Applikationsbericht

LCT Premier – Routine Automated Exact Mass Measurement

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Abstract

For LC-MS applications, the LCT Premier provides the user with the ability to acquire high resolution MS data and

therefore the ability to generate an exact mass measurement to within 3 ppm. Using the LockSpray ion source, traditional problems associated with exact mass measurement during LC-MS acquisitions are overcome - no multi point reference masses, no post-column addition of the lock mass, no ionization interference between your analytes and lockmass, and no nominal mass interferences. Having the ability to acquire an exact mass measurement aids determination of elemental composition for a particular analyte of interest, which provides the analyst a high degree of specificity and therefore a high level of confidence in the analytical result.

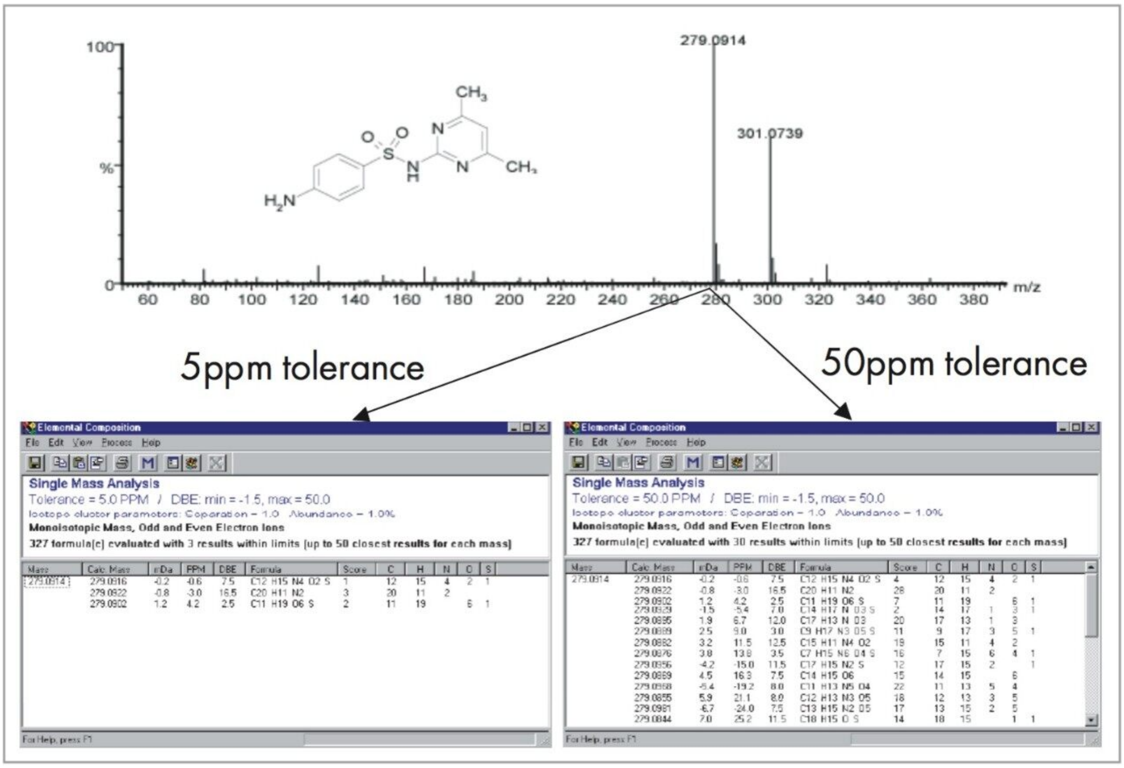
Benefits

- Determine the elemental compositions of analytes
- Distinguish between nominally isobaric components
- Distinguish between analyte and matrix related material
- Improve database search specificity
- Increase your confidence in analyte identification

Introduction

Why Exact Mass?

As with every analytical tool, the more accurate your measurement the more confident you can be with your results. For example, when carrying out a metabolism investigation following an in vivo experiment are you sure that a peak detected at *m/z* 342.1 is an actual metabolite or a matrix related component or even a solvent contaminant?
Mass measuring the peak of interest to 4 decimal places will give you that confidence. By reducing the error on a mass measurement, the possible elemental formulae that can fit a particular measurement significantly reduce, therefore providing better specificity. Figure 1 clearly shows this. Such mass measurement accuracy is not routinely available with typical scanning instruments such as quadrupole or ion trap mass spectrometers. For routine and easy-to-use exact mass measurement, orthogonal acceleration time-of-flight (oa-Tof) mass spectrometry provides the solution.



Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%
Monoisotopic Mass, Odd and Even Electron Ions
327 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	S
279.0914	279.0916	-0.2	-0.6	7.5	C12 H15 N4 O2 S	1	12	15	4	2	1
	279.0922	-0.8	-3.0	16.5	C20 H11 N2	3	20	11	2		
	279.0902	1.2	4.2	2.5	C11 H19 O6 S	2	11	19		6	1

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%
Monoisotopic Mass, Odd and Even Electron Ions
327 formula(e) evaluated with 30 results within limits (up to 50 closest results for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	S
279.0914	279.0916	-0.2	-0.6	7.5	C12 H15 N4 O2 S	4	12	15	4	2	1
	279.0922	-0.8	-3.0	16.5	C20 H11 N2	28	20	11	2		
	279.0902	1.2	4.2	2.5	C11 H19 O6 S	7	11	19		6	1
	279.0929	-1.5	-5.4	7.0	C14 H17 N O3 S	2	14	17	1	3	1
	279.0895	1.9	6.7	12.0	C17 H13 N O3	20	17	13	1	3	
	279.0889	2.5	9.0	3.0	C9 H17 N3 O5 S	11	9	17	3	5	1
	279.0882	3.2	11.5	12.5	C15 H11 N4 O2	19	15	11	4	2	
	279.0876	3.8	13.8	3.5	C7 H15 N6 O4 S	16	7	15	6	4	1
	279.0956	-4.2	-15.0	11.5	C17 H15 N2 S	12	17	15	2		1
	279.0869	4.5	16.3	7.5	C14 H15 O6	15	14	15		6	
	279.0968	-5.4	-19.2	8.0	C11 H13 N5 O4	22	11	13	5	4	
	279.0855	5.9	21.1	8.0	C12 H13 N3 O5	18	12	13	3	5	
	279.0981	-6.7	-24.0	7.5	C13 H15 N2 O5	17	13	15	2	5	
	279.0844	7.0	25.2	11.5	C18 H15 O S	14	18	15		1	1

Figure 1. Improving specificity through exact mass measurement.

Exact mass measurement on an oa-Tof can help to:

- Determine the elemental compositions of analytes
- Distinguish between nominally isobaric components
- Distinguish between analyte and matrix related material
- Improve database search specificity
- Increase your confidence in analyte identification

For LC-MS applications, the Waters Micromass LCT Premier benchtop oa-Tof Mass Spectrometer provides the user with the ability to acquire high resolution MS data and thus generate an exact mass measurement. In addition to the next generation of instrument electronics and oa-Tof optics, LCT Premier is equipped with LockSpray as standard. LockSpray is a novel dual electrospray ion source that was designed for dedicated exact mass measurement. Your LC column is connected to the analyte sprayer while the single point reference mass, used to exact mass measure your data, is introduced into the reference sprayer.

The eluent from each of the two sprayers is kept completely separate by a baffle that is indexed via software control for total data integrity. During an experiment, data from the reference sprayer is typically sampled once every 10 secs and the reference data is automatically applied to the analyte data to provide an exact mass measurement. LockSpray overcomes issues associated with traditional exact mass measurement, *i.e*. post-column addition of a lock mass and potential ionization interferences between analytes and lock mass etc.

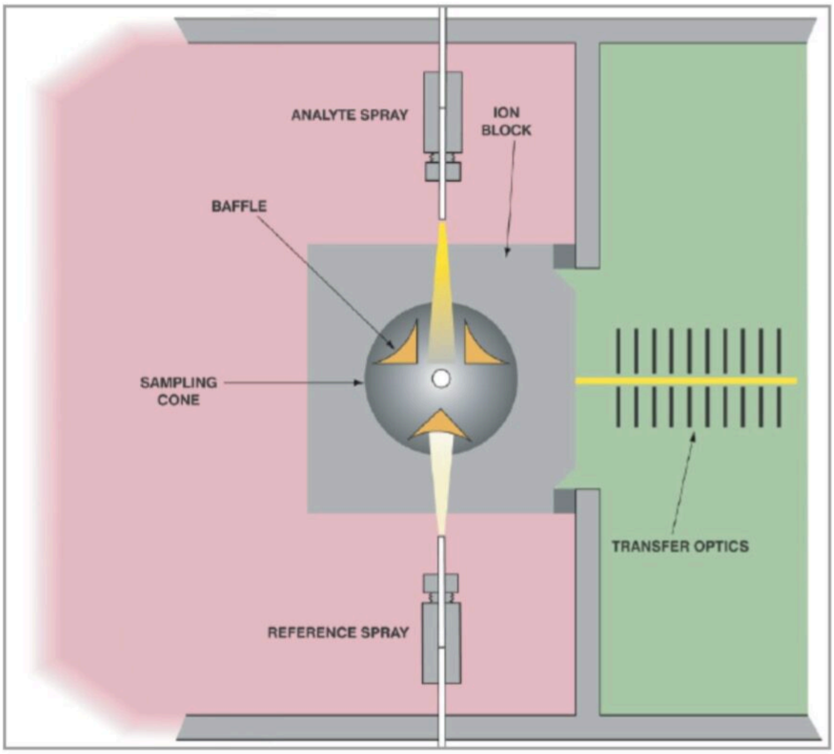


Figure 2. LockSpray in analyte position.

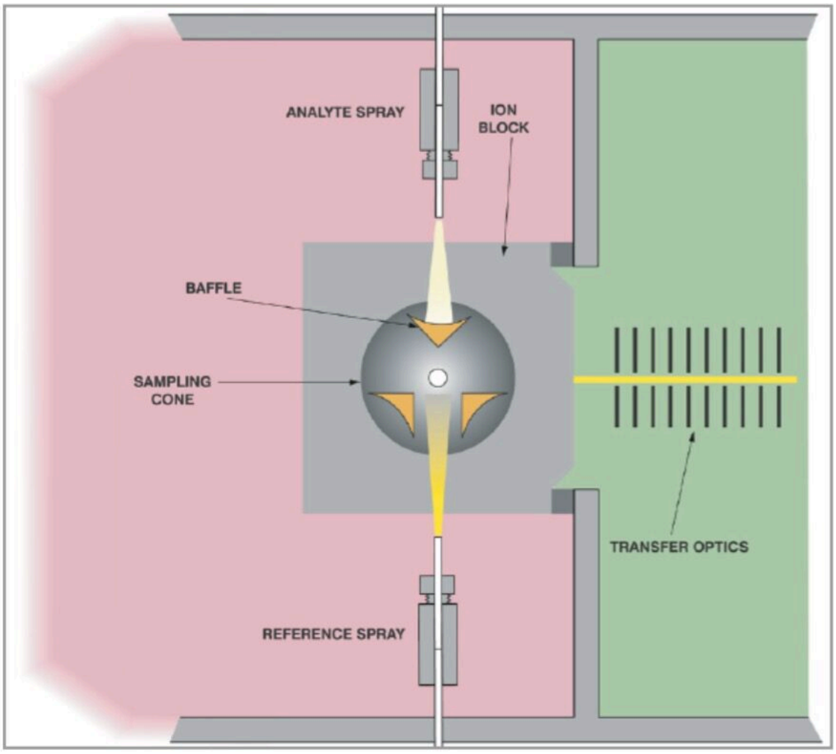


Figure 3. LockSpray in reference position.

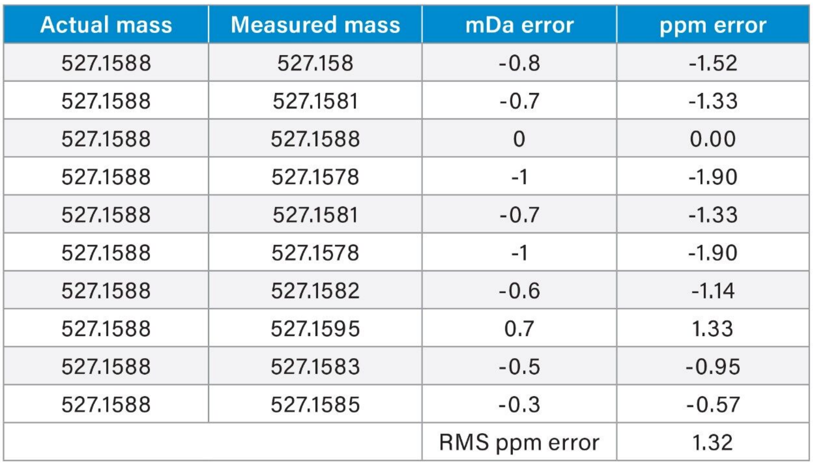
Results and Discussion

Examples of Mass Measurement Accuracy on LCT Premier

1) Single Analyte Exact Mass Measurement

- LockSpray with positive ion electrospray was used.
- Raffinose (500 pg/μL in 50/50 MeCN/H_2O) was infused into the analyte sprayer over 30 mins at 5 μL/min. The $[M+Na]^+$ ion was measured ($C_{18}H_{31}O_{16}Na$, *m/z* 527.1588)
- Leucine enkephalin (50 pg/μL in 50/50 MeCN/H_2O + 0.1% formic acid) was infused into the reference sprayer at 5 μL/min. The $[M+H]^+$ ion was used as the lock mass ($C_{28}H_{37}N_5O_7$, *m/z* 556.2771)
- The reference sprayer was sampled every 10 secs and 10 measurements were taken over the 30 min acquisition
- The spectral resolution was 10,000 FWHM

Table 1 below lists the exact mass measurements along with the calculated mDa and ppm errors from the theoretical. The RMS ppm error for all 10 measurements was calculated at 1.32 ppm.



Actual mass	Measured mass	mDa error	ppm error
527.1588	527.158	-0.8	-1.52
527.1588	527.1581	-0.7	-1.33
527.1588	527.1588	0	0.00
527.1588	527.1578	-1	-1.90
527.1588	527.1581	-0.7	-1.33
527.1588	527.1578	-1	-1.90
527.1588	527.1582	-0.6	-1.14
527.1588	527.1595	0.7	1.33
527.1588	527.1583	-0.5	-0.95
527.1588	527.1585	-0.3	-0.57
		RMS ppm error	1.32

Table 1. Exact Mass Measurement Results for raffinose.

2) Multiple Analyte Exact Mass Measurement during an LC-MS Experiment

- LockSpray with positive ion electrospray was used
- An 8 component analyte mixture was separated using the following conditions:

HPLC: Waters Alliance HT 2795

Mobile phase A: H_2O + 0.1% formic acid

Mobile phase B: MeCN + 0.1% formic acid

Gradient: 95% A to 5% A over 10 mins

Column: Waters Atlantis dC_{18}, 2.1 x 100 mm, 3 µm

Flow rate: 0.3 mL/min.

Injection volume: 10 µL

- Leucine enkephalin (50 pg/µL in 50/50 MeCN/H_2O + 0.1% formic acid) was infused into the reference sprayer at 5 µL/min. The $[M+H]^+$ ion was used as the lock mass ($C_{28}H_{37}N_5O_7$, m/z 556.2771)
- The reference sprayer was sampled every 10 secs.

Figure 4 shows a typical chromatogram obtained with the retention time for all 8 components. Table 2 lists the exact mass measurements obtained along with the calculated mDa and ppm errors from the theoretical. The RMS ppm error for all measurements was calculated at 1.8 ppm.

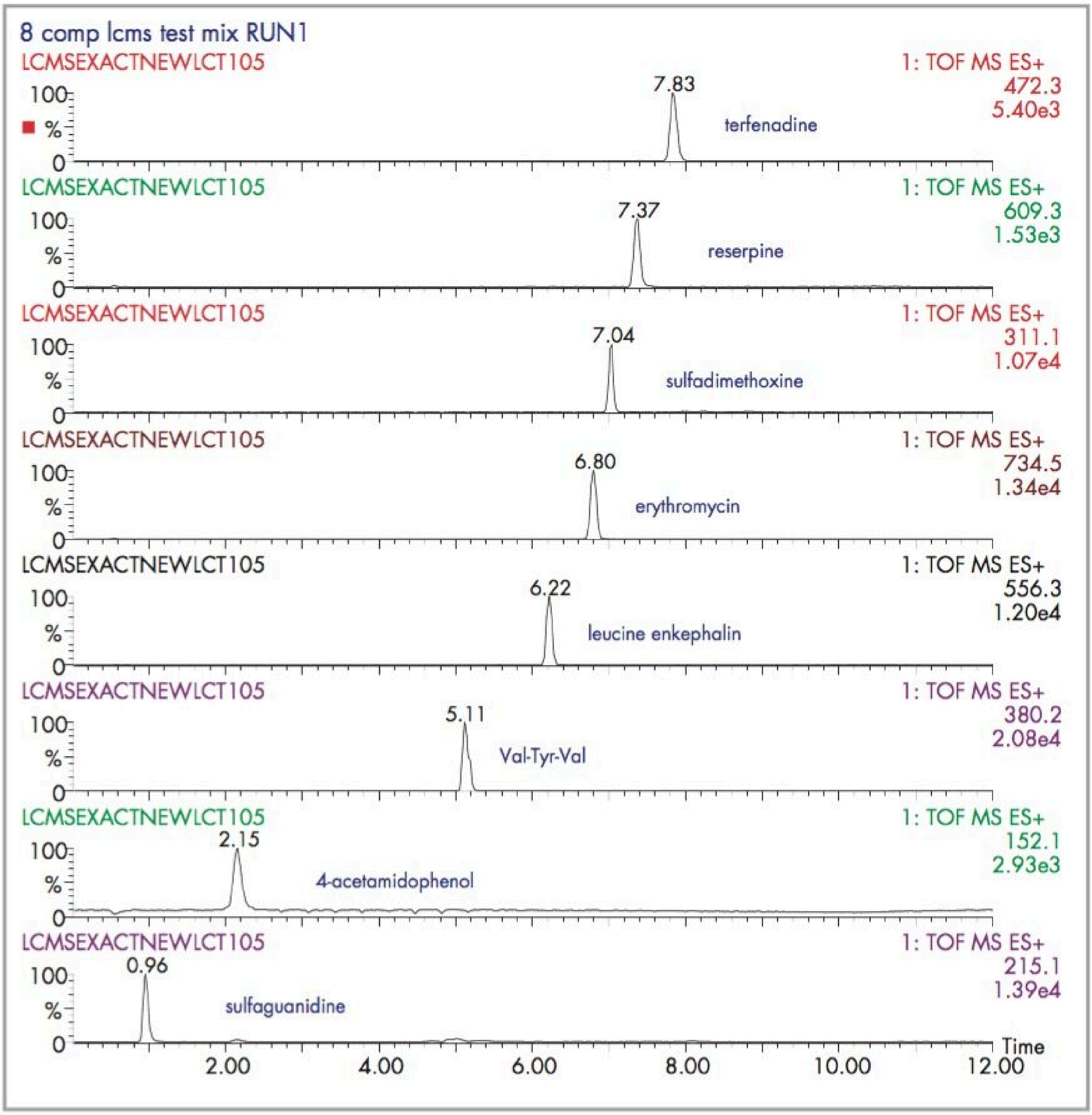
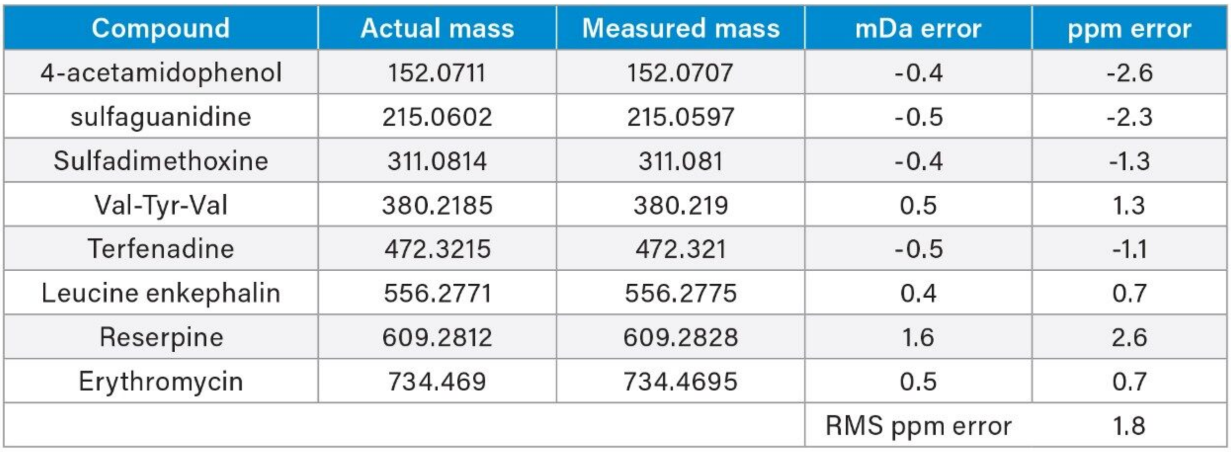


Figure 4. Retention times for all 8 components.



Compound	Actual mass	Measured mass	mDa error	ppm error
4-acetamidophenol	152.0711	152.0707	-0.4	-2.6
sulfaguanidine	215.0602	215.0597	-0.5	-2.3
Sulfadimethoxine	311.0814	311.081	-0.4	-1.3
Val-Tyr-Val	380.2185	380.219	0.5	1.3
Terfenadine	472.3215	472.321	-0.5	-1.1
Leucine enkephalin	556.2771	556.2775	0.4	0.7
Reserpine	609.2812	609.2828	1.6	2.6
Erythromycin	734.469	734.4695	0.5	0.7
			RMS ppm error	1.8

Table 2. Exact Mass Measurements of 8 Component Mix.

This example shows that the LCT Premier provides excellent mass measurement accuracy across a wide range of compounds with varying mass, physical properties and retention time during a reversed-phase LC gradient. This provides improved specificity during an LC-MS experiment providing the analyst with increased confidence in the analytical result.

3) Exact Mass Measurement during Positive/Negative Ion Mode Switching

Having the ability to carry out ionization mode switching in real time provides the analyst with an additional piece of information during the same LC-MS experiment. Ionization mode switching is often regarded as being useful for when the expected mode of ionization for a particular compound is unknown e.g. compound screening experiments. With the additional function of being able to generate an exact mass measurement during ion mode switching experiment in real time, further confidence in that the correct ion has been detected is obtained.

With significant improvements in electronics and detector hardware, the Waters Micromass LCT Premier now has the possibility to switch ionization mode during a real time LC-MS experiment in 300 msecs - providing an exact mass measurement and a sufficient number data points to define a typical chromatographic peak. The previous oa-Tof instrument, the LCT, was only able to switch ionization mode during a real time experiment in 1 second, due to the stabilization time required when the older generation high voltage electronics were switched polarity.

- LockSpray with positive/negative ionization mode switching was used
- An Inter Scan Delay (ISD) of 0.3 secs was used
- The reference sprayer was sampled every 5 secs
- Raffinose ($C_{18}H_{32}O_{16}$, 500 pg/µL in 50/50 MeCN/H_2O) was infused into the analyte sprayer at 5 µL/min

• [M+Na]$^+$ = 527.1588 *m/z*

• [M-H]$^-$ = 503.1612 *m/z*

- Leucine enkephalin ($C_{28}H_{37}N_5O_7$, 50 pg/μL in 50/50 MeCN/H_2O + 0.1% formic acid) was infused into the reference sprayer at 5 μL/min

• [M+H]+ = 556.2771 *m/z*

• [M-H]$^-$ = 554.2615 *m/z*

Figure 5 shows a typical positive and negative ion spectrum for raffinose that was acquired during the experiment. The exact mass measurements shown are representative of the data that is typically obtained during a positive/negative ion mode switching experiment with LCT Premier.

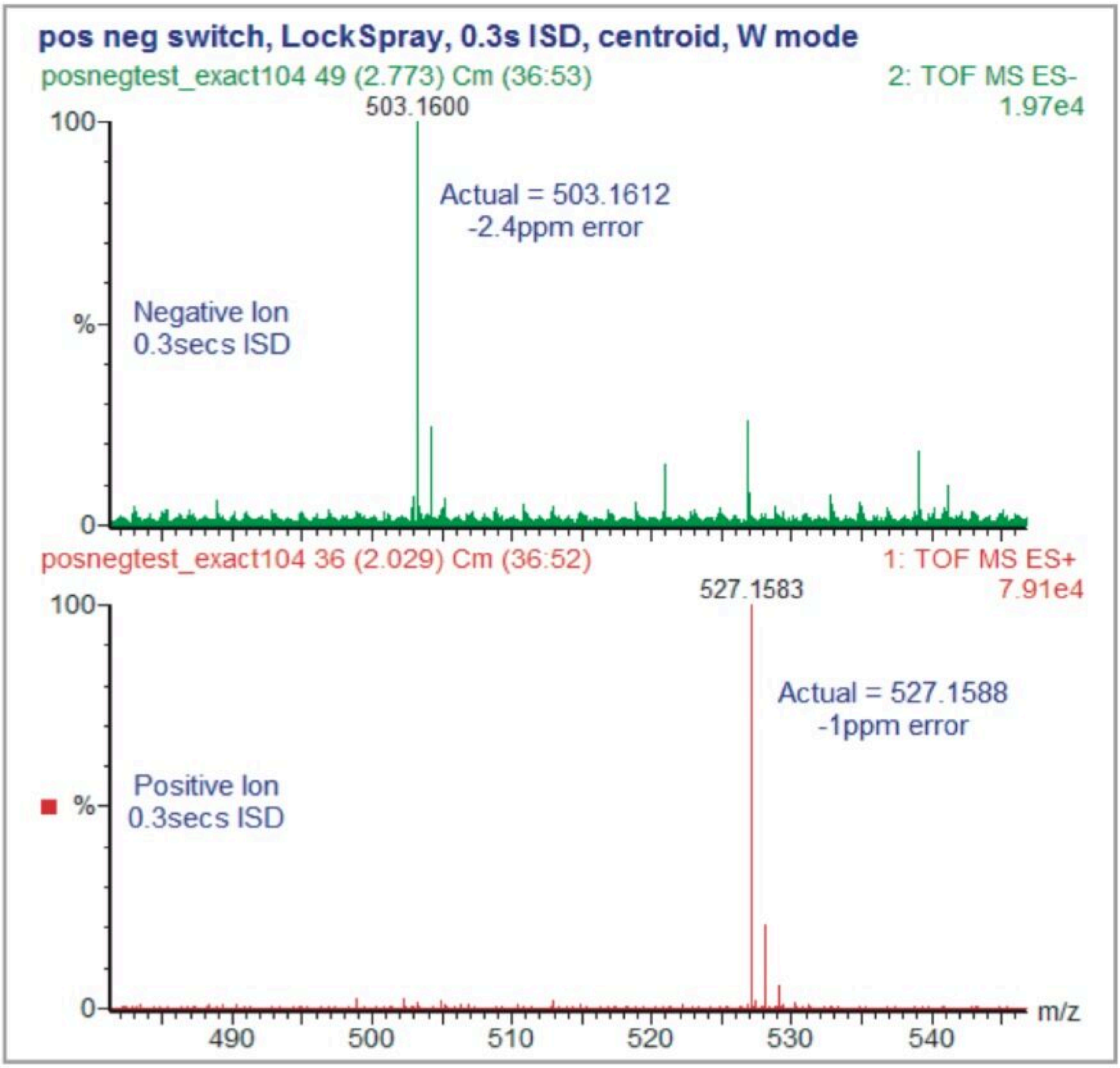


Figure 5. Positive/negative ion mode switching with lockSpray.

Conclusion

For LC-MS applications, the LCT Premier provides the user with the ability to acquire high resolution MS data and therefore the ability to generate an exact mass measurement to within 3 ppm. Using the LockSpray ion source, traditional problems associated with exact mass measurement during LC-MS acquisitions are overcome - no multi point reference masses, no post-column addition of the lock mass, no ionization interference between your analytes and lockmass, and no nominal mass interferences. Having the ability to acquire an exact mass measurement aids determination of elemental composition for a particular analyte of interest, which provides the analyst a high degree of specificity and therefore a high level of confidence in the analytical result.

LCT Premier – exact mass measurement made easy for LC-MS applications.

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