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Application News
SSI-LCMS-014

Liquid Chromatography Mass Spectrometry

A Fast Dual Ion Source (DUIS) Method for Analysis of Isobutrin and Butein in Rabbit Plasma Samples

LCMS-2020

Summary

Qualification and quantitation of isobutrin and butein in rabbit plasma samples were tested using the LCMS 2020 single quadrupole mass spectrometer. A LCMS method was developed and analyzed for these two standards.

Background

Butea monosperma (BM) is a known medicinal plant located in India and the tropics. Research has shown that standardized extract from the BM flower inhibits the inflammatory reactions in human mast cells. It is important to be able to monitor and quantitate the known constiuents founds in BME such as isobutrin and butein for their potential therapeutic value for the treatment of inflammatory and other diseases where mast cells are present (Rasheed et. al.).

Method

The standards for isobutrin and butein (structures shown in Figure 1) were at a concentration of 500 ppm (µg/mL). These were further diluted in water to 10 ppm for LCMS analysis. The Butea monosperma extract (BME) was diluted 10 fold for a final concentration of 1000 ppm. The remaining samples, control plasma, BME in control plasma, butein and isobutrin in control plasma, and three rabbit plasma samples were directly injected on the LCMS system.

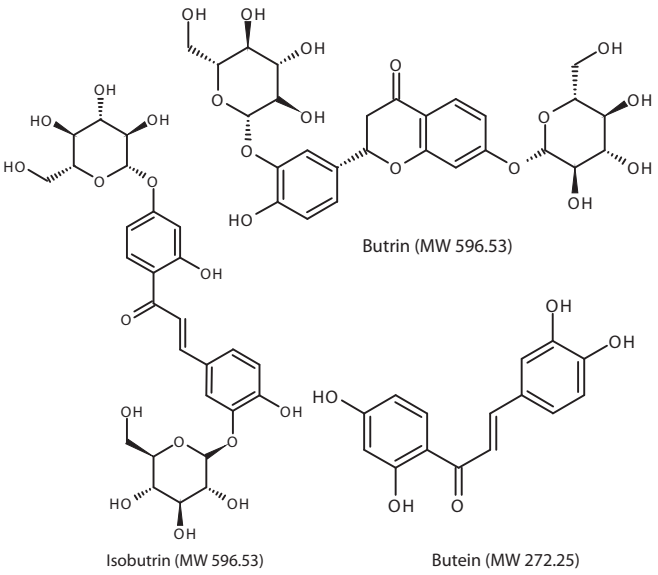


Figure 1: Structures of isobutrin, butrin and butein

Both selected ion monitoring (SIM) and scan methods were used for analysis. The details of these events are described in Table 1. Negative ion ESI plus simultaneous APCI (DUIS) was used for each component. For isobutrin the SIM m/z value was 595.1 and for butein the SIM m/z value was 271.1.

A ShimPack XR-ODSII (2.2 µm x 2.0 mm x 100 mm) column was used with a binary gradient consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The gradient conditions are shown in Table 2. The flow rate was 0.300 mL/min and the column temperature was 30 degrees C. The injection volume was 2 µL.

For quantitative analysis, dilutions of a mixture of isobutrin and butein were made as shown in Table 3. Calibrations curves were prepared and the unknown samples were analyzed for isobutrin and butein content.

Results and Discussion

The MS chromatogram (SIM) for the separation of the 10 ppm standards, isobutrin and butein, is shown in Figure 2. Figure 3 shows both the scan and SIM chromatograms for the butein and isobutrin spiked sample in control plasma. In the inset below the chromatogram, the MS spectra for both the isobutrin and butein peaks are shown. The isobutrin spetrum shows m/z 595.1 $[M-H]^-$ and m/z 641 $[M+formate]^-$. Butein shows a $[M-H]^-$ peak at 271 m/z.

The benefit of running in scan mode is that one is able to observe additional components based on m/z as illustrated in Figure 4. The top chromatogram is the total ion chromatogram (TIC) scan for the BME extract in control plasma. There are many peaks in this chromatogram, but with the use of the MS, the mass spectrum of each peak provides important identification clues. The MS spectra for

Scan (m/z)	150-1200
Scan Speed (u/sec)	5000
Event Time (sec)	0.25
SIM	595.1; 271.0
Mode	Negative, DUIS
Drying Gas (L/min)	15
Nebulizing Flow (L/min)	1.5
Interface Temp. (°C)	350
DL (°C)	250
Heat Block (°C)	400

Table 1: MS Parameters for the BME samples and standards.

Time	% B
0.0	10
7.0	75
7.5	100
8.5	100
8.5	10
10.0	10

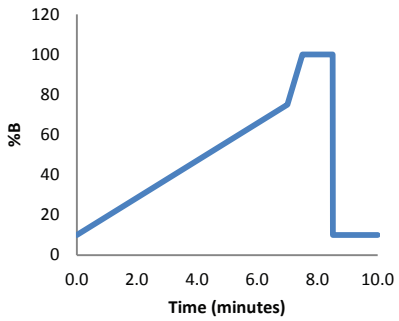


Table 2: Gradient Conditions

Level	ppm
1	10
2	3.33
3	1.11
4	0.37
5	0.123
6	0.041
7	0.014

Table 3: Calibration Levels

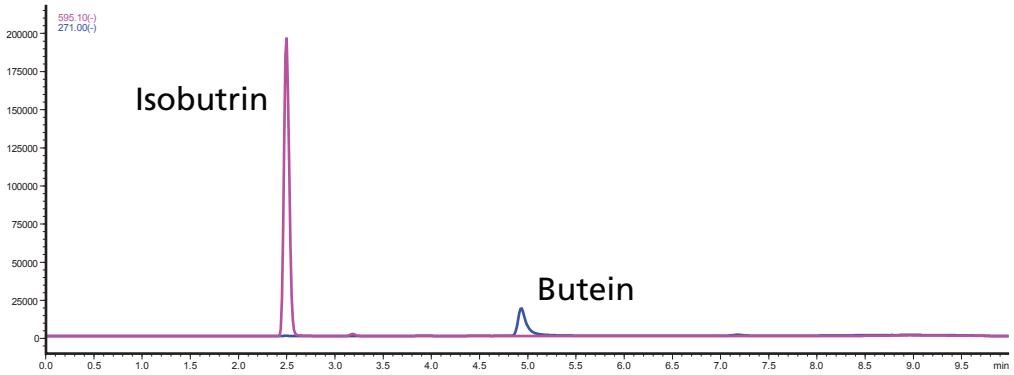


Figure 2: Selected ion monitoring (SIM) of 10 ppm standard mixture of isobutrin and butein.

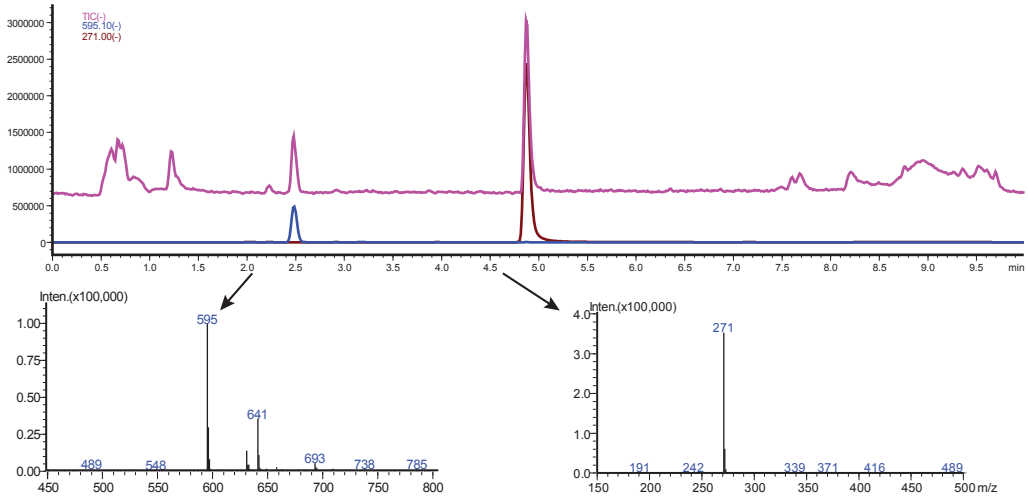


Figure 3: Scan (pink) and SIM chromatograms for isobutrin (blue) and butein (brown). Below: MS spectra for isobutrin (left) and butein (right).

three different peaks, labeled 1-3, are shown beneath the chromatogram. Peak 1 has a m/z of 433 which corresponds to the $[M-H]^-$ for the compound isocoreopsin (Rasheed et. Al.).

Figure 5 illustrates the scan and SIM modes for the BME extract sample. As noted on the figure, there are two peaks for the m/z of 595.1 (shown in blue). The first peak is identified as isobutrin based on mass and retention time. The second peak is most likely the compound butrin (shown in Figure 1) which is an isomer of isobutrin, thus having the same m/z in negative ion mode. This tentative identification could be confirmed by comparison with an authentic standard. Figure 6 shows the three rabbit plasma samples overlaid, in order to show the reproducibility.

The calibration curves were linear in the tested range for the two standards, isobutrin and butein. Each compound had at least five calibration points. The calibration curves for each standard are shown in Figure 7. The limit of detection (LOD, S/N>3) for both compounds was 14 ppb, which was the lowest concentration examined. The SIM for both standards, isobutrin at 14 ppb and butein at 123 ppb are shown in Figure 8. The SIM for the compounds isobutrin and butein found in the three rabbit plasma samples are shown in Figure 9.

The amount of butein present in the plasma samples was much lower than the isobutrin present but the MS was still able to detect the compound at low levels. The quantitative levels of the isobutrin and butein present in the real samples are shown in Table 4. Each sample was run three times and the average concentration for each compound is displayed in the table. The relative standard

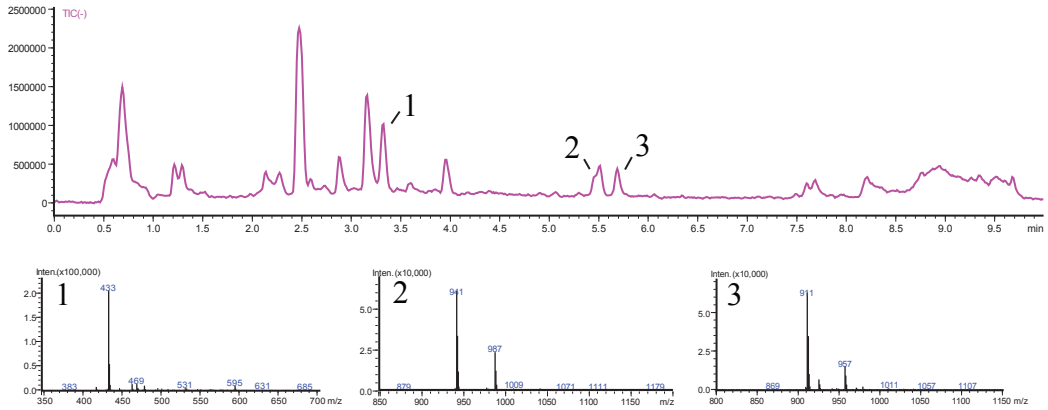


Figure 4: MS Scan chromatogram for the BME extract in control plasma. The respective MS spectra for peaks labeled 1, 2 and 3 are shown below.

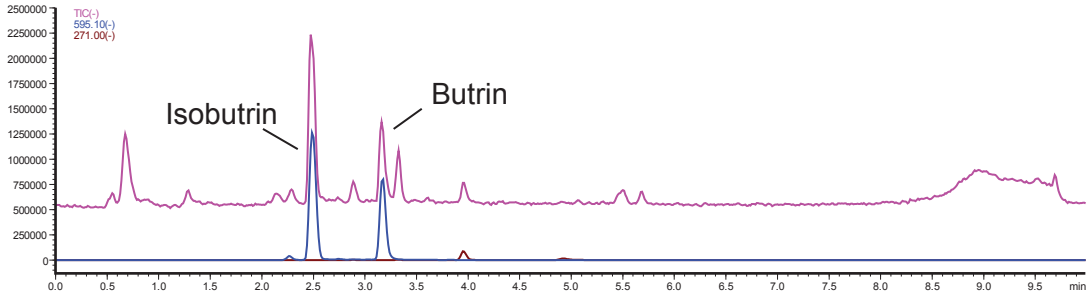


Figure 5: MS Scan and SIM for the BME extract sample.

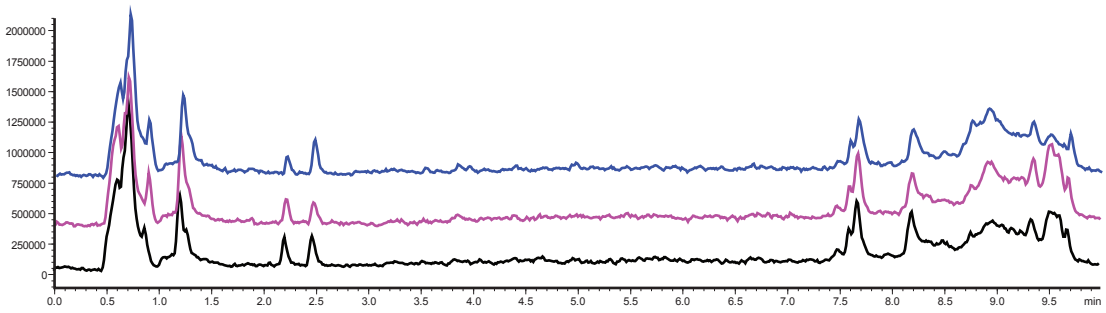


Figure 6: MS Scan for the three rabbit plasma samples. Blue (rabbit plasma 1), Pink (rabbit plasma 2), Black (rabbit plasma 3)

deviation for the isobutrin is very good with all samples under 3%. The RSD's are slightly higher for butein, but with the exception of rabbit plasma sample 3, the values are all less than 10%. In addition, the concentration for butein in the control plasma was not detected. For this demonstration, both scan and SIM modes were run simultaneously. The accuracy of the quantitation could be increased if only SIM mode was used for quantitation data.

Conclusion

The LCMS-2020 single quadruple instrument was able to demonstrate both scanning and selected ion monitoring methods for the identification and quantitation of isobutrin and butein in rabbit plasma samples.

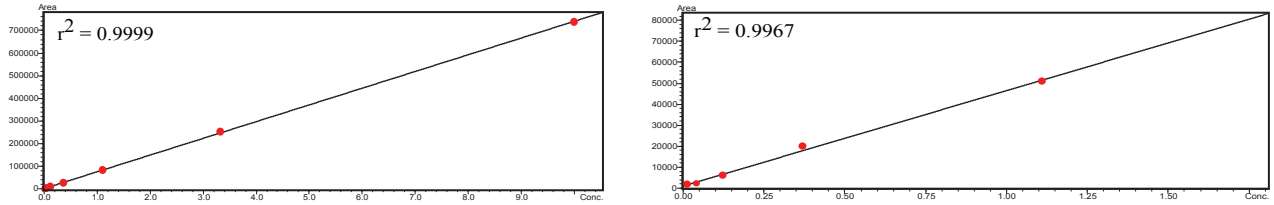


Figure 7: Calibration curves for isobutrin (left) and butein (right)

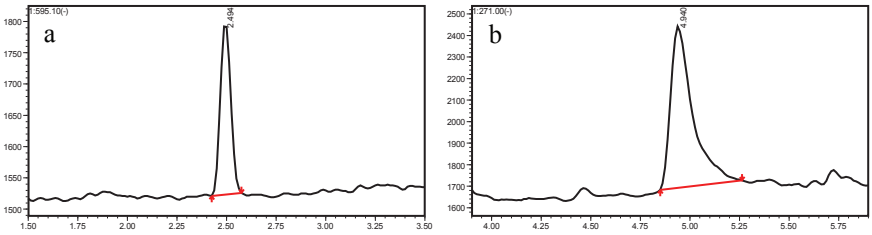


Figure 8: MS chromatograms for isobutrin standard at 14 ppb (a) and butein standard at 123 ppb (b)

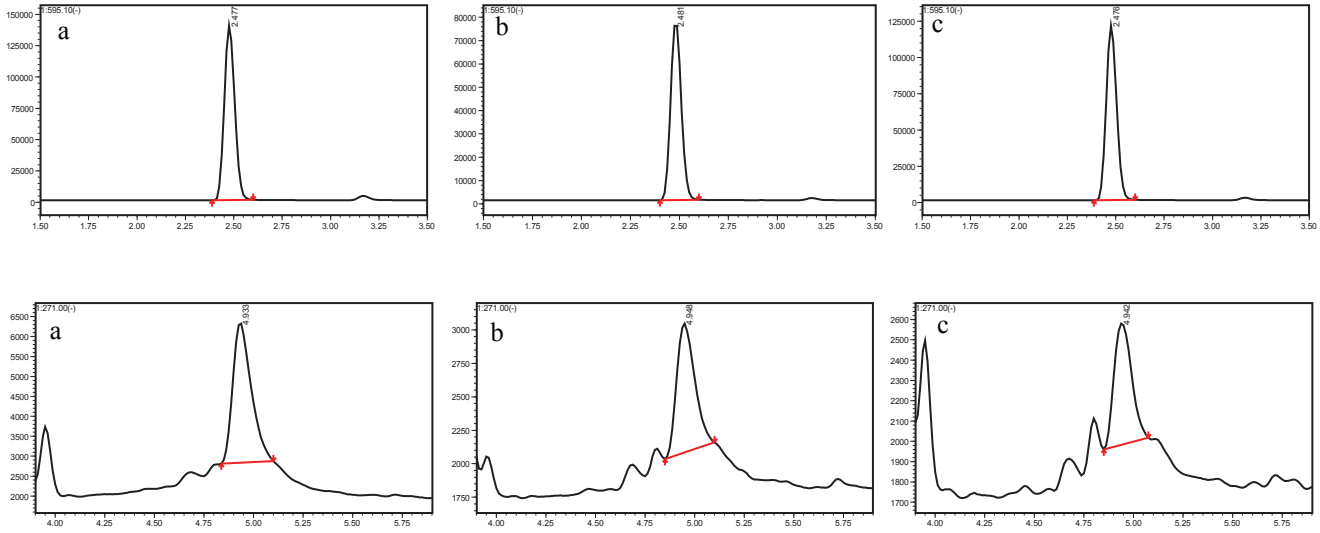
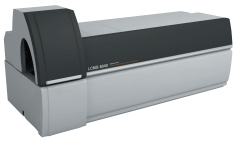


Figure 9: MS chromagrams for isobutrin (top) and butein (bottom) in rabbit plasma samples. (a) rabbit plasma 1 (b) rabbit plasma 2 (c) rabbit plasma 3

	Isobutrin			Butein		
Sample (n = 3)	Conc. (ppm) Average	St. Dev.	% RSD	Conc. (ppm) Average	St. Dev.	% RSD
Rabbit_Plasma 1	7.121	0.048	0.67%	0.464	0.040	8.52%
Rabbit_Plasma 2	3.907	0.064	1.63%	0.110	0.011	9.83%
Rabbit_Plasma 3	5.932	0.165	2.78%	0.055	0.012	20.97%
But_IsoB_in Control Plasma	26.185	0.058	0.22%	243.430	3.873	1.59%
BME in Control Plasma	102.894	1.754	1.70%	6.397	0.237	3.70%
BME Extract	75.751	0.127	0.17%	1.975	0.162	8.23%
Control Plasma	0.290	0.007	2.44%	ND	ND	ND

Table 4: Quantitative data for isobutrin and butein in the plasma samples.

ULTRA FAST MASS SPECTROMETRY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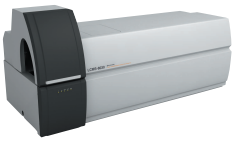
LCMS-8030


LCMS-8080


LCMS-2020


LCMS-IT-TOF

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