Increased Sensitivity of THC and Metabolites using β-Gone™ and Kinetex™ 2.6 µm C18 Column by LC-MS/MS

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Overview

THC, or tetrahydrocannabinol, is the chemical responsible for the psychological effects of cannabis. THC and its metabolites are tested in urine samples but need to undergo hydrolysis in the sample pre-treatment prior to extraction. The Dilute-and-Shoot method simply dilutes the sample after pre-treatment hydrolysis and the sample is then injected onto an LC-MS system. This method does not remove any matrix interferences that might exist which could clog an LC column due to exposure to a higher percentage of organic material during the gradient of the mobile phases in a chromatographic run. β-Gone targets and removes β-glucuronidase from hydrolyzed urine samples without requiring additional method development.

In this application note, we show improved sensitivity by an increase in intensity and signal to noise (S/N) ratio of THC and its metabolites using the β-Gone centrifuge tubes compared to the Dilute-and-Shoot method. The high efficiency Kinetex 2.6 µm C18 LC column was used for chromatographic separation.

Sample Preparation – Dilute-and-Shoot

Sample Pre-treatment:	Combine 200 µL of urine sample spiked with 20 µL analyte mixture, 60 µL hydrolysis buffer, and 20 µL of IMCSzyme® RT enzyme. Incubate at room temperature for 15 minutes.
Dilute:	20 µL from above 10 times with 0.1 % Formic acid in water.
Inject:	10 µL

Sample Preparation – β-Gone

Sample Pre-treatment:	Combine 200 µL of urine sample spiked with 20 µL analyte mixture, 60 µL hydrolysis buffer, and 20 µL of IMCSzyme RT enzyme. Incubate at room temperature for 15 minutes.
Combine:	200 µL from above with 133 µL 0.1 % Formic acid in methanol.
Load:	Sample in β-Gone 2 mL centrifuge tube (Part No.: 8N-S323-TUK). Mix by inverting tube 10 times.
Centrifuge:	For 10 min at 14800 rpm.
Inject:	10 µL supernatant.

LC Conditions

Column: Kinetex 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid in water
B: 0.1 % Formic acid in methanol

Gradient:

Time (min)	%B
0.25	68
5.25	70
7.75	80
7.95	100
8.95	100
9.15	68
11.0	68

Flow Rate: 3 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
LC System: Agilent® 1260 Infinity
Detection: MS/MS
Detector: SCIEX® API 4000 QTRAP®

MS Conditions

Ion Source: Positive or Negative
Source Temperature: 600 °C
GS1: 50
GS2: 50
CUR: 10
IS: +5500 or -4500

Table 1. MRM Transitions

Peak No.	Analyte	Q1 (m/z)	Q2 (m/z)
1	D9 THC-OH 1	331	193.1
1	D9 THC-OH 2	331	201.1
2	D9 THC-COOH 1	343	245
2	D9 THC-COOH 2	343	191
2	D9 THC-COOH 3	343	299.4
3	D9 THC	315.2	193.1
3	D9 THC ql 1	315.2	245
3	D9 THC ql 3	315.2	259.1
4	D8 THC	315.2	192.95
4	D8 THC ql 1	315.2	135.1

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Figure 1. Separation of THC and Metabolites using Dilute-and-Shoot Sample Preparation and the Kinetex™ 2.6 µm C18 Column

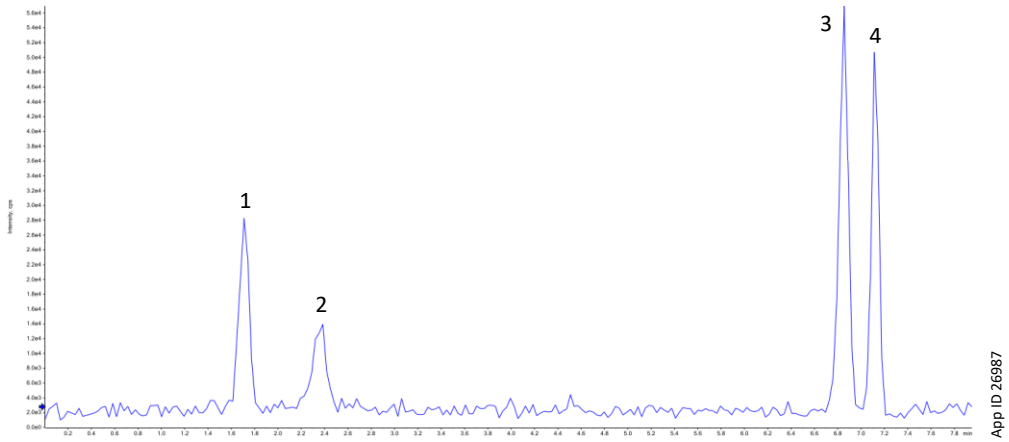

Figure 2. Separation of THC and Metabolites using β-Gone™ Sample Preparation and the Kinetex 2.6 µm C18 Column

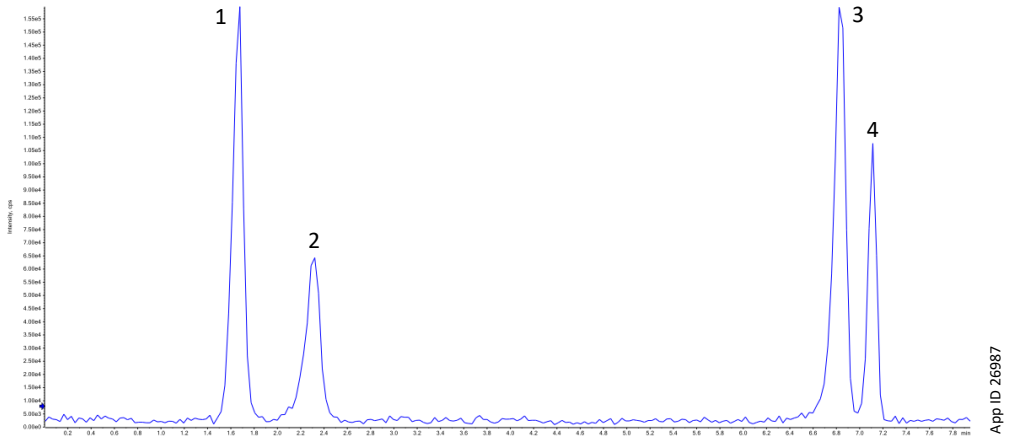

Table 2. Intensities and Signal to Noise Ratios of THC and Metabolites

		Dilute-N-Shoot	β-Gone	β-Gone/Dilute-and-Shoot
D9 THC-OH	Intensity	82525.8	528999.7	6.4
	% CV (n=3)	-	4.8	-
	S/N	218.7	484.3	2.2
D9 THC-COOH	Intensity	33604.2	196110.5	5.8
	% CV (n=3)	-	4.5	-
	S/N	33.9	135.5	4.0
D9 THC	Intensity	32885.1	140385.0	4.3
	% CV (n=3)	-	3.1	-
	S/N	20.0	76.9	3.8
D8 THC	Intensity	3587.9	7132.4	2.0
	% CV (n=3)	-	6.5	-
	S/N	3.5	12.0	3.4

Need a different column size or sample preparation format?

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