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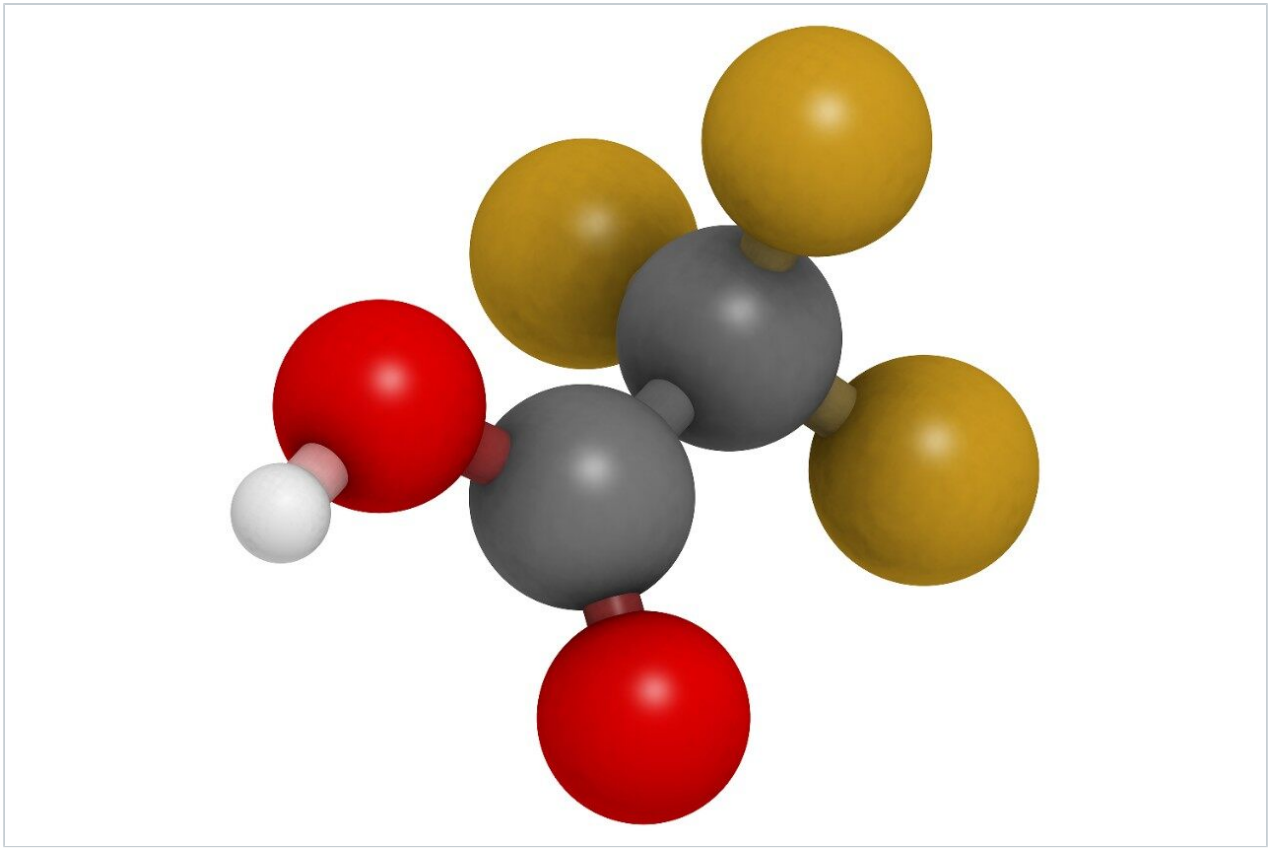
Nota de aplicación

Removal of TFA from Prep-LC Fractions using PoraPak Rxn CX; Isolation of the Purified Free-Base Product

Michael S. Young

Waters Corporation



Abstract

Medicinal compounds are often isolated and purified from reaction mixtures using preparative scale liquid chromatography (Prep-LC). In many cases, acidic modifiers, such as trifluoroacetic acid (TFA) are utilized to optimize the Prep-LC separation. However, residual TFA has been linked to degradation of basic compounds synthsized for medicinal investigration

We demonstrate the use of a PoraPak Rxn CX cartridge to remove TFA from a Prep-LC fraction of a reductive amination product, NMBA. The product was recovered exclusively in the elute fraction (>95% recovery) and all TFA was removed in the wash fractions. The product amine is eluted as a freebase in a methanol/ammonia solvent. Simple evaporation of this solvent yields a purified product free of TFA.

Introduction

Medicinal compounds are often isolated and purified from reaction mixtures using preparative scale liquid chromatography (Prep-LC). In many cases, acidic modifiers, such as trifluoroacetic acid (TFA) are utilized to optimize the Prep-LC separation. If the synthetic chemist simply removes the attendant mobile phase from the collected fraction, an equivalent amount of TFA will remain associated with any basic synthesized compound; the compound is recovered as a TFA salt. Residual TFA has been linked to degradation of basic compounds synthesized for medicinal investigation.

PoraPak Rxn CX is a polymeric strong cation-exchange resin. If a Prep-LC fraction is applied to a PoraPak Rxn CX cartridge, the basic medicinal compound is retained on the sorbent while the undesired TFA passes through and is discarded. The cartridge is washed with a mild formic acid/water solution to remove any trace TFA from the sorbent, and is then washed with methanol to remove water and any remaining acid from the previous wash.

Experimental

TFA Removal: A 4-mL Prep-LC fraction was collected, this fraction contained a product amine, N-

(naphthylmethyl)meth-oxybenzylamine (NMBA) synthesized using an amide coupling reaction. This fraction also contained 0.05% TFA in 50/50 methanol/water. Removal of TFA and water was accomplished using a PoraPak Rxn CX 6 cc 400 mg cartridge (Part Number: 186004541) according to the following protocol. All pro-cesses were accomplished using gravity flow. Approximately 2 mg of product NMBA was processed. The 400-mg cartridge has a maximum retention capacity of approximately 40 mg.

Steps:	Solvent
Condition:	4 mL 0.05% TFA in 50/50 methanol/water
Load:	4 mL of Prep-LC fraction
Wash 1:	4 mL of 2% formic acid in water
Wash 2:	4 mL of methanol
Elute:	4 mL 5% ammonia in methanol

UPLC-MS Confirmation of TFA Removal: A 10 µL portion of each of the fractions; load, wash and elute were diluted to 1 mL with 10/90 acetonitrile/water (0.01% formic acid). The resulting samples were analyzed using UPLC-MS(MS). This analysis was performed using a Waters ACQUITY UPLC System interfaced with a Waters Quattro Premier XE tandem mass spectrometer. Figure 1 gives UPLC conditions and MS transitions used for the determina-tion of NMBA product using positive electrospray ionization (ESI+) and for TFA using negative electrospray ionization ((ESI-). Figure 2 shows LC-MS chromatograms obtained for the load, wash and elute fractions, demonstrating that TFA was completely removed from the product using the PoraPak Rxn CX cartridge.

MS Transitions

Waters Quattro Premier™ XE

TFA 112.8 > 68.8 (ESI-)

NMBA 278.2 > 121.0 (ESI+)

UPLC Conditions

Column:	1.7 µm, 2.1 x 100 mm ACQUITY UPLC® BEH Shield RP18
Part Number:	186002854
Temperature:	30 °C
Injection Volume:	2 µL
Mobile Phase	A: 0.01 % formic acid
	B: 80:20 acetonitrile:water

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.42	95	5	Initial
7.00	0.42	1	99	6
7.60	0.70	1	99	1
7.80	0.42	95	5	1
8.30	0.60	95	5	1
8.80	0.42	95	5	6

Figure 1. UPLC-MS(MS) conditions for this study

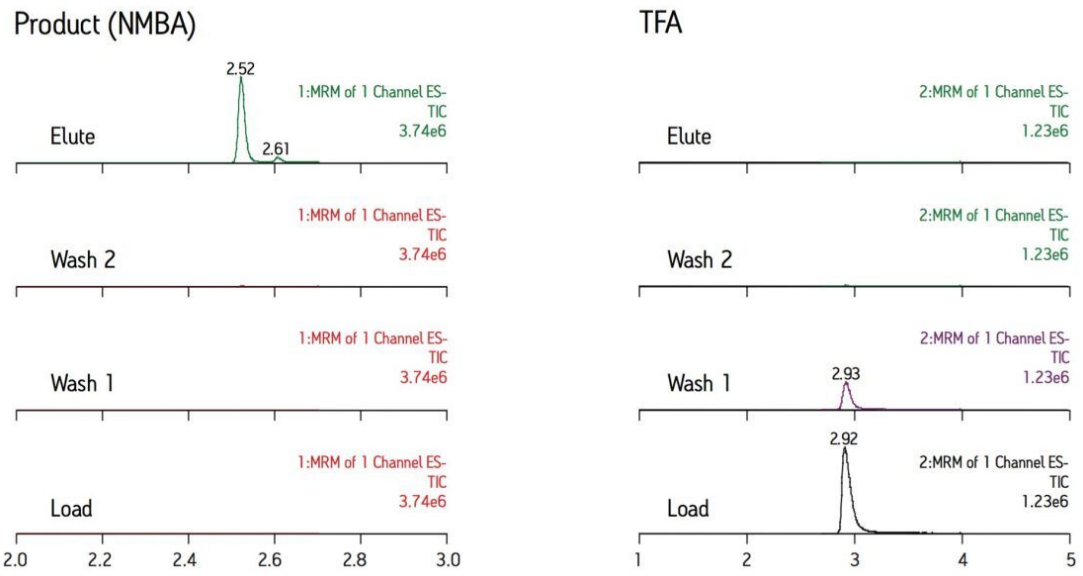


Figure 2. LC-MS(MS) chromatograms showing product only in the elute fraction and all TFA in the load and wash fractions

Conclusion

Residual TFA has been linked to degradation of basic compounds synthesized for medicinal investigation. A PoraPak Rxn CX Cartridge was used to remove TFA from a Prep-LC fraction of a reductive amination product, NMBA. The product was recovered exclusively in the elute fraction (> 95 % recovery) and all TFA was removed in the wash fractions.

The product amine is eluted as a freebase in a methanol/ammonia solvent. Simple evaporation of this solvent yields a purified product free of TFA.

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Available for purchase online:

PoraPak Rxn CX 6 cc Vac Cartridge <
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