

From Peptide Fractions to Pure, Dry Powders: Development of a Novel Automated Chromatographic Purification Process Supported by Solid-Phase Trapping

Pittcon 2016 1450-19

Robert Bucu¹, Tomoyuki Yamazaki¹, Tsutomu Okoba¹,
Eiichi Matsuo¹, Junichi Masuda¹, Yosuke Iwata¹,
Masayuki Nishimura¹, Tadayuki Nagashima²,
Sayoko Shigeto², Saori Saito², Patrick Reid², Keiichi Masuya²
¹Shimadzu Corporation, Kyoto, Japan;
²PeptiDream Inc., Tokyo, Japan

From Peptide Fractions to Pure, Dry Powders: Development of a Novel Automated Chromatographic Purification Process Supported by Solid-Phase Trapping

Introduction

There are many commercially available instruments that can be used to process preparative liquid chromatography (LC) fractions, recovering the pure compound of interest and discarding background from the mobile phase.

Some of these technologies utilize solid-phase trapping media to retain the compounds of interest while allowing the water, organic solvents and salt additives to be flushed to waste. Nearly two years ago, Shimadzu introduced a new instrument that leveraged this concept

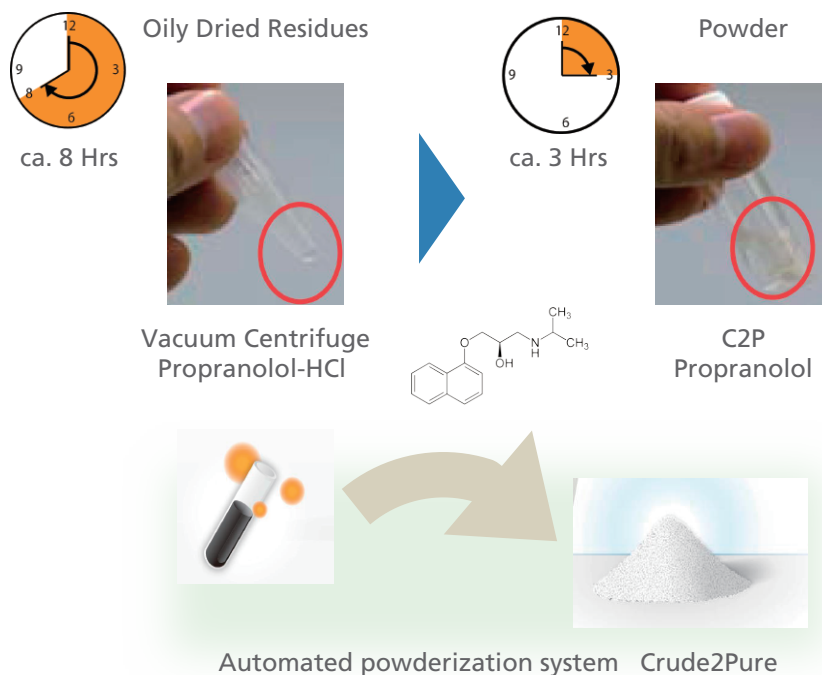
of solid-phase trapping and implemented a trapping column packed with a generically applicable resin that was appropriate for the retention of most small organic molecules. In addition to removing the mobile phase matrix background from the LC fraction, this instrument provided a mechanism to treat the retained compound on-column and recover it in a specific salt form as a highly pure and very dry powder, achieving better quality than traditional fraction dry-down techniques in significantly less time.

Chemical

Pharmaceutical
Medical

Fast Recovery of Highly Purified Powders from Liquid Prep LC Fractions

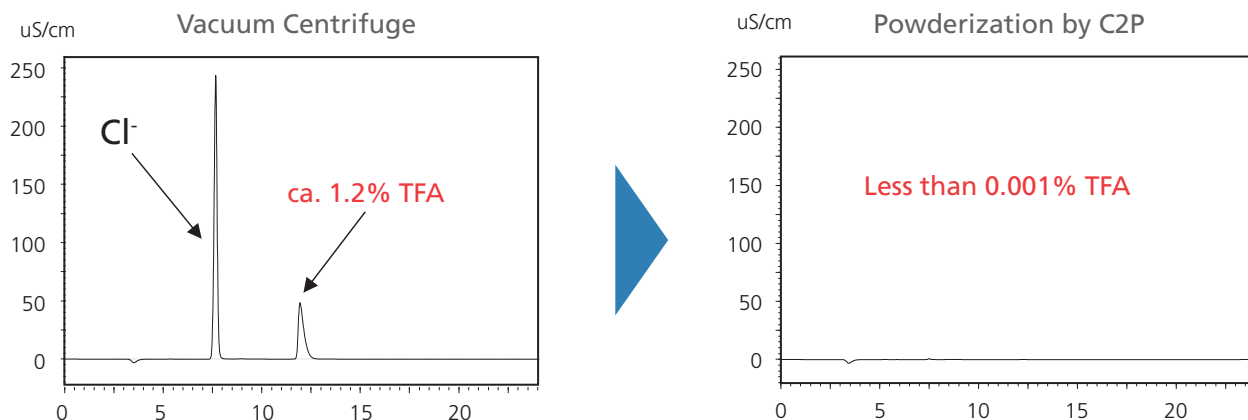
Liquid Prep LC fractions containing propranolol hydrochloride produce oily residues when dried using vacuum centrifugation, whereas C2P's process produces powder in less time.



From Peptide Fractions to Pure, Dry Powders: Development of a Novel Automated Chromatographic Purification Process Supported by Solid-Phase Trapping

Effective Removal of Residual Solvents and Counter-Ions in Liquid Fraction

Liquid fraction containing propranolol hydrochloride is concentrated and dried by vacuum centrifuge and C2P, respectively. Vacuum centrifuge obtained oily dried residues with 1.2% TFA left, whereas C2P realized highly-purified powder with almost no impurities.



Trapping and Recovery of Variety of Compounds with Wide LogP Range

C2P trapping columns achieve ca. 100% recovery regardless of compound polarity.



Hydrophobic



Hydrophilic

Compound	logP	C2P Recovery (%)
Verapamil	5.23	96
Dibucaine	3.79	96.1
Ketoprofen	3.29	95.1
Propranolol	3.03	96.3
Quinine	2.82	100.1
Metronidazole	-0.15	99.4

From Peptide Fractions to Pure, Dry Powders: Development of a Novel Automated Chromatographic Purification Process Supported by Solid-Phase Trapping

Materials and Methods

Crude2Pure (C2P) trapping and recovery systems (Shimadzu) were used to evaluate recovery rates and the extent of powderization for two different trapping column resins. 10 mg of Cyclosporin A in 5 mL of water (40) / acetonitrile (60) solution was loaded onto each column while being diluted with a solution of 0.1% TFA (44) / (DMSO (9) / Acetonitrile (1)) (56). The loaded

columns were washed with water (98) / acetonitrile (2) and then recovered with dichloromethane. Dried samples were analyzed by HPLC and/or weighed to calculate the recovery rate for each column.

Detailed characteristics of the trapping columns used are described in the next table.

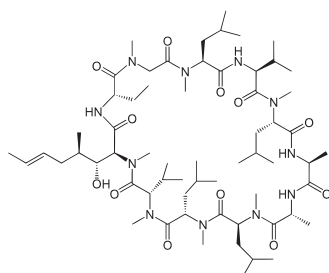
Parameter	Standard Resin	New Resin
Target	Low-molecular weight chemicals ► MW < 600	Peptides with higher molecular weight ► MW 1000 - 2000
Resin	Styrene-divinylbenzene polymer	Methacrylate polymer
Mean Diameter	20 - 30 μ m	20 - 30 μ m
Specific Surface Area	1100 m ² /g	500 m ² /g
Pore Volume	0.15 mL/g	1~2 mL/g
Pore Size	1 nm	20 – 30 nm

Results

The original C2P system utilizes a specially cross-linked styrene-divinylbenzene polymer with small pores as a standard column resin. It has a large specific surface area and therefore promotes increased interaction between the resin and target small molecule analytes. However, a model peptide (Cyclosporin A) demonstrated significantly

less binding to the column. The working hypothesis was that the molecular size of the peptide is too large compared to the resin pore size. A new resin with larger pore size was evaluated and demonstrated significantly improved recovery.

Model peptide	Characteristics	MW	Recovery Rate (Standard Resin)	Recovery Rate (New Resin)
Cyclosporin A	Hydrophobic Cyclic Peptide	1,202	42%	82% (n=9)



Cyclosporin A



Dried Sample (C2P)

From Peptide Fractions to Pure, Dry Powders: Development of a Novel Automated Chromatographic Purification Process Supported by Solid-Phase Trapping

Discussion and Conclusions

We demonstrated through this study that an optional trapping column using a new resin expands the capability of the C2P instrument to include peptides. Additional application expansions may be possible by choosing appropriate combinations of particle material and pore size.

Summary

C2P Instruments provide ...

- High capacity trapping columns that accommodate a wide range of analytes
- Efficient trapping and high recovery for both small organic molecules and peptides
- Purification of target compounds from Prep LC fractions, removing solvent-derived contaminants
- Fast recovery of targets as high purity and very dry powders, regardless of fraction's water content
- Powderization of peptides with large molecular weight using a new trapping column

First Edition: March, 2016



For Research Use Only. Not for use in diagnostic procedure.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, product/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation or its affiliates, whether or not they are used with trademark symbol "TM" or "®". Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services. Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.