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Improvements in Recovery, Reproducibility, and Matrix Effects with Oasis PRiME HLB, a Novel Solid Phase Extraction Sorbent

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APPLICATION BENEFITS

- Simpler SPE protocols that eliminate conditioning and equilibration steps.
- Higher and much more consistent recoveries with Oasis® PRiME HLB vs. competitive RP SPE devices.
- Removal of more than 90% phospholipids with a simpler protocol when compared to all competitors' RP SPE, significantly reduced matrix effects.
- The only commercial available reversed phase SPE designed to remove phospholipids from biological samples.

WATERS SOLUTIONS

Oasis PRiME HLB 10 mg Plate

CORTECS® UPLC® C_{18}, 90Å, 1.6 µm; 2.1 x 100 mm Column (p/n 186007095)

ACQUITY UPLC I-Class System

Xevo® TQD Mass Spectrometer

2 mL 96-well collection plates (p/n 186002482)

KEY WORDS

Synthetic cannabinoids, UPLC, forensic toxicology, whole blood, solid phase extraction, CORTECS, solid core, Oasis PRiME HLB, phospholipid removal

INTRODUCTION

Oasis PRiME HLB is a novel reversed-phase solid-phase extraction (SPE) sorbent 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. Superior analyte recoveries (90–110%), minimum %RSDs (3–7%) 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 (as low as 46%), higher %RSDs (as high as 41%) and higher matrix effects were obtained. For the cannabinoid JWH-203, all other SPE devices showed severe matrix effects with approximately 80% ion suppression, while Oasis PRiME HLB yielded minimum matrix effects of 11%. This was demonstrated to be directly related to phospholipid removal by Oasis PRiME HLB. Oasis PRiME HLB represents a successful next generation reversed SPE product that produces cleaner extracts with simpler protocols and faster processing time.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC I-Class
Column:	CORTECS UPLC C_{18} Column, 90Å, 1.6 µm; 2.1 x 100 mm (p/n 186007095)
Column temp.:	30 °C
Injection vol.:	5 µL
Flow rate:	0.6 mL/min
Mobile phase A:	0.1% formic acid in MilliQ water
Mobile phase B:	0.1% formic acid in ACN
Gradient:	Initial conditions started at 30% B. The %B was increased to 50% over 2 minutes, and held at 50% B for 1 minute, increased to 90% B over 4 minutes and then returned to 30% over 0.2 minutes. The system was allowed to re-equilibrate for 1.3 min. The entire cycle time was 8.5 min. The solvent gradient is listed in Table 1.

MS conditions

MS system:	Xevo TQD Mass Spectrometer
Ionization mode:	ESI Positive
Acquisition mode:	MRM (See Table 2 for transitions)
Capillary voltage:	1 kV
Collision energy (eV):	Optimized for individual compounds (See Table 2)
Cone voltage (V):	Optimized for individual compounds (See Table 2)

Data management

All data were acquired and analyzed using Waters MassLynx® Software v.4.1 (scn 855) and quantified using TargetLynx™ Software. MS conditions were optimized using IntelliStart™.

Materials

AM2233, JWH-015, RCS-4, JWH-203, RCS-8, JWH-210, JWH-073, and JWH-018 were purchased from Cerilliant (Round Rock, TX). All other compounds and metabolites were purchased from Cayman Chemical (Ann Arbor, MI).

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 whole separation.

Sample preparation for whole blood cannabinoid analysis:

Samples were extracted using Oasis PRiME HLB 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. The sample was directly loaded on the Oasis PRiME HLB 10 mg Plate without conditioning or equilibration. All wells were then washed with 2 x 500 µL 25:75 MeOH:water, and eluted with 2 x 500 µL 90/10 ACN/MeOH. The eluate was then evaporated under Nitrogen and reconstituted with 100 µL 30% ACN. 5 µL was injected onto the UPLC System.

Competitive SPE device format were also 96-well plates with 10 mg of sorbent per well. Sample pretreatment was identical to that used for Oasis PRiME HLB. SPE extraction on competitive devices included conditioning and equilibration with 1 mL methanol and 1 mL water, respectively, followed by the same loading, wash and elute steps used with Oasis PRiME HLB Plates. See Figure 1. For sample preparation details.

Analyte recovery was calculated according to the following equation:

$$\%\text{Recovery} = \left(\frac{\text{Area A}}{\text{Area B}}\right) \times 100\%$$

Where A equals the peak area of an extracted sample and B equals the peak area of an extracted matrix sample in which the compounds were added post-extraction.

Matrix effects were calculated according to the following equation:

$$\text{Matrix Effects} = \left(\left(\frac{\text{Peak area in the presence of matrix}}{\text{Peak area in the absence of matrix}}\right) - 1\right) \times 100\%$$

The peak area in the presence of matrix refers to the peak area of an extracted matrix sample in which the compounds were added post-extraction. The peak area in the absence of matrix refers to analytes in a neat solvent solution.

RESULTS AND DISCUSSION

Simplified 3-step protocol with Oasis PRiME HLB vs. standard 5-step protocol with other SPE devices

Previous work had demonstrated that only water wettable sorbents such as Oasis HLB can be used without conditioning and equilibration steps. These steps cannot be eliminated when using most types of SPE sorbents. To prove this, recoveries were compared between Oasis PRiME HLB, a silica-based C_{18} sorbent, and a competitive polymer-based sorbent. For this test,the micro-elution plate format and rat plasma samples were used. With the simplified protocol, the silica-based C_{18} and the competitive sorbents showed very low recoveries (Figure 2). In contrast, the Oasis PRiME HLB Sorbent performed very well with this 3-step protocol, indicating that non-water-wettable or partially water-wettable sorbents, even though they are polymer based, cannot be used for simple sample preparation in studies (For more details, please see application note 720005140EN). In order to get an unbiased performance comparison for synthetic cannabinoids, a 3-step protocol was used with Oasis PRiME HLB and 5-step protocols were applied to the other SPE devices.

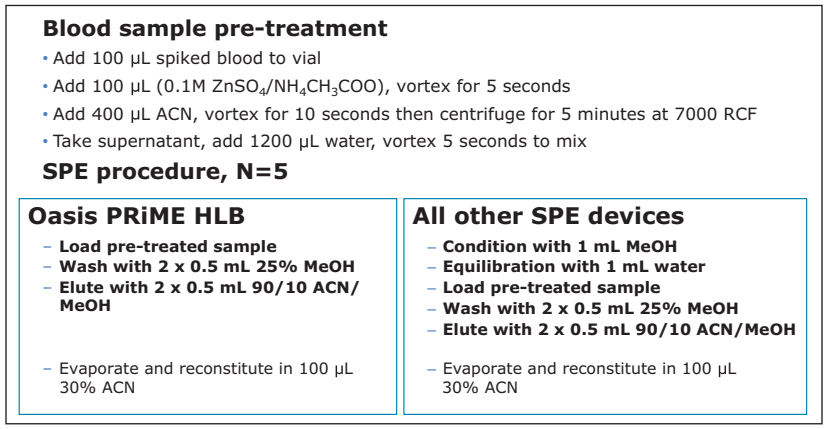

Figure 1. Sample pretreatment and SPE procedures for Oasis PRiME HLB and competitors' SPE.

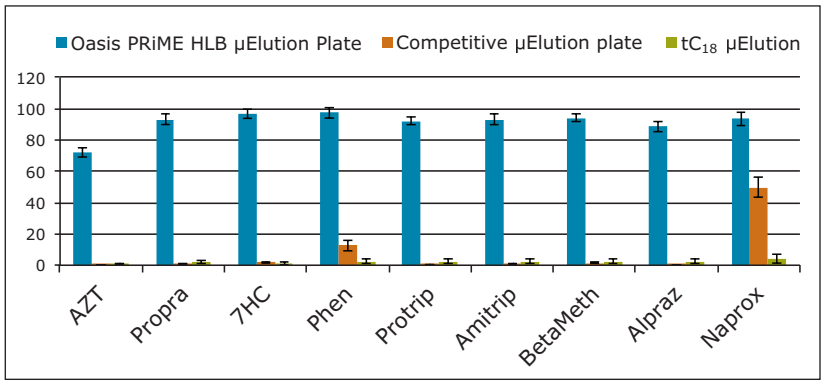

Figure 2. Recovery with the simplified 3 step protocol on Oasis PRiME HLB, a competitive polymer-based sorbent and silica-based C_{18}. All plates were evaluated in a micro plate format.

Time (min.)	Flow (mL/min.)	%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	0.6	70	30
8.5	0.6	70	30

Table 1. Mobile phase gradient. The compositions of MPA and MPB are listed in the Methods section.

Chromatography

The 22 compounds analyzed are listed in Table 2 and constitute a panel that includes various classes of forensically relevant synthetic cannabinoids. These include adamantoylindoles (AM 1248 and AKB48), napthoylindoles (JWH 022), phenylacetyl indoles (RCS-4 and RCS-8), and tetramethylcyclopropylindoles (UR-144 and XLR11). Major metabolites of JWH-073 and JWH-018 were also included, as some of these compounds are structural isomers with identical mass spectral fragments that require adequate chromatographic separation for accurate quantitation.

The design of the solid-core CORTECS particle, combined with optimal packing in the column, results in excellent chromatographic performance. A representative chromatogram of all compounds from a 20 ng/mL calibration standard is shown in Figure 3. Peak assignments are listed in Table 1. Using a CORTECS UPLC C_{18} Column (1.6 µm; 2.1 x 100 mm), all analytes were analyzed 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.

No.	Compound	Mol. Formula	Cone Voltage (V)	1°MRM Transitions	Collision Energy (eV)
1	AM2233	$C_{22}H_{23}IN_{20}$	40	459.2→98.05	34
2	RCS-4, M10	$C_{20}H_{21}NO_3$	40	324.2→121.0	22
3	RCS-4, M11	$C_{20}H_{19}NO_3$	36	322.2→121.0	22
4	AM 1248	$C_{26}H_{34}N_2O$	56	391.4→135.1	28
5	JWH-073 4-COOH	$C_{23}H_{19}NO_3$	50	358.2→155.1	26
6	JWH-073 4-OH met.	$C_{23}H_{21}NO_2$	50	344.2→155.1	22
7	JWH-018 5-COOH	$C_{24}H_{21}NO_3$	46	372.2→155.1	24
8	JWH-073 3-OH met.	$C_{23}H_{21}NO_2$	44	344.2→155.1	26
9	JWH-018 5-OH met.	$C_{24}H_{23}NO_2$	40	358.2→155.1	24
10	JWH-018 4-OH met.	$C_{24}H_{23}NO_2$	40	358.2→155.1	24
11	JWH-015	$C_{23}H_{21}NO$	42	328.2→155.1	24
12	RCS-4	$C_{21}H_{23}NO_2$	44	322.2→135.1	26
14	JWH-022	$C_{24}H_{21}NO$	50	340.2→155.1	26
13	JWH-073	$C_{23}H_{21}NO$	48	328.2→155.1	26
15	XLR-11	$C_{21}H_{28}FNO$	48	330.3→125.1	26
16	JWH-203	$C_{21}H_{22}ClNO$	46	340.2→125.0	26
17	JWH-018	$C_{24}H_{23}NO$	44	342.2→155.1	26
18	RCS-8	$C_{25}H_{29}NO_2$	42	376.3→121.1	26
19	UR-144	$C_{21}H_{29}NO$	46	312.3→125.1	24
20	JWH-210	$C_{26}H_{27}NO$	48	370.2→183.1	26
21	AB 001	$C_{24}H_{31}NO$	52	350.3→135.1	30
22	AKB 48	$C_{23}H_{31}N_3O$	38	366.3→135.1	22

Table 2. Molecular formulae, retention times, and MS/MS conditions for the synthetic cannabinoid compounds and metabolites in this application.

Recovery for Oasis PRiME HLB vs. competitors' RP SPE devices

The synthetic cannabinoids and metabolites in this application include compounds that are neutral, acidic and basic. Use of a reversed phase sorbent enabled the simultaneous extraction of all of the compounds and metabolites tested, regardless of their functionality. All recoveries for both Oasis PRiME HLB and competitors RP SPE devices were calculated according to the equations described in the experimental section and the results are shown in Figure 4a and Table 3. For Oasis PRiME HLB, 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 competitive RP devices. Competitor "EVA" recoveries ranged from 60–97% with %RSDs of 1–17%; Competitor "STX" recoveries ranged from 59–92% with %RSDs of 2–27% and competitor "PLX" recoveries ranged from 46–84% with %RSDs of 3–41%. For the last 7 analytes JWH-203, JWH-018, RSC-8, UR-144, JWH-210, AB-001 and AKB-48, the %RSDs for all competitors' SPE devices are unacceptably high (%RSD up to 17% for EVA, 27% for STX and 41% with PLX) compromising reliable quantification. In contrast, the high recoveries and low %RSDs for this panel of synthetic cannabinoids indicate that Oasis PRiME HLB should give similar results for other related compounds with a simple load-wash-elute protocol.

Matrix Effects and phospholipid removal

Matrix effects were calculated according to the equations described in the experimental section and the results are shown in Figure 4b. Matrix effects across the panel were excellent for Oasis PRiME HLB, with only three compounds exceeding 25%, and an average absolute matrix effect of only 11%. All competitors' SPE devices exhibited overall high variability for the later eluting compounds (RSC-8, UR-144, JWH-210, AB-001 and AKB-48). Furthermore, severe ion suppression was seen for JWH-203 with all competitors' SPE devices.

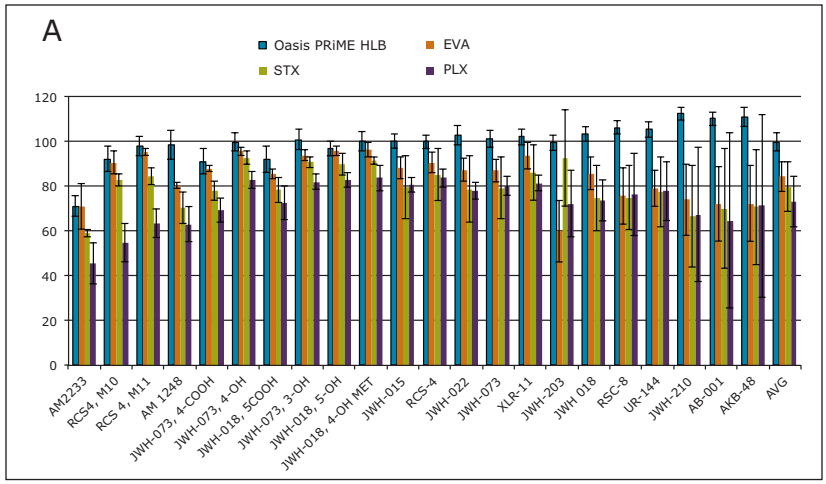

Figure 4a. Recoveries and standard deviations for Oasis PRiME HLB and competitors' SPE devices. Bars and error bars represent means and standard deviations (N=5), respectively. The values at the right of the chart represent average recoveries for all compounds for each device (labeled AVG).

10 mg Plate, N=5	% Recovery Range	Average % Recovery	% RSD Range	Average % RSD
Oasis PRiME HLB	**90–110% (AM2233=71%)**	**100**	**3–7**	**4**
EVA	60–97%	85	1–17	7
STX	59–92%	80	2–27	11
PLX	46–84%	73	3–41	11

Table 3. Recoveries and standard deviation ranges across all tested drug panel for Oasis PRiME HLB and competitors' SPE devices.

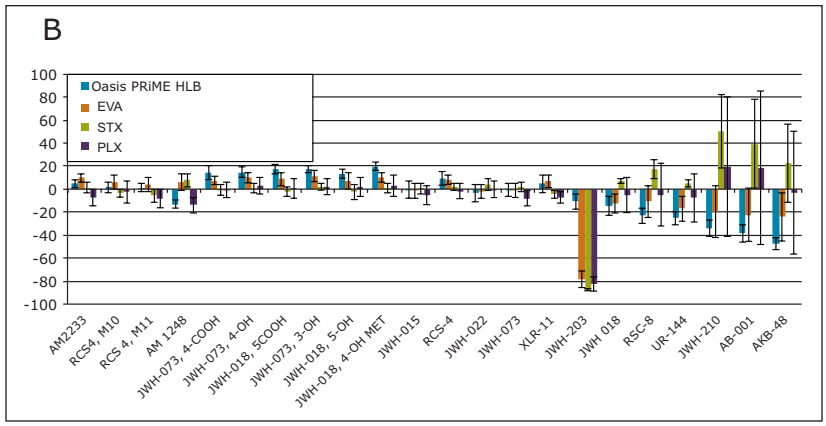

Figure 4b. Matrix effects (ME) and standard deviations obtained from Oasis PRiME HLB and competitors' SPE devices. Bars and error bars represent means and standard deviations (N=5), respectively.

This clearly indicated the presence of severe interferences in the extracts from the competitors' SPE devices. Examination of phospholipid traces revealed that JWH-203 co-eluted with residual phospholipid lysophosphatidycholine 18:0 (*m/z* 524.4), not removed by competitive SPE products. This co-elution is shown in Figure 5a. Figure 5b demonstrates the ion suppression for JWH-203 due to the co-elution with phospholipid 524.4. Figure 5c demonstrates the direct relationship between phospholipid area and ion suppression for this compound. Fitting a linear regression curve to the plot of matrix effects vs. relative phospholipid abundance yields an excellent correlation between these two factors with an R^2 value of 0.987.

A key attribute of Oasis PRiME HLB, the ability to deliver cleaner extracts than other sample preparation methods and remove more than 90% endogenous phospholipids compared to other SPE devices, represents a distinct advance in SPE technology. It is the only RP SPE sorbent that selectively removes phospholipids, eliminating the co-elution and associated ion suppression. Some compounds in this particular panel are structural isomers with identical mass spectral fragments that require adequate chromatographic separation for accurate quantitation. This makes it necessary to reduce the complexity of the sample matrix as chromatographic adjustments in a complex panel are often not practical. Removal of endogenous phospholipids with Oasis PRiME HLB results in a much cleaner extraction and leads to lower recovery variability and decreased matrix effects. Longer column lifetimes and less mass spectrometer source maintenance are also expected with using of Oasis PRiME HLB.

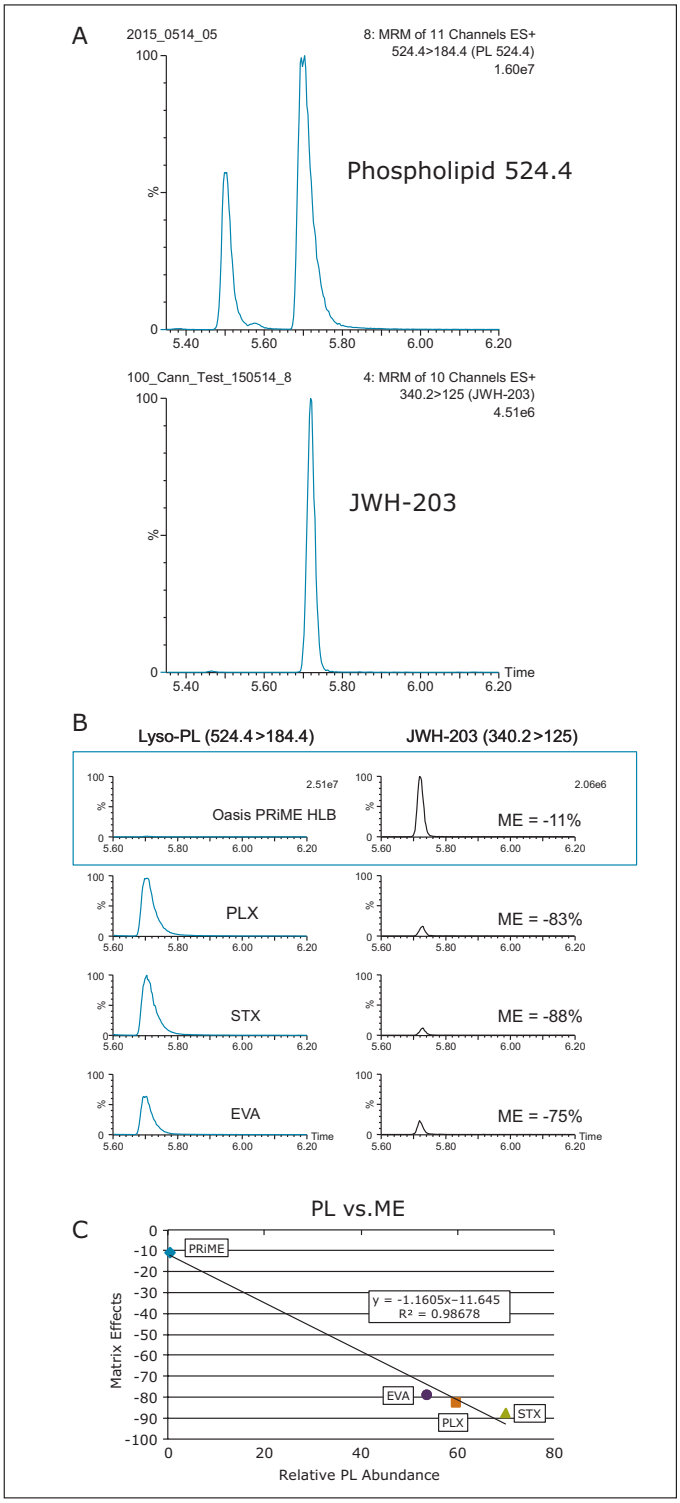

Figure 5a. Chromatograms for phospholipid 524 (Lysophosphatidylcholine 18:0) in the extract from a competitive SPE device (top panel), which coelutes with cannabinoid JWH-203 (bottom panel) at 5.74 minutes.

Figure 5b. Chromatograms of PL 524 (left) and whole blood samples post-spiked with JWH-203. Chromatographic scales are linked to show the relative abundance of PL 524 and the associated ion suppression of JWH-203. The values on the right indicate the degree of ion suppression calculated for each SPE device.

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

CONCLUSIONS

This application note highlights the comparison of Oasis PRiME HLB, a novel reversed-phase SPE sorbent, to several other of the most common commercially available reversed phase SPE devices, with respect to both analyte recovery and matrix effects. Oasis PRiME HLB is designed to enable simple and fast SPE protocols while nearly eliminating endogenous phospholipids with a simple load-wash-elute strategy. This comparison is based on the 3-step Oasis PRiME HLB protocol and the recommended 5-step protocol for other RP SPE devices, which do not work with the simple load-wash-elute 3-step protocol.

Higher recoveries and lower %RSDs were obtained with Oasis PRiME HLB; 21/22 compounds showed recoveries between 90–110% with all %RSDs ranged from 3–7%. Lower recoveries and higher overall variability were obtained with all other RP SPE devices, where recoveries were as low as 46% and %RSDs as high as 41%. Low and consistent matrix effects were obtained across the entire tested drug panel with Oasis PRiME HLB, while much higher matrix effects and variability were observed on other SPE devices, especially for the late eluting drugs. Oasis PRiME HLB also successfully demonstrated removal of 99% of the phospholipid with MW 524.4 relative to competitors' devices and nearly eliminated the ion suppression associated with this phospholipid. Oasis PRiME HLB represents a distinct advance in SPE technology with higher recovery, lower variability, and lower matrix effects. This should also translate to longer column lifetimes and less mass spectrometer source maintenance.

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