Microfluidic labeling of Peptides, Proteins and Antibodies

Nanotek Microfluidic synthesis system

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Isotope choices?

Nanotek users have successfully radiolabeled a variety of biological compounds using the following isotopes:

^{18}F, ^{64}Cu, ^{68}Ga, ^{89}Zr, ^{90}Y, ^{99m}Tc, ^{111}In and ^{211}At.

- Only one configuration of system is required for all isotopes listed.
- There has been no need to modify the principles of operation of the Nanotek when changing from small organic molecules to biomolecules whether they are peptides, proteins or antibodies.
- The small volume reactions possible using the system minimize the consumption of scarce, difficult to synthesize and expensive precursors both when optimizing reaction conditions and importantly provide the opportunity for enhanced effective specific activities .
- A Dose on Demand capability provides additional utility when dealing with pre clinical needs and patient situations

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Expectations using the Nanotek

- Reduced synthesis times
- Higher yields
- Cleaner radiosynthesis
- Reduced use of precursor
  - Higher relative specific activity
  - Reduced cost per optimization study and dose
- Automation of synthesis
  - Elimination of manual steps
- Dose on Demand capability
  - Simplifies multiple preclinical studies
  - Improves patient scheduling
- Integration with trap and release procedure
  - Maximize RAC
  - Reduce final product volume
  - Simplify multiple preclinical studies

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Recent Publications/ Presentations/ Posters

^{18}F-Labeled phosphopeptide-cell-penetrating peptide dimers with enhanced cell uptake properties in human cancer cells

Susan Richter, Vincent Bouvet, Melinda Wuest, Ralf Bergmann, Joerg Steinbach, Jens Pietzsch, Ines Neundorf, Frank Wuest.

Results: Isolated radiochemical yields ranged from 2% to 4% for conventional bioconjugation with [^{18}F]SFB. Significantly improved isolated radiochemical yields of up to 26% were achieved using microfluidic technology.

Rapid ^{18}F-radiolabeling of peptides from [^{18}F] fluoride using a single microfluidics device

Robin C. Cumming, Dag Erlend Olberg and Julie L. Sutcliffe

Single-step radiofluorination of peptides using continuous flow microreactor

Svetlana V. Selivanova, Linjing Mu, Johanna Ungersboeck, Timo Stellfeld, Simon M. Ametamey, Roger Schiblia and Wolfgang Wadsak

2-[^{18}F]-2-DEOXY-D-GLUCOSE ([^{18}F]-FDG) LABELING OF PEPTIDES USING A MICRO-FLUIDIC REACTOR

V. R. BOUVET and F. WUEST

Development of a ^{89}Zr radiolabeling protocol for an anti-GD2 antibody PET radiotracer using a microfluidics system

S Snyder

Preparation of technetium-99m bifunctional chelate complexes using a microfluidic reactor: a comparative study with conventional and microwave labeling methods

Ryan W. Simms, Patrick W. Causey, Darren M. Weaver, Chitra Sundararajan, Karin A. Stephenson, and John F. Valliant

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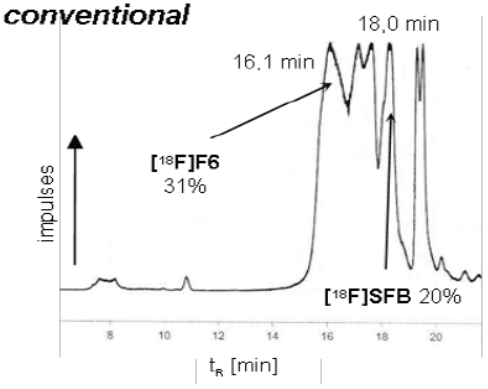
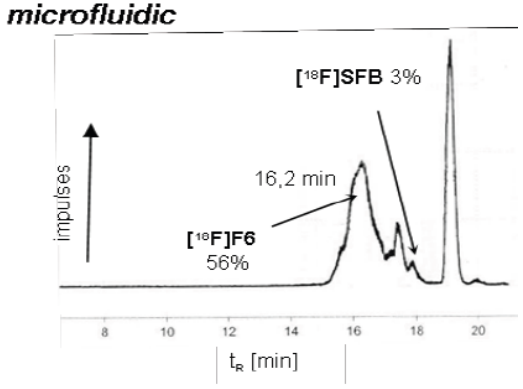
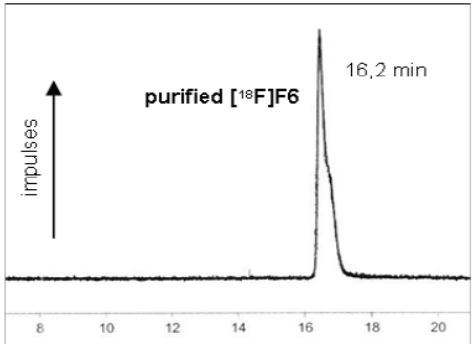
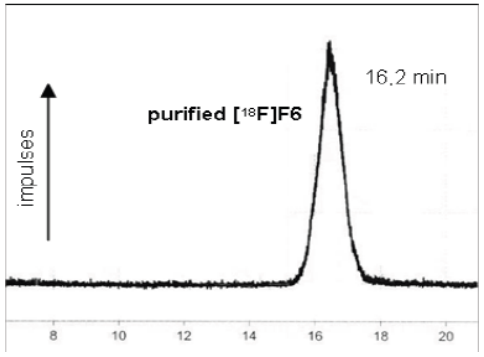
^{18}F-Labeled phosphopeptide-cell-penetrating peptide dimers with enhanced cell uptake properties in human cancer cells

The observed higher radiochemical yield can be explained by a significantly improved chemoselectivity of the acylation reaction toward the N-terminal end of peptide dimers. This is an important finding making microfluidic technology an ideal platform of chemoselective bioconjugations based on acylations reactions involving activated esters like [^{18}F]SFB. The aspect of significantly improved chemoselectivity is clearly demonstrated by comparing the HPLC traces of the reaction mixture using conventional and microfluidic-based radiolabeling with [^{18}F]SFB.









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Rapid ^{18}F-radiolabeling of peptides from [^{18}F] fluoride using a single microfluidics device

To our knowledge, this is the first report of a 2-step continuous microfluidic ^{18}F-peptide radiolabeling using a prosthetic group, from [^{18}F]Fluoride.

This approach offers an improvement in radiochemical yield, decreased reaction time from 1 hour to 8 min from anhydrous [^{18}F]Fluoride and implements an attractive automated approach for ^{18}F radio-labeling of peptides.

This can be seen as a method for ^{18}F-radiolabeling of amine-bearing peptides serving as a platform for radiolabeling of clinically relevant peptides in a rapid and automated fashion

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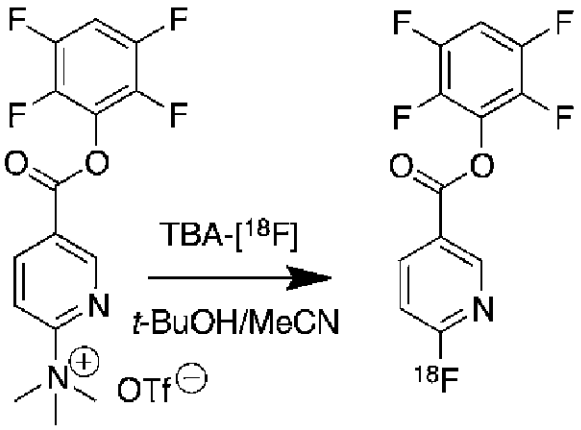
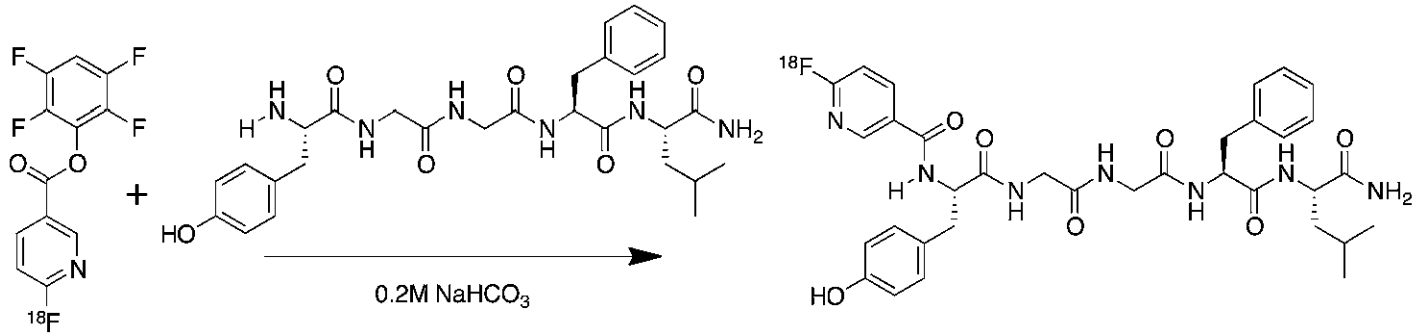
Rapid ^{18}F-radiolabeling of peptides from [^{18}F] fluoride using a single microfluidics device



The synthesis of the pendant group occurs in the first reactor.
The output of the first reactor is then sent to the inlet of the second reactor without any need for purification.
The peptide is then introduced and the pendant group rapidly reacts and the product is formed.



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Cumming *et* al, RSC Adv., 2014, 4, 49529

Single-step radiofluorination of peptides using continuous flow microreactor

Direct radiolabelling of peptides with ^{18}F-fluoride can be successfully achieved in a reproducible manner using the Advion NanoTek continuous flow microreactor.

Fast synthesis time and general module setup allows for rapid optimisation of the reaction conditions and multiple on-demand productions of a given radiolabelled peptide.

The amount of precursor required for the radiolabelling can be substantially decreased, in particular for peptides having a trimethylammonium leaving group, which facilitates purification and helps to save precious starting material.

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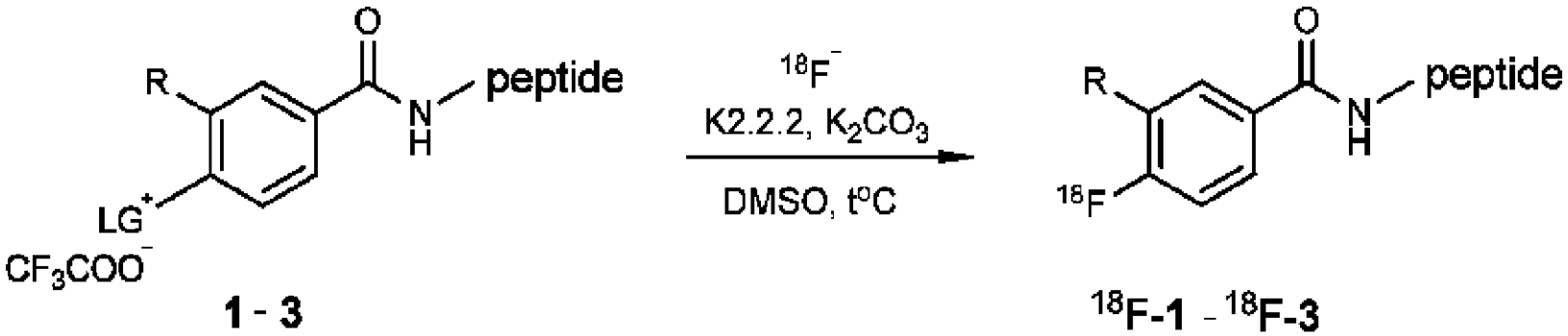
Single-step radiofluorination of peptides using continuous flow microreactor



LG: leaving group
R = CN or H

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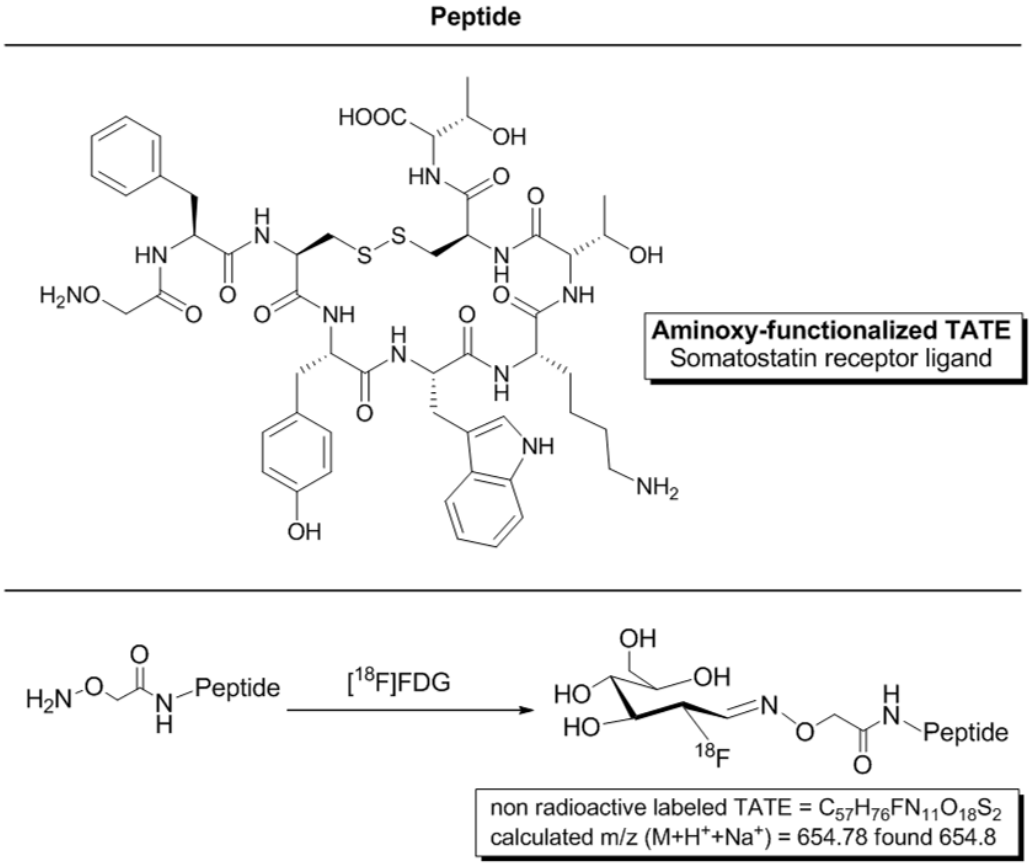
Selivanova *et al*, Org. Biomol. Chem., 2012, 10, 3871

2-[^{18}F]-2-DEOXY-D-GLUCOSE ([^{18}F]-FDG) LABELING OF PEPTIDES USING A MICRO-FLUIDIC REACTOR

- Optimal conditions (87% radiochemical yield) were obtained within **5 min** at 120 °C, with 12mg/mL of aminooxy-maleimide per mL of [18F]FDG solution.
- Manual labeling of aminoxy-TATE was completed in **70 min**. (3.6 µmol/mL, 90 °C)



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Development of a ^{89}Zr radiolabeling protocol for an anti-GD2 antibody PET radiotracer using a microfluidics system

Use of this system has several advantages:

The radioisotope can be stored in a reservoir and used as needed.

It is automated and can be set up for batch runs.

It simplifies the labeling protocol and decreases labeling time 10 fold.

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Preparation of technetium-99m bifunctional chelate complexes using a microfluidic reactor: a comparative study with conventional and microwave labeling methods

The microfluidic reactor allowed for a greater than 10-fold decrease in the amount of DTV to be labeled relative to conventional techniques.

The labeling yield of DTV-AHx-insulin (2.1mg/ml, 330 mm) at 37˚C using conventional conditions was 21% in 15.7min compared with 40% in the identically formulated microfluidic reactor.

In addition to the higher radiochemical yield, the radiochemical purity was significantly improved in the microfluidic reactor. The small volume and subsequent accurate control over the time and

temperature of reactions conducted in the microfluidic reactor improved the radiochemical yield and radiochemical purity for labeling reactions of the technetium tricarbonyl core with the DTV chelate and DTV-AHx-insulin.

The microfluidic reactor can be operated remotely, was easily shielded, and each reaction required a small amount of precursor, providing an efficient platform for rapidly optimizing the labeling of new molecules.

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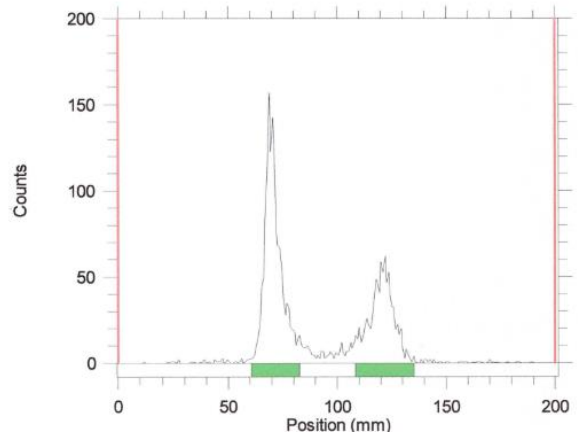
Radio Metal Performance

^{89}Zr Pertuzumab, DFO

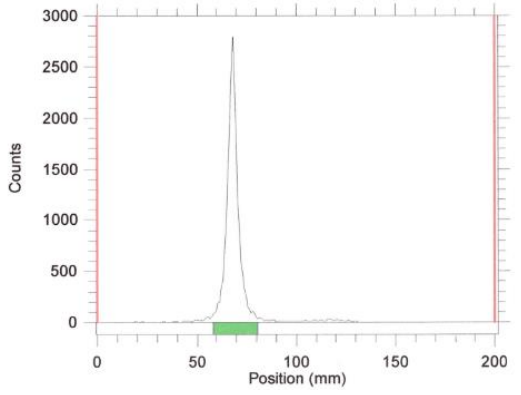
Using 1M HEPES
The highest incorporation yield was 62% using 40µl of 0.1mg/ml Antibody (4µg) in 1M HEPES and 20µl Isotope at a temperature of 37˚C
2 x 4M reactors and a residence time of 3 minutes.

Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	60.6	83.0	71.1	0.355	1375.0	1375.0	54.56	61.71
Rgn 2	108.7	135.2	120.4	0.602	853.0	853.0	33.85	38.29
2 Peaks					2228.0	2228.0	88.41	100.00



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	58.1	80.5	68.1	0.341	19890.0	19890.0	92.49	100.00
1 Peaks					19890.0	19890.0	92.49	100.00

Using 0.25M NaOAC ph 6.
The highest incorporation yield was 100%
Using 20µl of 0.5mg/ml Antibody (10µg) and 20µl Isotope at 37˚C ,
2 x 4M reactors in series and a residence time of 1.25 minutes



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Radio Metal Performance

^{68}Ga / ^{111}In

Trastuzumab PCTA
POC Non optimized only three expts undertaken 72% yield.
40µl antibody , 40 µl isotope
37˚C, 3 minutes residence time, 4M reactor. NaOAC, 0.25M pH=6.

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Injection Date:        08/11/2012
Acq. Operator:         Gemma
Method:                C:\CHEM32\1\METHODS\ANTIBODY_GD.M
Method Info:           Size Exclusion: Herceptin
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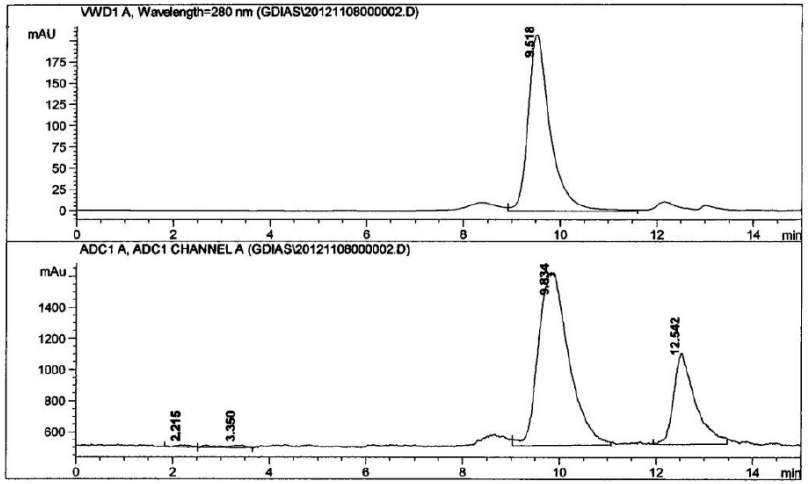
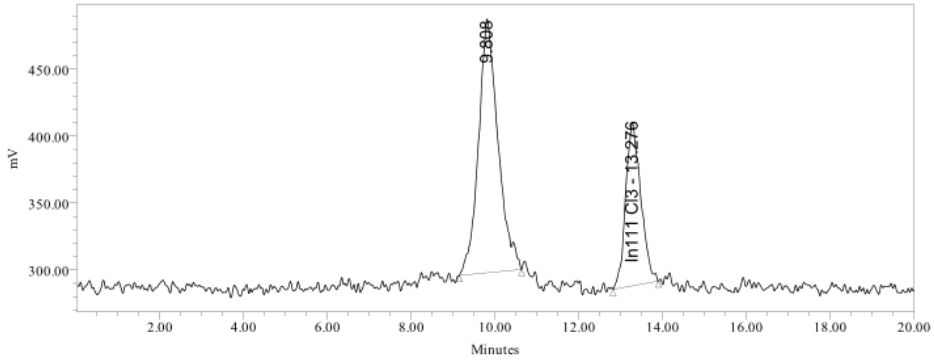


Confidential antibody, DTPA chelator.
4M reactor. Stop flow reaction reagents (15 µl Antibody and 15µl isotope) held in loop for 35 minutes at 45˚C .



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Cleaning of NanoTek

Cleaning of the NanoTek after synthesis is accomplished using standard macros provided with the unit

The typical cleaning of the unit for use with F-18 and C-11 tracers is accomplished with Acetonitrile. For other isotopes a combination of solvents is used. Other isotopes than those seen in the table below can be easily adapted.

Isotope(s)	Primary Cleaning Solvent	Second Solvent	Third Solvent	Storage Solvent
Fluorine-18 (Human Production)	Sterile Water	Ethanol	Acetonitrile	Acetonitrile
Copper-64	1% EDTA	Metal Free Water		Acetonitrile
Iodine-125	0.1N NaOH	Water	Acetonitrile	Acetonitrile

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Cleaning of NanoTek

Measured cross contamination using standard method when changing isotope / precursor

Analysis following F-18 labeling for radioactive and chemical carryover. Both radioactive and chemical levels were < 0.1%

The decay corrected radioactive carryover was determined to be <0.065%

Chemical carryover was <0.09% for F-18

After cleaning following Cu-64 and I-125 incorporation there was <3 X Background activity measured

The NanoTek can be effectively cleaned using standard cleaning macros.

The cleaning procedure takes between 20 – 30 minutes to complete.

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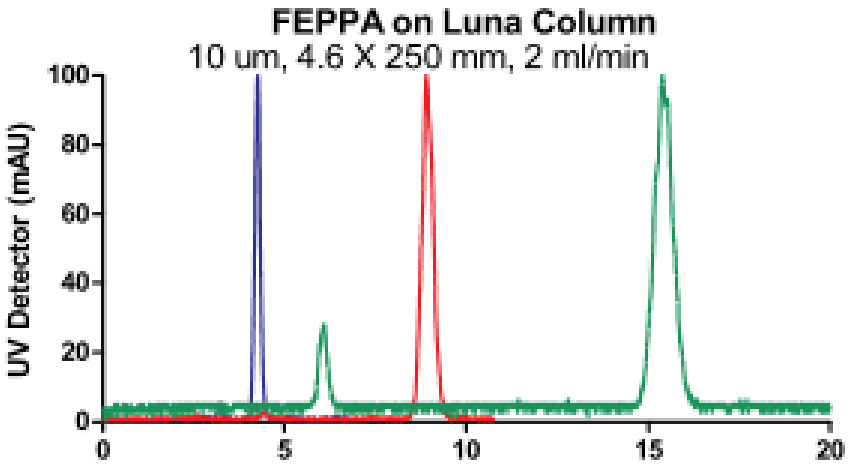
“trap and release” system and analytical HPLC columns to purify radiotracers for small animal imaging and to purify materials which possess close eluting impurities.



HPLC chromatograms of FEPPA, using a Luna C18 ODS (4.6 X 250 mm, 10 µm) and 3 different ethanol and 0.1 M ammonium acetate based mobile phases. (60% Ethanol, Blue line), (40%, Ethanol Red line) and (20% Ethanol, Green line).

Table 1. Example Compounds

Radiotracers	Precursor mass (mg)	Semi-prep Volume (ml)	This method (ml)
FLT [4]	10	7	0.75
FMISO [3]	4	5	0.6
Fallypride	2	10*	0.23
FEPPA [2]	5	10*	0.95
SFB [5]	10	10*	0.72
T807 [1]	1	10*	0.65

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