Waters™

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Liquid chromatography mass spectrometry (LC-MS) analysis of complex biological samples often requires extensive sample cleanup to remove the undesirable components from the sample matrix, which otherwise will inadvertently affect the analysis, often resulting in MS ion signal suppression or enhancement. Mixed-mode solid-phase extraction (SPE) is a commonly used sample preparation technique for LC-MS analysis. However, developing mixed-mode SPE methods can be a challenging task. Not knowing where to start or which sorbents to choose can be daunting. Further adding to the complexity, most SPE workflows involve several steps for pipetting and transferring samples, reagents, and solvents. Automation of these pipetting and transfer workflows using expensive liquid handlers often involve complex programming and require expertly trained and dedicated personnel to perform the task. Performing workflows manually can be extremely tedious and prone to errors and

requires good analytical skills to produce reproducible results. An automated SPE workflow for a quick, reliable, and reproducible mixed-mode SPE sorbent selection method development, using an Andrew+ Pipetting Robot connected and operated using OneLab, an easy-to-use browser-based software, is described in this application brief. The ease of Andrew+ automated mixed-mode SPE sorbent selection is demonstrated by extracting five analytes from spiked human blood plasma using Waters Oasis 96-Well µElution Plate.

Benefits

- Automation with the Andrew+ Pipetting Robot is used for simplifying the mixed-mode SPE sorbent selection process, resulting in a quick, reliable, and reproducible method for optimum analyte recovery, without requiring an expert user
- The Andrew+ Pipetting Robot is configured with labware that are commonly used in laboratory settings for convenient setup and execution
- An easy-to-apply OneLab Protocol for the entire mixed-mode SPE sorbent selection workflow in a µElution plate format
- SPE vacuum manifold with the IKA VACSTAR control vacuum pump (IKA Works Inc., USA) connected and controlled by the Andrew+ Pipetting Robot ensures precise pressure controls for each of the Load, Wash, and Elution steps in the protocol
- Load, Wash, and Elution steps of the SPE workflow are automated, manual intervention is only required to change the SPE sample collection plate from the vacuum manifold between the wash and elution steps of the protocol
- Automation of the Oasis 2x4 SPE sorbent selection method development workflow produces reproducible results while saving the analyst approximately two to three hours of manual effort

Introduction

Quantitative analysis of complex biological samples using LC-MS requires extensive sample cleanup to remove undesirable matrix components that may otherwise coelute and adversely affect the quantitation of the target analyte. Mixed-mode SPE, where the sorbent exhibits both reversed-phase and ion-exchange properties, is an effective and well proven technique for sample cleanup. However, developing mixed-mode SPE methods for the

quantitation of analytes can be a tedious process. It requires expert understanding of the physical and chemical properties of both the target analyte and the SPE sorbent, in order to select the most suitable sorbent for effective removal of undesired matrix components while affording highest analyte recovery.

Waters Oasis 96-Well µElution Plate (p/n: 186004475 < https://www.waters.com/waters/partDetail.htm?partNumber=186004475>) simplifies this sorbent selection process, wherein it includes all four different mixed-mode ion-exchange sorbent chemistries (MCX, MAX, WAX, and WCX) in a single plate format and utilizes just two optimized and proven protocols (2x4 Sorbent Selection Method) (p/n: WA60090 <https://www.waters.com/waters/library.htm?cid=511436&lid=1532071&lcid=1532072>) to develop a good SPE method. Thus, the Oasis Method Development Plate, combined with these protocols, provides a streamlined and simplified solution to mixed-mode SPE sorbent selection, that will undoubtedly result in high analyte recovery. Furthermore, automation of this Oasis 2x4 Sorbent Selection Protocol by using the Andrew+ Pipetting Robot, controlled and programmed by the user-friendly, browser based OneLab Software, makes the entire sorbent selection method development rapid, accurate, reproducible, and robust.

In this application brief, a rapid, accurate, reproducible, and robust mixed-mode SPE sorbent selection method development is demonstrated using human blood plasma spiked with four analytes (imipramine, 1-decanesulfonic acid, ketoprofen, and valethamate) for ion-exchange retention and one analyte (prednisone) for reversed-phase retention.

Experimental

The below steps are performed by the Andrew+, unless specified.

- The Andrew+ Workbench is arranged manually based on guidance provided in OneLab, as shown in Figure 2.
- The automated workflow starts with the initial vacuum setup of the SPE vacuum manifold, wherein the IKA VACSTAR control vacuum pump is automatically turned ON for 1 min, allowing the user to visually inspect and ensure the pressure reaches 700 mbar in 1 min, this is done with the SPE and waste collection plate inserted into the SPE vacuum manifold.
- This is followed by conditioning and equilibration steps of the SPE protocol wherein 200 µL of methanol and water are pipetted respectively into the SPE plate, and the vacuum profile corresponding to the conditioning and equilibration steps are applied.

- The pre-spiked-pre-treated plasma (analyte spiked plasma diluted 1:1 with 4% H_3PO_4 for sorbents MCX and WAX and 4% NH_4OH for sorbents WCX and MAX) and post-spiked-pre-treated plasma (blank solvent spiked plasma diluted 1:1 with 4% H_3PO_4 for sorbents MCX and WAX and 4% NH_4OH for sorbents WCX and MAX) samples are then loaded into the SPE plates in four-replicates and the vacuum profile for the LOAD step is applied.
- The WASH solvents (2% formic acid in water and 5% ammonium hydroxide in water) are then transferred for both the Protocol-1 and Protocol-2, respectively, into the appropriate wells of the SPE plate, the corresponding vacuum profile is then applied.
- After the WASH step, a *user action required* prompt will appear to remind the user to replace the waste collection plate in the SPE manifold with the ELUTE-1 collection plate.
- This is followed by pipetting ELUTE-1 solvent (methanol) for both Protocol-1 and Protocol-2 into the appropriate wells of the SPE plate and the corresponding vacuum profile is then applied. This pipetting and vacuum application is performed twice (Note: ELUTE-1 is collected only when the analyte is retained by reversed-phase mechanism for the SPE sorbents).
- After ELUTE-1 step, a *user action required* prompt will appear to remind the user to replace the ELUTE-1 collection plate in the SPE manifold with the ELUTE-2 collection plate.
- This is followed by pipetting the elution solvents (5% ammonium hydroxide in methanol and 2% formic acid in methanol for the Protocol-1 and Protocol-2 respectively into the appropriate wells of the SPE plate, and the corresponding vacuum profile is applied. This pipetting and vacuum application is performed twice.
- After the completion of the ELUTE-2 step a *user action required* prompt will appear to remind the user to manually keep the ELUTE-1 and the ELUTE-2 collection plate for post spiking the solvent or standard.
- After post spiking, the ELUTE-1 and ELUTE-2 plates are vortexed for 15 min at 1200 rpm and analyzed by LC-MS.

Results and Discussion

The Oasis 2x4 SPE Method Development Workflow (Figure 1) is automated using the Andrew+ Pipetting Robot with labware that are commonly used in laboratory settings for convenient setup and execution.

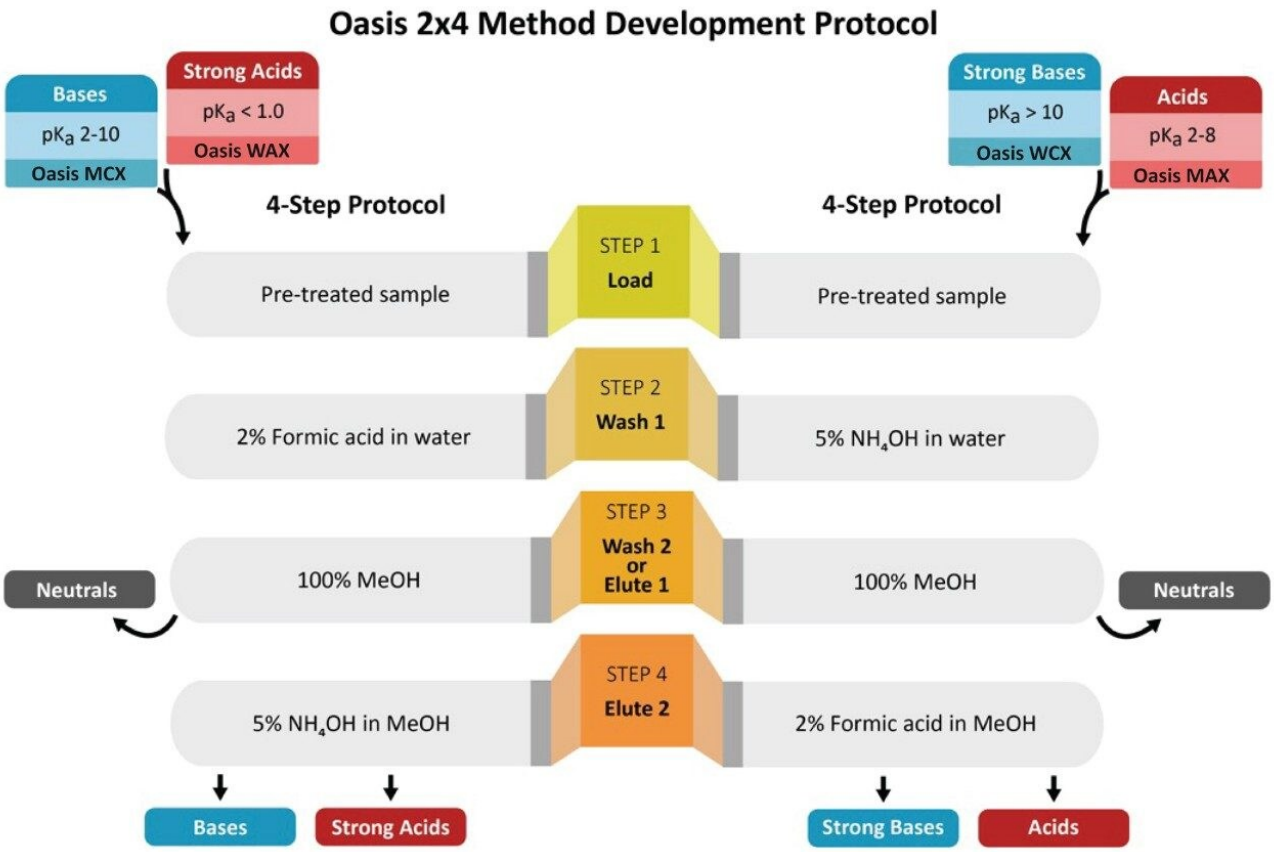

Figure 1. The Oasis 2x4 SPE Sorbent Selection Protocols for method development.

Figure 2. Andrew+ Pipetting Robot workbench layout and configuration for automating the Oasis 2x4 SPE Sorbent Selection Method, as shown in OneLab.

The solvents, reagents, and the spiked human blood plasma samples required for the workflow are prepared manually prior to the execution of the Andrew+ Protocol.

Figure 3 shows the recovery of analytes from spiked human blood plasma samples for imipramine, 1-decanesulfonic acid, ketoprofen, and valethamate that are retained by ion-exchange MCX, WAX, MAX, and WCX sorbents respectively and prednisone that is retained by reversed-phase mechanism by all four sorbent chemistries using the Andrew+ Automated Oasis 2x4 SPE Sorbent Selection Method, demonstrating quick and easy sorbent selection method development, combined with good reproducibility and saving approximately two-to-three hours of manual effort.

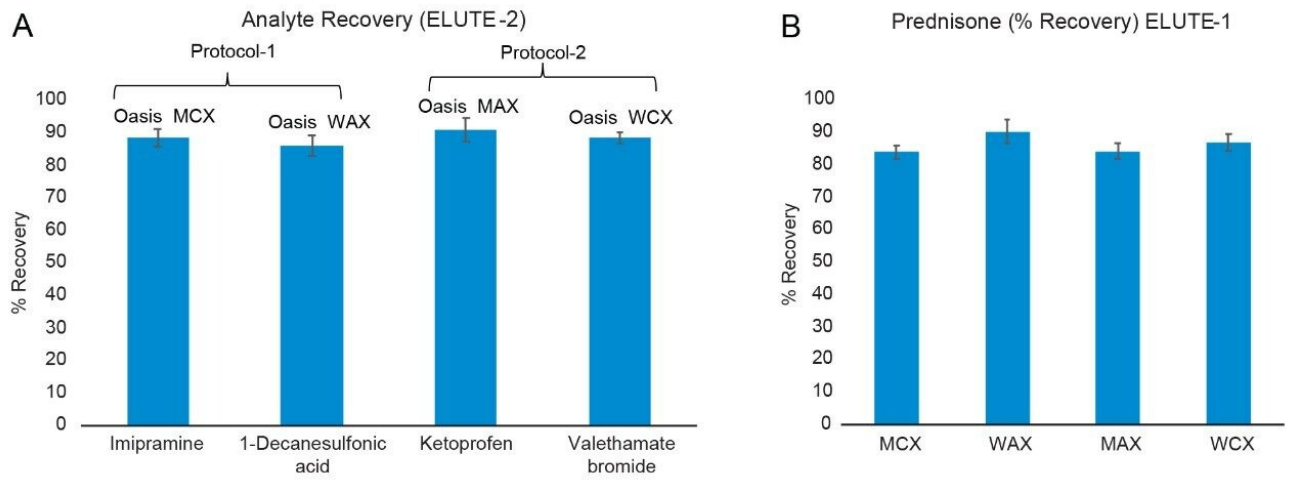

Figure 3. Percentage recovery of the analytes from four replicate spiked human blood plasma for the four Oasis mixed-mode SPE sorbents using (A) ELUTE-2 and (B) ELUTE-1 steps of the protocol.

Conclusion

An automated mixed-mode SPE sorbent selection method development workflow is demonstrated using the Andrew+ Pipetting Robot for quick, reliable, and easy method optimization of the analyte recovery, combined with good reproducibility using mixed-mode SPE.

References

1. Oasis 2x4 Method: Proof of Concept. Waters Application Note, WA60090 < https://www.waters.com/webassets/cms/library/docs/oasis210.pdf> , June 2007.
2. Oasis Sample Extraction Products: Methodology. Waters Application Note, 720003679 < https://www.waters.com/nextgen/global/library/application-notes/2010/oasis-sample-extraction-products-methodology.html> , September 2010.

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