

Rapid Purification of Fluorine-18 Containing Synthons Using Molecularly Imprinted Polymer Cartridges

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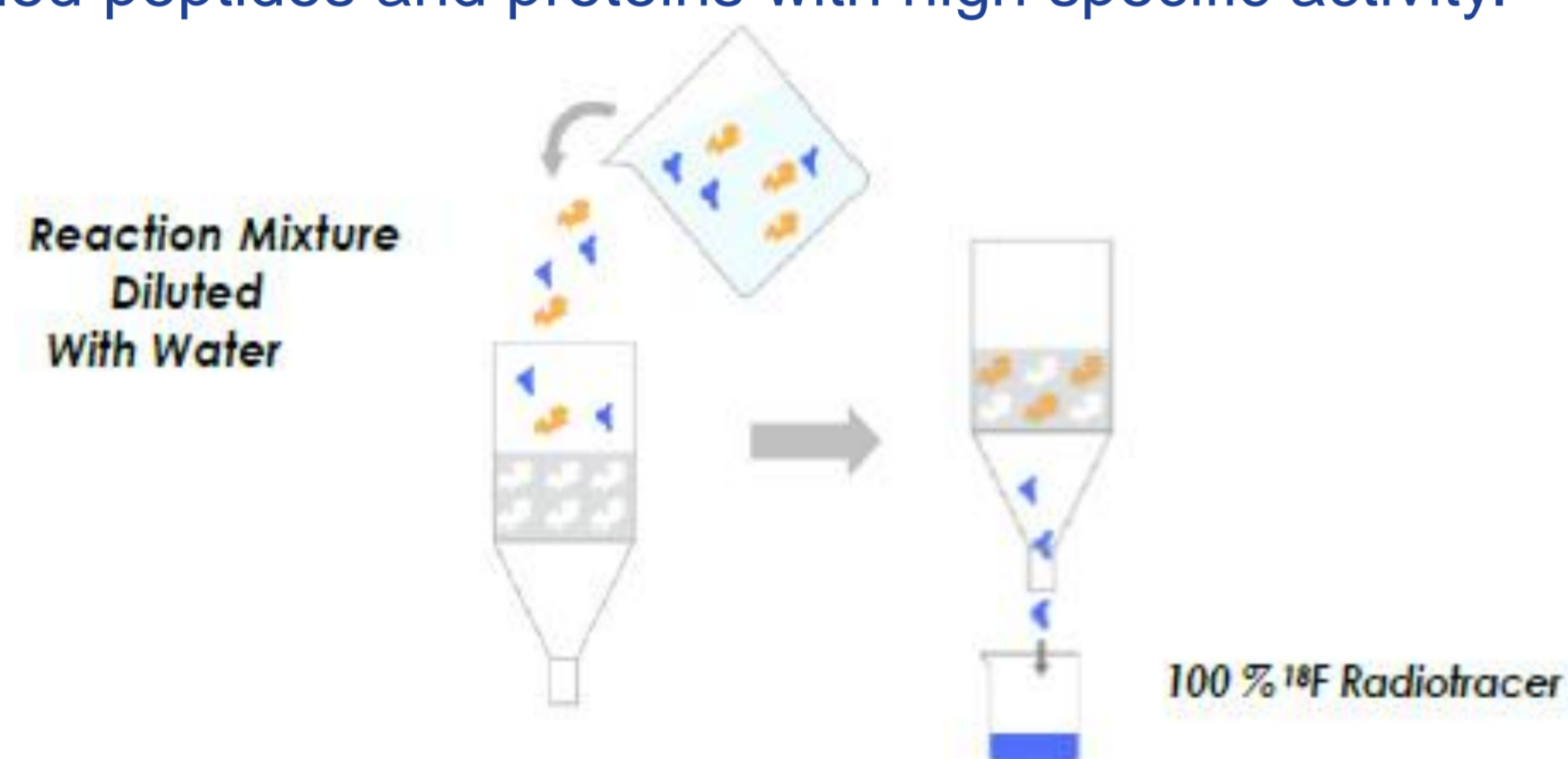
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Introduction

The synthesis of fluorine-18 containing radiotracers on automated platforms is increasing being conducted using solid phase extraction (SPE) cartridges for tracer purification in place of conventional high performance liquid chromatography (HPLC). SPE cartridges enable a rapid, cheap and disposable means of purifying radiolabelled molecules. A key benefit is that it reduces operator input or and facilitates the development of fully automated processes resulting in robust, repeatable processes for purification of radiotracers.

Molecularly imprinted polymers (MIPs) are comprised of highly cross linked polymers that are designed to recognise a specific molecule or molecular moiety. When combined with conventional SPE sorbents, MIP phases offer a new and powerful tool in the purification of radiotracers.

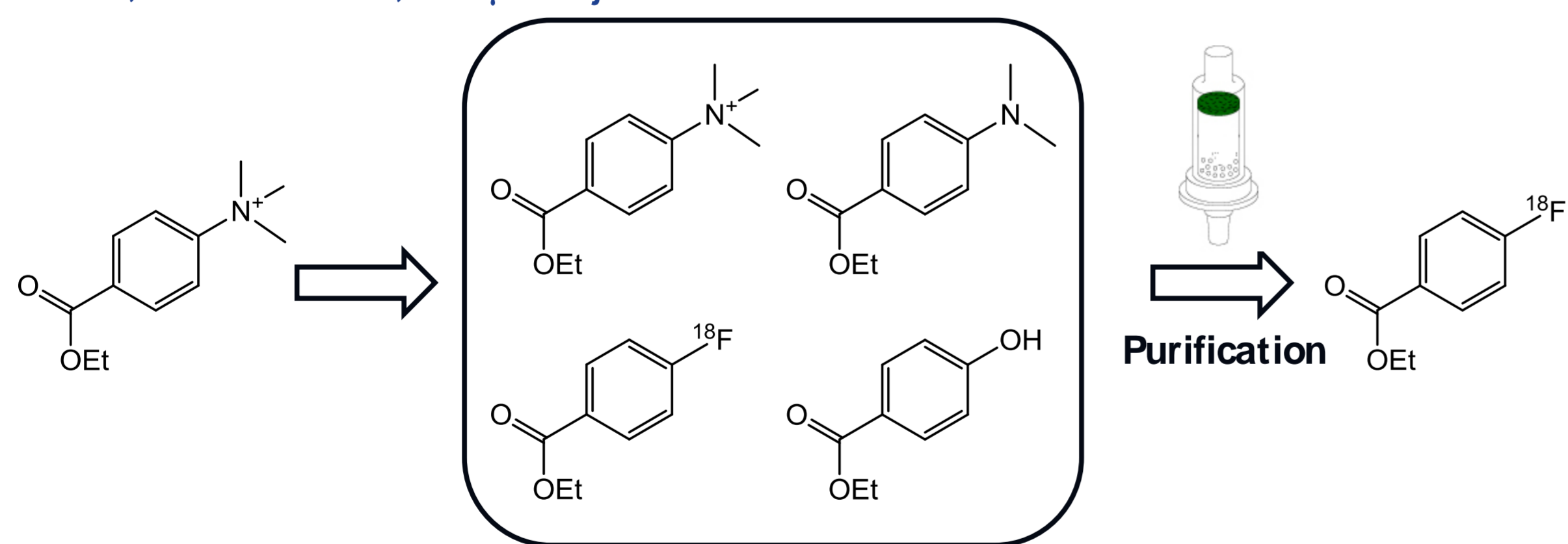
We have investigated use of POLYINTELL AFFINIMIP®SPE 18F (a new MIP/SPE cartridge) for the purification of the reaction mixtures created from the reaction of [¹⁸F]fluoride with N,N,N-trimethylbenzenaminium precursors. Low chemical content formulations of synthons produced from these precursors are critical in the formation of radiolabelled peptides and proteins with high specific activity.



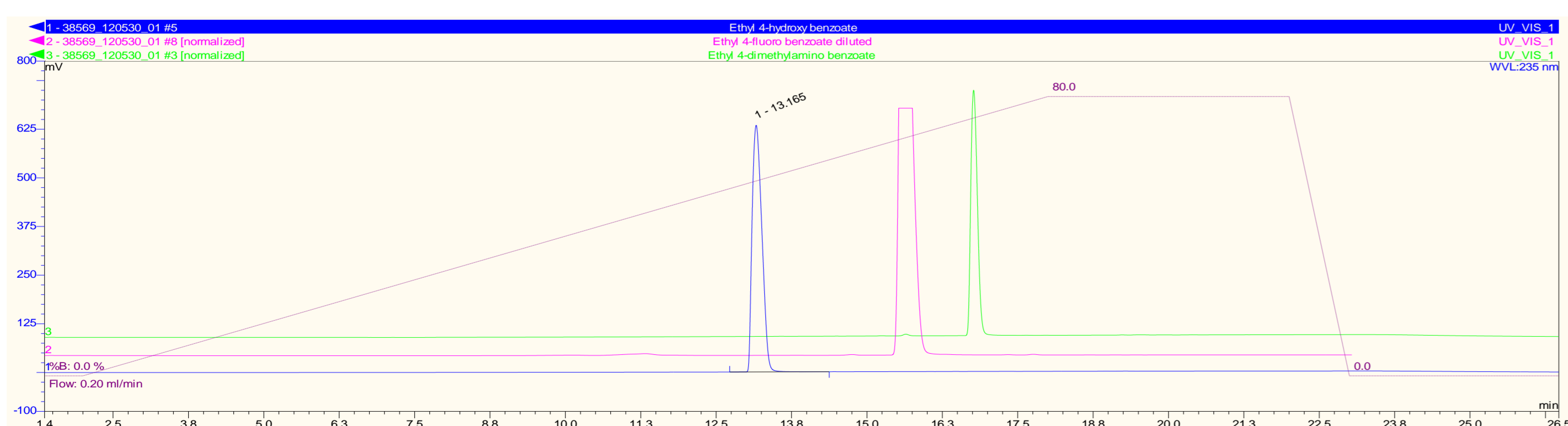
Fluorination reaction mixtures are diluted with water and passed through AFFINIMIP® SPE 18F cartridges, followed by the elution with ethanol. Impurities are retained and only the target compound is eluted in the case of ethyl 4-[¹⁸F]fluorobenzoate and 4-[¹⁸F]fluorobenzaldehyde

Methods

Peptide/protein labelling synthons 4-[¹⁸F]fluorobenzaldehyde (FBA) and ethyl 4-[¹⁸F]fluorobenzoate (a precursor to [¹⁸F]SFB) were produced using conventional Kryptofix® potassium carbonate chemistry and [¹⁸F]fluoride starting activities ranging from 368 MBq- 90 GBq from 4-formyl-N,N,N-trimethylbenzenaminium triflate and 4-(ethoxycarbonyl)-N,N,N-trimethylbenzenaminium triflate respectively (2 mg – 20 mg studied). Radiolabelling was conducted either on a custom manual rig or on a GE TRACERlab FX F-N depending on starting activity. Purification was conducted using a mixed mode AFFINIMIP®SPE 18F cartridge. Following radiolabelling in DMSO or MeCN at 110°C, for 10 minutes, reaction mixtures were diluted to reduce the organic solvent content to 20% and loaded onto a conditioned AFFINIMIP®SPE 18F. The cartridge was washed with 80:20 H₂O:(EtOH or MeCN) then eluted with neat solvent (EtOH or MeCN). Samples from each of the labelling and purification stages were then subjected to analytical HPLC. Thermo Scientific Hypersil GOLD column (50 × 2.1 mm, 1.9 μm), A= H₂O (+0.1% formic acid), B= MeCN, 0-2 minutes 0% B, 2-18 minutes 0%-80% B, 0.2 mL/min, 20 μL injection.



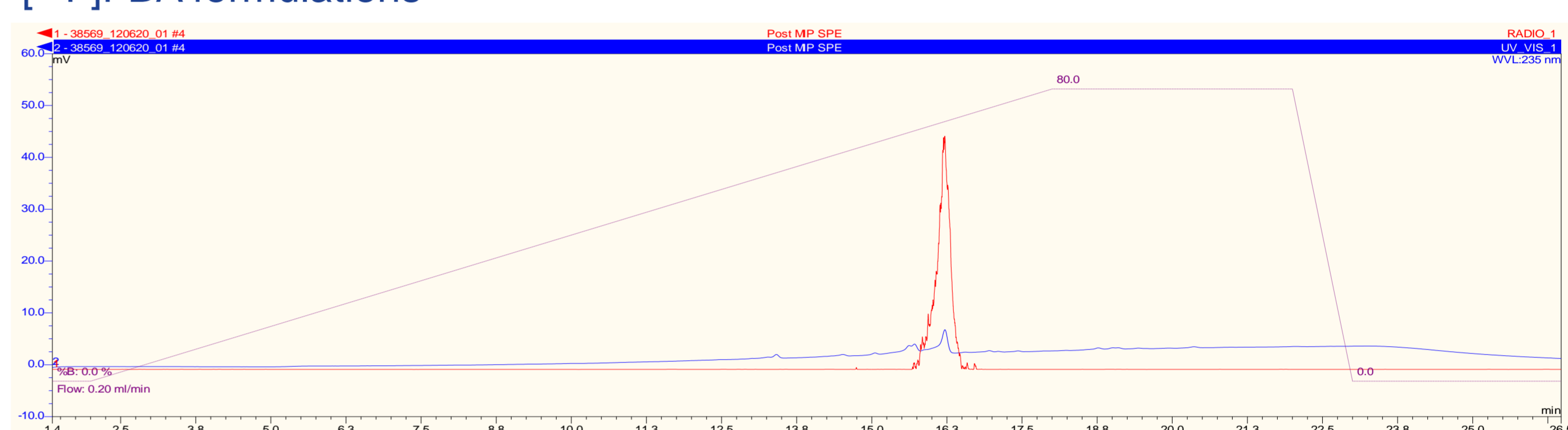
The synthesis of compounds such as ethyl 4-[¹⁸F]fluorobenzoate produces several key impurities alongside the target compound. These and unreacted [¹⁸F]fluoride were found to be removed using AFFINIMIP®SPE 18F cartridges.



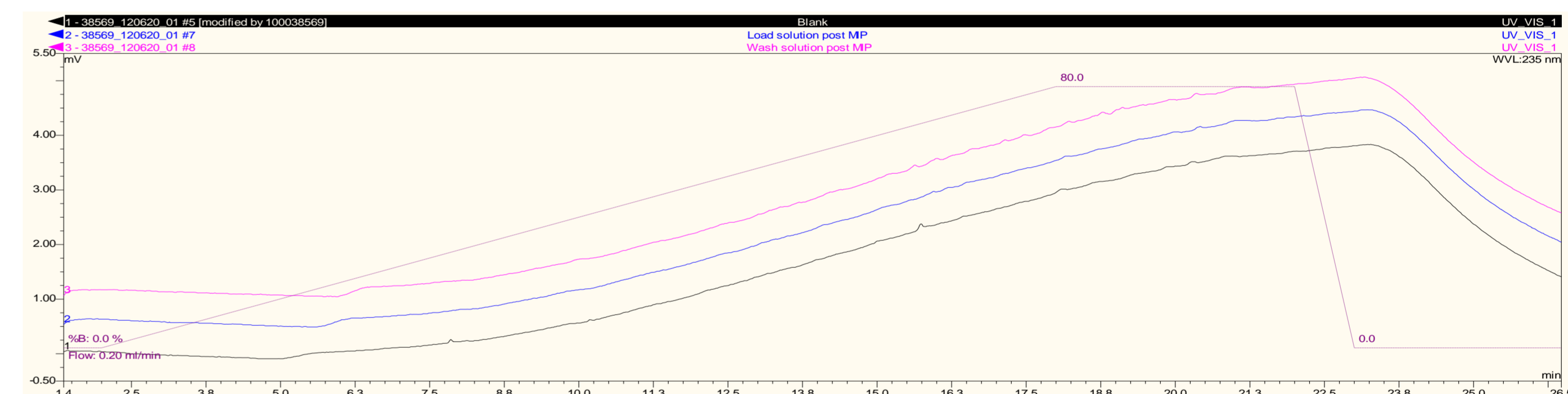
Analytical HPLC methods were developed to study the main impurities in the ethyl 4-[¹⁸F]fluorobenzoate and ethyl 4-[¹⁸F]fluorobenzaldehyde synthesis. Blue: ethyl 4-hydroxybenzoate, Pink: ethyl 4-fluorobenzoate, Green: ethyl 4-dimethylaminobenzoate.

Results

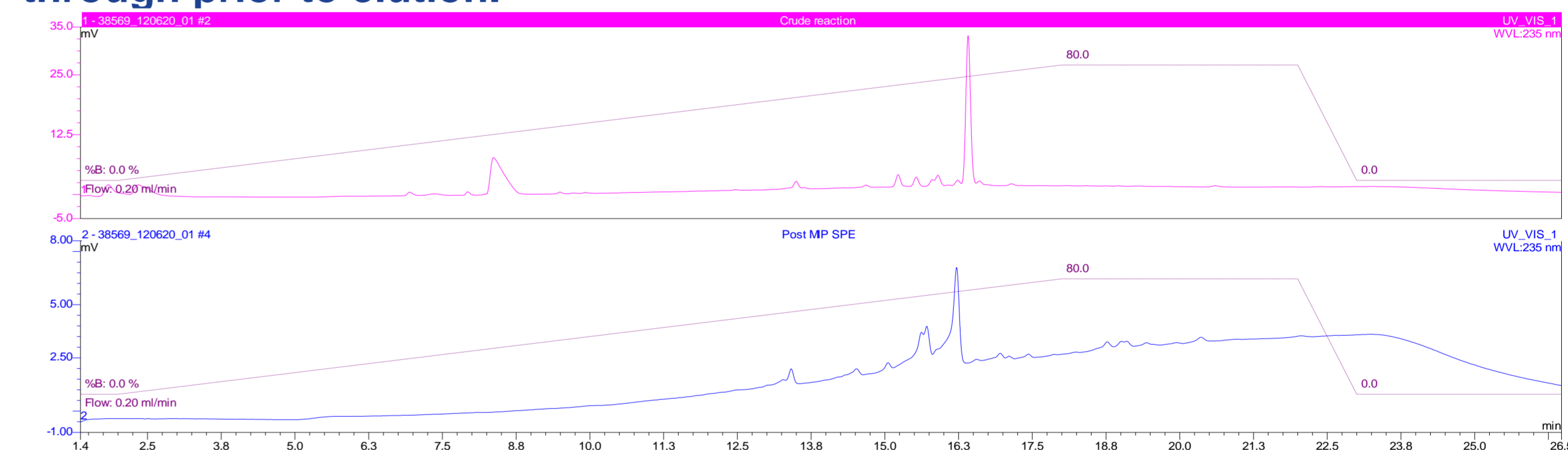
[¹⁸F]Synthons were produced from the corresponding N,N,N-trimethyl-benzenaminium using between 2 to 20 mg of precursor (typically 2 mg). Following labelling, the crude reaction mixtures were diluted with water and then passed through a POLYINTELL AFFINIMIP®SPE 18F cartridge. Controlled studies demonstrated elution with ethanol or acetonitrile resulted in >99% [¹⁸F]synthon recovery. Residual activity on the cartridge was determined to be unreacted [¹⁸F]fluoride by performing trap, wash, elute studies with semi-preparative HPLC purified [¹⁸F]synthons. During high activity runs (up to 90 GBq) the POLYINTELL AFFINIMIP®SPE 18F continued to trap all unreacted [¹⁸F]fluoride. Furthermore, trace 4-[¹⁸F]fluorobenzoic acid that were observed in the high activity production of ethyl 4-[¹⁸F]fluorobenzoate was shown to be completely removed during the cartridge wash step by analysis of this fraction. HPLC analysis demonstrated typically 90 μg of chemical content in the final purified samples of ethyl 4-[¹⁸F]fluorobenzoate and less than 95 μg/ml chemical content in 4-[¹⁸F]FBA formulations



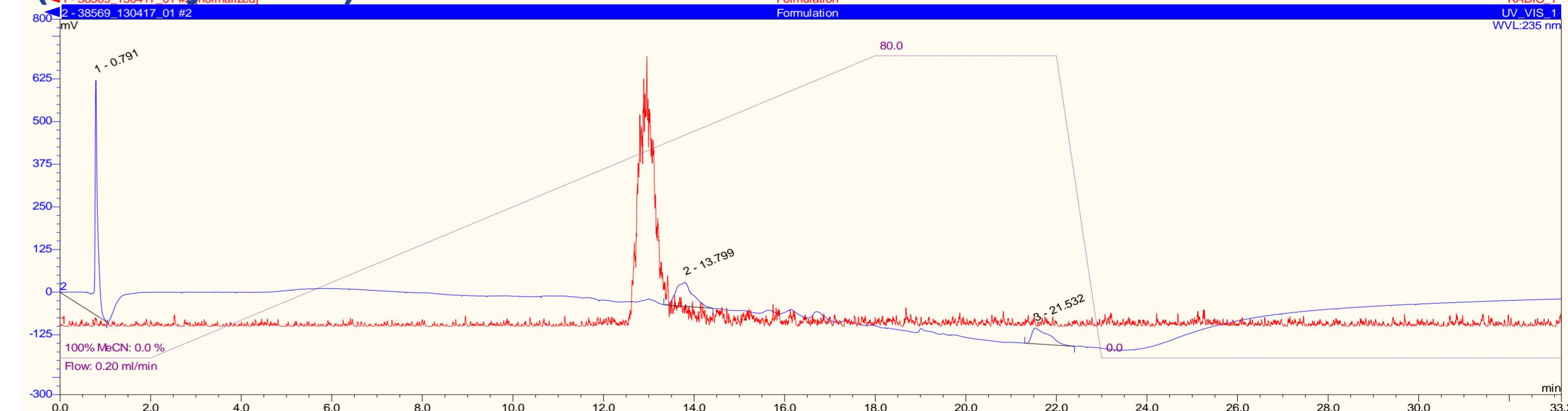
Radiochemical purity (RCP) of >99% was observed in purified samples of ethyl 4-[¹⁸F]fluorobenzoate (red) at the range of starting activities studied.



Analytical HPLC of samples of the crude load solution post AFFINIMIP® SPE 18F (blue), AFFINIMIP®SPE 18F wash solution (pink) and a blank (black) showed that no chemical impurity, target product or [¹⁸F]fluoride broke through prior to elution.



Chemical content was largely removed (blue) compared with crude reactions (diluted sample in pink). 90 μg chemical content remaining in the purified mixture was found to be a combination of cold compound formed through trace ¹⁹F contamination and breakthrough of trace ethyl 4-(dimethylamino)benzoate.



Similarly the above trace demonstrates that 4-[¹⁸F]FBA was produced with an RCP>99%. Total chemical content in the synthon formulation (based on a 4-(dimethylamino)benzaldehyde HPLC standard) was 15.7 μg/ml.

Conclusions

AFFINIMIP®SPE 18F cartridges have been demonstrated to offer excellent purification of the synthons 4-[¹⁸F]FBA and ethyl 4-[¹⁸F]fluorobenzoate. The purification process has been fully automated and the levels of chemical content present in final formulations offer the potential to conjugate to a wide range of peptides/proteins and provide fluorine-18 containing PET tracers with high specific activity. These results demonstrate the benefits of these MIP-based cartridges and highlight the scope for their wider use in F-18 chemistry.

References

- [1] Olberg, D.E., Hjelstuen, O.K. (2010), Curr. Topics Med. Chem., 10(16), 1669-1679.
- [2] Tamayo, F. G., Turiel, E., Martin-Esteban, A (2007), J. Chromatogr. A 1152, 32–40.