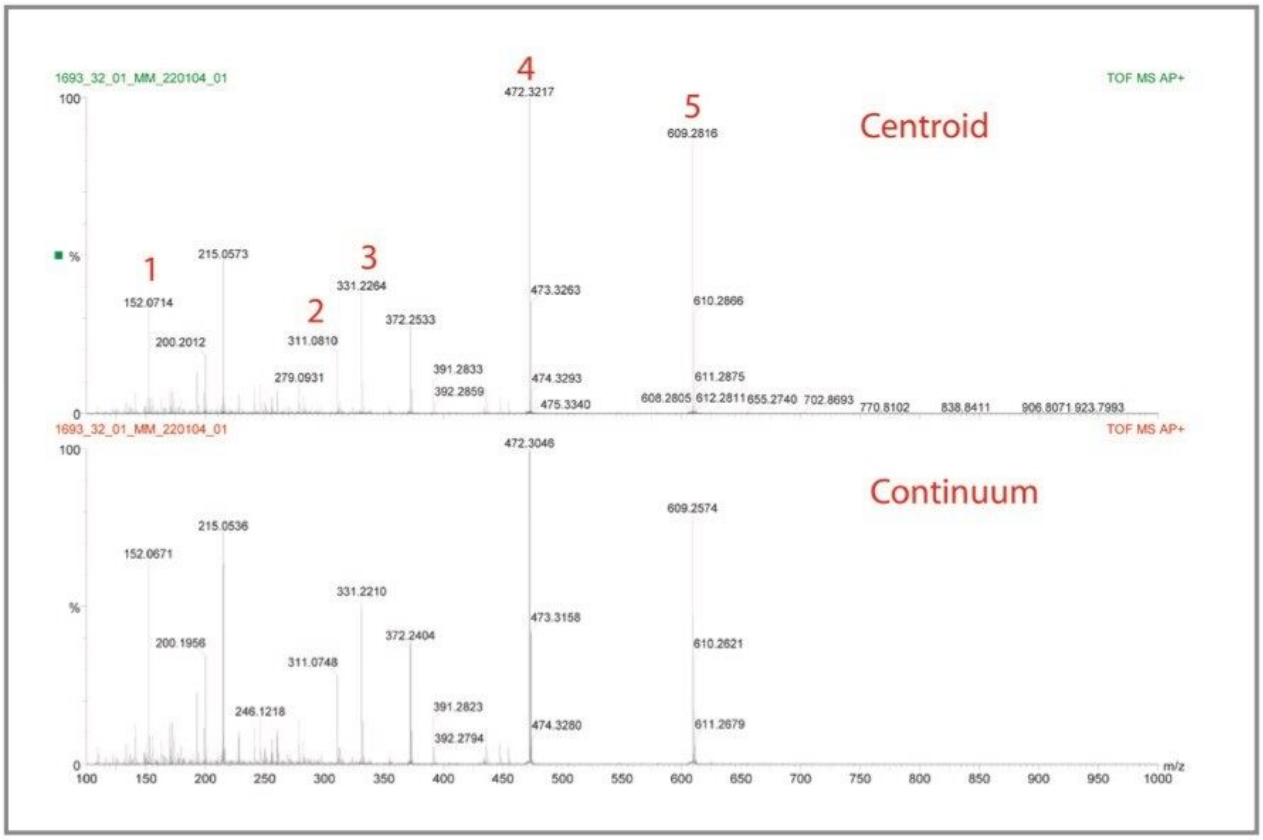

Figure 6. APCI continuum mode exact mass measurement performance.

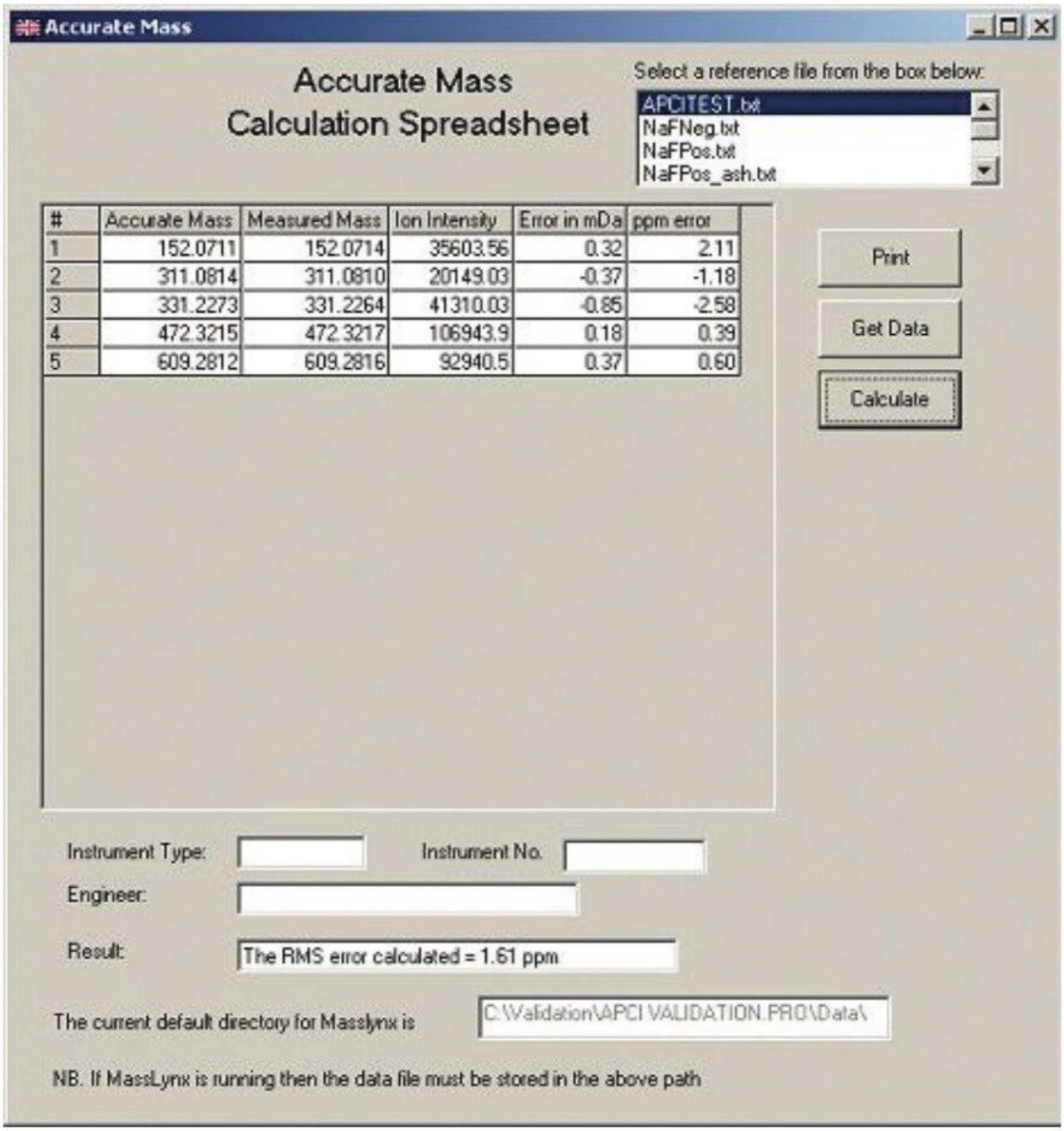

Figure 7. APCI continuum mode RMS error = 1.61 ppm obtained between m/z 100 - 700.

The RMS ppm errors for six consecutive injections of each compound are shown in Table 2. The minimum RMS ranged from 1.05 ppm for *m/z* 152 to 3.24 ppm for *m/z* 331.

Continuum PPM Error

Injection Number	Acetamid-ophen	Sulphadi-methoxine	17 DP	Terfenadine	Reserpine
1	0.9	-3.04	-3.87	-1.68	-0.6
2	0.9	-2.55	-2.4	0.06	0.9
3	0.3	-4.41	-4.05	3.1	2.5
4	2.01	-0.02	-2.67	-1.42	-0.3
5	0.9	-1.86	-3.5	1.1	1.2
6	0.32	-1.18	-2.58	0.39	0.6
RMS	1.05	2.58	3.24	1.62	1.24

Table 2. RMS ppm errors obtained in APCI continuum mode for six consecutive loop injections of five standards.

Mass Measurement Accuracy in Centroid Mode The system was tested further by repeating the experiment in centroid mode. Presented in Figure 8 is an example of the exact mass measurement obtained for APCI centroid mode. The five standards used are labelled 1 to 5. Standard number 3 (17α hydroxyprogesterone) was selected and entered into the elemental composition calculator to generate the most probable elemental composition and the mass measurement error. As can be seen from Figure 9, only one probable elemental composition was produced, with a mass measurement error of only 1.1 ppm.

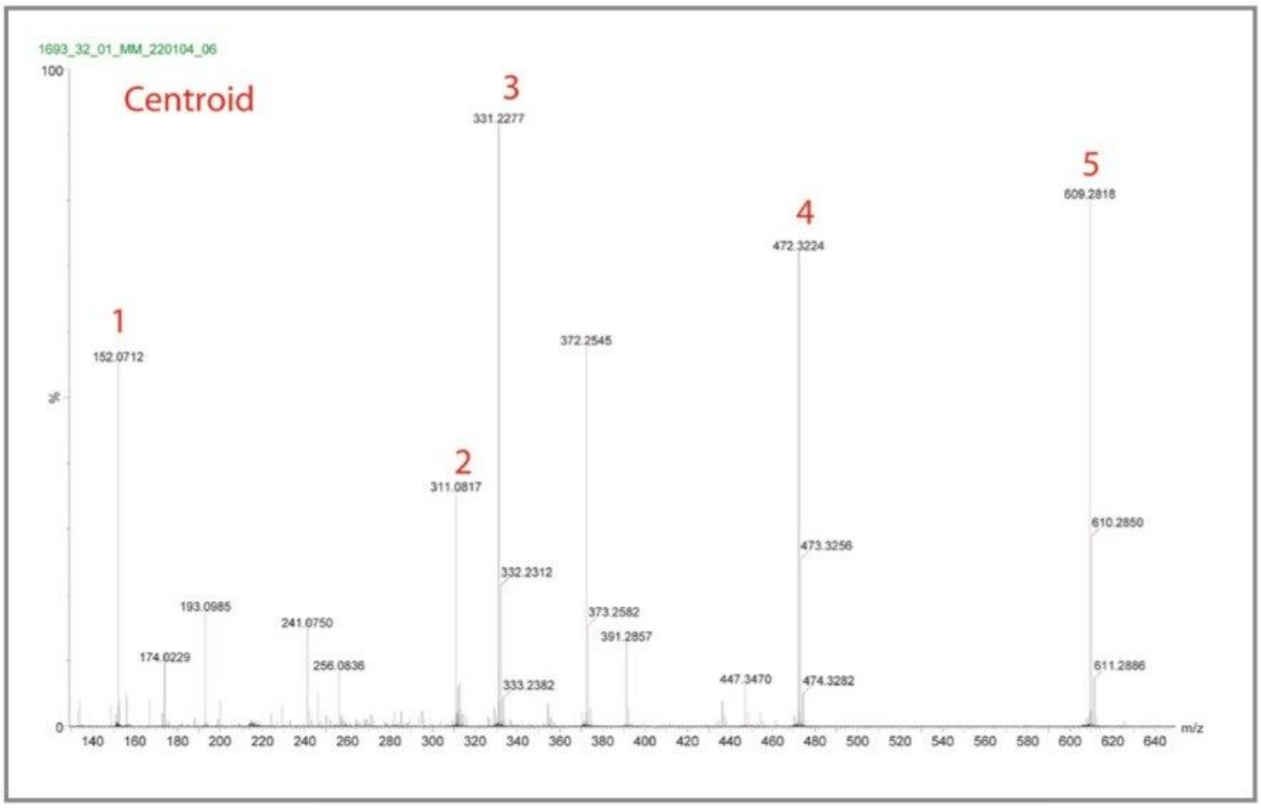

Figure 8. APCI centroid mode exact mass measurement performance.

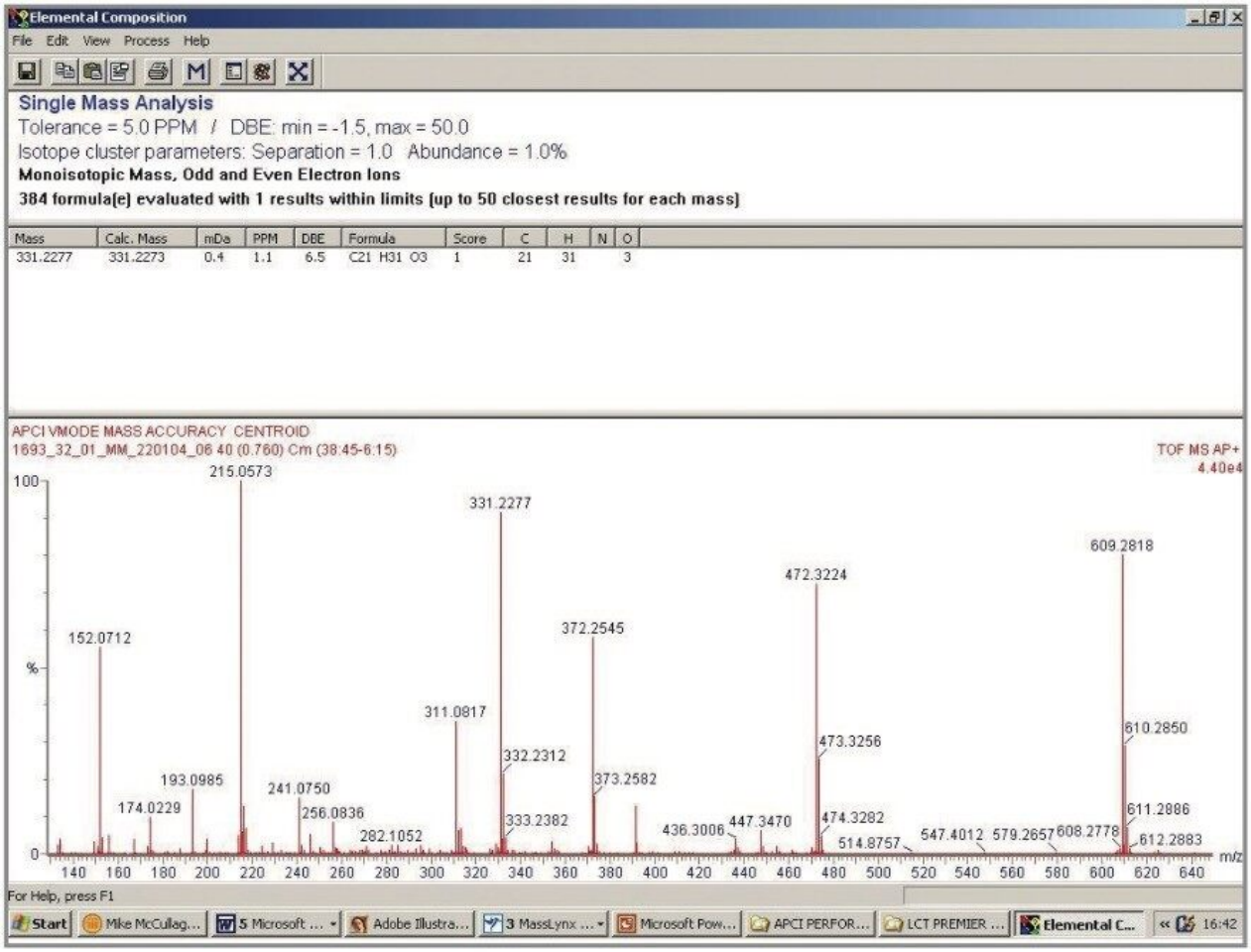

Figure 9. Elemental composition determined for 17α hydroxyprogesterone mass 3.

It can be seen in Figure 10 that excellent exact mass measurement has been obtained for all five standards. The calculated accurate masses are shown alongside the mass measured. The error varied from 0.8 ppm to 1.87 ppm, giving an RMS error of only 1.25 ppm over the mass range acquired. The RMS ppm errors for six consecutive injections of each compound are shown in Table 3. The minimum RMS ranged from 3.7 ppm for *m/z* 152 to 0.9 ppm for *m/z* 609.

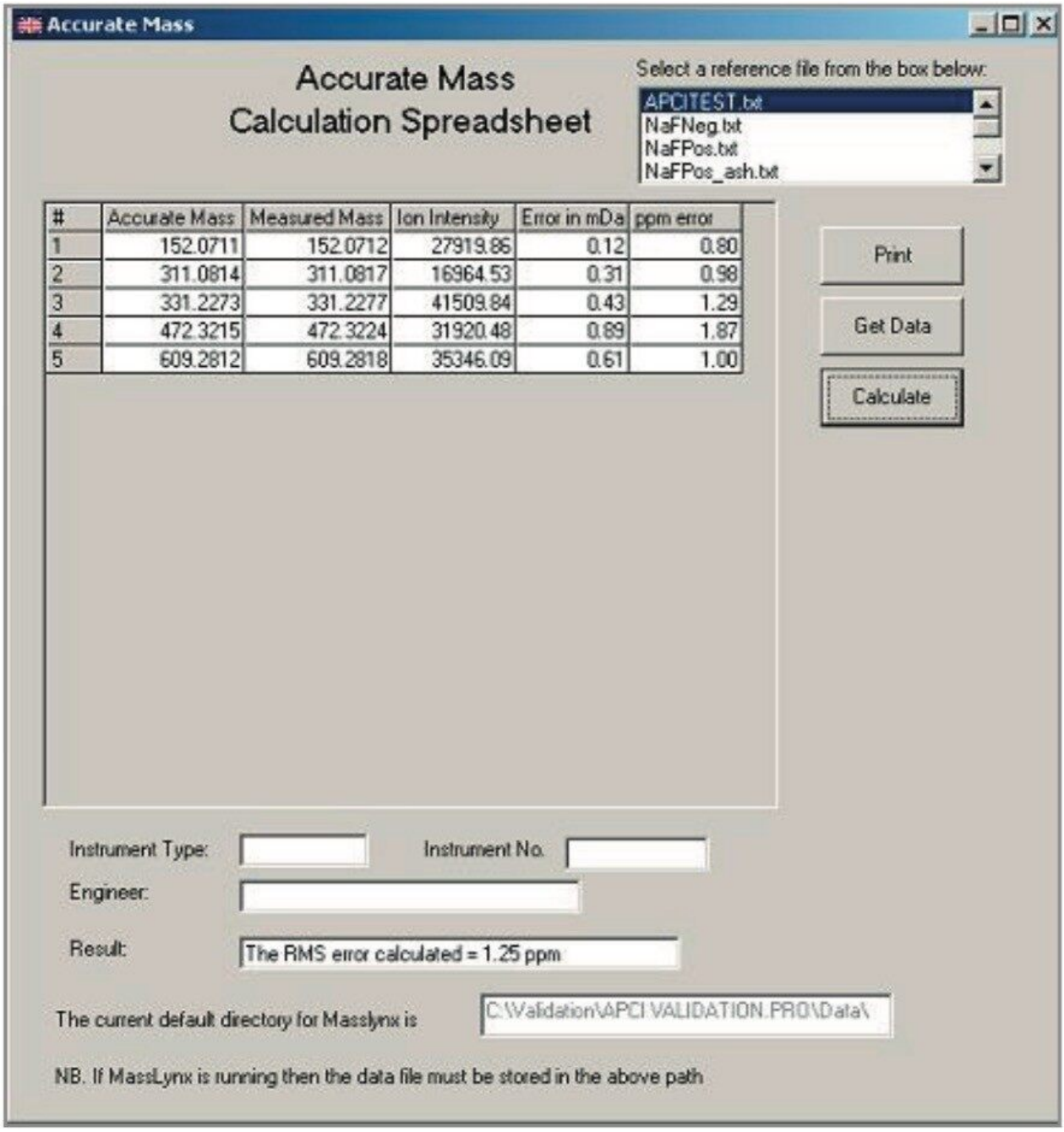

Figure 10. APCI centroid mode RMS error = 1.25 ppm obtained between 100 and 700 Da.

Centroid PPM Error

Injection Number	Acetamid-ophen	Sulphadi-methoxine	17 DP	Terfenadine	Reserpine
1	0.80	0.98	1.29	1.87	1.0
2	4.52	1.67	2.95	1.87	0.9
3	4.72	2.65	2.58	0.71	0.70
4	3.71	2.75	4.24	1.49	1.1
5	4.21	3.04	3.87	1.29	0.7
6	2.71	0.2	0.64	0.13	-1.2
RMS	3.7	2.144	2.89	2.61	0.9

Table 3. RMS ppm errors obtained in APCI centroid mode for six consecutive loop injections of five standards.

Dynamic Range Shown in Figure 11 is an illustration of the linear response obtained for sulphadimethoxine using DRE (Dynamic Range Enhancement). Four orders of magnitude dynamic range are shown for sulphadimethoxine, where concentrations ranging from 1 pg/µL to 10000 pg/µL were injected on column. A correlation coefficient of 0.9997 was achieved.

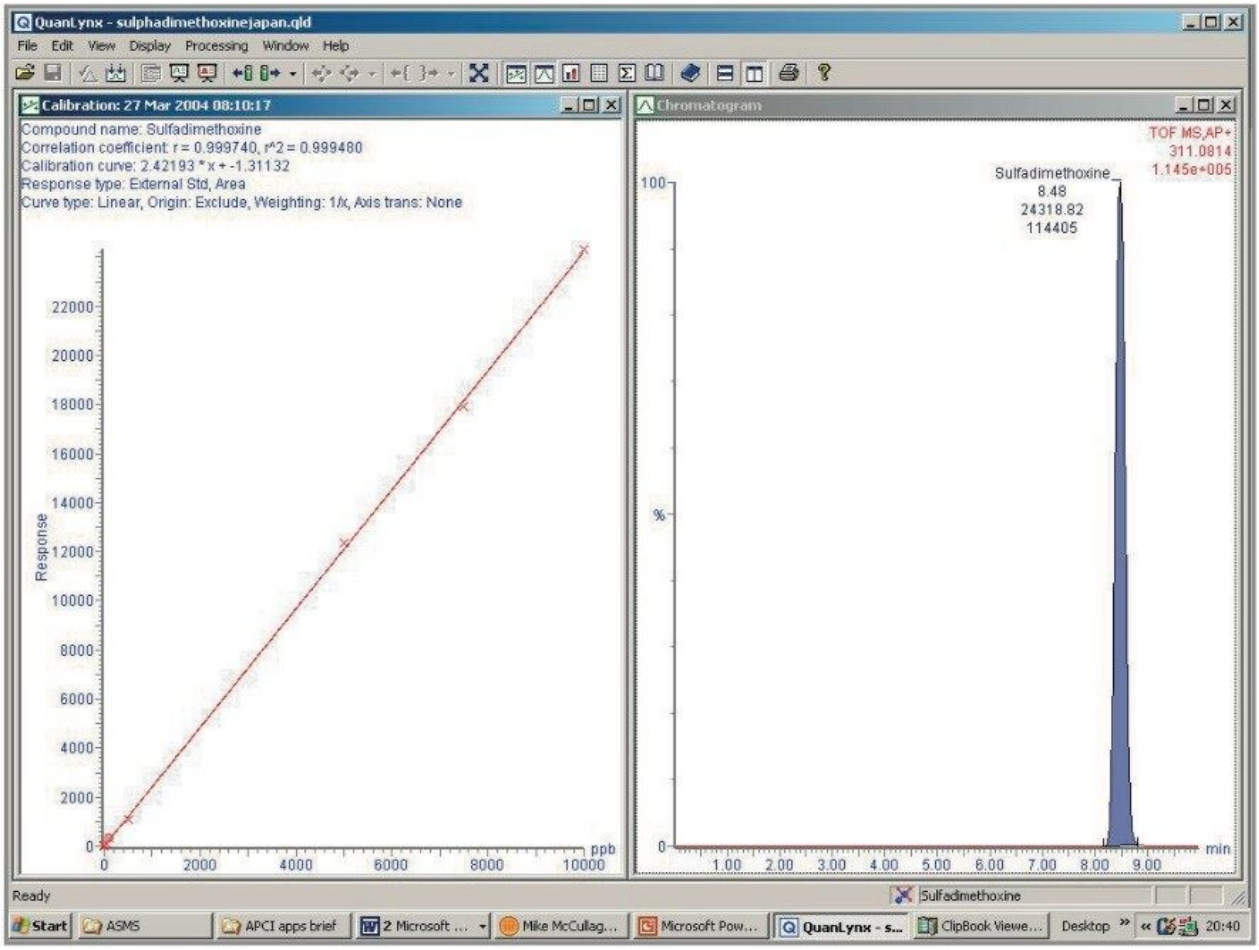

Figure 11. APCI DRE linearity plot for sulphadimethoxine (1 pg/µL to 10,000 pg/µL).

Conclusion

- Instrument calibration over a mass range of *m/z* 100–1000 has been shown, with residuals of 0.08 mDa.
- For positive ion mode APCI an average S/N of 452:1 for 10 ng of 17α hydroxyprogesterone was acquired.
- For negative ion mode APCI an average S/N of 2992:1 for 10 ng of sulphadimethoxine was acquired.
- Exact mass measurement in continuum mode for a mass range of *m/z* 100–700 Da produced an RMS error of 1.61 ppm.
- Exact mass measurement in centroid mode for a mass range of *m/z* 100–700 produced an RMS error of 1.25 ppm.
- Consecutive loop injections of five standards in continuum acquisition mode gave RMS errors ranging

from 1.05 ppm (*m/z* 152 acetamidophen) to 3.24 ppm for 17α hydroxyprogesterone (*m/z* 331).

- Consecutive loop injections of six standards in centroid acquisition mode gave RMS errors ranging from 0.9 ppm (*m/z* 609 reserpine) to 3.7 ppm for acetamidophen (*m/z* 152).
- Using sulphadimethoxine (concentration range 1pg/µL to 10,000 pg/µL), four orders of linearity have been presented.

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720001074, April 2005