HPLC Application

ID No.: **24536**

Q1 Scan showing sample cleanliness of Oral fluid SPE from ntercept buffer using Starta-X-C cartridge

Column: Kinetex® 2.6 µm C18 100 Å, LC Column 50 x 3 mm, Ea

Dimensions: 50 x 3 mm ID

Order No: 00B-4462-Y0

Elution Type: Gradient

Eluent A: 0.1% formic aid in water

Eluent B: 0.1% formic acid in MeOH

Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	90	10
2	4	5	95
3	5.5	5	95
4	5.51	90	10
5	7	90	10

Flow Rate: 500 µL/min

Col. Temp.: ambient

Detection: Tandem Mass Spec (MS-MS) @ (ambient)

Detector Info: <a target="_blank" href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<,

Products used in this application:

KINETEX®

Analyst Note:

SPE Product: Strata-X-CW, 30 mg/3 mL cartridge

Part# 8E-S035-TBJ

Sample pre-treatment

Transfer 1 mL oral fluid collected on an applicator tip in its preservative buffer (Intercept). Leave it for 2 hours followed by centrifugation for 15 mins at 600g. Remove 0.5 mL of supernatant to combine with 1 mL 1% formic acid, mix/vortex 5-10 secs to load on Strata X-CW 30mg/3 mL

Condition: 1 mL Methanol

Equilibrate: 1 mL DI Water

Load: Combine 0.5mL of pretreated sample with 1 mL 1% formic acid, mix/vortex 5-10 sec and load

Wash 1: 1 mL 1% Formic acid/DI Water

Wash 2: 1 mL DI Water

Dry Down: 5-6 minutes at maximum vacuum (20" Hg or higher)

Elute: 2 x 500 µL methylene chloride/Isopropanol/30% Ammonium Hydroxide (80:18:2)

Dry Down: Evaporate to dryness under gentle nitrogen and 45-50°C.

Reconstitute: With 167 uL initial mobile phase

SPE Product: CEREX OFX 3mg

Part# 633-003I NBE, 96 well NBE plate 3mg/1Ea

Sample pre-treatment

Transfer 1 mL oral fluid collected on an applicator tip in its preservative buffer (Intercept). Leave it for 2 hours followed by centrifugation for 15 mins at 600g. Remove 0.5 mL of supernatant to combine with 0.3 mL 0.1M HCl, mix/vortex 5-10 secs to load on SPEware cartridge

Condition: 500 uL methanol

Equilibrate: 500 uL water

Load: Sample from pre-treatment step

Wash1: 700 uL 0.1M HCl

Wash2: 2x0.8 mL D.I. water

Dry: 10 mins under 45050 psi

Elution: 300 uL of Dichloromethane:Isopropanol: 30%ammonium hydroxide (80/18/2)

Drydown: Evaporate to dryness under gentle nitrogen and 45-50°C.

Reconstitute:167 µL initial mobile phase

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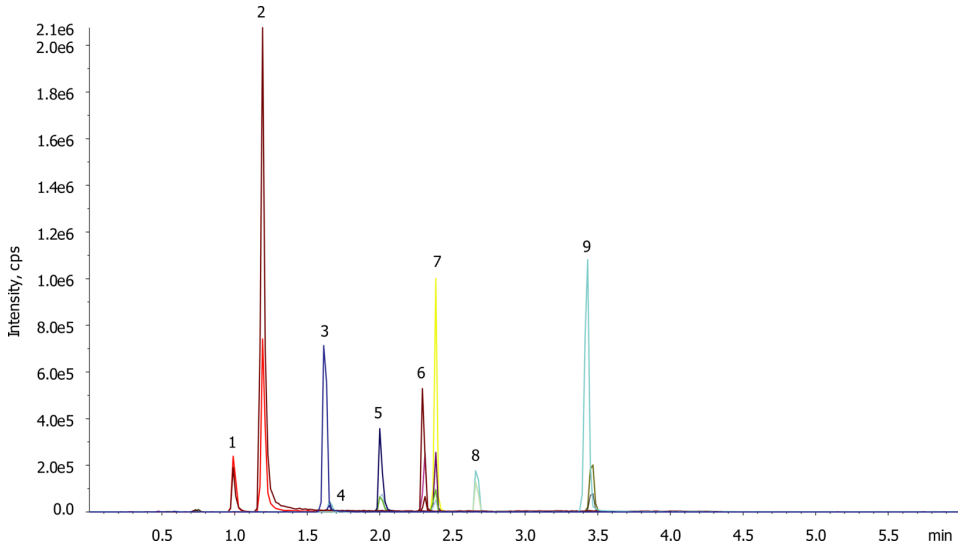

ANALYTES:

1 Intercept-i2 Oral Fluid devise