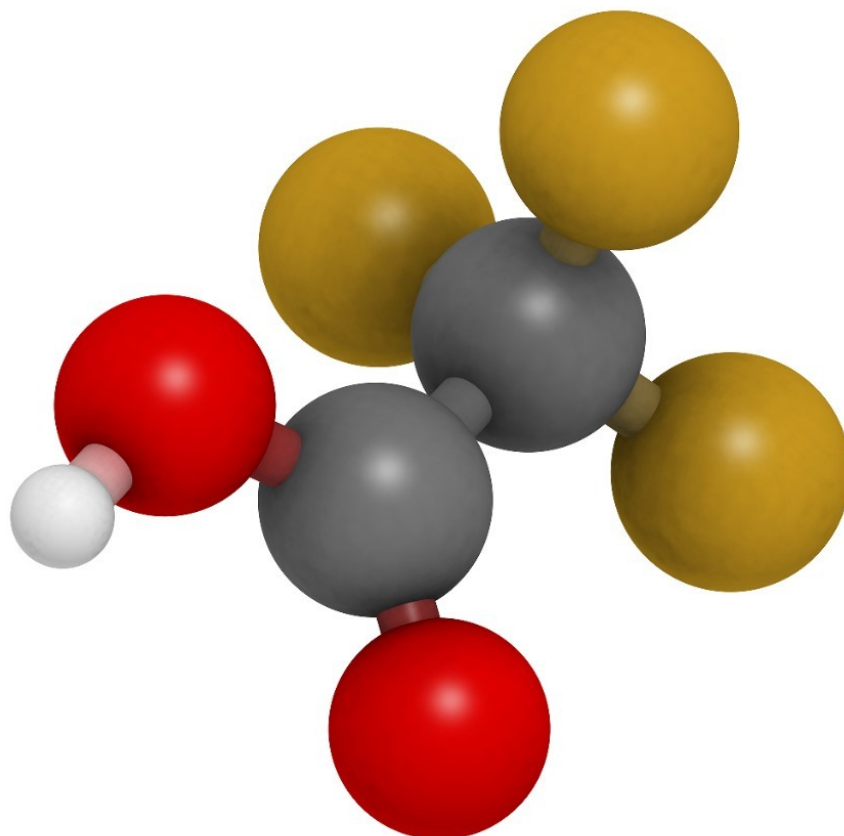


## Removal of TFA from Prep-LC Fractions using PoraPak Rxn CX; Isolation of the Purified Free-Base Product

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### Abstract

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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 synthesized for medicinal investigation

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)methoxybenzylamine (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 processes 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  $\mu$ L 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 determination 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

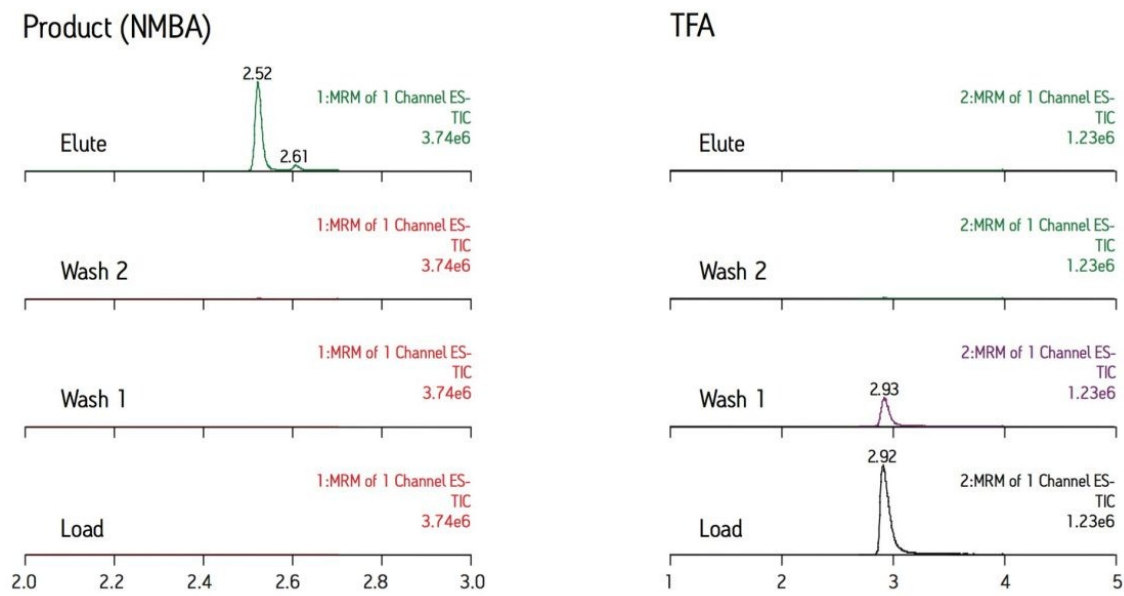


Figure 2. LC-MS(MS) chromatograms showing product only in the elute fraction and all TFA in the load and wash fractions

## 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.

## Featured Products

- [ACQUITY UPLC System <https://www.waters.com/514207>](https://www.waters.com/514207)

Available for purchase online:

- [PoraPak Rxn CX 6 cc Vac Cartridge <https://www.waters.com/waters/partDetail.htm?partNumber=186004541>](https://www.waters.com/waters/partDetail.htm?partNumber=186004541)

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