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Note d'application

Evaluation of LCT Premier Negative Ion Mode ESI Exact Mass Measurement Performance

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Abstract

The aim of the study presented is to illustrate the enhanced performance of the Waters Micromass LCT Premier oa-Tof Mass Spectrometer. The data presented shows mass accuracy achieved over a twelve-hour period. Using negative ion mode electrospray, very good mass accuracy is achieved routinely with real time centroid data acquisition and lockmass correction. Acquisition of UV data in parallel is performed. The system used is comprised of a Waters Alliance HT 2795 Separations Module, 2996 PDA Detector, Symmetry C_{18} Column, and Micromass LCT Premier oa-Tof Mass Spectrometer.

Benefits

- Using plant extract samples, the flavonoids isoorientin, orientin, and isovitexin have been successfully detected over a period of twelve hours
- Increased confidence in analyte confirmation is achieved using exact mass measurement

Introduction

The LCT Premier is a newly developed benchtop oa-Tof (orthogonal acceleration time of flight) mass spectrometer. New hardware and software control technology has been incorporated to meet the increased analytical demands in the pharmaceutical, environmental, and clinical applications arenas. This study utilizes a plant extract at concentrations of 100 ng/µL. The extract contains a plethora of major and minor components of which exact mass measurement can be performed in one analysis. The high duty cycle of Tof is utilized for qualitative studies, generating full spectra at high mass accuracy (<3 ppm RMS). The highly specific data generated provides an extra degree of information that aids interpretation of the data. Using real time exact mass centroid data acquisition, the evaluation of electrospray negative ion mode performance of the LCT Premier has been performed. The LCT Premier oa-Tof is presented in Figure 1 and the schematic of the LCT Premier with the analyzer in V geometry is illustrated in Figure 2.

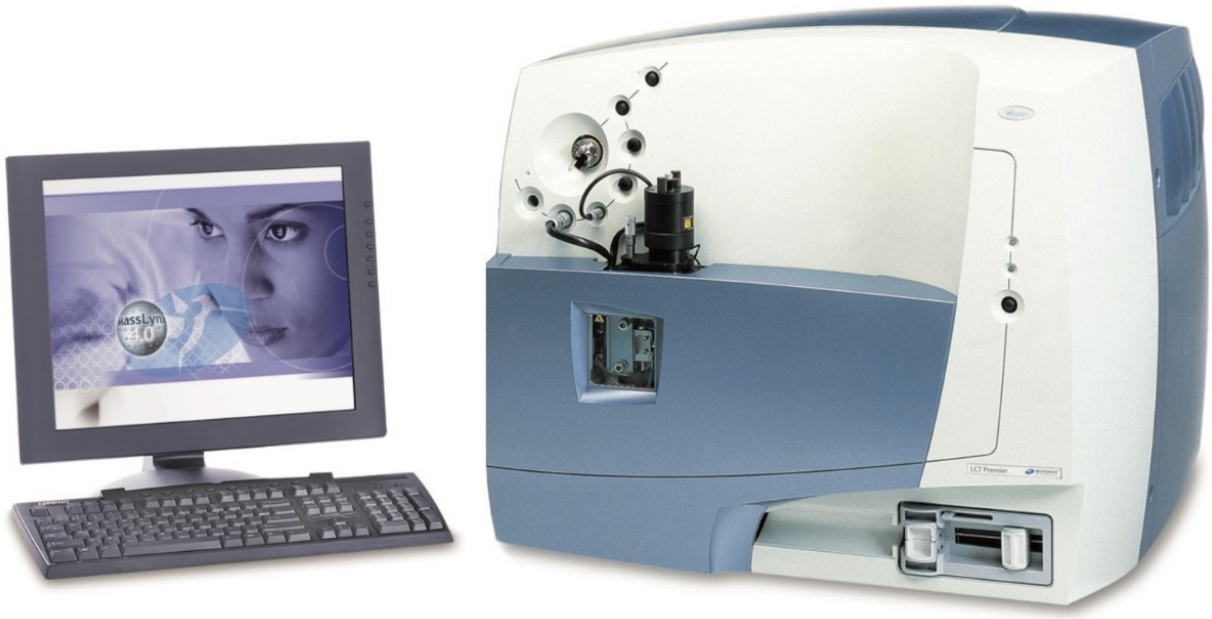

Figure 1. LCT Premier incorporating integral LockSpray.

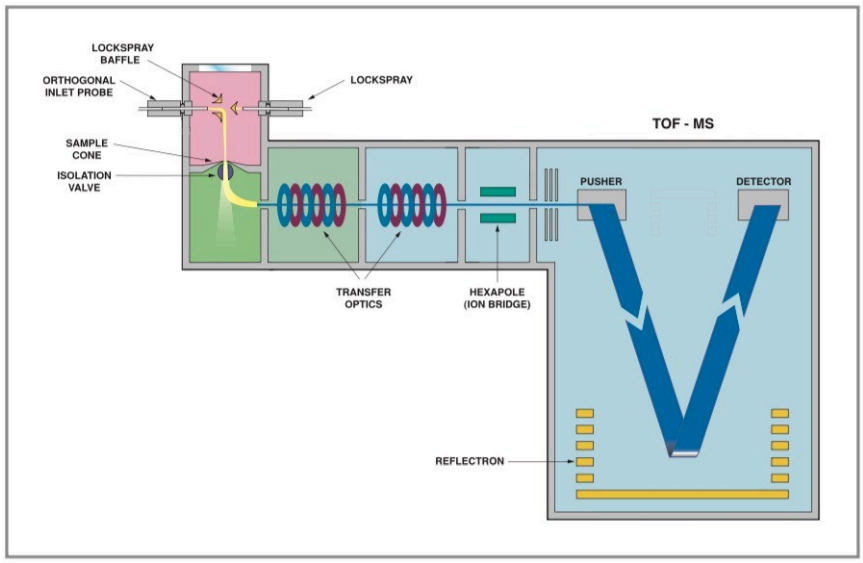

Figure 2. oa-Tof schematic (V-mode > 5000 FWHM).

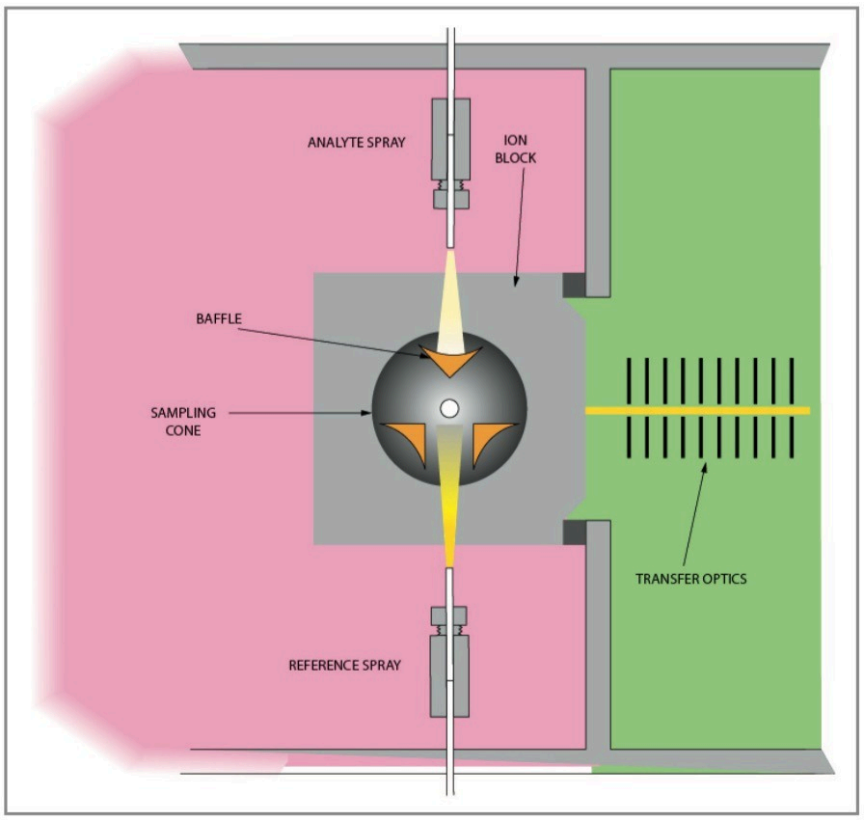

Figure 3. Schematic of the LCT Premier LockSpray electrospray source.

Experimental

HPLC Conditions

HPLC:	Alliance HT 2795 Separations Module
Column:	Waters Symmetry C_{18} (250 mm x 4.6 mm, 5 µm particles) with guard column (2 mm x 3.9 mm, 5 µm particles)

Column temp.:	35 ˚C
Flow:	1 mL/min - split 1:4
Mobile phase:	A: H_2O (0.2% HCOOH), B: MeCN
Gradient:	0-10 min:15% B; 10-40 min: 15-30% B; 40-50 min: 30-15% B

MS Conditions

Mass spectrometer:	Micromass LCT Premier oa-Tof
Ionization mode:	ESI Voltage -ve = 2.7 kV
Sample cone voltage:	100 V
Reference mass:	Leucine enkephalin, [M-H]=554.2615
Acquisition parameters:	100 –1000 Da 1 spectrum/second 5500 FWHM 0.1 second inter scan delay

Results and Discussion

Two plant extracts (*Passiflora caerulea* and *edulis*) were selected for analysis to evaluate the LCT Premier negative mode exact mass measurement performance. The BPI chromatograms obtained for the negative mode analysis of the two species are shown in Figure 4, where *Passiflora edulis* (A) and *Passiflora caerulea* (B) are presented. Visually comparing the two chromatograms, the difference in the profile of the two species is evident. This highlights the advantage of oa-Tof technology, where good sensitivity can be obtained with full exact mass spectral acquisition. A plethora of major and minor components have been detected in both

extracts, and acquiring full spectra has ensured that a maximum amount of specific information can be obtained.

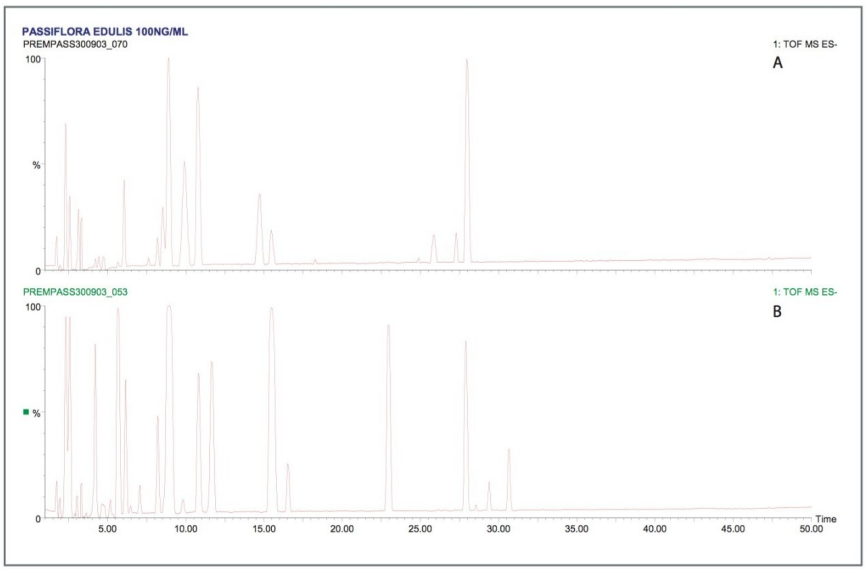

Figure 4. Negative mode BPI chromatograms, acquired for analysis of a 100 ng/µL plant extract of Passiflora edulis (A) and caerulea (B).

The data was acquired in centroid mode and mass corrected in real time. The reference mass Leucine enkephalin was sampled independently using the integral LockSpray source. The reference compound Leucine enkephalin ionizes in negative mode to produce ($[M-H]^-$=554.2615), and is sampled independently from the analyte spray to provide a lockmass. As shown in Figures 5 and 6, it is not seen in the analyte mass spectrum. Lockmass correction takes place automatically in real time and the independent sampling enhances the mass accuracy obtained. Both plant extracts were consecutively injected six times, each analysis time taking sixty minutes. This allowed the negative ion mode performance of the LCT Premier to be evaluated over a period of twelve hours. In Figure 5, examples of the exact mass spectra acquired over the twelve-hour period are illustrated for isoorientin. The manually selected spectra illustrate data where exact mass measurement has been achieved within –0.1 mDa to 0.2 mDa. In Figure 6, data acquired for orientin in the two plant extracts is shown. Example exact mass spectra acquired over the twelve-hour period are illustrated where exact mass measurement has been achieved within –0.2 mDa to 0.2 mDa range.

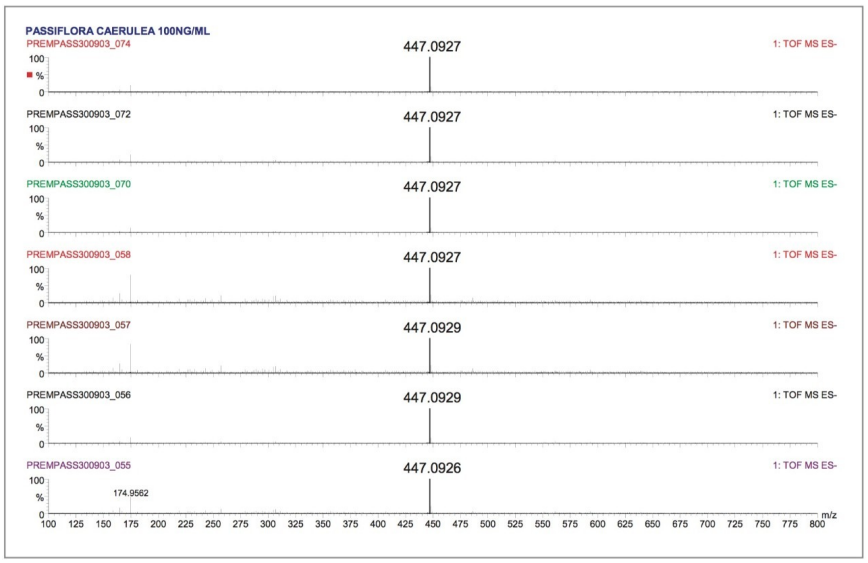

Figure 5. Examples of negative mode exact mass spectrum of isoorientin acquired for the repeat analysis of Passiflora caerulea and edulis isoorientin over a period of twelve hours.

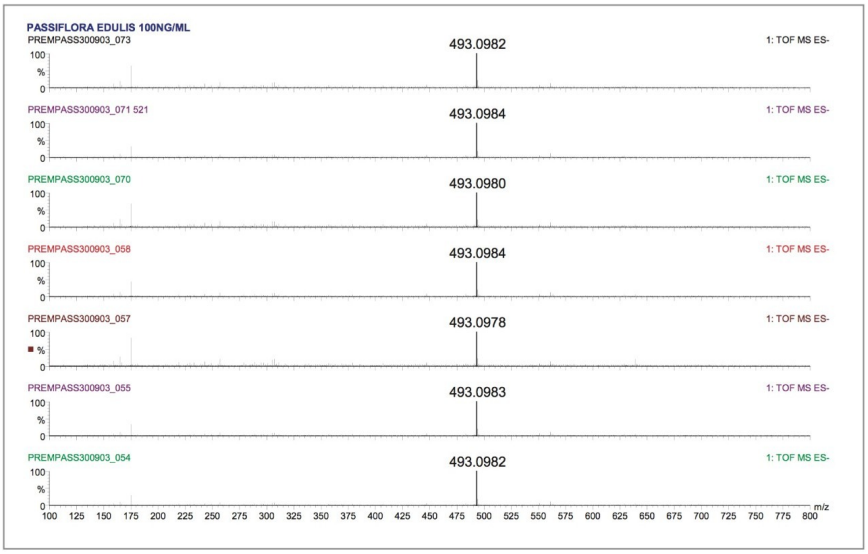

Figure 6. Examples of negative mode exact mass spectrum of orientin acquired for the repeat analysis of Passiflora caerulea and edulis over a period of twelve hours.

Tables 1, 2, and 3 show the masses measured, ppm errors and RMS ppm errors for isoorientin, orientin, and isovitexin respectively. The data processing was performed automatically using OpenLynx. RMS ppm errors of less than 3 ppm were obtained in negative ion mode during the analysis period.

Analysis Number	Total Analysis Time Mins	Isoorientin [M-H] Calculated Mass	Isoorientin [M-H] Measured Mass	Error mDa	Error ppm
1	60	447.0927	447.0937	1	2.1
2	120	447.0927	447.0924	-0.3	-0.7
3	180	447.0927	447.0923	-0.4	-0.9
4	240	447.0927	447.0939	1.2	2.7
5	300	447.0927	447.0931	0.4	1
6	360	447.0927	447.0918	-0.9	-2.1
7	420	447.0927	447.0933	0.6	1.2
8	480	447.0927	447.0932	0.5	1.1
9	540	447.0927	447.0931	0.4	0.8
10	600	447.0927	447.0914	-1.4	-3.1
11	660	447.0927	447.0926	-0.1	-0.2
12	720	447.0927	447.0914	-1.4	-3.1
RMS PPM ERROR					1.82

Table 1. Exact mass measurement obtained over a twelve-hour period for isoorientin determined to be present in the plant extracts of Passiflora caerulea and edulis.

Analysis Number	Total Analysis Time Mins	Orientin [M-H+CH2O2] Calculated Mass	Orientin [M-H+aCH2O2] Measured Mass	Error mDa	Error ppm
1	60	493.0982	493.0973	-0.9	-1.8
2	120	493.0982	493.0969	-1.4	-2.7
3	180	493.0982	493.0982	0.0	0.0
4	240	493.0982	493.0969	-1.4	-2.7
5	300	493.0982	493.0979	-0.3	-0.6
6	360	493.0982	493.0976	-0.6	-1.3
7	420	493.0982	493.0975	-0.7	-1.5
8	480	493.0982	493.0994	1.2	2.5
9	540	493.0982	493.0967	-1.5	-3.0
10	600	493.0982	493.0986	0.4	0a.8
11	660	493.0982	493.0974	-0.8	-1.7
12	720	493.0982	493.0900	-1.1	-2.2
RMS PPM ERROR					1.95

Table 2. Exact mass measurement obtained over a twelve-hour period for orientin determined to be present in the plant extracts of Passiflora caerulea and edulis.

Analysis Number	Total Analysis Time Mins	Isovitexin [M-H] Calculated Mass	Isovitexin [M-H] Measured Mass	Error mDa	Error ppm
1	60	431.0978	431.0979	0.1	0.2
2	120	431.0978	431.0993	1.5	3.4
3	180	431.0978	431.0963	-1.6	-3.6
4	240	431.0978	431.0968	-1.0	-2.3
5	300	431.0978	431.0987	0.9	2.1
6	360	431.0978	431.0966	1.2	-2.8
7	420	431.0978	431.0978	0	0
8	480	431.0978	431.0970	-0.8	-1.8
9	540	431.0978	431.0977	-0.1	-0.2
10	600	431.0978	431.0958	-2.0	-4.7
11	660	431.0978	431.0970	-0.8	-1.8
12	720	431.0978	431.0961	-1.8	-4.1
RMS PPM ERROR					2.7

Table 3. Exact mass measurement obtained over a twelve-hour period for isovitexin determined to be present in the plant extracts of Passiflora caerulea and edulis.

Conclusion

- Using plant extract samples, the flavonoids isoorientin, orientin, and isovitexin have been successfully detected over a period of twelve hours
- Analysis has been performed in centroid acquisition mode and exact mass measurement performed in real time
- 1.83 RMS ppm error obtained for negative mode acquisition of isoorientin
- 1.95 RMS ppm error obtained for negative mode acquisition of orientin

- 2.70 RMS ppm error obtained for negative mode acquisition of isovitexin
- The LCT Premier oa-Tof with integral LockSpray and independent reference mass acquisition enables the routine acquisition of highly specific data
- Increased confidence in analyte confirmation is achieved using exact mass measurement

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