Improving Compound Integrity: Accelerated Extraction of Compounds from Non-volatile HPLC Eluents and In Situ Freebasing of TFA Salts

Application Note

Authors

Paul Boguszewski and Jane Wheeler
Agilent Technologies, Inc.

Introduction

As the dependence on preparative HPLC for final compound purification has grown in recent years, the pharmaceutical industry is experiencing a new bottleneck: compound isolation. Using current techniques, large volumes of HPLC fractions have to be evaporated under high vacuum and elevated temperatures, in some cases for as long 16-24 hours. The effects of this technique on compound integrity are of concern to the pharmaceutical industry.

The use of ion-pairing reagents is common in most drug molecule purifications as they often aid loadability, selectivity and resolution in a chromatographic system. One of the most popular ion-pairing reagents is trifluoroacetic acid (TFA). This organic acid is regarded as the universal ion-pair reagent in most preparative separations of amine containing or basic polar compounds.

While TFA is an excellent choice for chromatographic efficacy, it does have some limitations once compound isolation is required. TFA is relatively non volatile, which means that during eluent evaporation, the overall concentration of TFA will increase, posing a risk of hydrolysis for certain functional groups or structures contained within the molecule of interest.

However, these issues are overcome by using FlowTrap columns. FlowTrap columns are ultra retentive, high capacity, hydrophobic columns for the capture and release of small pharmaceutical molecules. Once trapped, the desired analyte can be back eluted using a much smaller volume of solvent. FlowTrap columns are highly effective in removing TFA and reducing dry down times.

Agilent Technologies

Materials and Reagents

940-LC fitted with a Column Valve Module
Detection: UV 280 nm
Trapping Column: FlowTrap, 4.6 x 150 mm
(p/n PL1560-3M07)

TFA Removal from Caffeine and Metronidazole Containing Fractions

Loading Conditions

Loading Eluent: (12 mL)
Metronidazole: 2 mg/mL in water + 0.1% TFA
Caffeine: 2 mg/mL in water + 0.1% TFA

Wash Conditions

Method A:

R.O. Water Flow Rate: 4 mL/min for 4 min

Method B:

2M NH_3, Flow Rate: (4 mL/min) for 2 min then
R.O. Water, Flow Rate: (4 mL/min) for 4 min
Elution: 100% CH_3CN over 5 min flow rate: (4 mL/min)

A series four flow trapping runs were conducted with the two compounds. Once the compound was immobilized onto the column two different washing methods were evaluated; A) a standard aqueous wash and B) a basic wash using 2M NH_3 aqueous solution (Figure 1). Each sample was isolated and evaporated to dryness before undergoing ^{19}F NMR analysis to determine the relative content of residual TFA compared to the original loading solution.

Results and Discussion

Results gained from the ^{19}F NMR analysis show that that an on-column washing protocol is very effective in removing TFA from the mobile phase.

Compound	Washing Protocol	Amount of TFA Removed
Metronidazole	A: Water	88%
Metronidazole	B: Base	>99%
Caffeine	A: Water	94%
Caffeine	B: Base	>99%

In the case of metronidazole and caffeine, even a simple water wash was good enough to remove the majority of, and presumably unbound, TFA in the sample. Moving to the base wash protocol yielded freebase samples, with no detectable TFA in the NMR analysis.

One other interesting observation is that the back elution peak profile is much cleaner and narrower once the compound has undergone freebasing in situ. This means that much tighter elution volumes can be achieved in end sample collection.

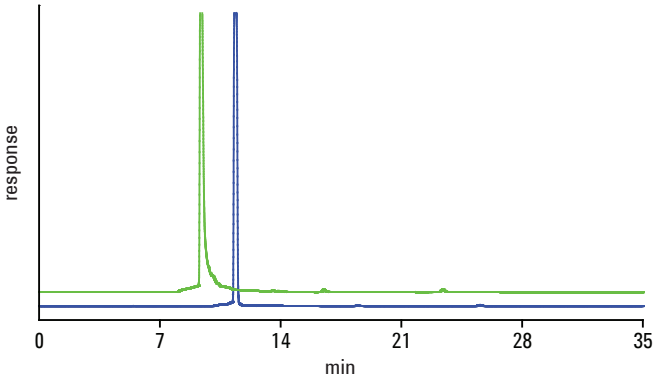

Figure 1. Elution chromatograms of a 26 mg Metronidazole load after the A) Water washing method (green) and B) Base washing method (blue). The elution peak of the freebase Metronidazole is significantly sharper.

Conclusion

FlowTrap columns offer significant productivity enhancements by reducing dry down times of HPLC fractions affording much simpler compound isolation. If the HPLC separation requires an ion-pairing reagent such as TFA, then by adopting the simple washing protocol, more than 99% of TFA can be removed from the system. This is highly advantageous in applications where TFA salts are to be avoided due to potential compound degradation issues. This technology is particularly attractive to users who then go on to store their compound in a repository environment where it is commonplace to re-solvate into a solution of DMSO.

www.agilent.com/chem

This information is subject to change without notice.

Published in UK, June 3, 2011
5990-8368EN

Agilent Technologies