Improved Recovery, Reproducibility and Matrix Effects with an Advanced Technology in SPE – Oasis PRiME HLB

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INTRODUCTION

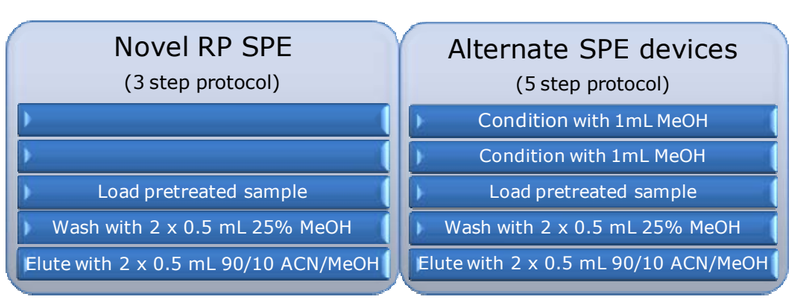
A novel reversed-phase solid phase extraction (SPE) sorbent (Oasis PRiME HLB) has been developed to enable simpler and faster SPE protocols, while at the same time generating cleaner extracts than other sample preparation methods. In this application, a 3 step load-wash-elute SPE protocol, eliminating conditioning and equilibration, was successfully employed to extract 22 synthetic cannabinoids and metabolites from whole blood samples using this novel reversed phase SPE sorbent. Superior analyte recoveries, with low %RSDs and modest matrix effects (ME) were achieved across the entire panel of compounds. At the same time, parallel extractions were conducted with other reversed phase (RP) SPE devices using recommended 5 step SPE protocols. Lower recoveries, higher % RSDs and higher matrix effects were obtained. In addition, this new SPE sorbent removes 90% more phospholipids when compared to the other RP SPE devices, resulting in the near elimination of matrix effects for particular analytes.

METHODS

Individual stocks (1 mg/mL) were prepared in methanol, DMSO, or 50:50 DMSO:methanol. A combined stock solution of all compounds (10 µg/mL) was prepared in methanol. Working solutions were prepared daily in 40% methanol. Pre-spiked samples for recovery determination were prepared by spiking working solutions directly into whole blood.
Common phospholipids were monitored during the analysis.

SPE Procedure

Samples were extracted using this novel reversed phase SPE 10 mg plates. 0.1 mL of a solution of 0.1 M zinc sulfate/ammonium acetate was added to 0.1 mL whole blood and vortexed for 5 seconds to lyse the cells. All samples were then precipitated by adding 400 µL ACN. The entire sample was vortexed for 10 seconds and centrifuged for 5 min at 7000 rcf. The supernatant was then diluted with 1.2 mL water prior to loading to all SPE devices
As the alternate devices do not work with 3 step simplified protocol (details see Waters application note PN# 720005140EN), a 3-step protocol was used with this novel SPE and 5-step protocols were applied to the all the alternate SPE devices to get an unbiased performance comparison.
All the elutions were evaporated and reconstituted in 100 µL 30% acetonitrile. 5 µL was injected onto the UPLC-MS/MS system. Other alternate SPE devices also consisted of 96-well plates with 10 mg of sorbent per well.



Chromatographic and MS Conditions

UPLC System:	ACQUITY UPLC® I-Class
Column:	ACQUITY UPLC® CORTECS UPLC C18, 1.6 µm 2.1 x 100 mm
Mobile Phase A:	0.1% formic acid in water
Mobile Phase B:	0.1% formic acid in acetonitrile
Column Temp:	30 °C
Sample Temp:	10 °C
Gradient:	See table 1

Table 1—UPLC Gradient

Time	Flow	%A	%B
0	0.6	70	30
2.0	0.6	50	50
3.0	0.6	50	50
7.0	0.6	10	90
7.2	1.6	70	30
8.5	0.6	70	30

Waters Xevo® TQ-S Conditions, ESI+

Capillary Voltage:	1 kV
Desolvation Temp:	550 °C
Desolvation Gas Flow:	900 L/Hr
Source Temp:	150 °C
MRM transitions, ESI+:	See Table 2

Table 2 MS/MS conditions

No.	Compound	Cone Voltage (V)	1˚MRM Transitions	Collision Energy (eV)
1	AM2233	40	459.2→98.05	34
2	RCS-4, M10	40	324.2→121.0	22
3	RCS-4, M11	36	322.2→121.0	22
4	AM 1248	56	391.4→135.1	28
5	JWH-073 4-COOH	50	358.2→155.1	26
6	JWH-073 4-OH met.	50	344.2→155.1	22
7	JWH-018 5-COOH	46	372.2→155.1	24
8	JWH-073 3-OH met.	44	344.2→155.1	26
9	JWH-018 5-OH met.	40	358.2→155.1	24
10	JWH-018 4-OH met.	40	358.2→155.1	24
11	JWH-015	42	328.2→155.1	24
12	RCS-4	44	322.2→135.1	26
14	JWH-022	50	340.2→155.1	26
13	JWH-073	48	328.2→155.1	26
15	XLR-11	48	330.3→125.1	26
16	JWH-203	46	340.2→125.0	26
17	JWH-018	44	342.2→155.1	26
18	RCS-8	42	376.3→121.1	26
19	UR-144	46	312.3→125.1	24
20	JWH-210	48	370.2→183.1	26
21	AB 001	52	350.3→135.1	30
22	AKB 48	38	366.3→135.1	22

RESULTS AND DISCUSSION

Chromatogram (Figure 1)

Using a Waters CORTECS UPLC C_{18} column (2.1 x 100 mm; 1.6 µm), all analytes eluted within 7.5 minutes with a total cycle time of 8.5 minutes. Peak shape was excellent for all compounds, with no significant tailing or asymmetries, and all peak widths were under 3 seconds at 5% of baseline.

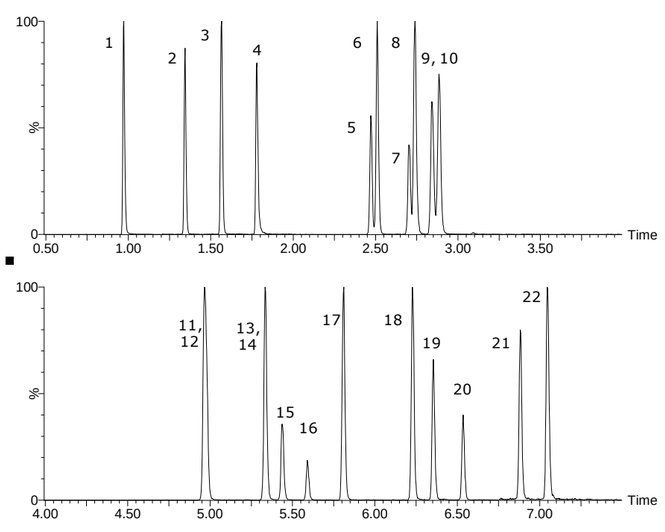


Figure 1. *UPLC-MS/MS chromatogram for 22 synthetic cannabinoids and metabolites*

Recovery (Figure 2 and Table 3)

With this novel SPE device, 21/22 compounds had recoveries between 90-110% (AM2233 at 71% recovery), with all %RSDs ranged from 3-7%. Much lower recoveries and overall higher variability were obtained with other RP-SPE devices.

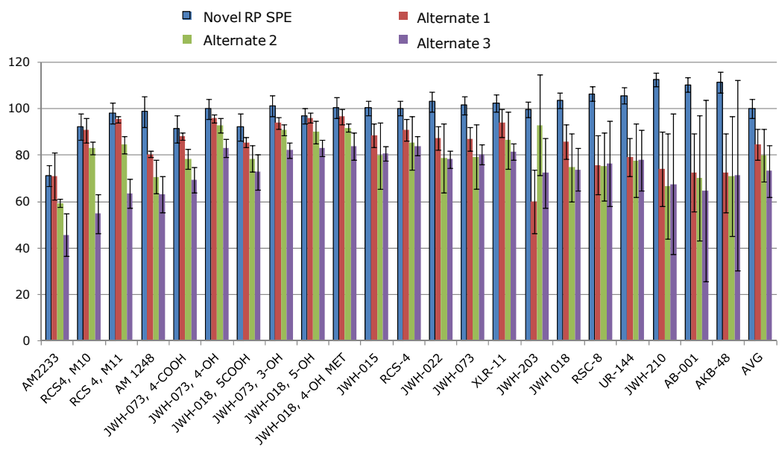


Figure 2. *Recovery and SD for the novel RP SPE device vs. all other SPE devices (n=5)*

Table 3 Recoveries and standard deviation ranges

N=5	% Rec Range	AVG % REC	% RSD Range	AVG % RSD
Novel RP SPE	**90-110%**	**100**	**3-7**	**4**
Alternate 1	60-97%	85	1-17	7
Alternate 2	59-92%	80	2-27	11
Alternate 3	46-84%	73	3-41	11

Matrix Effect (ME)

Matrix effects across the panel were excellent for this novel SPE device, with only three compounds exceeding 25%, and an average absolute matrix effect of only 11%. All other SPE devices exhibited overall high variability for the later eluting compounds.

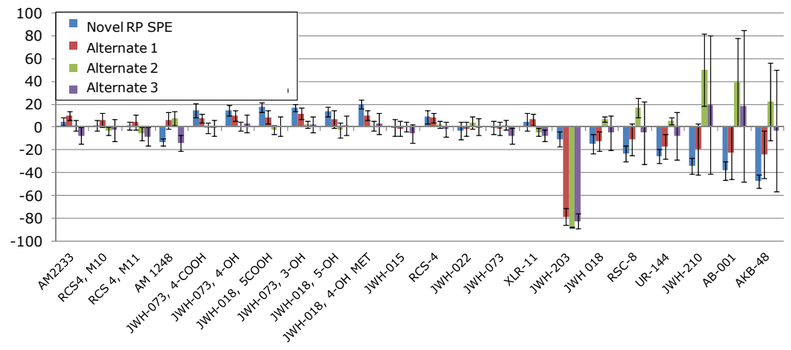


Figure 3. *Matrix effects (ME) and standard deviations obtained from the novel RP SPE and* alternate *SPE devices. Bars and error bars represent means and standard deviations (N=5), respectively*

Examination of phospholipid traces revealed that JWH-203 co-eluted with residual phospholipid lysophosphatidycholine 18:0 (m/z 524.4) causing the severe matrix effect with all other SPE products. This novel RP SPE device removed >90% phospholipids and thus eliminated matrix effect and variability. (***Figure 4 and Figure 5***)

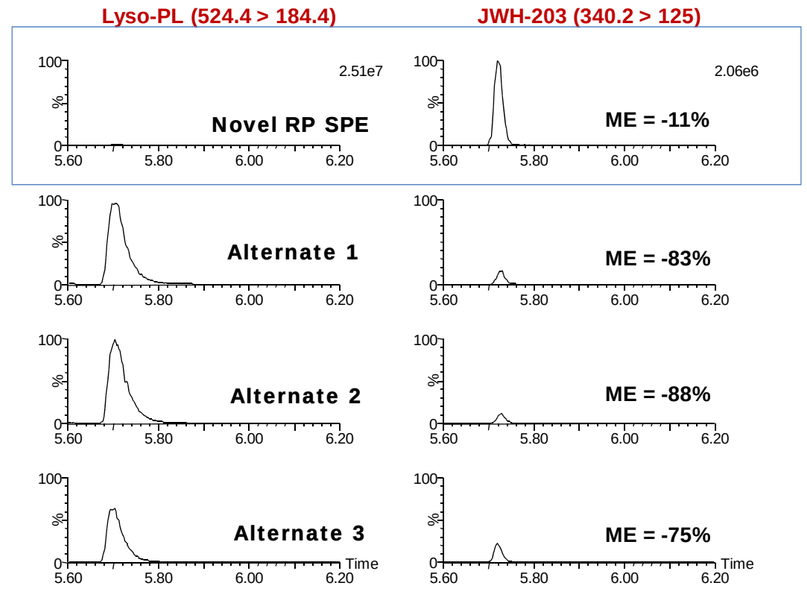


Figure 4. *Chromatograms of PL 524 (left) and whole blood samples post-spiked with JWH-203(right). The ME values on the right indicate the degree of ion suppression calculated for each SPE device.*

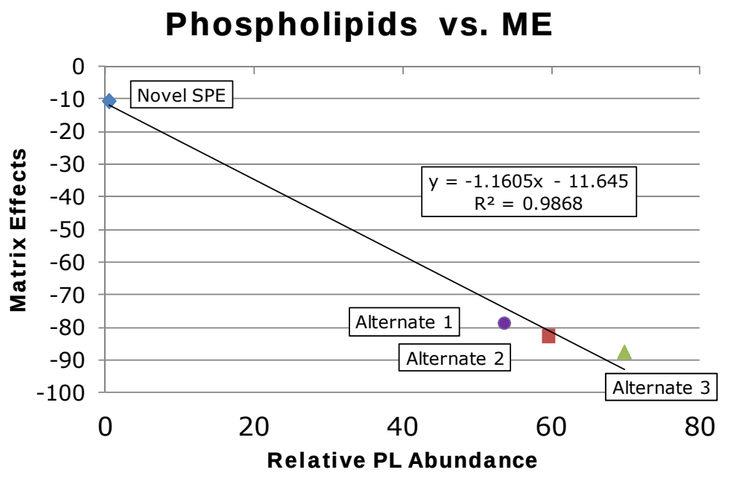


Figure 5. *Relationship between Phospholipid 524 abundance and ion suppression for JWH-203, showing the direct correlation between this coeluting phospholipid and the ion suppression for JWH-203*

CONCLUSIONS

- Simpler SPE protocol eliminates conditioning and equilibration steps with this new reversed phase SPE device.
- Removing interference with this novel RP SPE device results in a higher and much more consistent recoveries even with simplified protocol
- The ability to remove >90% phospholipids efficiently prevented the coelution with analytes of interest and thus significantly decreased matrix effect.
- Longer column lifetimes and less mass spectrometer source maintenance are expected with using of this novel RP SPE device

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