Fast Multiplexed Analysis of Cannabinoids and their Metabolites in Urine Using MassHunter StreamSelect LC-MS System

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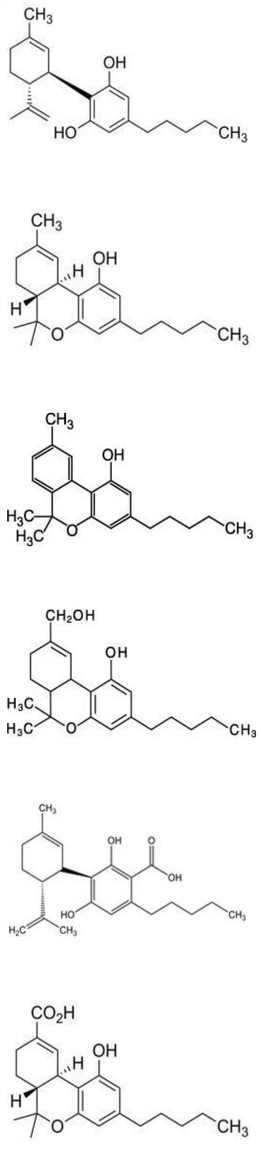
Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is the ideal solution for the simultaneous analysis of multiple cannabinoids and metabolites due to the high specificity and analytical sensitivity of the instrumentation. Many of these compounds have a similar molecular structure (Figure 1), and some are even isobaric. Chromatographic separation of analytes from isobaric compounds and other biological interferences allows for the mass spectrometer to easily and accurately quantitate panels of analytes. However, this chromatographic separation is often the bottleneck in the sample throughput of a LC-MS system. Agilent's MassHunter StreamSelect LC-MS software can increase sample throughput up to four times by running simultaneous, staggered chromatographic separations and only collecting mass spectrometry data for the portion of the run that contains the analytes of interest (Figure 2).



Cannabidiol (CBD)
MW 314.22
$C_{21}H_{30}O_2$

Δ^9-Tetrahydrocannabinol (THC)
MW 314.22
$C_{21}H_{30}O_2$

Cannabinol (CBN)
MW 310.19
$C_{21}H_{26}O_2$

11-hydroxy-Δ9-Tetrahydrocannabinol (THC-OH)
MW 330.23
$C_{21}H_{30}O_3$

Cannabidiolic Acid (CBDA)
MW 358.21
$C_{22}H_{30}O_4$

11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol (THC-COOH)
MW 344.20
$C_{21}H_{28}O_4$

Figure 1. Cannabinoid Structures

A highly selective analytical method has been developed for the analysis of six cannabinoids using liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS). A 6-minute chromatographic method was developed to separate the analytes – cannabidiol (CBD), cannabidiolic acid (CBDA), cannabinol (CBN), tetrahydrocannabinol (THC), nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH) – using an Agilent 1290 Infinity II Liquid Chromatograph. Quantitative data was acquired using an Agilent's 6470 triple quadrupole mass spectrometer. Sample throughput was nearly quadrupled by running four simultaneous, staggered chromatographic analyses on a single mass spectrometer using Agilent's MassHunter StreamSelect LC-MS software.

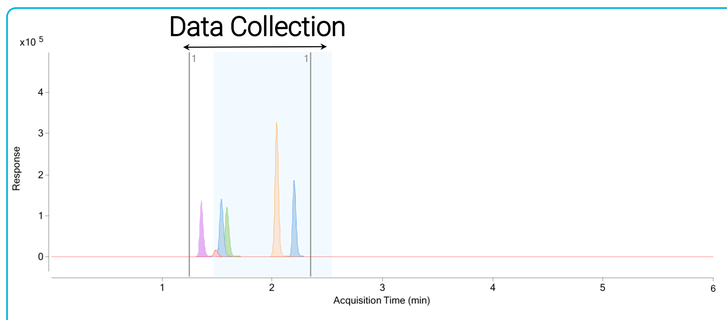


Figure 2. Data Collection Interval of 6 min Method

Experimental

Reagents and Standards

Labeled and unlabeled standards were purchased from Cerilliant, Round Rock, TX. Mass Spect Gold Urine (MSG5000) was purchased from Golden West Biologicals, Temecula, CA. LC-MS grade methanol and formic acid were purchased from Sigma-Aldrich, St. Louis, MO. Ammonium Formate 5M solution was purchased from Agilent Technologies

Sample preparation

Calibrators ranging from 5 ng/mL to 5000 ng/mL were prepared by spiking clean urine with 6 cannabinoid standards and performing serial dilutions. A large batch (n = 1000) of identical samples was made by spiking clean urine with 6 cannabinoid standards at 100 ng/mL. An additional set of samples at 200 ng/mL were prepared to test the quantitative performance of the system. Calibrators and samples were prepared with a simple dilution; 100uL of each sample was diluted with 900 uL of 70% methanol:water containing labeled internal standards at 100 ng/mL. Deuterated internal standards were used for all analytes, except for CBDA (not available at the time of publication).

Experimental

Method Parameters

Parameter	Value
Analytical Columns	Agilent Poroshell 120 EC-C18, 2.1x50mm, 2.7µm (P/N 699775-902T)
Column Temp	50°C
Injection Volume	5 µl
Autosampler Temp	4°C
Needle Wash	50% Methanol
Mobile Phase A	MilliQ Water with 5 mM Ammonium Formate and 0.01% Formic Acid
Mobile Phase B	Methanol with 0.01% Formic Acid
Flow Rate	0.4 mL/min
Pump Gradient	Time (min.) %B 0 70.0 2.0 82.5 4.0 98.0 4.1 70.0 6.0 70.0

Table 1. UHPLC Parameters

Parameter	Value
Ion mode	AJS ESI+
Gas temperature	300 °C
Drying gas (nitrogen)	10 L/min
Nebulizer gas (nitrogen)	45 psi
Sheath gas (nitrogen)	375 °C
Sheath flow	12 L/min
Capillary voltage	3500V
Nozzle voltage	500V
Q1/Q3 Resolution	0.7 unit
Dwell time	18 ms
Delta EMV	200V

Table 2. MS Parameters

Name	ISTD	Precursor Ion	Product Ion	Fragmentor	CE	CAV
Cannabidiol		315.2	193.1	110	24	4
Cannabidiol		315.2	123.1	110	36	4
Cannabidiol-d3	x	318.2	196.1	105	24	4
Cannabinol		311.2	223.1	110	24	4
Cannabinol		311.2	178.0	110	76	4
Cannabinol-d3	x	314.2	223.1	125	20	4
CBDA		359.2	219.1	75	36	4
CBDA		359.2	341.2	75	12	4
THC		315.2	193.1	110	24	4
THC		315.2	123.1	110	36	4
THC-d3	x	318.2	196.1	120	24	4
THC-COOH		345.2	299.1	110	20	4
THC-COOH		345.2	193.1	110	32	4
THC-COOH-d9	x	354.3	307.9	120	20	4
THC-OH		331.2	313.2	100	12	4
THC-OH		331.2	193.1	100	28	4
THC-OH -d3	x	334.2	196.1	90	28	4

Table 3. MRM Parameters

Instrumentation

The Agilent StreamSelect LC/MS system (Figure 3) is completely integrated, and consists of a triple quadrupole mass spectrometer coupled to four UHPLC streams, all controlled by a single software application. For this application, the Agilent 6470 triple quadrupole LC/MS equipped with Agilent Jet Stream (AJS) technology was coupled with four Agilent 1290 Infinity II high speed pumps, four Agilent 1290 Infinity II multicolumn thermostats, and a StreamSelect RSI (PAL3) autosampler.

Figure 3. The Agilent StreamSelect LC/MS System

Data acquisition

MassHunter StreamSelect LC-MS software was configured for shared-stream, independent calibration analysis. In this mode, each stream is calibrated independently, and samples are split between all four streams.

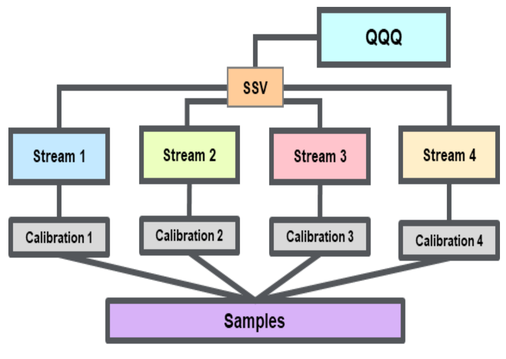


Figure 4. Diagram of Samples Distribution

Data Analysis

MassHunter Quantitative Analysis (10.0) was used for data analysis. A 1/x weighting factor was applied during linear regression of the calibration curves. The quantitation using MassHunter Quantitative software was performed by chromatographic peak area ratio to a known concentration of the internal standards. Each analyte was quantitated with its own deuterated internal standard, except for CBDA, which was quantitated with CBD-d3. Samples and calibrators were grouped and quantitated based on the stream on which they were acquired. Combined calibration curves using all calibrators from all streams were also used to quantitate as a comparison.

Results and Discussion

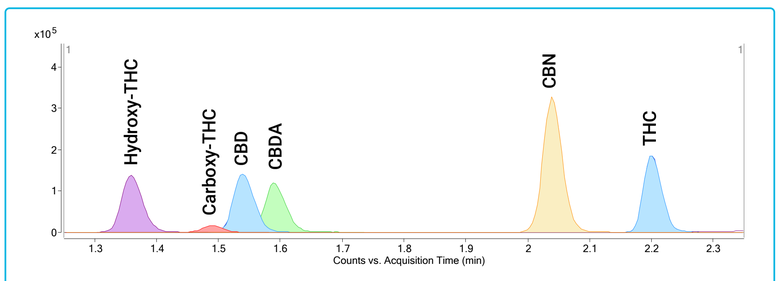


Figure 5. Cannabinoids Chromatogram

1000 Samples study

StreamSelect acquired data for 894 samples over a period of 24-hours, which equates to 97 seconds per analysis. Compared to a 6-minute runtime for the same analysis using traditional LC-MS, this results in a 3.7x increase in sample throughput.

The chromatography (Figure 5) remained robust and reproducible with this increased sample throughput. Over the course of nearly 27 hours, 1000 identical urine samples containing 200 ng/mL of each of the six analytes and their respective internal standards were run across the four LC streams. Retention time %RSDs were excellent, ranging from 0.43% to 1.54% (Table 4).

Analyte	RT %RSD	Analyte	RT %RSD
THC	0.53	CBD	1.43
THC d3	0.50	CBD-d3	1.40
THC-COOH	1.26	CBDA	0.85
THC-COOH-d9	1.31	CBN	0.46
THC-OH	1.53	CBN-d3	0.43
THC-OH-d3	1.54		

Table 4. 1000 Samples Retention Time %RSD's

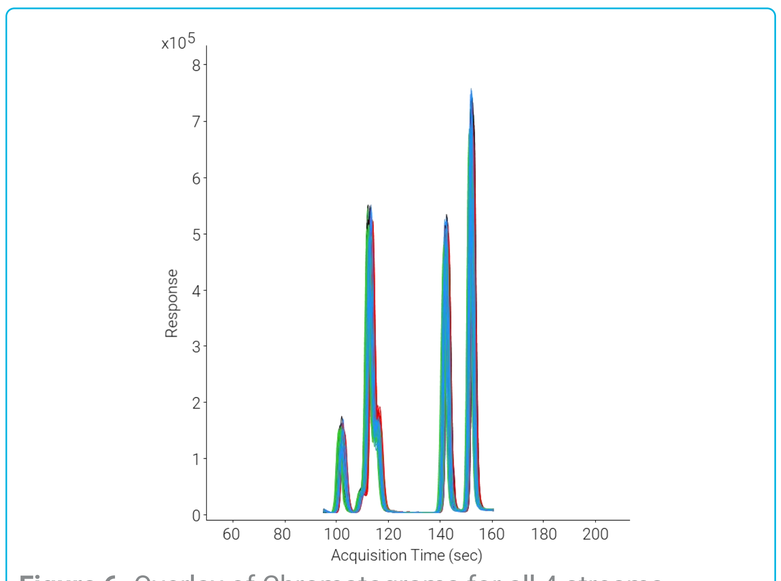


Figure 6. Overlay of Chromatograms for all 4 streams

Reliable Quantitative Results

Whether quantitation was performed by combining all four streams (Figure 7) or treating each stream independently (Figure 8), quantitation was accurate and precise. Results ranged from 95.3 to 109.1% for a 200 ng/mL sample, depending on analyte.

Calibration curves for all analytes were linear from 5 to 5,000 ng/mL with R^2 values >0.996 across all four streams. Four curves for THC (Figure 8) are representative of what was observed for each of the analytes. Furthermore, combined calibration curves composed of calibrators from all four streams had R2 values between 0.993 to 0.997, showing that all four streams are quantitatively equivalent. Quantitative results for the 200 ng/mL sample were highly reproducible across all four streams. The average results for all six analytes ranged from 190.6 to 218.2 ng/mL with %RSDs ranging from 0.47 to 2.79 %.

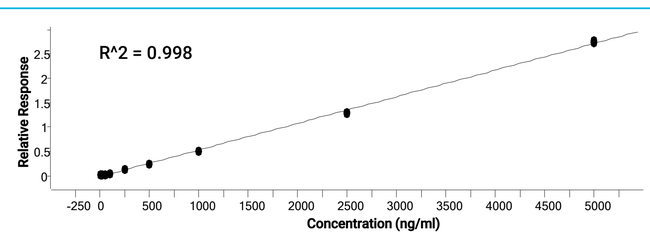


Figure 7. Representative Combined THC Calibration Curves

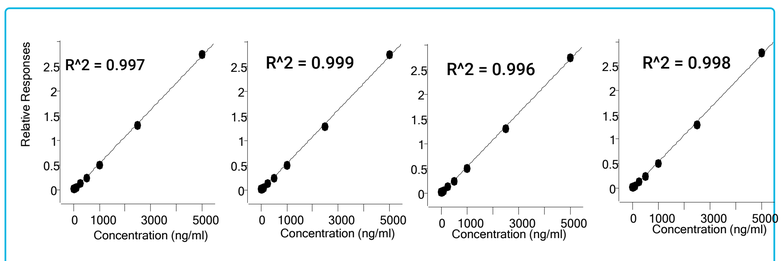


Figure 8. Representative Individual THC Calibration Curves

Conclusions

Agilent's StreamSelect LC-MS system has been shown to increase sample throughput up to four times when compared to convectional LC-MS. This is achieved without compromising data integrity, producing consistent and reliable result for routine lab operations. Calibration curves for each analyte displayed excellent linearity and retention time reproducibility between all four chromatographic streams was excellent. Quantitation across all streams for all analytes was accurate with average results ranging from 95.3 to 109.1% for a 200 ng/mL sample.