RP-HPLC
Report

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PREP-RP-HPLC: ADDITIONAL/ SURROGATE STATIONARY PHASES (ASP/ SSP) BOUND TO REVERSED PHASE COLUMNS RESULT IN 10-FOLD INCREASE IN SAMPLE LOADABILITY

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Abstract

There are only two ways to increase the amount of sample that can be purified by preparative reversed phase high performance liquid chromatography (Prep-RP-HPLC) in a single run in spite of recent advances in the production of reversed phase derivatized silica stationary supports: (1) The traditional approach is to use a bigger column (greater amount of stationary phase); and (2) Use displacement chromatography which (while labor intensive to develop) uses the stationary phase more effectively. This invention describes a unique Prep-RP-HPLC technique that uses a C-18/ C-8 derivatized silica coated with a hydrophobic quaternary ammonium salt or quaternary phosphonium salt to result in 7 to 12 fold increase in sample loading (of the crude mixture of organic compounds including synthetic crude peptides) in contrast to the conventional Prep-RP-HPLC technique. This increase in sample loading capacity and output is due to the additional surrogate stationary phase characteristic of the C-18/ C8 adsorbed (bound) quaternary salt. The quaternary surfactant is bound to the C-18/ C-8 chains of the stationary phase via Van der Waals forces (hydrophobic interactions) and ionic interactions with the residual silanols of the stationary phase.

Introduction

Reversed phase high performance liquid chromatography (RP-HPLC) is used ubiquitously in academic institutions, forensic laboratories, fine chemicals, and pharmaceutical industries etc. for the analysis, characterization, separation, purification and/or isolation of small organic molecules, natural products, and biologically active molecules such as polypeptides, proteins, and nucleotides. In the pharmaceutical industry, analytical RP-HPLC is used for the release and characterization of raw materials, intermediates, and active pharmaceutical ingredients (APIs). Preparative reversed phase high performance liquid chromatography (Prep-RP-HPLC) is used for the commercial production of Peptide APIs, and most other complex APIs that are not amenable to crystallization.

Results and Discussion

Preparative RP-HPLC in the elution mode is limited by the loading capacity of the analyte. In the elution preparative RP-HPLC mode, the typical loading capacity of synthetic peptides is in the range of 1 to 2 mgs per ml of packed column volume (viz., 0.1% to 0.2% with respect to total column volume). Table 1 lists the performance of surrogate stationary phase aided Prep-RP-HPLC in contrast to standard Prep-RP-HPLC using crude Leuprolide as example, and tetra-n-butylammonium hydrogen sulphate (TBAHS) as ASP/ SSP.

Table 1

Purification of Leuprolide: Comparison of the Surrogate Stationary Phase aided Prep-RP-HPLC with the Standard Prep-RP-HPLC

Entry #	Prep RP-HPLC Method	Column Dimensions (ID x L)	Total Column Volume (mL)	Input Crude API (g)	Output Pure API (g)	% Yield	% Purity by HPLC (USP Method)	Relative Loading Capacity
1	Standard RP-HPLC [Comparitive Example]	YMC, ODS-AQ (50 mm x 250 mm, C18, 10 u, 120 Å pore diameter)	491.0	4.0 g	1.20 g	30.0 %	99.86 %	1.0
2	SSP-Purification Method [TBAHS-SSP]	Waters Symmetry (19 mm x 50 mm, C8, 5 u, 100 Å pore diameter)	14.2	1. 4 g	0.42 g	30.0 %	99.79 %	12.1
3	SSP-Purification Method [TBAHS-SSP]	Discovery Bio Wide Pore (10 mm x 250 mm, C8, 5 u, 300 Å pore diameter)	19.6	1. 2 g	0.32 g	26.7 %	99.73 %	6.7

The purified product (Leuprolide) output of the standard Prep-RP-HPLC is 2.45 mg/ mL of column volume: In contrast the purified product output of the surrogate stationary phase aided Prep-RP-HPLC is 29.6 mg/ mL of column volume (table 1, entry 2) and 16.3 mg/ mL of column volume (table 1, entry 3). These results suggest that loadings of 7 to 12 times capacity of conventional prep-RP-HPLC are achievable with the processes described in the present invention.