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Application Note

Otto SPEcialist Positive Pressure Manifold Enables Quick, Easy, and Reproducible Peptide Method Development and Quantification

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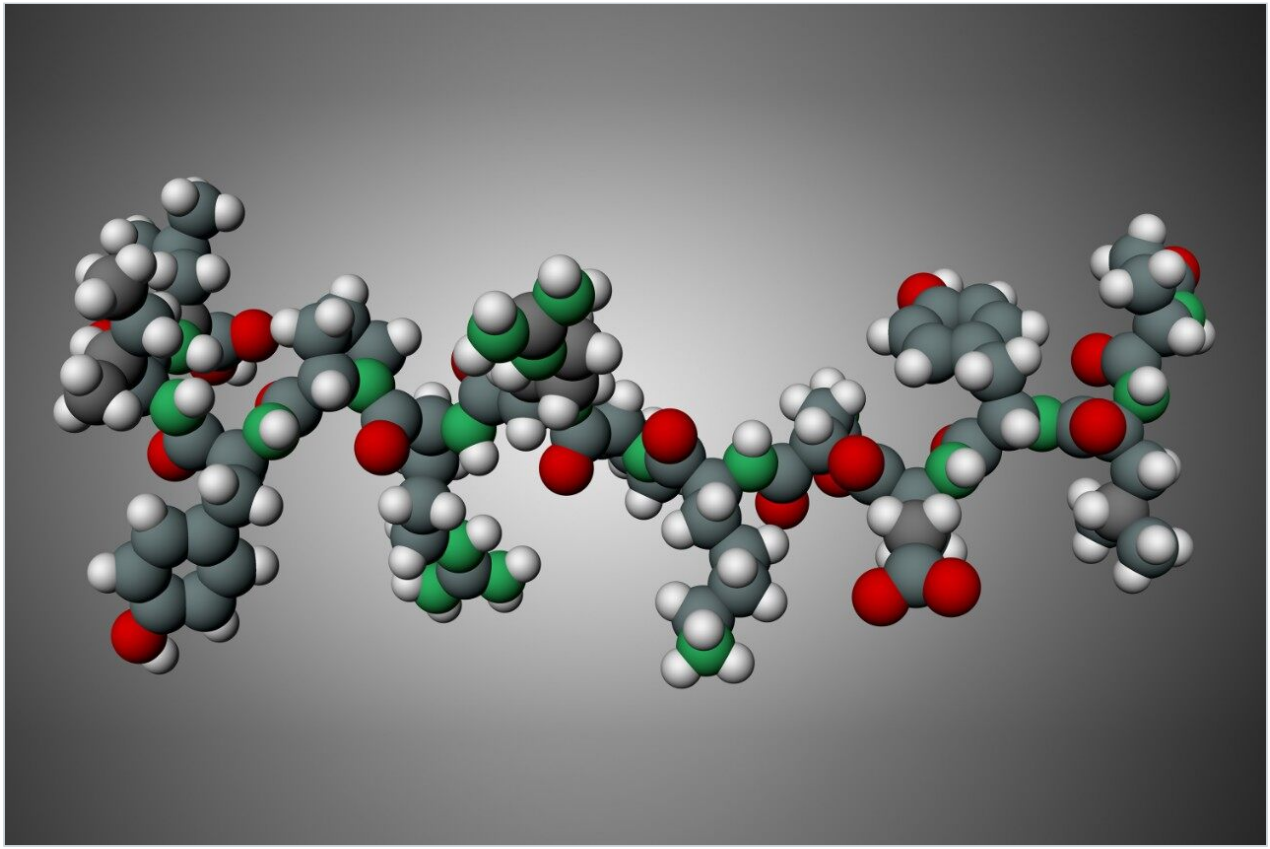

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The Otto SPEcialist Positive Pressure Manifold enables reproducible extraction of therapeutic and endogenous peptides from neat solution and biological matrices. Comparable results for peptide extraction reproducibility were observed as compared to a manual positive pressure manifold operated by an expert user.

Benefits

- Semi-automated SPE enables highly reproducible extraction of therapeutic and endogenous peptides
- Simple and reproducible method transfer

Introduction

Therapeutic and endogenous peptides are notoriously difficult to extract from biological matrices. It can be especially difficult for novice users to reproducibly extract peptides via solid phase extraction (SPE) due to the need to carefully control load, wash, and elution steps. Vacuum and positive pressure manifolds can be used for processing SPE, but these methods rely on the user to carefully control the flow of solvents and samples. Slow and reproducible flow rates are critical for the successful extraction of peptides, therefore inconsistent flow across sample preparations and users can lead to irreproducible results. The Otto SPEcialist positive pressure manifold automates the pressure control of each SPE step and is able to reproduce the same extraction conditions every time. In this work, we demonstrate the ability of the Otto SPEcialist to extract therapeutic peptides from both neat solutions and biological matrices with high reproducibility.

Results and Discussion

Four therapeutic peptides – Leuprolide, Goserelin, Bivalirudin, and Pramlintide – were prepared in neat solution and extracted using Waters OASIS Peptide Separation Technology (PST) SPE protocols for two ion exchange sorbents, MAX and WCX.[1] These anion and cation exchange sorbents are suitable for SPE

purification of most therapeutic and endogenous peptides. The peptides were prepared as a mixture at 50 ng/mL each and acidified with phosphoric acid. To assay recovery of these peptides, samples were either spiked with sample prior to SPE or post-SPE. A PST method development µElution SPE plate was conditioned with methanol and water, then loaded with the acidified samples (n=8). Samples were washed with ammonium hydroxide followed by an aqueous solution of acetonitrile and eluted with a mixture of acetonitrile, water, and trifluoroacetic acid. The recovery of these peptides on both MAX and WCX sorbents can be seen in Figure 1. Table 1 indicates the recoveries and reproducibility of each extraction using the two different sorbents. The sorbent of choice for each peptide is highlighted. These results are consistent with previous experiments performed with positive pressure by an experienced SPE user.

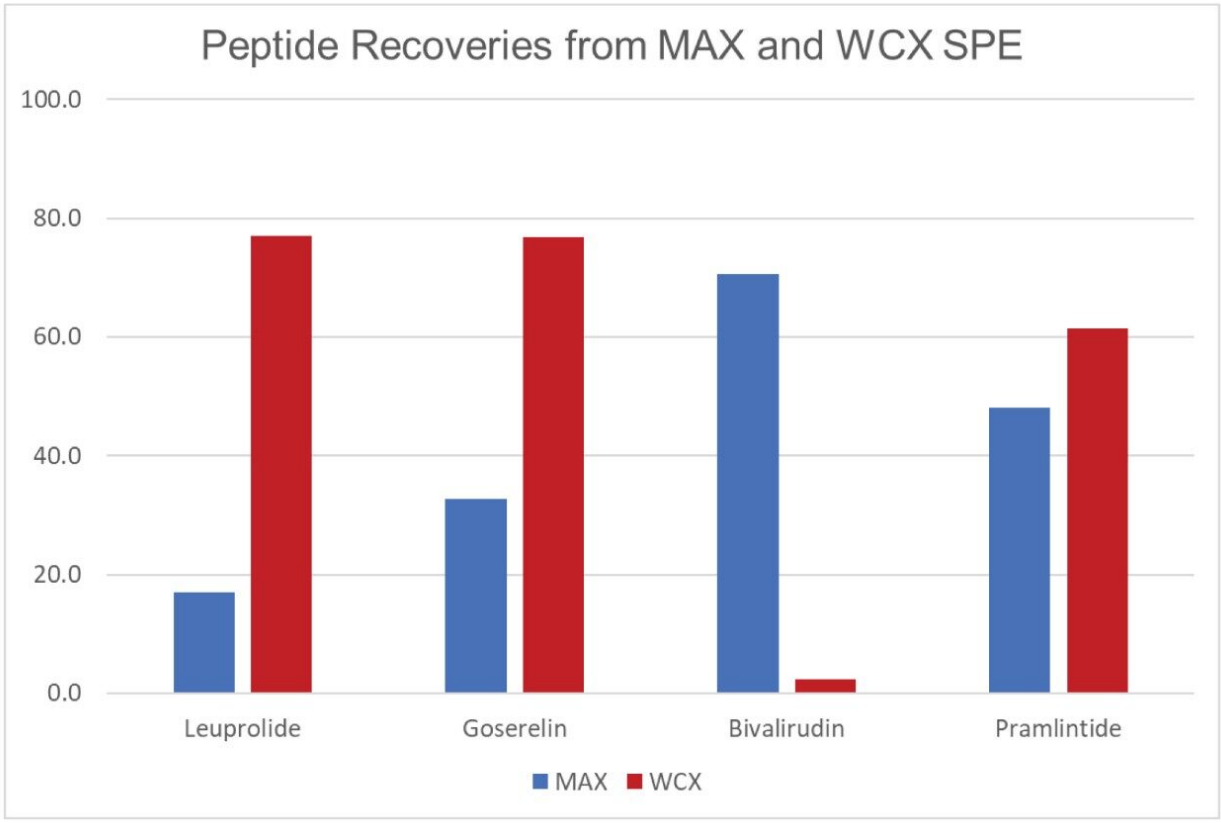

Figure 1. Leuprolide, Goserelin, Bivalirudin, and Pramlintide were extracted via MAX and WCX SPE using Otto SPEcialist.

Otto SPEcialist recoveries and % RSDs (n=8)					
SPE sorbent		Leuprolide	Goserelin	Bivalirudin	Pramlintide
MAX	% Recovery	17.0	32.8	70.6	48.1
	% RSD	26.0	14.5	6.4	10.0
WCX	% Recovery	77.0	76.8	2.3	61.5
	% RSD	2.7	12.3	81.9	0.8

Table 1. Leuprolide, Goserelin, Bivalirudin, and Pramlintide were extracted via MAX and WCX SPE. The %RSDs for each extraction show high reproducibility for the selected optimal sorbents for each peptide.

The Otto SPEcialist can also aid in reproducible extraction of peptides prepared in biological matrices. Pramlintide was prepared in rat plasma over the concentration range of 50–50,000 pg/mL. Quality control (QC) samples were prepared at low (75 pg/mL), medium (2,500 pg/mL), and high (40,000 pg/mL) levels (n=6) to assay extraction reproducibility across the entire standard curve range. Using a previously optimized method, WCX SPE was used to extract samples using both the Otto SPEcialist and a manual positive pressure manifold.[2] Figure 2 demonstrates the precision of the QC sample extraction. All %RSDs were <15%, easily meeting bioanalytical assay guidelines. The Otto SPEcialist achieved lower %RSDs <5% than the positive pressure manifold <10%. The Otto SPEcialist achieved comparable %RSDs to the positive pressure manifold, and in some cases better, such as at the low QC level of 75 pg/mL.

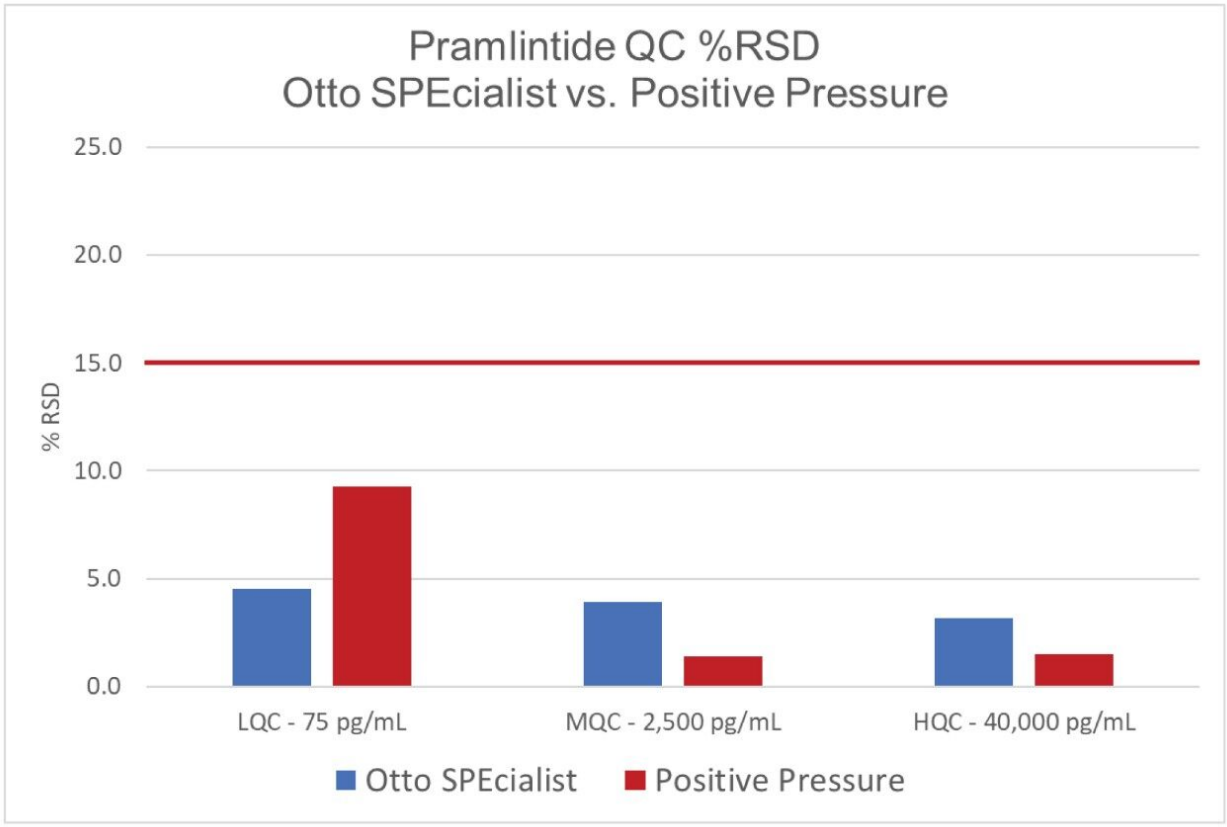

Figure 2. Pramlintide was extracted at low, medium, and high QC levels from rat plasma using Otto SPEcialist and a manual positive pressure manifold. %RSDs for each QC level were comparable between extraction methods.

Conclusion

This work demonstrates the ability of the Otto SPEcialist positive pressure manifold to achieve comparable results to a manual positive pressure manifold for the extraction of therapeutic peptides.

- The Otto SPEcialist achieved comparable recoveries and %RSDs for the extraction of therapeutic peptides from neat solution and biological matrix
- It is easily programmed and allows for simple method transfer across users and laboratories

References

1. Oasis Peptide Bioanalysis Reference Card. Waters Literature 720006298EN < https://www.waters.com/webassets/cms/library/docs/720006298en.pdf> .
2. Dunning CM, Lame M, Wrona MD, Haynes K. Development of a SPE LC-MS/MS Method Utilizing QuanRecovery Sample Plates with MaxPeak Performance Surfaces for the Bioanalytical Quantification of Pramlintide from Serum. Waters Application Note 720006527EN < https://www.waters.com/nextgen/us/en/library/application-notes/2019/spe-lc-ms-ms-method-quanrecovery-sample-plates-maxpeak-performance-surfaces-pramlintide-from-serum.html> .

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