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Improved Peak Shape Using MaxPeak™ Premier Columns and the Impact on SPE Results in bioanalytical workflows

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Abstract

Non-specific adsorption can occur when analytes interact with exposed metal surfaces of an LC column or system.[1-2] This interaction can cause sample loss due to adsorption onto the metal surfaces, as well as peak tailing and generally poor peak shape. This is particularly troublesome for critical assays such as the detection of compounds after solid-phase extraction (SPE). SPE is often employed to not only remove unwanted material from a sample such as phospholipids or proteins, but also is used to concentrate a sample to make it more readily detected and quantified. Non-specific adsorption for post-SPE samples can lead to poor recovery or high variability, even if the samples have been properly cleaned to remove any interferences. This work shows an SPE experiment where nicotine and three metabolites are spiked into collected oral fluid and analyzed on both a stainless-steel column and a MaxPeak Premier High-Performance Surface (HPS) Column. Higher variability seen with the stainless-steel column, along with poor peak shape for nornicotine highlights the benefit of using MaxPeak Premier Columns for these types of samples to eliminate doubt and ensure accurate results are achieved after SPE.

Benefits

- No SPE method development using Oasis™ PRiME MCX sorbent and 4-step protocol.
- Improved reproducibility for percent recovery calculation
- Sharper, more symmetrical peak shape in LC-MS allowing better integration and quantitation

Introduction

Non-specific adsorption (NSA) can have a drastic impact on LC-MS applications.[3-8] From lower peak areas to poor peak shape affecting integration, NSA can be extremely detrimental to critical assays. Mitigating NSA can be done a variety of ways, including but not limited to, passivating the system with sample matrix or "sacrificial sample", using passivation agents in the mobile phase like medronic acid, or even using PEEK or PEEK-lined columns and system tubing. While these techniques have their uses, the downsides to all of them can be worse than NSA. Passivation agents like medronic acid act as ion exchangers, can have an effect on LC-MS signal by affecting analyte ionization. Passivating a system and column with matrix or sample will work in the short-term but is costly and also requires repeating the process at regular intervals to maintain the performance. Lastly, PEEK and PEEK-lined columns have higher variability between columns due to less control in manufacturing making them problematic for longer lifetime methods.

The creation of MaxPeak Premier High-Performance Surface (HPS) Technology addresses all of the concerns around NSA, by employing a hybrid organic-inorganic surface modification to the stainless-steel components of the column and system hardware. This modification prevents the analytes from interacting directly with the metal surface, improving peak shape and reducing sample loss due to adsorptive effects. This technology is particularly useful in bioanalytical workflows, where solid-phase extraction and low analyte concentrations are often common. Many bioanalytical workflows employ solid-phase extraction (SPE) to reduce matrix interferences and improve analyte concentration, however even after SPE, low analyte concentrations are common. NSA is known to have a greater impact on sample adsorption at lower analyte concentrations, making limit of detection and limit of quantitation determinations more challenging in bioanalytical workflows.[1,8] MaxPeak Premier HPS columns reduce NSA allowing more accurate quantitation of analytes. To demonstrate this, nicotine and three metabolites were spiked into collected oral fluid samples and processed using Oasis PRiME MCX sample prep devices. Then the samples were analyzed using both stainless-steel and MaxPeak Premier

HPS hardware columns packed with the same batch of BEH™ C_{18} 2.5 µm particles.

Experimental

Sample Description

Stock solutions of the compounds made at 1 mg/mL in water (nicotine and cotinine). Stocks of hydroxycotinine and nornicotine purchased from Cerilliant at 1 mg/mL in methanol. Working solutions for solid-phase extraction (SPE) prepared in water at 6.25 µg/mL (pre-spike solution) and 2.08 µg/mL (post-spike solution).

Sample Preparation

Quantisal Oral Fluid Collection Device used to collect an oral fluid sample from a non-smoker, to be used as a blank. Sample collected and used the same day to avoid degradation of sample. Oasis PRiME MCX µelution plate (p/n: 186008914 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186008914-oasis-prime-mcx-96-well--elution-plate-2-mg-sorbent-per-well-30-.html>) used for sample cleanup. A portion of collected oral fluid was spiked with analytes and pre-treated (1:1) with 4% phosphoric acid in water. Oasis PRiME MCX 4-step clean-up protocol followed including washes of 100 mM ammonium formate 2% formic acid and methanol.[9] Elution using 5% ammonium hydroxide in methanol was used and immediately placed on system for injection. Volumes used for all steps based on SPE format, in this case the µElution plate.[10] Final sample concentration for LC-MS analysis was 50 ng/mL for each of the four analytes.

LC Conditions

LC System:	ACQUITY™ UPLC H-Class PLUS System with PDA and QDa Detector
Detection:	SIRs (see Figure 1)
Columns:	XBridge™ Premier BEH C_{18} Column 2.5 µm, 2.1

	x 50 mm (p/n: 186009827) XBridge BEH C_{18}, 2.5 µm, 2.1 x 50 mm (p/n: 186006029)
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	2.0 µL (UPLC)
Flow rate:	0.5 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase D:	200 mM Ammonium Hydroxide in water
Gradient conditions:	constant 5% mobile phase D throughout the gradient. 5–95% mobile phase B in 4.90 minutes. Hold at 95% B for 0.82 minutes, return to starting conditions and hold for 2 minutes.

Data Management

Chromatography Software:	Empower™ 3 Feature Release 5

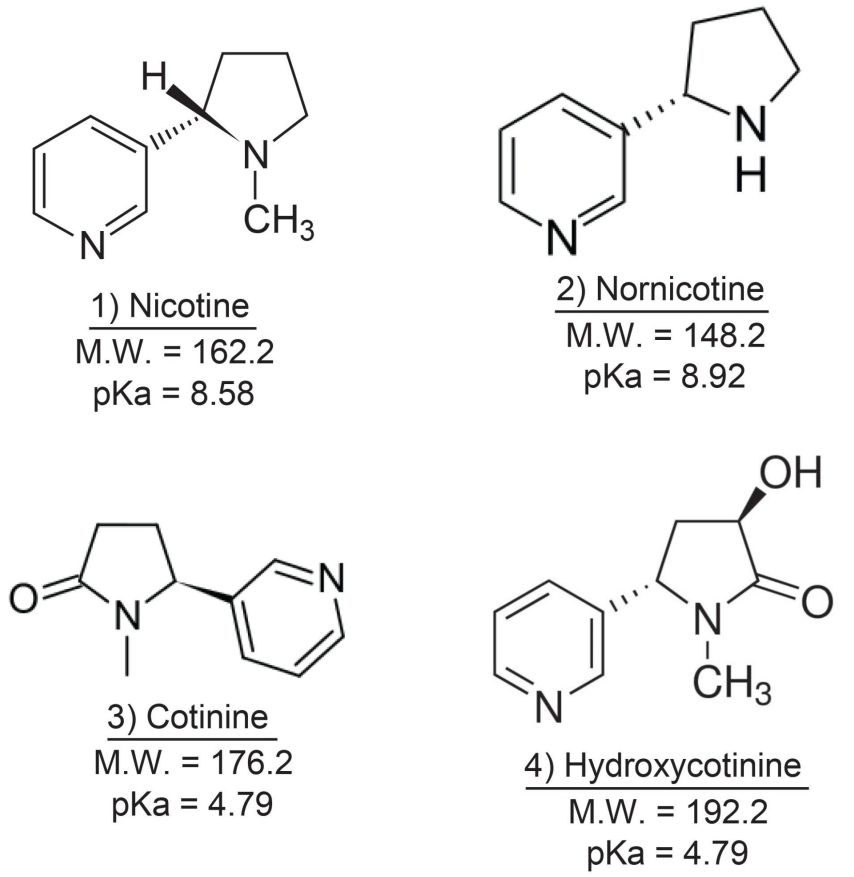

Figure 1. Chemical structures, molecular weights, and pKa values for the four compounds of interest.

Results and Discussion

Nicotine is a commonly known chemical present in tobacco-based products such as cigarettes, cigars, and even vape liquids. The monitoring of nicotine and metabolites in oral fluid has been used as part of cessation therapy or to assess the level to which an individual smokes. Oral fluids are typically collected in a non-invasive manner using devices like Quantisal Oral Fluid Collection devices. The collection involves placing a piece of the device under the tongue and waiting for enough of the fluid to be collected. Then the device is placed into a storage solution for transport and analysis. The storage

solution usually contains stabilizing buffers to ensure no sample degradation occurs.

To highlight the benefits of MaxPeak Premier HPS Technology for bioanalytical workflows, oral fluid was collected from a non-smoker. This would act as blank matrix as the individual has never smoked and would not have any nicotine or metabolites in their system. After collection with a Quantisal device, the samples were subjected to SPE using the Oasis PRiME MCX protocol. The Oasis PRiME MCX 96 well µElution plate was used for this work as sample was limited and the intent was to concentrate the analytes from the oral fluid. Since nicotine and all three metabolites, nornicotine cotinine and hydroxycotinine, are charged at low pH and neutral at high pH, the cation exchange SPE plate was preferred. Following the Oasis PRiME MCX 4-step protocol the samples were processed and subsequently analyzed using both an XBridge BEH C_{18} and XBridge Premier BEH C_{18} Column. Both columns were packed with the same batch of 2.5 µm BEH C_{18} particles into 2.1 x 50 mm hardware. The only difference between the columns is that use of MaxPeak Premier HPS Technology hardware. BEH C_{18} particles were chosen as the analytes are better retained and separated at high pH, and the BEH particle technology is specifically designed to be stable at high pH.

After SPE and subsequent LC-MS analysis, percent recovery of the analytes was calculated using both columns and is shown in Figure 2.

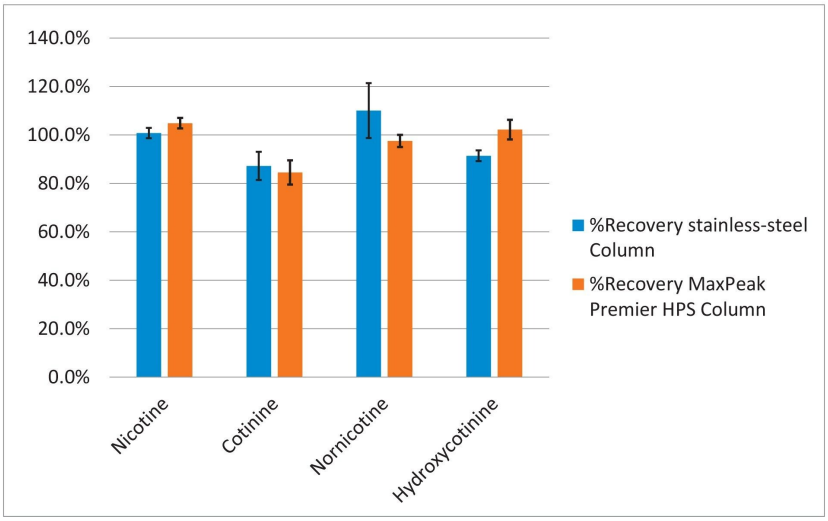

Figure 2. Percent recovery calculations using a stainless-steel column and a MaxPeak Premier HPS Column. Error bars denote 1 standard deviation for %Recovery calculated from three replicate injections. No internal standard correction was used for %Recovery calculations.

While the same samples were analyzed on the same day, there is still some variation between the data collected with the stainless-steel column and the MaxPeak HPS Column. While all analytes have %Recovery between 80–120 with reasonable error bars, the data for nornicotine on the stainless-steel column has considerably higher standard deviations (11%) compared to the same samples on the MaxPeak HPS Column (2.5%). The other three compounds show comparable results between the two columns. Investigating the reason for the higher standard deviation of recovery of nornicotine, we can see that the chromatography shows a distinct difference between the two columns, Figure 3.

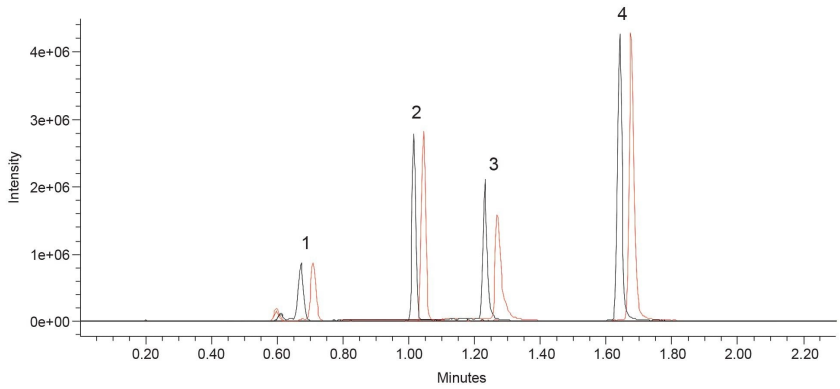

Figure 3. Overlay of Single Ion Recording (SIR) of the four analytes of interest. Black trace is the MaxPeak Premier HPS Column, Red trace the Stainless-Steel Column. 1) Hydroxycotinine, 2) Cotinine, 3) Nornicotine, and 4) Nicotine.

As seen in the red trace, the stainless-steel column shows a wider and slightly tailing peak for nornicotine (3) compared to the MaxPeak Premier HPS Column. The slightly worse peak shape means that integration and quantitation of the peak is harder and less reliable than for a symmetrical peak. As such the %Recovery and standard deviations are slightly more variable for the wider, asymmetrical peak. The stainless-steel column shows comparable peak shapes for the other analytes compared to the MaxPeak Premier HPS column. In this instance, using MaxPeak Premier HPS Columns allowed for more accurate quantitation of SPE samples, which is crucial in bioanalytical workflows. While not tested in this work, limit of detection and quantitation determination using stainless-steel columns can lead to higher thresholds due to sample loss. The use of MaxPeak Premier HPS columns provides an accurate measurement of the sample by eliminating non-specific adsorption, allowing lower levels of analyte to be detected and properly integrated and quantified by improving peak shape.

Conclusion

MaxPeak Premier High-Performance columns mitigate secondary interactions between the analytes and any exposed metal surfaces in the system and column. These secondary interactions typically present as

peak shape distortions and lower peak areas due to non-specific adsorption and analyte loss. In the case of post-SPE analysis, the lower peak areas or poor peak shape can affect integration and quantitation leading to poor recovery or wide error bars. This can lead to higher uncertainty in the results and a poor representation of the SPE protocol' s effectiveness at sample clean-up.

Nicotine and metabolites were spiked into oral fluid collected with Quantisal collection devices. Samples were processed using the recommended Oasis PRiME MCX 4-step protocol and a µElution plate. By using this recommended protocol, no SPE method development is required providing clean samples for further analyses. Post-SPE, the eluates were analyzed on both a stainless steel XBridge BEH C_{18} Column, and an XBridge Premier BEH C_{18} Column which uses MaxPeak HPS Technology. While both columns show reasonable recovery and error bars, the stainless-steel column results showed considerably wider error bars for nornicotine. In looking at the chromatography it is seen that nornicotine has poor peak shape due to tailing when analyzed on the stainless-steel column. The MaxPeak Premier HPS Column shows considerably better peak shape, allowing more accurate peak integration and therefore more consistent results. By using MaxPeak Premier HPS ACQUITY to analyze post-SPE samples, more reliable and accurate data can be obtained, eliminating any doubt in the SPE effectiveness, and providing a clearer picture for the analysis.

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