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ANALYSIS OF COMPLEX NATURAL PRODUCTS USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH TIME OF FLIGHT MASS SPECTROMETRY

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INTRODUCTION

Phytomedicines are of interest as a natural resource for the discovery of new drug therapies from the plant life in the surrounding environment. An increase in the interest in analysis of herbal extracts has also taken place due to changes in legislation throughout Europe, North America and Asia. The ability to derive drugs from botanical sources accounts for 25% of prescription drugs in the US alone.

A comparison of the response and chromatographic separation obtained using conventional HPLC and UPLC™ coupled with oa-TOF has been performed. Utilising UPLC technology and columns packed with particles <2 µm, high efficiency separations can be achieved at high flow rates, allowing superior resolution and sensitivity to be obtained in a shorter analysis time. UPLC generates chromatographic peaks that are typically only a few seconds in width, which places demands upon the detection technique to acquire data at an appropriate rate. The fast duty cycle of oa-TOF technology allows 20 spectra/second to be acquired generating sufficient sampling points across a peak to produce a representative response. The natural product application area has been selected, where previous studies have been performed in order to illustrate the reduced retention times, improved chromatographic resolution and increased MS response.

Using the Micromass® LCT Premier™ bench top oa-TOF mass spectrometer (Figure 1) coupled with HPLC, a plethora of components were determined to be present and elemental compositions derived. A twenty fold increase in sensitivity was achieved over previous HPLC oa-TOF studies using the LCT™ mass spectrometer. The integral LockSpray™ (Figure 2) source enabled real time exact mass measurements to be used to identify the numerous major and minor components present in four different species in order to obtain a complete profile of the four species profiled. Due to the nature of natural product extracts containing non-polar and polar compounds, long HPLC gradient analysis times result, to enable better analyte separation. Using UPLC, faster analysis times and better sensitivity have been achieved than was obtained using conventional HPLC.

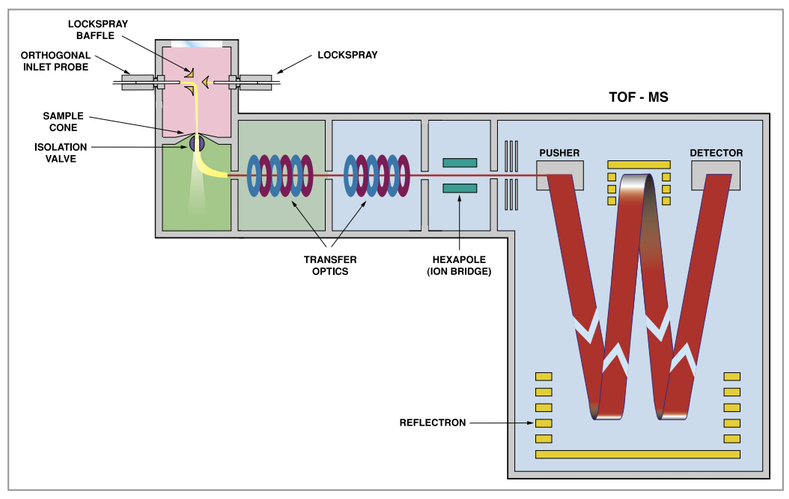

Figure 1. Schematic of dual resolution geometry LCT Premier oa-TOF (W mode >10000 FWHM).

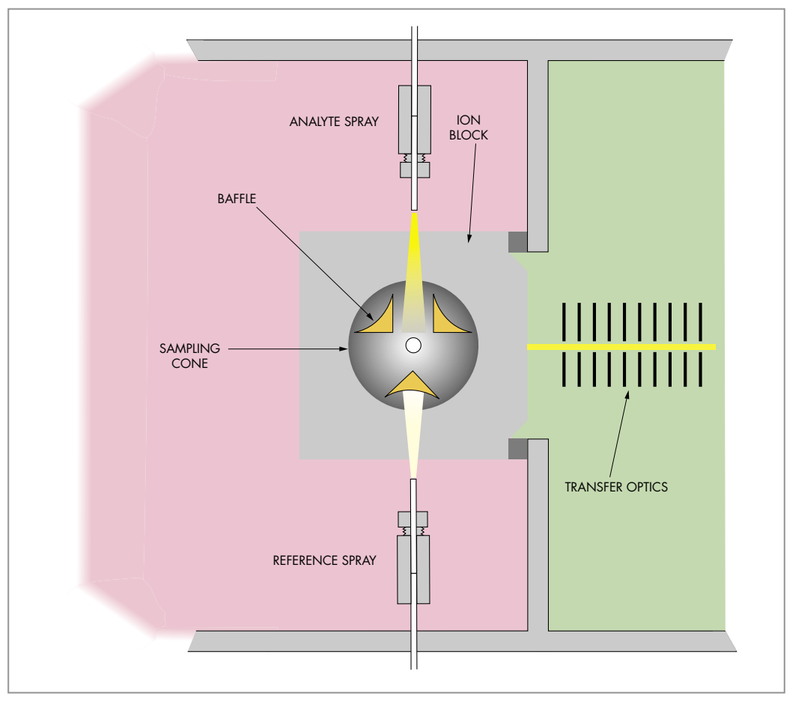

Figure 2. Schematic of the LCT Premier LockSpray source.

UPLC CONDITIONS

UPLC System:	Waters® ACQUITY UPLC™
Column:	Waters ACQUITY UPLC BEH C_{18} (100 mm x 2.1 mm x 1.7 µm)
Mobile phase:	A: H2O (0.2% HCOOH) B: MeCN
Gradient:	0–1 min: 5% B 1–10 min: 20% B 10–13 min: 20% B 13–14 min: 30% B 14–19 min: 30% B 19.1–27 min: 5% B
Column temperature:	40 °C
Flow:	0.6 mL/min

HPLC CONDITIONS

HPLC System:	Waters Alliance® HT 2795
Column:	Waters Symmetry® C_{18} (250 mm x 4.6 mm x 5 µm) with guard column (2 cm x 3.9 mm x 5 µm)
Mobile phase:	A: H2O (0.2% HCOOH) B: MeCN
Gradient:	0–10 min: 15% B 10–40 min: 15–30% B 40–50 min: 30–15% B
Column temperature:	35 °C
Flow:	1 mL/min split 1:4

MS CONDITIONS

MS:	Waters Micromass® LCT Premier oa-TOF
Capillary Voltage:	2600 V (-VE)
Ionisation mode:	Positive and negative electrospray
Resolution:	5500 FWHM (V mode) and 12000 FWHM (W mode)
Reference lock mass:	Leucine Enkephalin [M-H]-= 554.2615
LockSpray switch time:	10 spectra HPLC 30 spectra UPLC
Acquisition time:	1 spectra/second HPLC 1 spectra/ 0.2 seconds UPLC

RESULTS

Presented in Figure 3 is an example of the high standard of chromatography that can be achieved using HPLC and Symmetry column technology for natural product profiling studies. In Figure 3 the 50 minute analysis of *Passiflora edulis* is presented with the negative ion electrospray BPI chromatogram and the corresponding 10 minute analysis UPLC BPI chromatogram is shown in Figure 4. From the HPLC and UPLC analyses the m/z 431 extracted mass chromatograms are illustrated in Figure 5, along with the overlaid expanded chromatography obtained with HPLC and UPLC for the group of flavonoid isomers determined to be present. The exact mass and elemental composition obtained for flavonoid isomer A is presented in Figure 6.

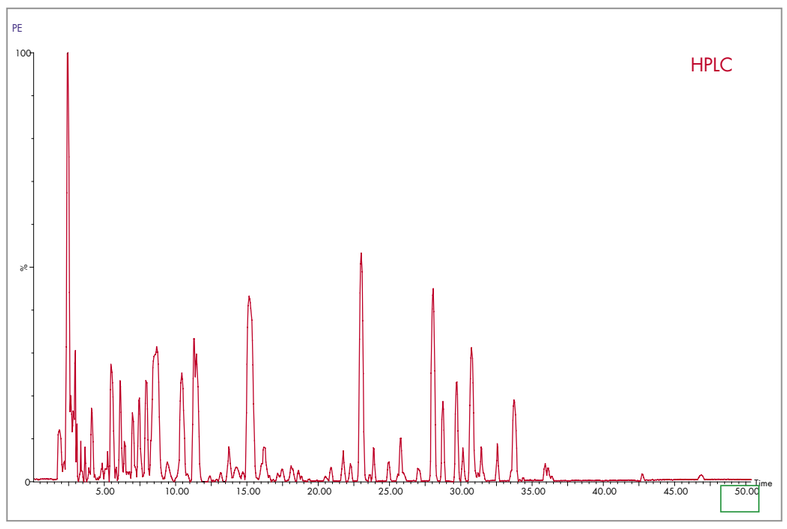

Figure 3. Negative ion electrospray base peak ion chromatogram (BPI) for the analysis of plant extract Passiflora edulis *using HPLC oa-TOF-MS.*

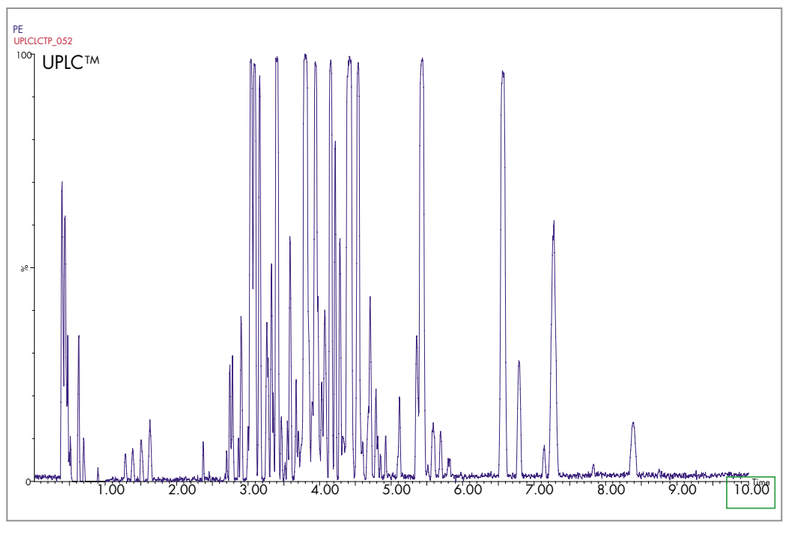

Figure 4. Negative ion electrospray base peak ion chromatogram (BPI) for the analysis of plant extract Passiflora edulis *using UPLC oa-TOF-MS.*

DISCUSSION

Using HPLC combined with the LCT Premier oa-TOF, an increase of greater than twenty times improvement in sensitivity was observed. Achieving separation of the closely related flavonoids is a challenging analysis and excellent separation has been achieved using Symmetry column technology and HPLC. For natural product profiling, the nature of the sample make up can typically consist of hundreds of components ranging from polar to non-polar. Using UPLC, analysis of *Passiflora edulis* is completed in less than nine minutes. This compares with 50 minutes required for HPLC and represents a reduction in analysis time of eighty percent. It can be seen from Figure 5 a series flavonoid isomers have been selected, for both the UPLC and HPLC oa-TOF-MS analyses. From the analysis performed using UPLC, it is clear the chromatographic resolution has been maintained. From these examples it can be seen that the lower plate height produced using 1.7 µm particles and a 2.1 mm id column at flow rates of 0.6 mL/min has improved the peak efficiency, resulting in narrower chromatographic peaks. With improved peak efficiency comes better resolution, due to narrower chromatographic peaks and hence an increase in peak capacity. For flavonoid isomer A, the base peak widths were 0.83 minutes (HPLC) and 0.14 minutes (UPLC), indicating a 6 fold decrease in peak width for the natural product profiling application. The reduction in peak width has been utilized to enable faster analysis times whilst maintaining resolution. The natural product profiling study required analysis of four extracts, where a total time of 200 minutes was previously required, all four extracts were profiled using UPLC oa-TOF-MS in forty minutes, less time than required to profile one extract using HPLC oa-TOF-MS. Oa-TOF-MS enables fast acquisitions to be performed at 20 spectra per second allowing sufficient points across the superior efficient narrow peaks obtained with UPLC oa-TOF-MS acquisition. The combination of low analyte detection with full spectra acquisition and the highly specific nature of exact mass is required to produce complete confidence in analyte identification. UPLC and oa-TOF-MS offers an unequalled means of increasing sample throughput with full exact mass spectral information being acquired.

CONCLUSION

- UPLC oa-TOF-MS analysis for natural product profiling has been performed with an 80% increase in speed of analysis .
- For natural product profiling, UPLC data indicating a 6 fold decrease in peak width has been shown enabling time efficient analysis to be achieved without compromising resolution.
- Increased peak efficiency has produced narrower chromatographic peaks and therefore improved peak resolution.
- The LCT Premier with integral LockSpray and independent reference sampling enables the routine acquisition of highly specific data in combination with UPLC.
- Exact mass measurement with <3 ppm error is achieved routinely.

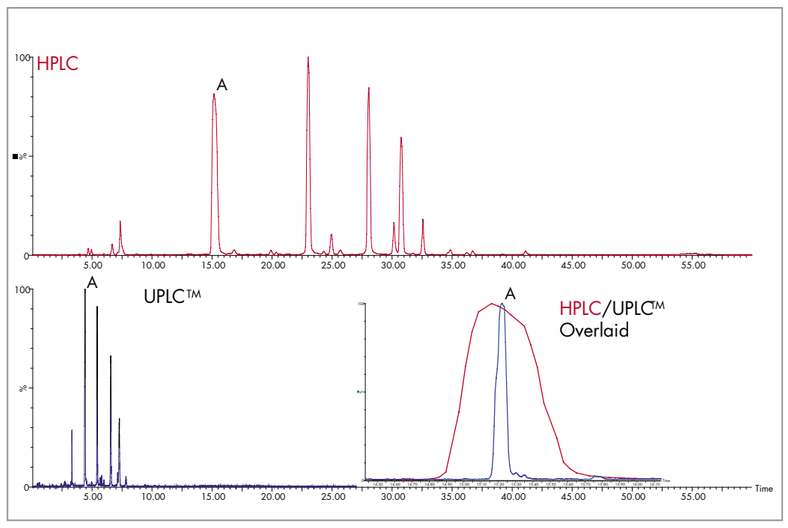

Figure 5. Negative ion electrospray m/z 431 extracted mass chromatograms for the analysis of Passiflora edulis *using UPLC /HPLC oa-TOF-MS and the overlaid UPLC/HPLC chromatographic peaks obtained for flavonoid A.*

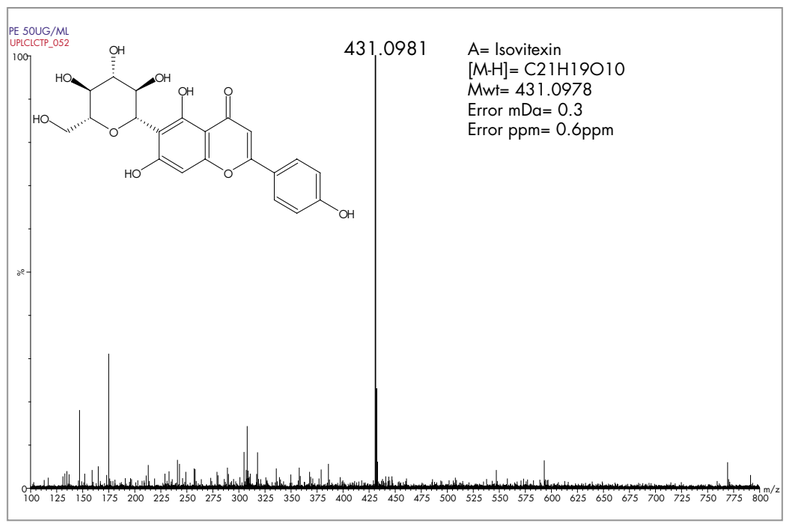

Figure 6. Exact mass spectrum of flavonoid A shown in Figure 5.