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Applikationsbericht

Automation of Peptide SPE for Bioanalytical Method Development

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

Abstract

Bioanalysis requires careful method development in order to define working parameters for robust and reliable sample preparation and extraction of compounds. Automating SPE sample preparation development with Andrew+™ pipetting robot enables users to focus on more complex tasks.

Figure 1. Andrew+ pipetting robot deck layout.

Benefits

- Automation with the Andrew+ pipetting robot simplifies the mixed-mode SPE sorbent selection process by using the Oasis™ Peptide Method Development 96-well µElution Plate, resulting in a quick, reliable, and reproducible method for optimum analyte recovery. Integrating with Waters™ Xevo™ Triple Quadrupole Mass Spectrometer and Waters™ ACQUITY™ Premier UPLC™ Systems, provides an end-to-end robust solution for peptide quantitation
- An easy-to-apply OneLab™ Protocol for the SPE workflow in a µElution plate format enables high sample throughput
- The Andrew+ pipetting robot leverages labware that are commonly used in laboratory settings for convenient setup and execution

Introduction

LC-MS/MS analysis of biological samples requires extensive sample clean-up procedures to remove matrix components that may adversely affect the quantitation of the target analyte. Peptide and protein-based

therapeutics bring their own specific method development challenges which can be time consuming and tedious.

This work demonstrates an automated SPE (Solid Phase Extraction) protocol using Oasis Peptide Method Development 96-well µElution Plate for developing reproducible and robust extraction methods for peptides from plasma samples. The 96-well plate format contains six rows each of two Oasis mixed-mode, ion-exchange chemistries: a mixed-mode anion exchanger (MAX), which is ideal for peptides with pKa >2–8; and a weak cation exchanger (WCX), which is usually preferred for peptides with pKa >10. Sample preparation method development can be quickly and easily achieved with the use of a single SPE protocol.

Results and Discussion

All the steps in the SPE sorbent selection method development are automated by the Andrew+ pipetting robot scripted in the OneLab software. This includes sample dilution, loading, equilibration, washing, elution, post spiking of reference samples, as well as controlling the vacuum of the SPE manifold. The SPE extracted samples in this example were quantified by LC-MS/MS. Automation of the SPE sorbent selection protocol using the Andrew+ pipetting robot makes the entire process rapid, reproducible, and robust with minimal manual intervention. Additionally, automating the approach enables multiple variables (e.g., different compositions for wash and elution solvents or variable vacuum settings, for better recoveries) to be tested in a logical and reproducible manner. PST-SPE workflow for the solid phase extraction of peptides from plasma samples are shown in Figure 2.

SPE Reagents

Pretreatment:	4% Phosphoric acid in water, by volume.
Wash 1:	5% Ammonium hydroxide in water, by volume.
Wash 2:	20% Acetonitrile in water, by volume.
Elution:	75/25% Acetonitrile/water containing 1%

trifluoroacetic acid, by volume.

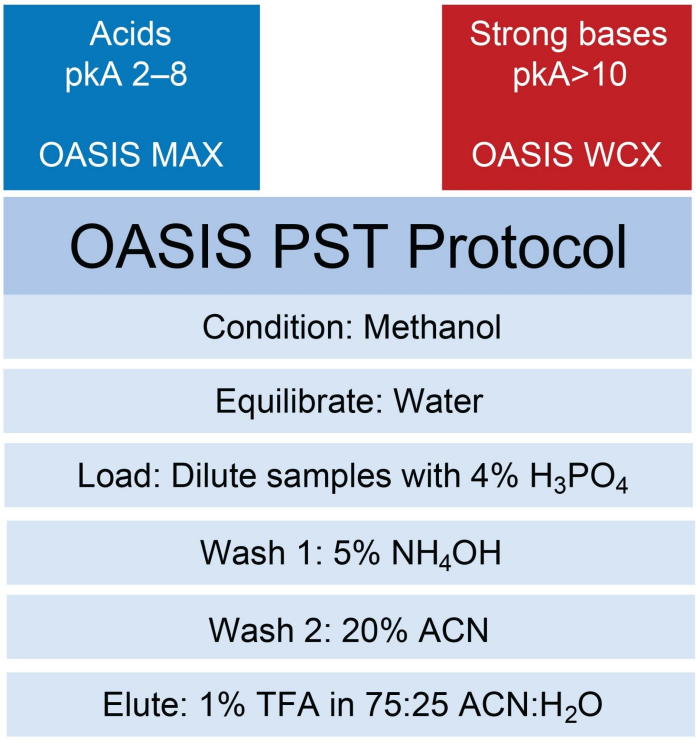

Figure 2. Oasis Peptide Separation Technology SPE protocol.

				MRM transitions used	
Name	**Sequence**	**Mol. wt**	**pI**	**Precursor (*m/z*)**	**Product (*m/z*)**
Goserelin	XHWSYXLRP	1269.4	8.75	635.7	249.3
Leuprolide	XHWSYLLRP	1209.4	8.75	605.7	299.4
Bivalirudin	FPRPGGGGNGDFEEIPEEYL	2180.3	3.91	1091.1	1531.6
Pramlintide	KCNTATCATQRLANFLVHSSNNFGPILPPTNVGSNTY	3949.0	8.90	988.4	968.1

Table 1. Peptides used in this work.

In this protocol, the automation of the sorbent selection method using Oasis Peptide Method Development 96-

well µElution Plate is demonstrated by extracting four analytes Leuprolide, Goserelin, Pramlintide, and Bivalirudin from spiked human plasma samples. Peptides typically produce good analyte recovery specific to one of the two Oasis mixed-mode sorbent chemistries WCX and MAX depending on characteristics of the peptide. In this case Leuprolide, Goserelin, and Pramlintide for which the average Pi is >8.00 showed good recoveries using the WCX sorbent, whereas Bivalirudin with average Pi of 3.91 showed better recovery using the MAX sorbent as shown in Figure 3.

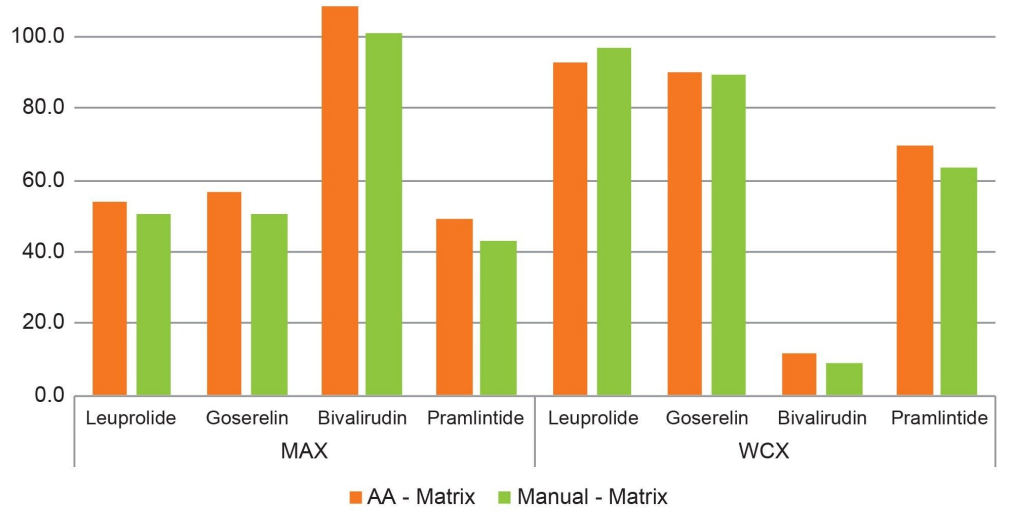

Figure 3. Graphical representation of peptide recoveries on MAX and WCX sorbents with manual vs automation process.

To demonstrate the reproducibility and robustness of the method transfer from manual process to automation, this protocol was performed both manually and also automated using the Andrew+ pipetting robot. The results generated with manual sample treatment vs automated process shown in Table 2 demonstrates that this protocol can be adapted onto the Andrew+ pipetting robot with ease and reliability. Narrow %RSD (<5%) between replicates (n=4) indicates pipetting precision of Andrew+ pipetting robot. Difference of <10% (5.4% max) between manual processing and automation gives the confidence to transfer methods onto the robot.

		Andrew+		Manual		Andrew+ vs manual
Sorbent	Peptide	Accuracy (%)	%RSD (n=4)	Accuracy (%)	%RSD (n=4)	%Difference
MAX	Bivalirudin	106.8	1.5	101.4	5.1	5.4
WCX	Leuprolide	92.8	4.8	96.7	4.5	3.9
	Goserelin	89.9	1.5	89.5	6.0	0.3
	Pramlintide	69.4	2.0	66.8	2.7	2.6

Table 2. Comparison of automation vs manual sample handling of peptides using PST-SPE protocol.

The Microplate vacuum+ extraction manifold with auto-controlled vacuum connected to the IKA VACSTAR pump provides stepwise and controlled pressures (pressure gradient) within the OneLab software. This is particularly useful for peptide SPE, where controlling the rate/time of adsorption and elution can be critical for good recovery. To demonstrate this, an experiment was performed where single setting of vacuum (60 sec at 700 mBar negative pressure) was used instead of stepwise increase during sample loading and elution steps (e.g., 20 sec at 950 mbar +30 sec at 800 mbar +10 sec at 700 mbar negative pressure). Complete protocol can be found in the OneLab protocol library under the title "Peptide SPE Method Development < https://onelab.andrewalliance.com/library/peptide-spe-method-development-pVnV50DW> ". Results shown in Table 3 demonstrates that single setting (non-gradient) pressure gives less recovery and more variable data when compared to auto-controlled gradient pressure.

		Gradient vacuum		Non-gradient vacuum	
Sorbent	Peptide	Accuracy (%)	%RSD (n=4)	Accuracy (%)	%RSD (n=4)
MAX	Bivalirudin	106.8	1.5	68.7	35.0
WCX	Leuprolide	92.8	4.8	63.1	13.1
	Goserelin	89.9	1.5	56.4	6.1
	Pramlintide	69.4	2.0	48.0	15.9

Table 3. Comparison of gradient vs non-gradient vacuum control of PST-SPE protocol.

Conclusion

This work demonstrates the ability of the Andrew+ pipetting robot to easily automate and execute a SPE method for therapeutic peptides.

- Excellent recovery was achieved for all peptides using the optimal SPE sorbent
- Narrow %RSD (<5%) between replicates (n=4) indicates precision in pipetting
- Difference of <10% (5.4% max) between manual processing and automation gives the confidence to transfer methods onto an Andrew+ system
- Auto-controlled vacuum enables better method control, resulting in improved recovery and less variability between replicates
- OneLab software is very user friendly and without much expertise one can easily write scripts and execute laboratory protocols. It is compliant-ready and protocols can be securely shared with fellow lab members or even collaborators in other labs, anywhere in the world
- The Andrew+ pipetting robot along with the newly developed script for Peptide SPE method development in the OneLab software allows for simple method transfer across users and laboratories

References

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