アプリケーションノート

LockSpray: Automated Exact Mass Measurement for Electrospray Applications

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief details on how LockSpray allows exact mass measurement for LC-MS applications to be performed with ease.

Introduction

What is LockSpray?

LockSpray is a novel patented ion source designed specifically for exact mass measurement with electrospray ionization using Waters LC-time-of-flight (LC-Tof) mass spectrometers. Whether analyzing drug candidates, environmental pollutants, residues or drugs of abuse, LockSpray allows exact mass measurement for LC-MS applications to be performed with ease.

How Does LockSpray Work?

The LockSpray ion source is designed specifically for the Waters ZSpray API interface and is used for LC-MS analysis with electrospray ionization. The source is arranged with dual sprayers-one sprayer is connected to the LC eluent and the other sprayer is used to introduce a reference solution for exact mass measurement. The eluent from each of the sprayers is kept completely separate by a rotatable baffle and is indexed via MassLynx Software control. By keeping the information produced by each sprayer completely separate, data integrity is maintained.

The process of gaining an exact mass measurement using LockSpray is automatic. During your analysis, a reference solution is constantly infused into the reference sprayer. The reference solution is set up prior to any analysis with a known compound (or compounds) which act as a 'lock mass' during the experiment. By providing a known reference mass, you can be sure that your exact mass measurement will have a high degree of accuracy and therefore provide you with the confidence in your answer.

During the LC-MS analysis, the reference sprayer is sampled periodically which maintains a very high duty cycle on the analyte sprayer. By sampling the reference sprayer once every 10–20 seconds, a 'snapshot' of the reference sprayer information is used to produce your exact mass measurement. Using LockSpray constantly validates any exact mass measurement performed, providing you with assurance that your answer is correct. Figure 1 shows a schematic representation of the LockSpray ion source with the two graphics showing both the analyte and reference sprayer positions.

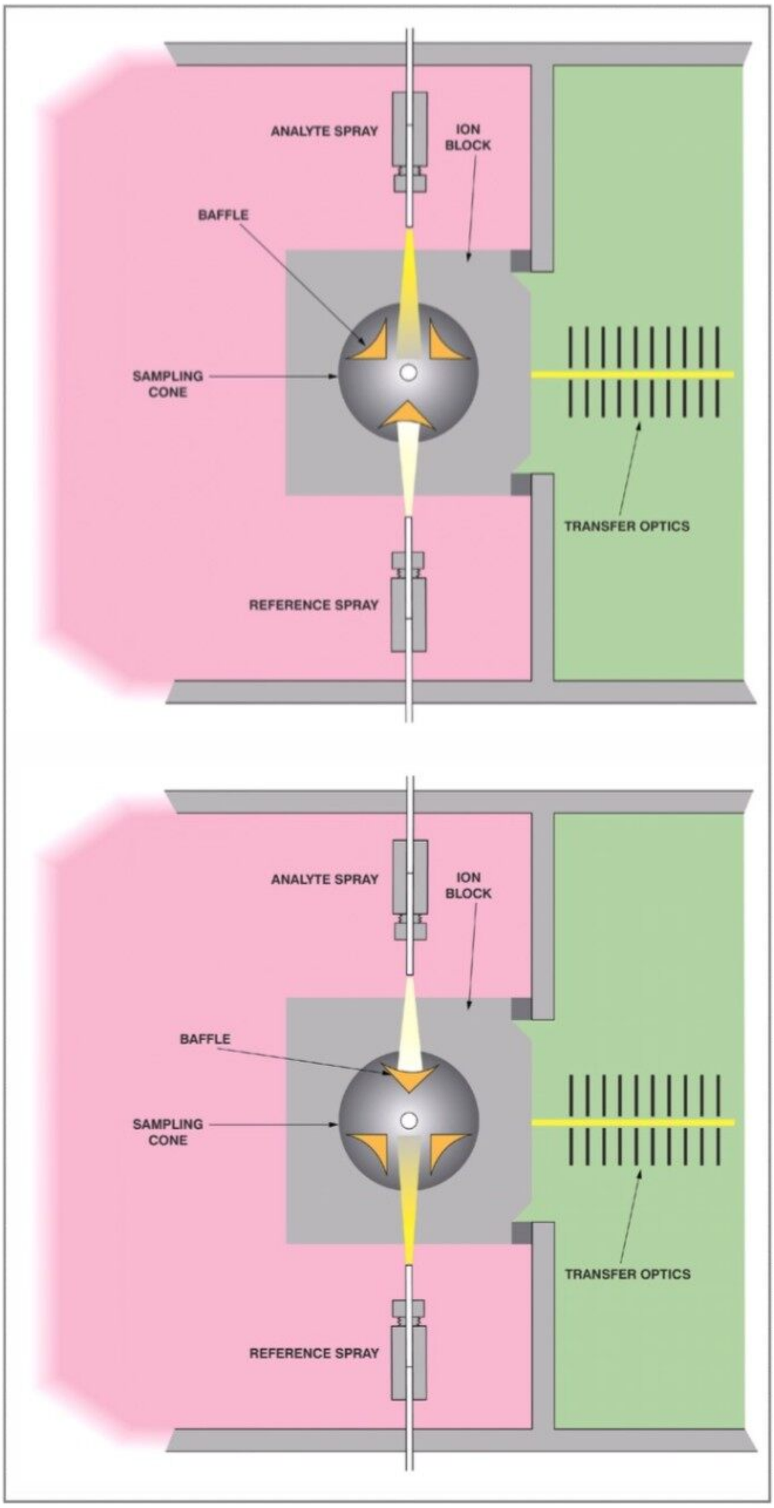


Figure 1. Exact mass measurement made easy using LockSpray.

Results and Discussion

The Benefits of using LockSpray

Traditional methods of performing exact mass measurement would often cause difficulties during analysis. During LC-MS experiments, the reference mass would have to be infused directly into the eluent post-column but this could often result in :

- Instability of the reference solution due to plumbing issues
- Variability of the reference mass ion current due to LC gradient changes
- Possibility of mass interferences during the exact mass experiment
- Suppression of the lock mass ion current due to electrospray charge competition

Through the use of LockSpray, these difficulties are removed. Infusing a lock mass solution directly into the separate sprayer ensures that there are no practical set up problems, no variability when carrying out an LC gradient and completely removes the possibility of mass interferences as the reference sprayer information is kept completely separate from the analyte data.

Some other commercially available LC-Tof Mass Spectrometers provide the facility of a dual sprayer arrangement for LC-MS analysis. However, such ion sources allow the two sprayers to mix prior to sampling by the mass spectrometer. Through mixing the two sprays, the experimental problems listed above are still potential issues -particularly the variability of the reference mass ion current, the possibility of mass interferences, and potential of ion suppression.

Figure 2 highlights the issue of introducing the reference mass post-column directly into the eluent stream, as in traditional methods of performing exact mass measurement. Running a normal LC-MS experiment with electrospray ionization and an LC gradient from high aqueous to high organic modifier can change the ion current of the reference mass causing signal instability. The upper trace is the ion current produced by the reference mass and it can be seen that over the course of the gradient, the signal produced by the ion varies considerably resulting in experimental difficulties. Figure 3 shows the same LC gradient but now using LockSpray. The upper trace is again the reference mass, and it remains completely constant because the analyte and reference sprays are kept separate but indexed via the rotatable baffle.

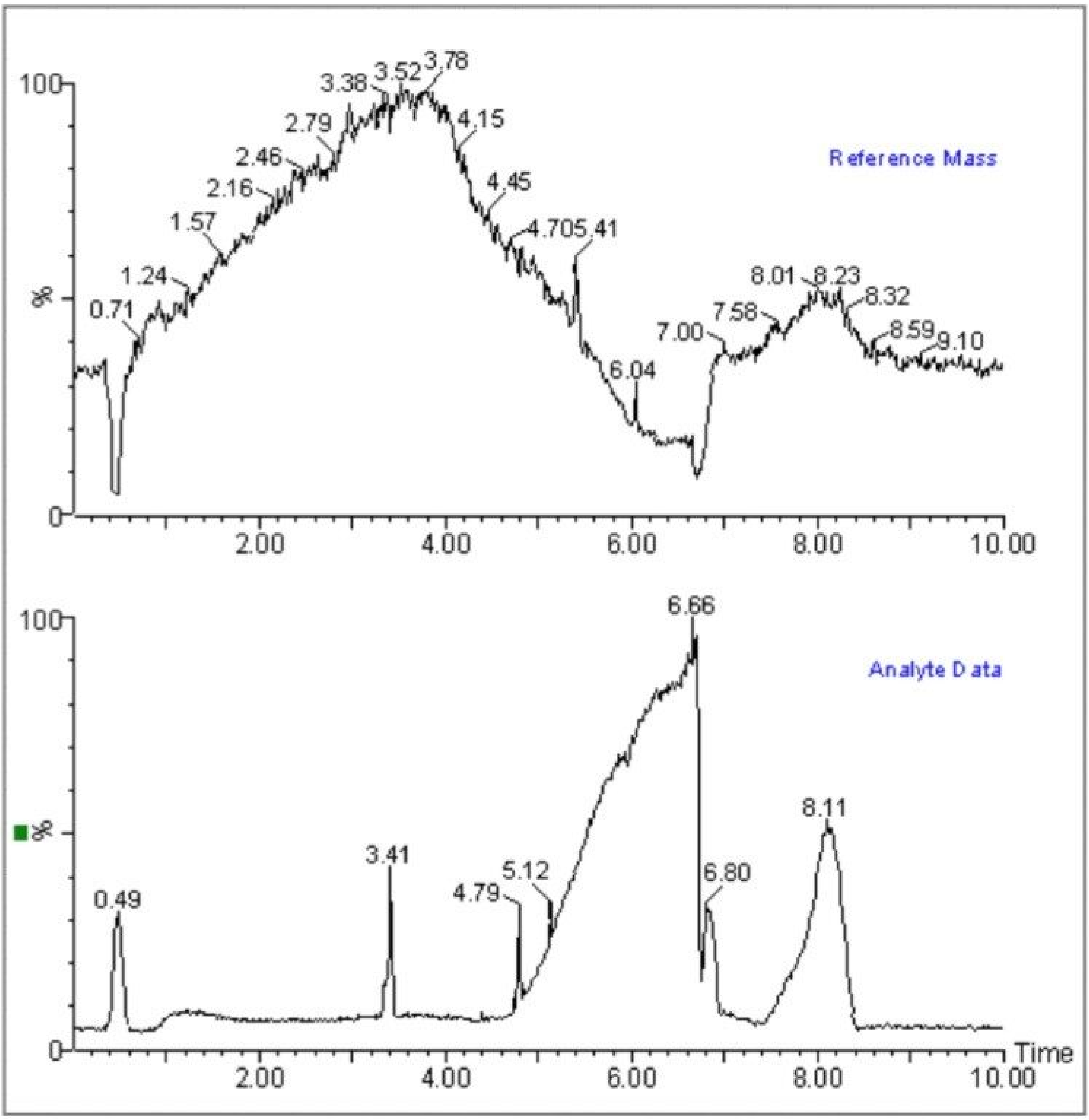


Figure 2. Introduction of a reference mass post-column during an LC gradient without LockSpray.

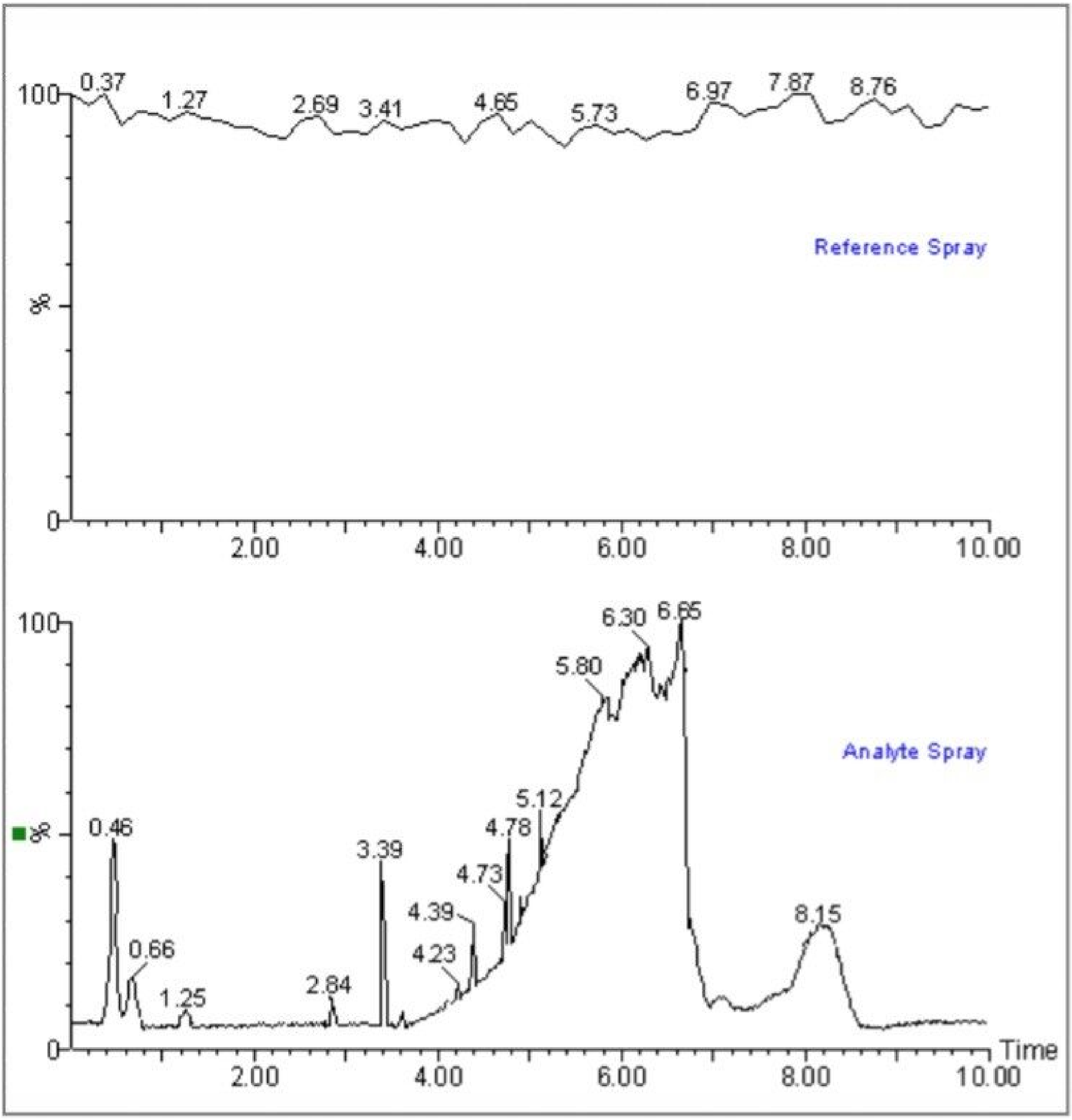


Figure 3. Introduction of a reference mass during an LC gradient with LockSpray.

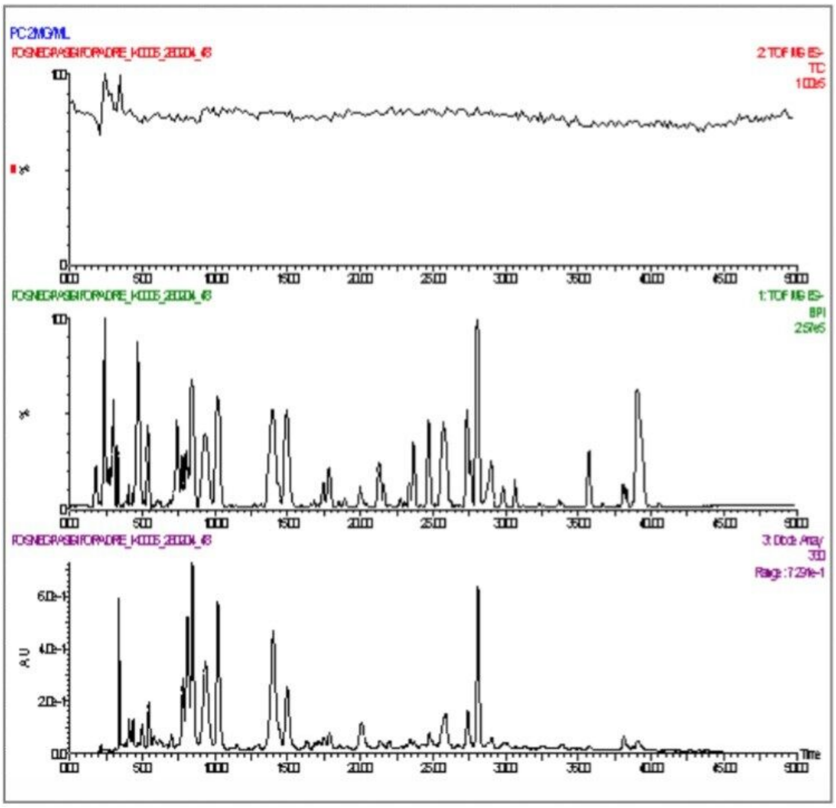


Figure 4. Effectiveness of the LockSpray ion source during an extended LC-MS analysis.

Exact Mass Measurement Results Using LockSpray

LockSpray with the Waters Micromass LCT Premier Mass Spectrometer have been used to analyze an 8 component mixture of ‘pharmaceutical-like’ compounds. Each compound was analyzed by LC-MS and an exact mass was generated for each peak. Figure 5 shows a typical LC-MS chromatogram obtained, and Table 1 shows the exact mass measurements for each of the peaks. The RMS ppm error for all the exact mass measurements was 1.6 ppm.

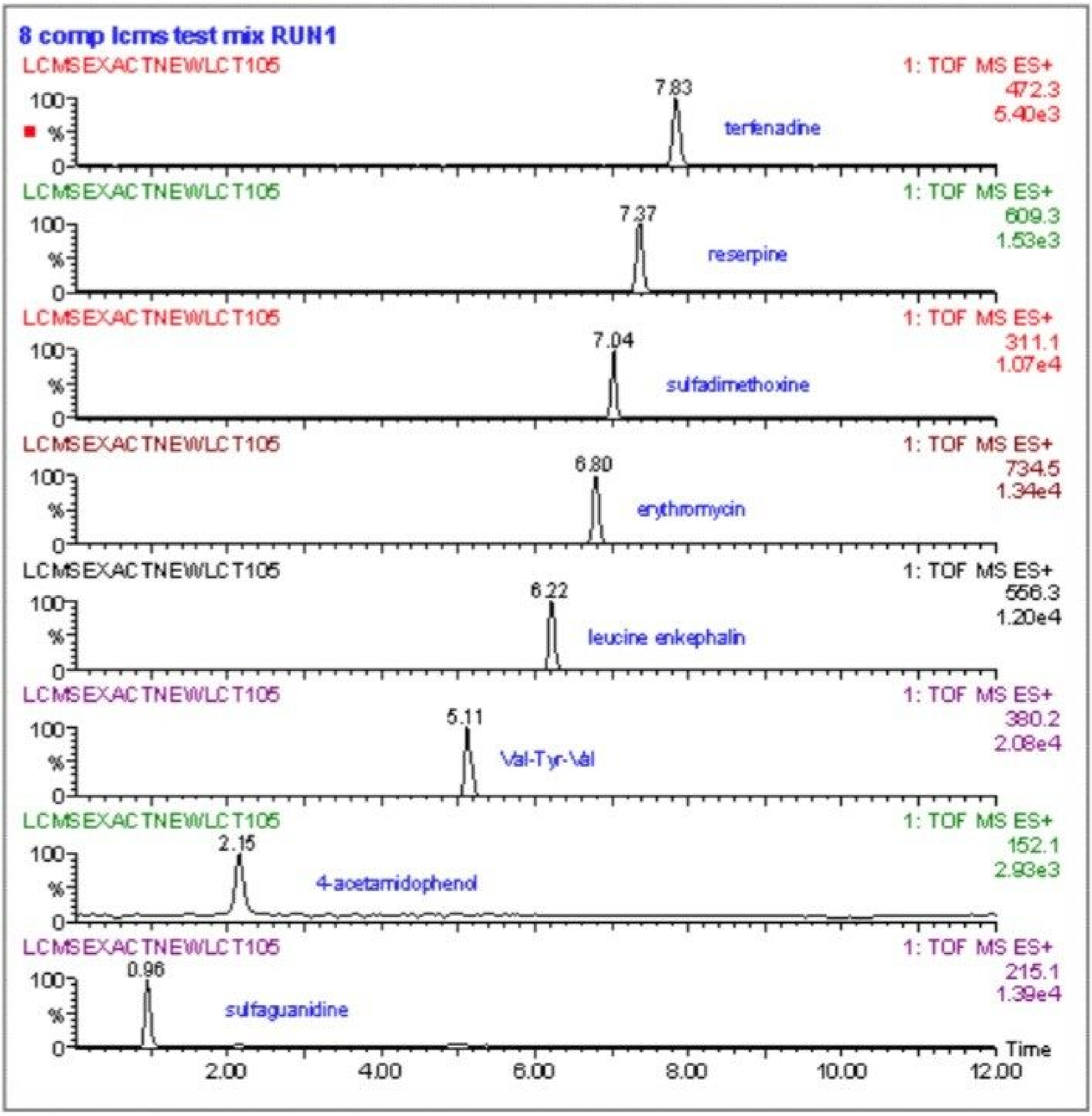
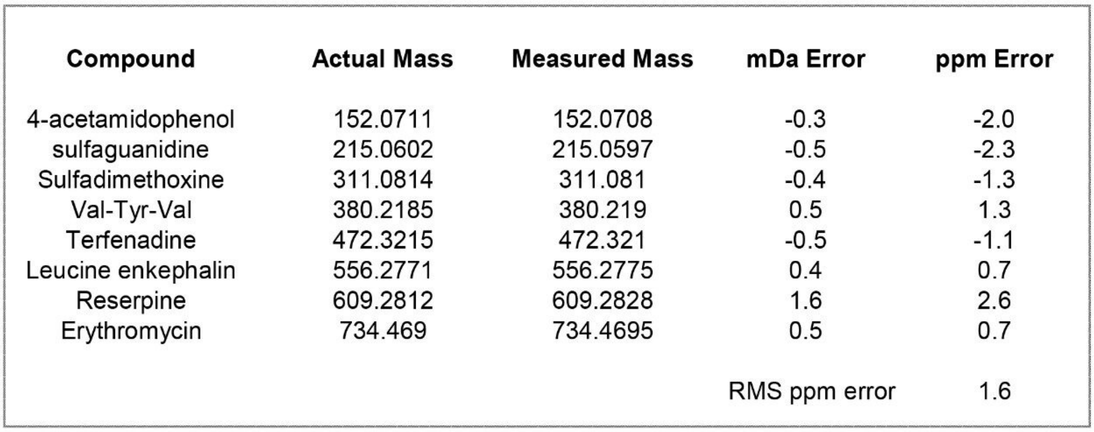


Figure 5. Exact mass measurement of 8 'pharmaceutical-like' components.



Compound	Actual Mass	Measured Mass	mDa Error	ppm Error
4-acetamidophenol	152.0711	152.0708	-0.3	-2.0
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	1.6

Table 1. Exact mass measurement results of 8 components during LC-MS analysis.

This example shows that the LCT Premier gives 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 yields improved specificity during an LC-MS experiment, providing the analyst with increased confidence in the analytical result.

The LCT Premier also has the capability of carrying out positive/negative ionization per sample, and maintaining exact mass measurement. Positive/negative ionization is ideal for compound screening applications where the particular mode of ionization is not known. Figure 6 shows an exact mass measured spectrum of raffinose in both positive ($[M+Na]^+$) and negative ($[M-H]^-$) ionization with an inter-acquisition delay of only 200 milliseconds.

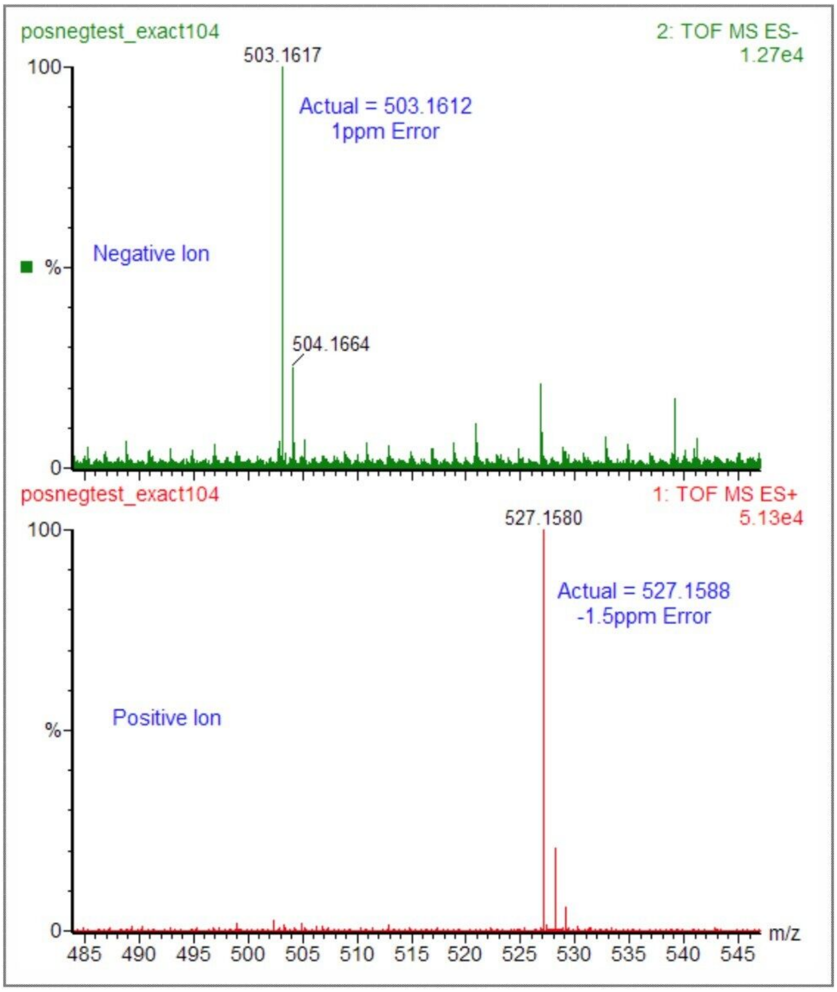


Figure 6. Positive/negative switching analysis of raffinose.

The use of LockSpray is also key for exact mass measurement over extended periods of time. LockSpray is not only designed to provide routine exact mass measurement over extended LC run times, as highlighted in Figure 4, but it is also designed to provide reliable measurements for several hours, days and months. Figure 7 shows repeat exact mass measurement of a natural product sample over 10 days and over 3 orders of dynamic range. As can be seen, the data shows that exact mass measurements using LockSpray are constant over the time period of the study, providing very accurate and reliable results.

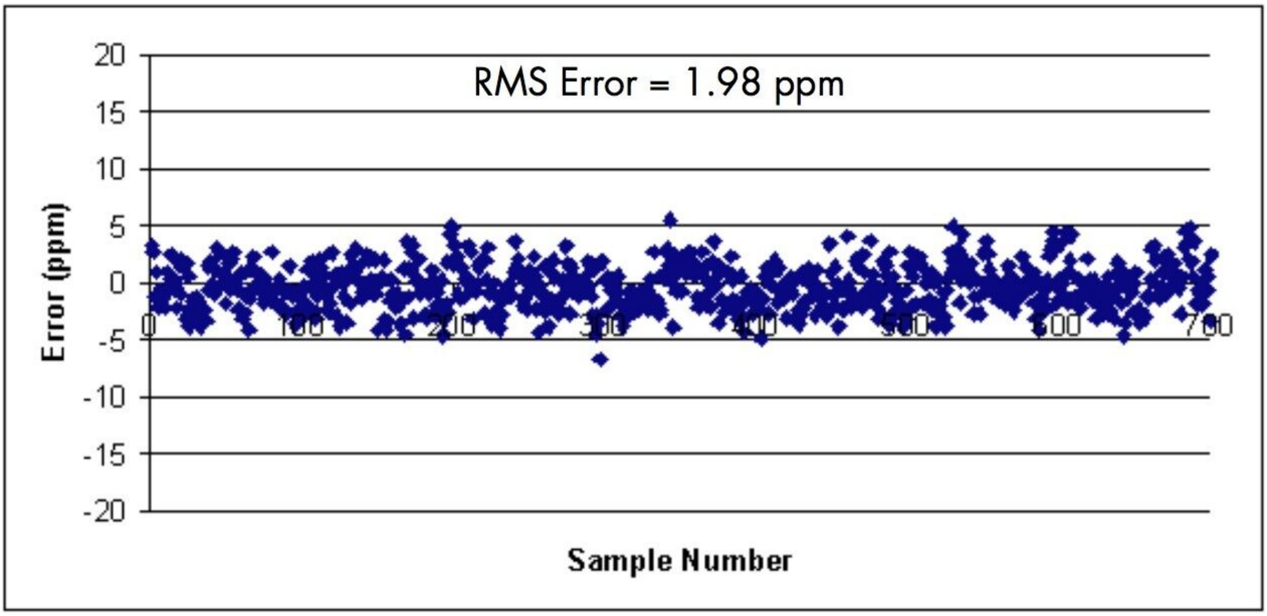


Figure 7. Exact mass measurement over extended periods using LockSpray.

Conclusion

LockSpray is specifically designed to make exact mass measurements easy. It provides a simplistic experimental setup for exact mass measurement in conjunction with electrospray ionization and LC-MS applications. LockSpray removes any practical setup issues but more importantly, any possibility of experimental problems that may effect the ionization of the reference mass. By providing a reference mass continuously during an LC-MS experiment, all exact mass measurements can be validated without having to rely on stable environmental or instrument conditions. With the use of LockSpray and LCT Premier, exact mass measurements are routine and reliable, even for extended periods of LC-MS analysis.

Featured Products

LockSpray Exact Mass Ionization Source <https://www.waters.com/1000396>

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