

Analysis of Cannabinoids and Their Metabolites in Urine Using the MassHunter StreamSelect LC/MS System

Authors

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Abstract

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

All calibration curves displayed excellent linearity with an $R^2 > 0.996$. Retention time reproducibility between all four chromatographic streams was between 0.43 and 1.54 % ($n = 1,000$), depending on the analyte. 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.

Introduction

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

Experimental

Reagents and standards

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

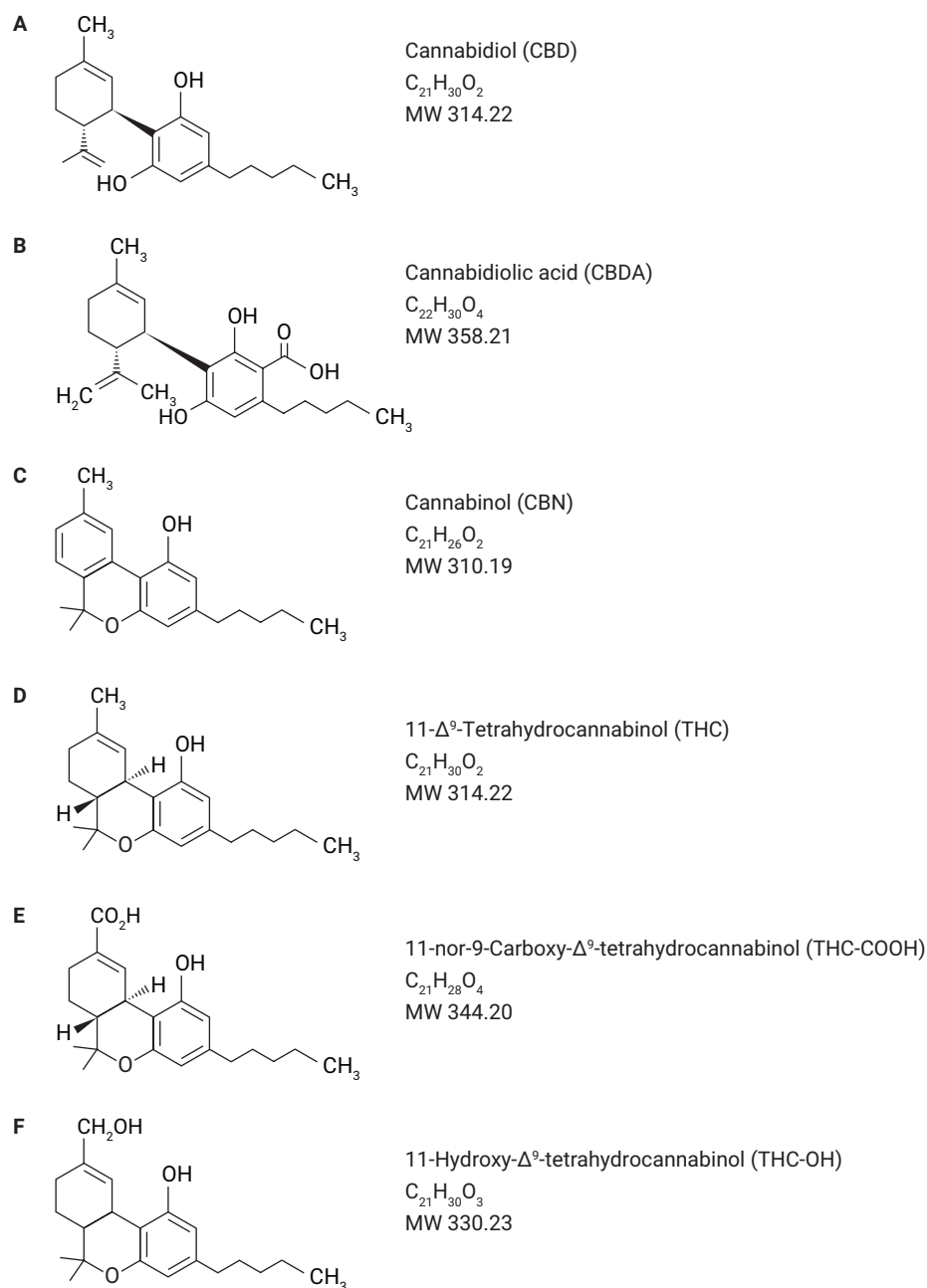


Figure 1. Structures of (A) cannabidiol, (B) cannabidiolic acid, (C) cannabinol, (D) tetrahydrocannabinol, (E) nor-9-carboxy- Δ^9 -tetrahydrocannabinol, and (F) 11-hydroxy- Δ^9 -tetrahydrocannabinol.

Instrumentation

The Agilent StreamSelect LC/MS system 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.

Sample preparation

Calibrators ranging from 5 to 5,000 ng/mL were prepared by spiking clean urine with six cannabinoid standards and performing serial dilutions. A large batch of identical samples (n = 1,000) was made by spiking clean urine with six 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; 100 µL of each sample were diluted with 900 µL 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).

LC Method

Table 1. LC Parameters.

Parameter	Value
Analytical Columns	Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (p/n 699775-902T)
Column Temperature	50 °C
Injection Volume	5 µL
Autosampler Temperature	4 °C
Needle Wash	50 % Methanol
Mobile Phase A	Milli-Q 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
	2.0 70.0
	4.0 82.5
	4.1 98.0
	6.0 70.0

MS Method

Table 2. MS 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	3,500 V
Nozzle Voltage	500 V
Q1/Q3 Resolution	0.7 unit
Dwell Time	18 ms
Delta EMV	200 V

Table 3. MRM Transitions.

Name	ISTD	Precursor Ion	Product Ion	Fragmentor	CE	CAV
Cannabidiol		315.2	193.1	110	24	4
Cannabidiol		315.2	123	110	36	4
Cannabidiol-d ₃	X	318.2	196.1	105	24	4
Cannabinol		311.2	223	110	24	4
Cannabinol		311.2	178	110	76	4
Cannabinol-d ₃	X	314.2	223	125	20	4
CBDA		359.2	219	75	36	4
CBDA		359.2	341.2	75	12	4
THC		315.2	193	110	24	4
THC		315.2	123	110	36	4
THC-d ₃	X	318.2	196.1	120	24	4
THC-COOH		345.2	299.1	110	20	4
THC-COOH		345.2	193	110	32	4
THC-COOH-d ₃	X	354.3	307.9	120	20	4
THC-OH		331.2	313.2	100	12	4
THC-OH		331.2	193	100	28	4
THC-OH-d ₃	X	334.2	196.1	90	28	4

Data acquisition

MassHunter StreamSelect LC/MS software was configured for shared-stream, independent calibration analysis (Figure 2). In this mode, each stream is calibrated independently, and samples are split between all four streams.

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. A combined calibration curve using all calibrators from all streams was also used to quantitate as a comparison.

Results and discussion

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

The chromatography (Figure 3) remained robust and reproducible with this increased sample throughput. Over the course of nearly 27 hours, 1,000 identical urine samples containing 100 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 and Figure 4).

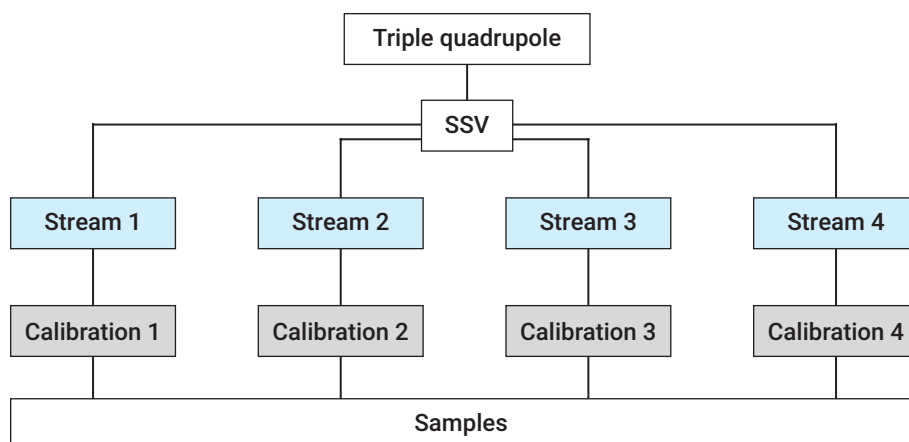


Figure 2. Sample distribution for StreamSelect configured for shared-stream, independent calibration analysis.

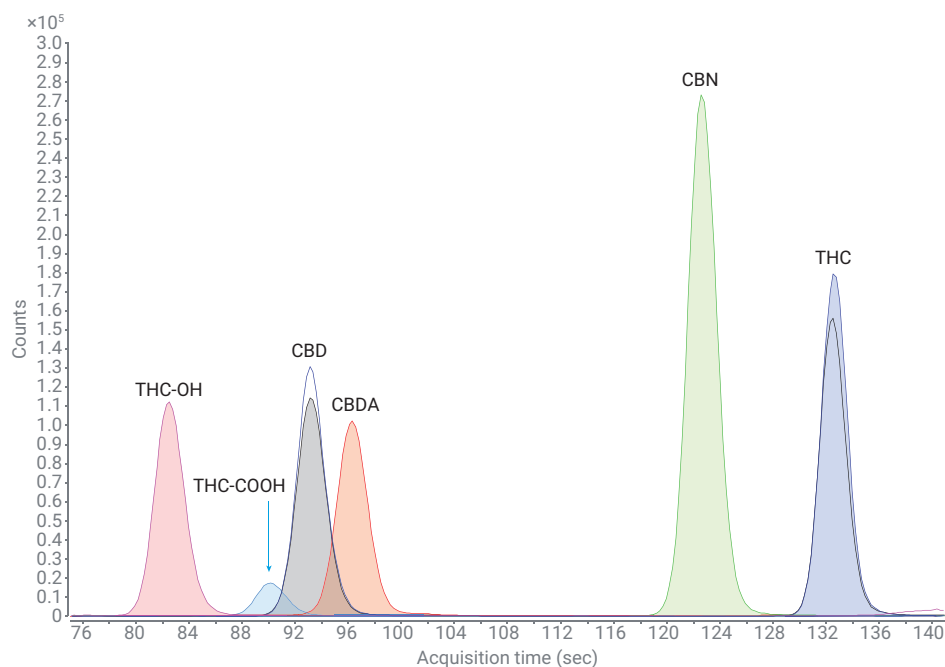


Figure 3. Chromatographic separation of six cannabinoid metabolites.

Table 4. Retention time reproducibility (n = 1,000).

Analyte	RT %RSD	Analyte	RT %RSD	Analyte	RT %RSD
CBD	1.43	CBN-d ₃	0.43	THC-COOH-d ₃	1.31
CBD-d ₃	1.40	THC	0.53	THC-OH	1.53
CBDA	0.85	THC d ₃	0.50	THC-OH-d ₃	1.54
CBN	0.46	THC-COOH	1.26		

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 5) are representative of what was observed for each of the analytes.

Furthermore, combined calibration curves composed of calibrators from all four streams (Figure 6) had R^2 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 %.

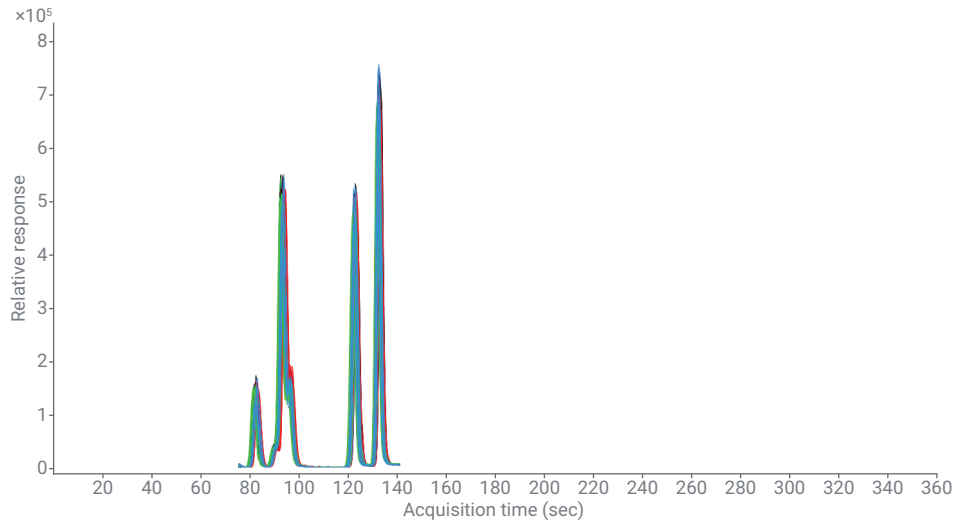


Figure 4. Overlaid chromatograms from streams one (black), two (red), three (green), and four (blue).

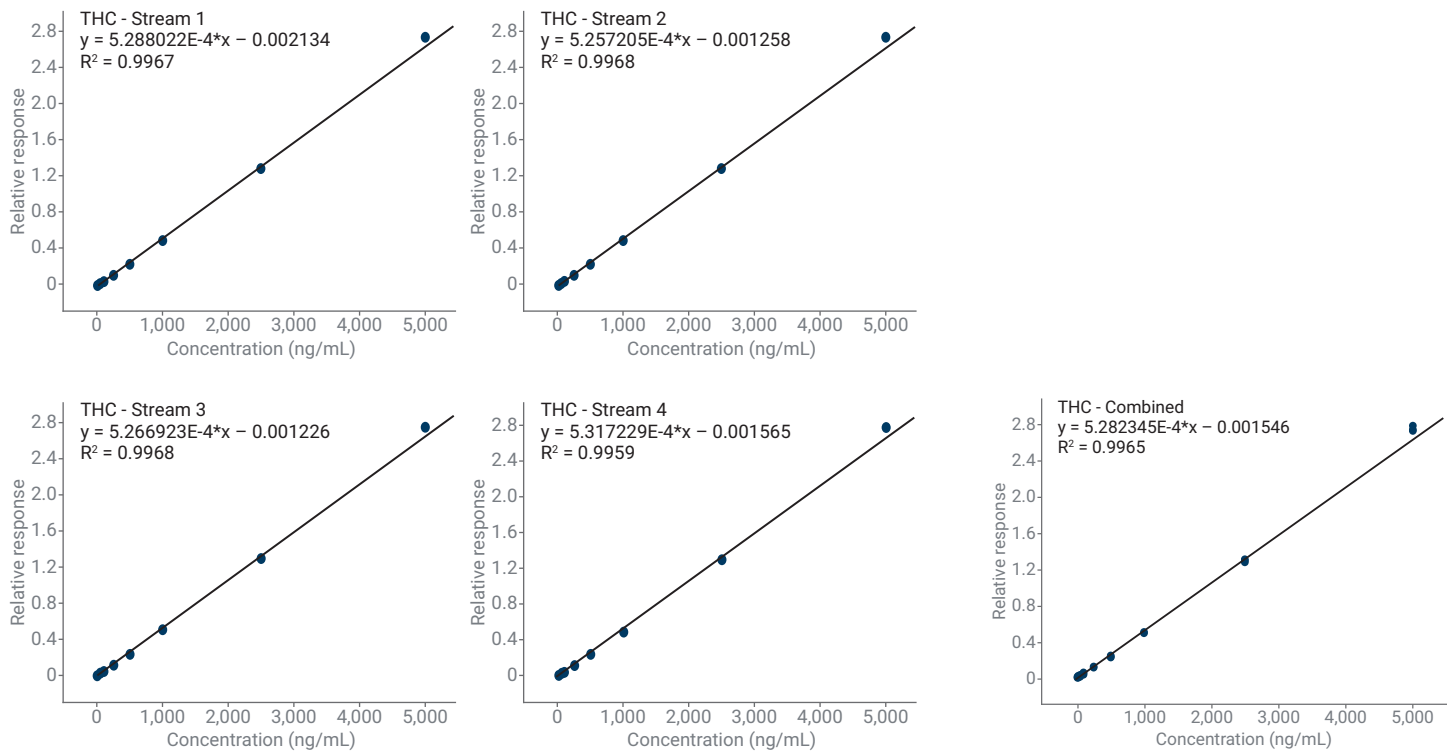


Figure 5. Calibration curves for THC across each of the four streams.

Figure 6. Combined calibration curve for THC, composed of calibrators from all four streams.

Conclusion

A robust and accurate LC/MS/MS method has been developed for the analysis of six cannabinoids. By running this method on a four-stream StreamSelect instrument, data acquisition has been reduced to 1.5 minutes per sample. Quantitative results and retention times were highly reproducible across all four streams, meaning reliable results will be achieved regardless of which stream is used to analyze a sample. Furthermore, calibration curves from each stream showed excellent agreement, and resulted in equivalent quantitative results when treated individually or combined into a single calibration curve.

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