

Application Note

## Rapid Purification of Positron Emission Tomography (PET) Agent with Short Half Lives by Preparative SFC

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## Abstract

In this application note we use a RAC/DMRAC mixture as an example to demonstrate the use of preparative SFC for the rapid purification of raclopride. The advantages of SFC for purifying radiolabeled imaging agents are discussed, including speed, fast dry down, and the ability to alter the elution order for high purity compound recovery.

### Benefits

- Through the purification of a PET agent, raclopride, from its precursor, it is demonstrated that supercritical fluid chromatography (SFC) offers a wide range of selectivity, often enabling a desirable elution order for high purity target compound recovery.
- Furthermore, SFC provides shorter overall process time, including both chromatography and post purification dry down, resulting in a significant gain in the final yield of the high value product.
- SFC holds great promise to become an integral part of radio-pharmaceutical research, as well as for other purification applications demanding high speed and purity.

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## Introduction

Positron Emission Tomography (PET) is an imaging technique for the diagnosis and treatment of a variety of diseases, as well as for drug development. The imaging scans involve the use of radioactive tracer materials containing  $^{11}\text{C}$ ,  $^{15}\text{O}$ ,  $^{13}\text{N}$ , or  $^{18}\text{F}$ , all with relatively short half lives. To minimize the decay loss, the radiotracers need to be synthesized, purified, and sterilized within two to three half lives before being administered to the human or animal subject.

Raclopride is a commonly used PET ligand for studying the  $D_2$  receptor system in the brain.<sup>1</sup> The positron emitting isotope  $^{11}\text{C}$  ( $t_{1/2} = 20.4$  min) is typically incorporated into the raclopride (RAC) molecule via methylation of its precursor desmethyl raclopride (DMRAC), as shown in Figure 1. Currently, raclopride is purified by preparative reverse phase liquid chromatography (RPLC).<sup>2-4</sup>

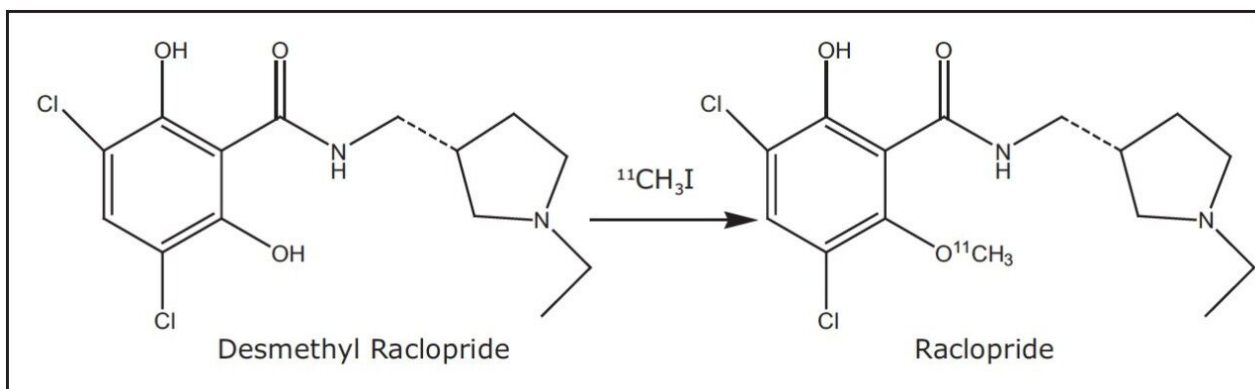


Figure 1. Reaction scheme for  $^{11}\text{C}$ -labeled synthesis of raclopride from desmethyl raclopride.

Herein, we use a RAC/DMRAC mixture as an example to demonstrate the use of preparative SFC for the rapid purification of raclopride. The advantages of SFC for purifying radiolabeled imaging agents are discussed, including speed, fast dry down, and the ability to alter the elution order for high purity compound recovery.

## Experimental

### Analytical SFC method conditions

Co-solvent:	Methanol with 3% (v/v) 7N $\text{NH}_3$ /methanol
Temp.:	40 °C
Back pressure:	120 bar
Injection vol.:	10 $\mu\text{L}$
Flow rate:	3.0 mL/min
PDA/UV:	220 to 300 nm

Isocratic:	30%
Columns:	Diol, Propylpyridyl Urea (PPU), Cyano and Phenyl (4.6 x 150 mm, 5 µm)

### Preparative SFC method conditions

Co-solvent:	Methanol with 3% (v/v) 7N NH <sub>3</sub> /methanol
Temp.:	40 °C
Back pressure:	120 bar
Injection vol.:	80 µL
Flow rate:	8.6 mL/min
PDA/UV:	239 nm
Isocratic:	22%
Column:	Cyano (7.8 x 75 mm, 5 µm)

#### Instrumentation:

Analytical method development was done on a Waters Resolution SFC MS System controlled by MassLynx Software. The system consists of the following components: Fluid Delivery Module (FDM), Automated Back Pressure Regulator (ABPR), Alias Autosampler, 10-port Analytical-2-Prep Column Oven, 2998 Photodiode Array (PDA) Detector, and 3100 MS Detector.

All preparative experiments were performed on an Investigator SFC System controlled by ChromScope Software. The system consists of the following components: FDM, ABPR, Alias Autosampler, 10-port Analytical-2-Prep Column Oven, 2998 PDA Detector, make-up pump, and six-position Fraction Collection Module.

#### Chemicals:

Raclopride and desmethyl raclopride were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and NH<sub>3</sub>/methanol solution were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA).

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## Results and Discussion

Alkylation of a non-radioactive precursor is a common synthetic route to incorporate the radiolabels into the radiotracers, as in the case of the synthesis of raclopride shown in Figure 1. As a result, the radiolabeled final product is more hydrophobic than the precursor. For example, the LogPs for desmethyl raclopride (precursor) and raclopride (product) are 1.93 and 2.20, respectively. Furthermore, in order to drive the completion of the limiting radiolabel agent, a large excess of precursor is often used. The resulting crude reaction mixture, therefore, contains a relatively low quantity of radioactive product in a large excess of unreacted precursor.

With a classic C<sub>18</sub> column in RPLC, desmethyl raclopride elutes off the column before raclopride. However, due to the wide dynamic range of the crude mixture, there is a high likelihood that desmethyl raclopride tails into the raclopride peak; thus, contaminating the final formulation. The issue is further compounded by the time constraint imposed by the short half life of <sup>11</sup>C. It is, therefore, highly desirable to have raclopride elute off the column before desmethyl raclopride. Obviously, this can be achieved by normal phase liquid chromatography (NPLC), but the use of toxic organic solvents in NPLC is prohibitive for its adoption in such purification. Hydrophilic interaction chromatography (HILIC) was attempted, but there was no success for the RAC/DMRAC mixture.<sup>3</sup> Gillings *et al.* demonstrated the elution order reversal in RPLC by using a bonded C<sub>16</sub> alkylamide column in RPLC to induce stronger polar interactions. The total chromatography time was 20 min.<sup>4</sup>

To that end, SFC has the potential to resolve all aforementioned challenges. By primarily using CO<sub>2</sub> and alcohols as the mobile phase, SFC eliminates the use of toxic organic solvents and is deemed a "greener" normal phase chromatography. SFC has also proven advantageous in speed and fast dry down in purification. Figure 2 shows the SFC/UV chromatograms of the RAC/DMRAC mixture using four different columns under isocratic conditions. The peaks were identified using MS (results not shown).

Clearly, all four columns yielded baseline resolutions between RAC and DMRAC in less than 5 min. The elution order varied depending on the stationary phases. When a phenyl column was used, shown in Figure 2A, the elution order is the same as those observed with classic RPLC.<sup>2</sup> However, when polar stationary phases were used – such as diol, PPU, and Cyano, shown in Figure 2B-D – all three chromatograms

exhibited the desired elution order, *i.e.*, target compound before the precursor. The stationary phases used in SFC encompass both polar and non-polar phases, offering a great deal of flexibility in altering the elution order of the analytes.

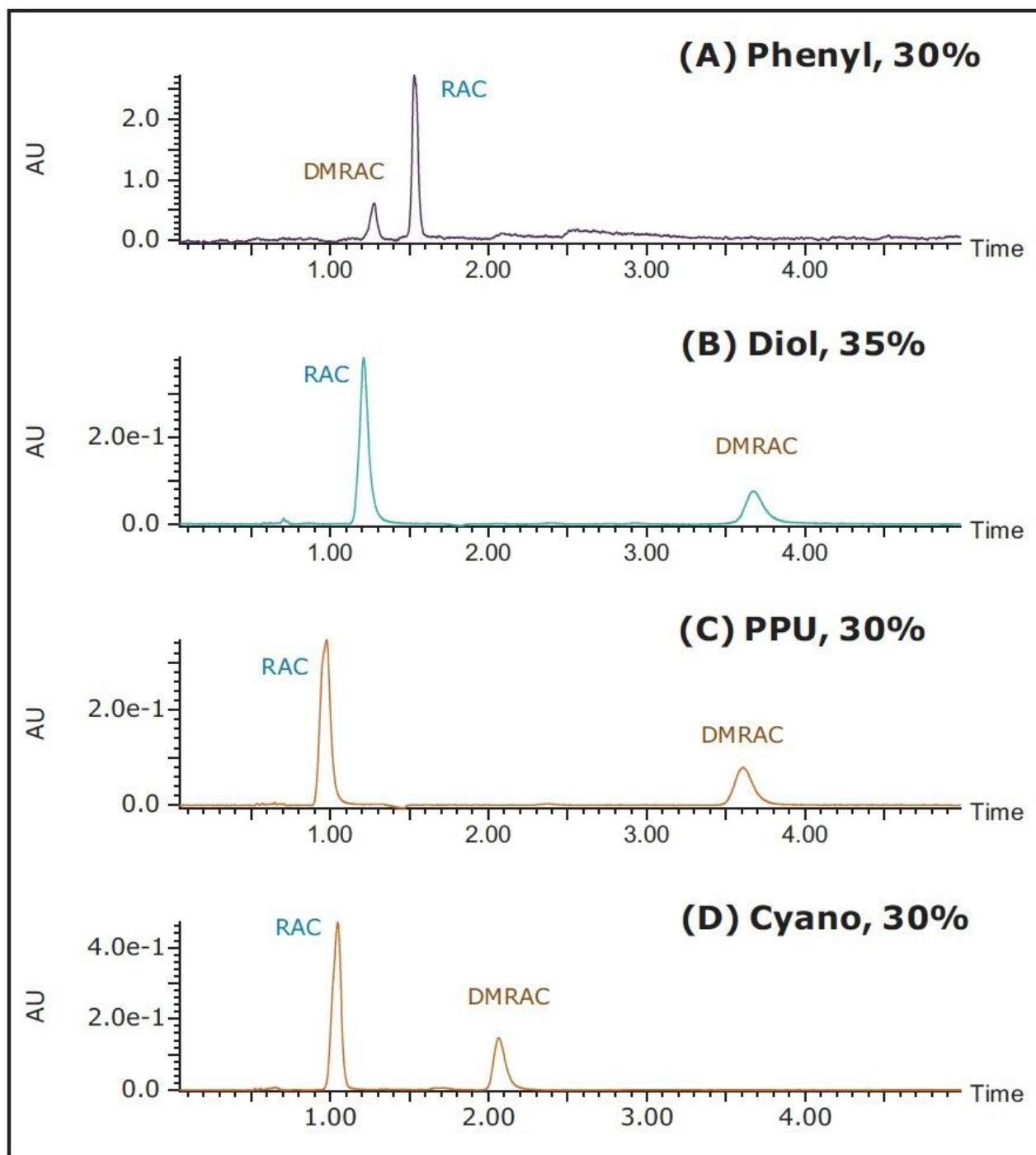


Figure 2. SFC/UV chromatograms of a RAC/DMRAC mixture using different columns. Key experimental parameters are listed in the experimental section.

Based on the analytical runs, the Cyano column was selected for scale-up purifications, due to shorter run time and relatively lower co-solvent percentage. To further reduce the preparative run time, a short Cyano

column (75 mm in length) was used, and the co-solvent percentage was adjusted accordingly. Figure 3 is a representative chromatogram for the purification of a RAC/DMRAC mixture. Ensuing fraction analyses indicated 100% purity for both fractions. It is noteworthy that NH<sub>3</sub>/methanol was used as the additive in the mobile phase based on its lower toxicity and higher volatility.

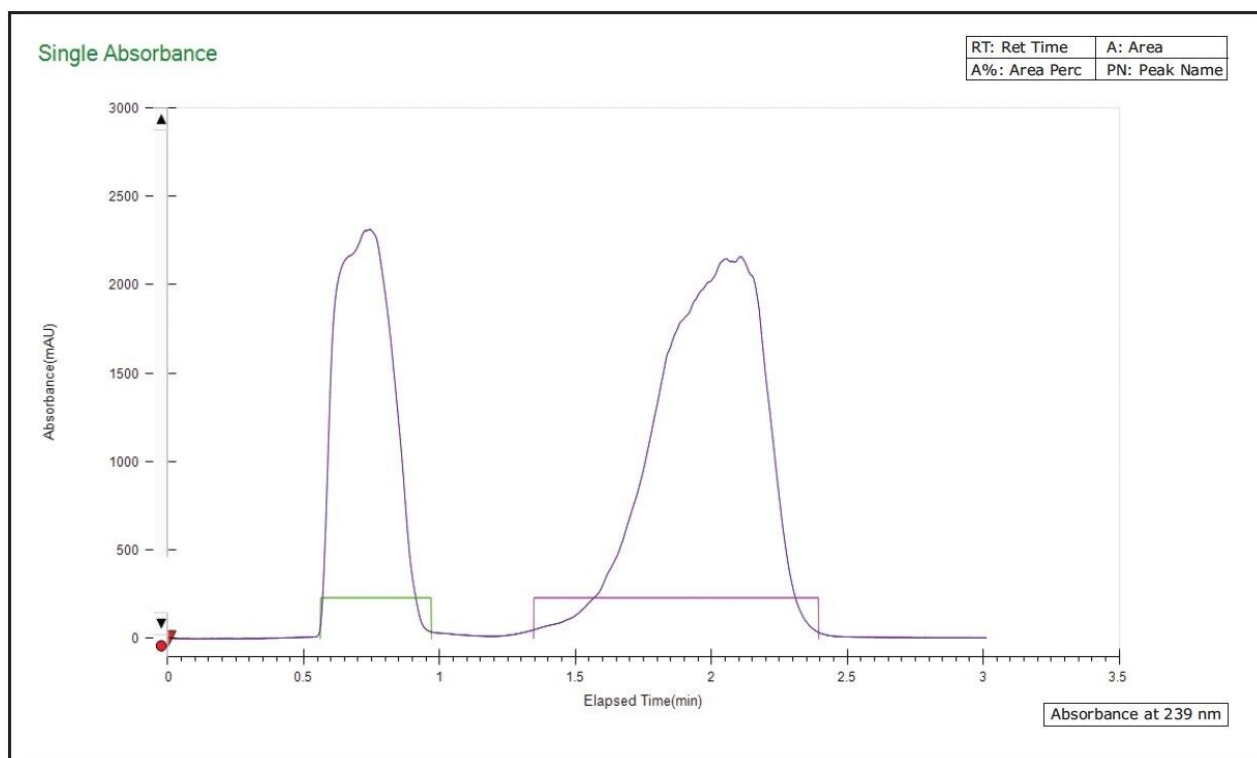


Figure 3. A preparative SFC/UV chromatogram of a RAC/DMRAC mixture using a Cyano column. Key experimental parameters are listed in the experimental section.

Table 1 illustrates a comparison between the SFC method and two reported HPLC methods. Clearly, the SFC method offers the shortest run time. In addition, post purification dry down was shorter because fractions were collected in a small volume of volatile methanol; whereas, in the two HPLC methods, a majority of the mobile phase (>70%) was aqueous buffer. Compared to the two HPLC purifications, the overall process time using SFC was substantially shorter, which can lead to a significant gain in the final yield of the valuable <sup>11</sup>C-labeled product.



	Mobile phase	Column	t <sub>r</sub> of product (min)	t <sub>r</sub> of precursor (min)	Total run time (min)	Post purification dry down (min)
HPLC (Ref. 2)	H <sub>3</sub> PO <sub>4</sub> /acetonitrile (70/30)	RP18	10.50	7.5	15.0	6
HPLC (Ref. 4)	0.025 M citrate buffer (pH 3.0)/ ethanol 96% (75/25)	Suplex pKb100	5.80	16.6	20.0	N/A
SFC	MeOH with 3% NH <sub>3</sub> /MeOH	Cyano	0.75	2.0	2.5	3

Table 1. Comparison between the SFC method and two reported HPLC methods.

## Conclusion

Raclopride can be readily separated from its precursor, desmethyl raclopride, in the desired elution order, on a variety of stationary phases using SFC. SFC offers a wide range of selectivity, which allows for easy maneuvering of the elution order of the analytes. This is particularly useful for the purification of low concentration analytes in a mixture of wide dynamic range. For raclopride/desmethyl raclopride purification, the described SFC method also provided shorter overall process time, including both chromatography and post purification dry down, critical for the purification of radiolabeled agents with short half lives. As SFC continues to make inroads in pharmaceutical and associated industries as a viable chromatographic technique for purification, it also holds great promise to become an integral contributor to radio-pharmaceutical research.

## References

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