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HPLC methods for the purification of [^{11}C]-labelled radiopharmaceuticals: reversal of the retention order of products and precursors

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Preparative HPLC methods have been developed for a number of [^{11}C]-methylated PET tracers, which enable elution of the labelled compounds prior to their precursors, thus reducing the overall synthesis time and avoiding contamination of the final product with precusor. This reversal of retention order has been achieved for [^{11}C]DASB, [^{11}C]raclopride, [^{11}C]FLB 457, [^{11}C]carfentanil, and 2-fluoro-[*N*-methyl-^{11}C]apomorphine, enabling collection of the purified radiopharmaceuticals from the HPLC system after 5–7 min. Furthermore, by using ethanol as the organic modifier, residual solvent analysis prior to human injection could be avoided and three of the radiopharmaceuticals could be injected directly following simple dilution and sterile filtration.

Keywords: PET; carbon-11; methylation; HPLC

Introduction

Positron emission tomography (PET) is a non-invasive biomedical imaging technique, whereby the distribution of tracer molecules, labelled by positron emitting isotopes, can be studied quantitatively in the living body. PET is widely used in clinical practice, and has proven to be a powerful diagnostic technique in neurology, psychiatry, cardiology, and oncology and is also a valuable tool for studying drug interactions and receptor function.

Carbon-11 is often used for labelling of neuroreceptor ligands for PET studies. Owing to its short half-life (20.4 min), the synthesis of [^{11}C]-labelled radiopharmaceuticals should be completed within a short period of time. By far the most utilized method for labelling compounds with carbon-11 is methylation with [^{11}C]methyl iodide or [^{11}C]methyl triflate, followed by preparative HPLC purification and formulation to obtain an injectable solution. Attention is often paid to optimizing the labelling reaction, while the rest of the procedure (i.e. HPLC separation and formulation) is not always fully optimized. Efforts are made to find the best conditions for a given reaction, on the other hand, a long separation and formulation can considerably decrease the overall yield and specific radioactivity. This can be of great importance for neuroreceptor studies where high specific activity radiopharmaceuticals are often required.

The introduction of a methyl group generally increases the lipophilicity of a compound. This normally results in elution of the nor-methyl precursor prior to the methylated product under reverse phase HPLC conditions. For [^{11}C]-labelled radiopharmaceuticals the precursor is in large excess in the crude reaction mixture. This often results in severe tailing of the precursor peak into the product peak, thus contaminating the final product. Especially for neuroreceptor ligands this can be detrimental if the precursor also has affinity for the receptor system being studied. In such cases a very good HPLC separation of the precursor and product is required, thus increasing the overall synthesis time and consequently reducing the radiochemical yield and the specific radioactivity of the product. It is therefore highly desirable if the radiopharmaceutical elutes prior to its precursor during HPLC purification. This can be achieved using normal phase HPLC columns but this is undesirable due to the use of toxic organic solvents. Furthermore, many intravenous pharmaceuticals are rather polar molecules, having basic or acidic groups, which limits their solubility in pure organic solvents.

The aim of this work was to develop preparative reverse phase HPLC methods for purification of [^{11}C]-labelled radiopharmaceuticals with the labelled products eluting prior to their precursors. This was successful for [^{11}C]DASB,[1] [^{11}C]raclopride,[2] [^{11}C]FLB 457,[3] [^{11}C]carfentanil,[4] and 2-fluoro-[*N*-methyl-^{11}C]apomorphine[5,6] (Scheme 1). In addition to reversal of retention order, we also focused on the use of ethanol as the organic modifier, as this minimizes residual solvent analysis prior to human injection and can in some cases allow direct injection of the HPLC eluent following dilution/pH adjustment and sterile filtration.

Results and discussion

In reversed phase HPLC, disregarding the steric effects, there are two types of column–sample interaction, namely hydrophobic

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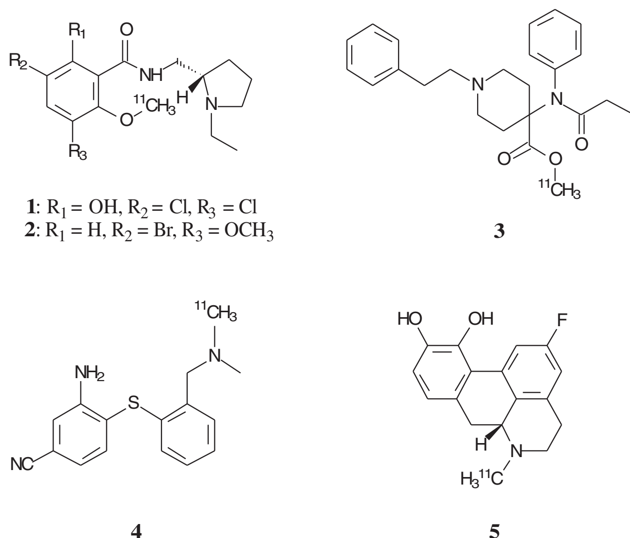
and polar interactions.[7] Thus, a dual retention mechanism has to be accounted for. As all the methylated products investigated are more lipophilic than their respective precursors, a change in retention order may be achieved if the polar activity of the stationary phase is utilized. By careful choice of the chromatographic conditions it is possible to increase the influence of the polar interactions compared with the hydrophobic interactions. If the polar interactions are sufficiently strong, and these forces are stronger between the stationary phase and precursor than between the stationary phase and methylated product, then a reversal of the retention order will result.



Scheme 1.

Highly basic HPLC columns

[^{11}C]raclopride (**1**), [^{11}C]FLB 457 (**2**), and [^{11}C]carfentanil (**3**) differ essentially from their labelling precursors, being phenoxy and carboxylic esters, respectively. These pairs tend to be separated readily under conventional reversed phase conditions; however, the retention order follows the order of lipophilicity. Hence, in order to change the retention order we utilized the fact that the precursors are considerably more acidic than the labelled products. By using a column of basic character (base deactivated column), the polar interactions between the precursors and the stationary phase increase their retention on the column. If this interaction is strong enough, it can exceed the effects of the hydrophobic interaction. For [^{11}C]raclopride and [^{11}C]FLB 457 a Supelcosil^{TM} Suplex pKb100 column proved successful in the required retention reversal. (Figure 1A and B). This column is deactivated for basic compounds providing shorter retention time and higher efficiency for bases. In addition, the stationary phase consists of a bonded C_{16} alkylamide chain, which is less lipophilic than the conventional octadecyl (C_{18}) chains, thus allowing for the use of a lower organic solvent content in the eluent. In case of [^{11}C]raclopride the long retention time can be attributed to the two acidic functional groups, compared with one for [^{11}C]FLB 457.

For the purification of [^{11}C]carfentanil (**3**) the Suplex pKb100 column turned out to be unsuitable: the polar interactions between the acidic precursor and the basic column did not compensate the huge difference in lipophilicity. Even though a change in retention order could be achieved, the separation was not sufficient for isolation of the labelled product. A more basic column was discovered (Adsorbosphere XL C8-B), which did, however, enable a reversal of the retention order with a suitable resolution (Figure 2). [^{11}C]carfentanil elutes well before its precursor with an acceptable peak shape, while the precursor peak is rather broad with considerable tailing.

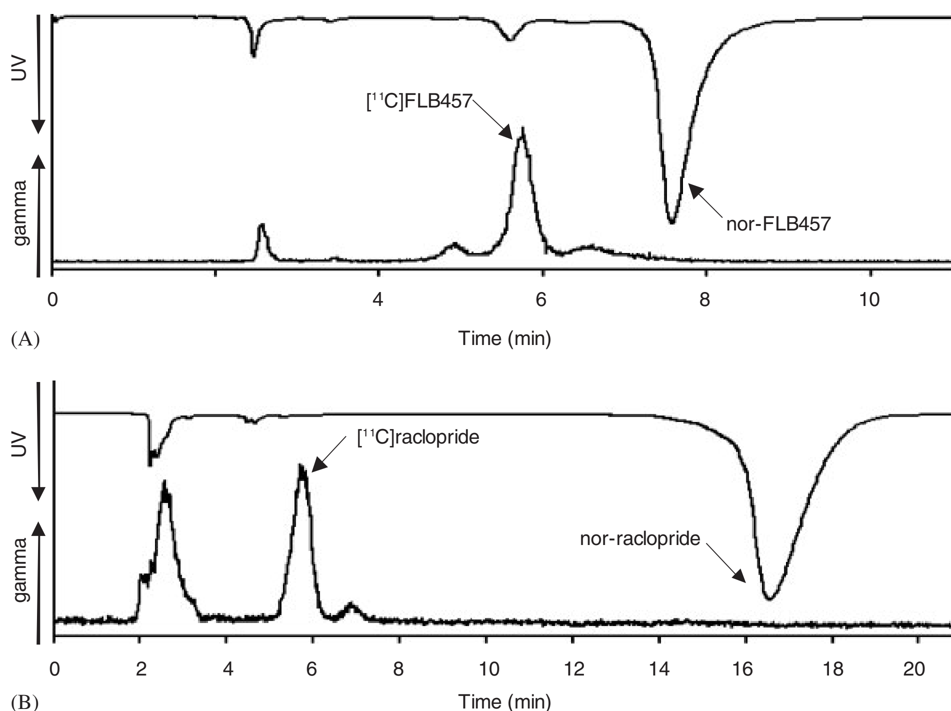


Figure 1. Preparative HPLC chromatograms: (A) [^{11}C]FLB 457 and (B) [^{11}C]raclopride.

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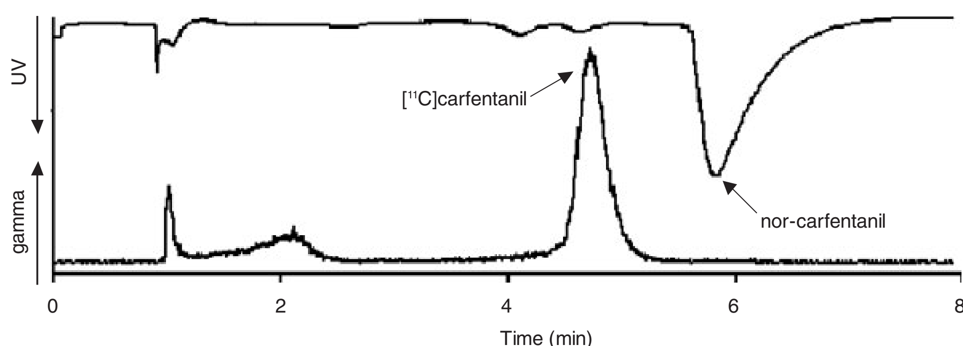

Figure 2. Preparative HPLC chromatogram of [^{11}C]carfentanil purification.

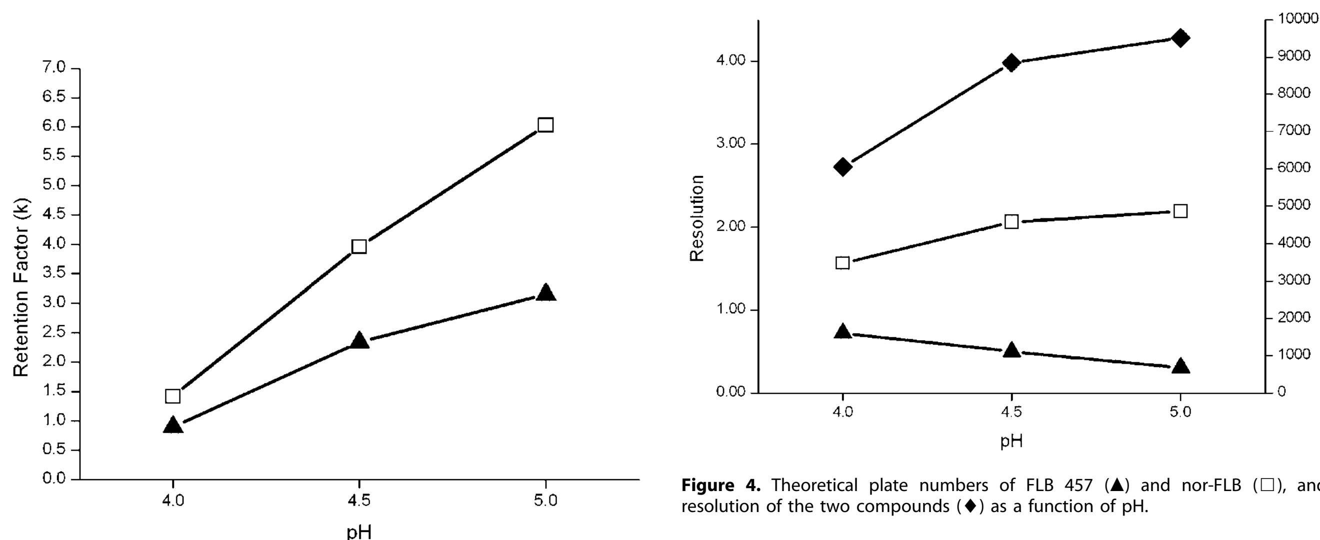

Figure 3. Retention factors of FLB 457 (▲) and nor-FLB (□) as a function of pH.

Figure 4. Theoretical plate numbers of FLB 457 (▲) and nor-FLB (□), and resolution of the two compounds (♦) as a function of pH.

However, since the product elutes first this tailing is of no real consequence.

It should be noted that while these HPLC methods are advantageous for our purposes, they cannot be considered to be robust. We are taking advantage of interactions and column effects, which were probably not designed during the column development. Therefore, relatively small changes in pH and eluent composition, or indeed changing to a new column, can lead to differences in performance. Figure 3 shows the retention factors ($k = t_R - t_0/t_0$) of [^{11}C]FLB 457 and its precursor as a function of pH. As the ionization forms of the [^{11}C]FLB 457 and its precursor remain the same (this pH interval is far from their pK_a value of >9), the considerable changes in the interactions between the column and the analytes can most probably be attributed to the column. Not only retention times can change with the pH, but also resolution and peak shape. Figure 4 shows the theoretical plate numbers of [^{11}C]FLB and nor-FLB 457 as a function of pH. The low theoretical plate number of nor-FLB 457 can be attributed to the tailing (the acidic compound on a base-deactivated column behaves the same as a basic compound usually behaves on a conventional column). The non-acidic [^{11}C]FLB 457 has a much higher plate number due to its better peak shape. Using an eluent at pH 4.5 is a compromise: the separation is good enough to isolate [^{11}C]FLB 457 from its precursor, while retaining an acceptable peak shape.

Cyanopropyl HPLC column

For the purification of [^{11}C]DASB (**4**) and 2-fluoro-[*N*-methyl-^{11}C]apomorphine (**5**) a different approach was required. A cyanopropyl column was used with an acetate buffer at pH 5.0 and a high ethanol content.

At pH 5.0 both the silanol groups and the analytes are partially ionized, thus favouring polar interactions. The high ethanol content enables good solubilization of the bonded cyano-propyl groups, thus increasing the number of available silanol groups.[7]

Figure 5 shows the log of retention factors of nor-DASB and DASB as a function of ethanol content. For nor-DASB, polar interactions dominate at very high ethanol concentrations, thus the retention time starts to increase with increasing ethanol content, while for DASB a linear relationship is seen. Therefore, at high ethanol concentrations a reversal in retention order is achieved (Figure 6) and the resolution is sufficient for isolation of the product. Reversal of retention order for 2-fluoro-[*N*-methyl-^{11}C]apomorphine and its precursor was achieved using identical conditions.

The described phenomenon is slightly similar to hydrophilic-interaction chromatography (HILIC[8]), where a hydrophilic column is eluted with hydrophobic, mostly organic, mobile phase, and the retention increases with the hydrophilicity of the solutes. This technique is frequently used for the analysis of

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hydrophilic compounds, for example sugars, but there are only a few applications for separation of more lipophilic compounds. This approach was only successful in changing the retention order of tertiary amines and their secondary amine precursors. Attempts to change the retention order of several primary/secondary amine pairs were unsuccessful. The method was also unsuccessful for [^{11}C]flumazenil, which is an amide.

Experimental

Materials

Citric acid monohydrate, trisodium citrate dihydrate, sodium acetate, acetic acid, ascorbic acid (Unikem, Copenhagen, Denmark), sterile water, 96% ethanol, 2% phosphate buffer pH 7 (Hospital Pharmacy, Rigshospitalet, Copenhagen, Denmark), acetonitrile (anhydrous), DMF (anhydrous), and tetrabuthylammonium hydroxide 1 M in CH_3OH (Sigma-Aldrich). DASB, racropride, FLB 457, carfentanil, and their precursors (ABX, Radeberg, Germany).

Radiosynthesis

[^{11}C]methyl iodide or [^{11}C]methyl triflate were produced by gas phase iodination[9] of cyclotron produced [^{11}C]methane using a fully automated system[10] with only minor modifications of previously published procedures. The precise reaction conditions used are summarized in Table 1. HPLC retention times for all compounds are shown in Table 2.

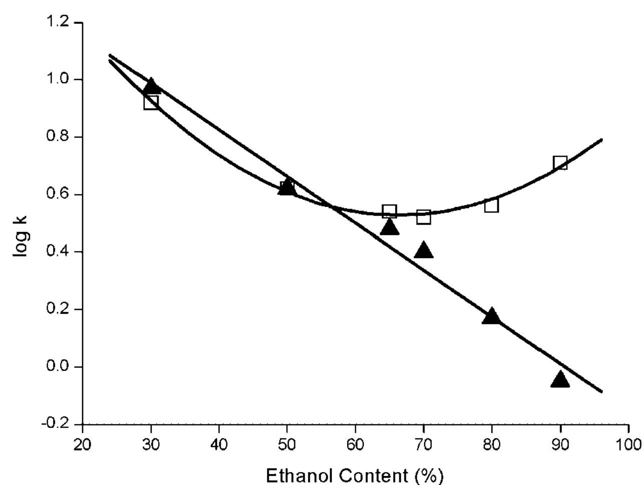

Figure 5. Logarithm of retention factors (*k*) of DASB (▲) and nor-DASB (□) as a function of ethanol content.

*[^{11}C]raclopride (**1**)*

Following [^{11}C]-methylation, the reaction mixture (300 μl) was diluted with 1.0 ml citrate buffer and subsequently injected onto a Supelcosil™ Suplex pK_b100 HPLC column (5 μm, 10 × 250 mm, Supelco, USA). Eluent: 0.025 M citrate buffer pH 3.0/ethanol 96% (75:25); flow rate 6.0 ml/min; wavelength $\lambda = 250$ nm. The fraction containing the product was collected within 1 min (6.0 ml) and passed through a sterile filter into a 30 ml sterile vial containing 9.0 ml 0.1 M phosphate buffer pH 7, giving a final solution for injection containing 10% ethanol.

*[^{11}C]FLB 457 (**2**)*

Following [^{11}C]-methylation, the reaction mixture (300 μl) was diluted with 4.5 ml acetate buffer and subsequently injected onto a Supelcosil™ Suplex pK_b100 HPLC column (5 μm, 10 × 250 mm, Supelco, USA). Eluent: 0.025 M acetate buffer pH 4.5/ethanol 96% (85:15); flow rate 6.0 ml/min; wavelength $\lambda = 250$ nm. The fraction containing the product was collected in within 30 s (3.0 ml) and passed through a sterile filter into a 30 ml sterile vial containing 12.0 ml 0.1 M phosphate buffer pH 7, giving a final solution for injection containing 7% ethanol.

*[^{11}C]carfentanil (**3**)*

Following [^{11}C]-methylation, the reaction mixture (300 μl) was diluted with 4.5 ml phosphoric acid and subsequently injected onto an Adsorbosphere XL C8-B HPLC column (5 μm, 10 × 150 mm Alltech, USA). Eluent: 0.1% phosphoric acid/ethanol (90:10); flow rate 10.0 ml/min; wavelength $\lambda = 250$ nm. The fraction containing the product was collected within 40 s (6.0 ml) and passed through a sterile filter into a 30 ml sterile vial containing 9.0 ml 0.1 M phosphate buffer pH 7, giving a final solution for injection containing 5% ethanol.

*[^{11}C]DASB (**4**) and 2-fluoro-[*N*-methyl-^{11}C]apomorphine (**5**)*

Following [^{11}C]-methylation, the reaction mixture (300 μl) was diluted with 1.0 ml eluent and was injected onto a Luna-CN 5 μm, 10 × 250 mm preparative column (Phenomenex, Torrance, USA). Eluent: 0.025 M acetate buffer pH 5.0/ethanol 96% (20:80); flow rate 6.0 ml/min; wavelength $\lambda = 250$ nm. The fraction containing the product was diluted with 100 ml sterile water and subsequently passed through a C_{18} SepPak cartridge (Waters, Milford, USA) preconditioned with 10 ml 96% ethanol

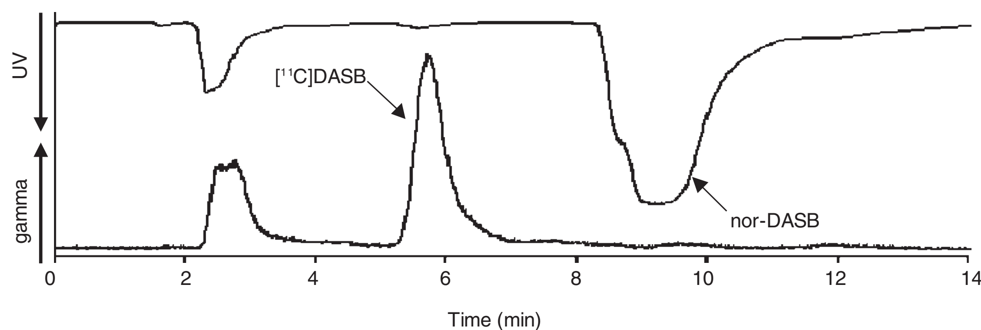

Figure 6. Preparative HPLC chromatogram of [^{11}C]DASB purification.

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Table 1. Reaction conditions for preparation of the [^{11}C]-labelled radiopharmaceuticals

Compound	Precursor (mg)	Solvent	Base	Methylating agent	Temperature (°C)
[^{11}C]raclopride (**1**)	1.0	DMF	Aq. NaOH	[^{11}C]CH_3I	85
[^{11}C]FLB 457 (**2**)	0.5	MeCN	Aq. NaOH	[^{11}C]CH_3OTf	20
[^{11}C]carfentanil (**3**)	0.5	MeCN	Anh. TBAH	[^{11}C]CH_3OTf	20
[^{11}C]DASB (**4**)	1.0	DMF	—	[^{11}C]CH_3I	85
2-fluoro-[*N*-methyl-^{11}C]apomorphine (**5**)	1.0	DMF	Dry $NaHCO_3$	[^{11}C]CH_3I	140

Table 2. Preparative HPLC retention data

Compound	t_R of product (min)	t_R of precursor (min)
[^{11}C]raclopride (**1**)	5.8	16.6
[^{11}C]FLB 457 (**2**)	5.7	7.6
[^{11}C]carfentanil (**3**)	4.7	5.9
[^{11}C]DASB (**4**)	5.7	9.3
2-fluoro-[*N*-methyl-^{11}C]apomorphine (**5**)	4.7	6.8

and 20 ml sterile water. The collected activity was eluted from the SepPak with 3 ml 96% ethanol followed by 3 ml phosphate buffer (2 mM, pH 7) and passed through a sterile filter into 30 ml sterile vial containing an additional 12.0 ml phosphate buffer, giving a final solution for injection containing $<20\%$ ethanol.

Conclusions

In conclusion, we have developed reversed phase HPLC methods for rapid purification of 5 [^{11}C]-labelled radiopharmaceuticals. With the proper choice of eluents and columns we were able to elute these radiopharmaceuticals from the HPLC column prior to their less lipophilic precursors. This is useful for reducing the overall synthesis time and avoiding contamination of the final product with precursor. Reduction of the overall synthesis time enables radiotracers to be produced in better yields and with higher specific radioactivities. Moreover, by using ethanol and aqueous buffers suitable for human injection in the eluent systems, some radiopharmaceuticals could be formulated by a simple dilution of the collected HPLC fraction, without the need for lengthy evaporation or solid phase extraction procedures for removal of organic solvents.

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