"[18F]Fluoromisonidazole synthesis method based on solid-phase extraction (SPE) purification"

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Background and goal of the present work

[18F]FMISO is highly specific especially in hypoxia of gliomas, hypoxia in lung cancer, head and neck carcinomas and other hypoxic tumors. So far, two methodologies have been developed for the production of [18F]FMISO. One method is known as standard and use a synthesis module connected to HPLC purification unit and the second recent developed method use a synthesis module with solid-phase extraction (SPE) cartridges. SPE offers advantages such as simple and faster purification, usage of commercial cartridges, easily adaptable, practical, fast and low cost.

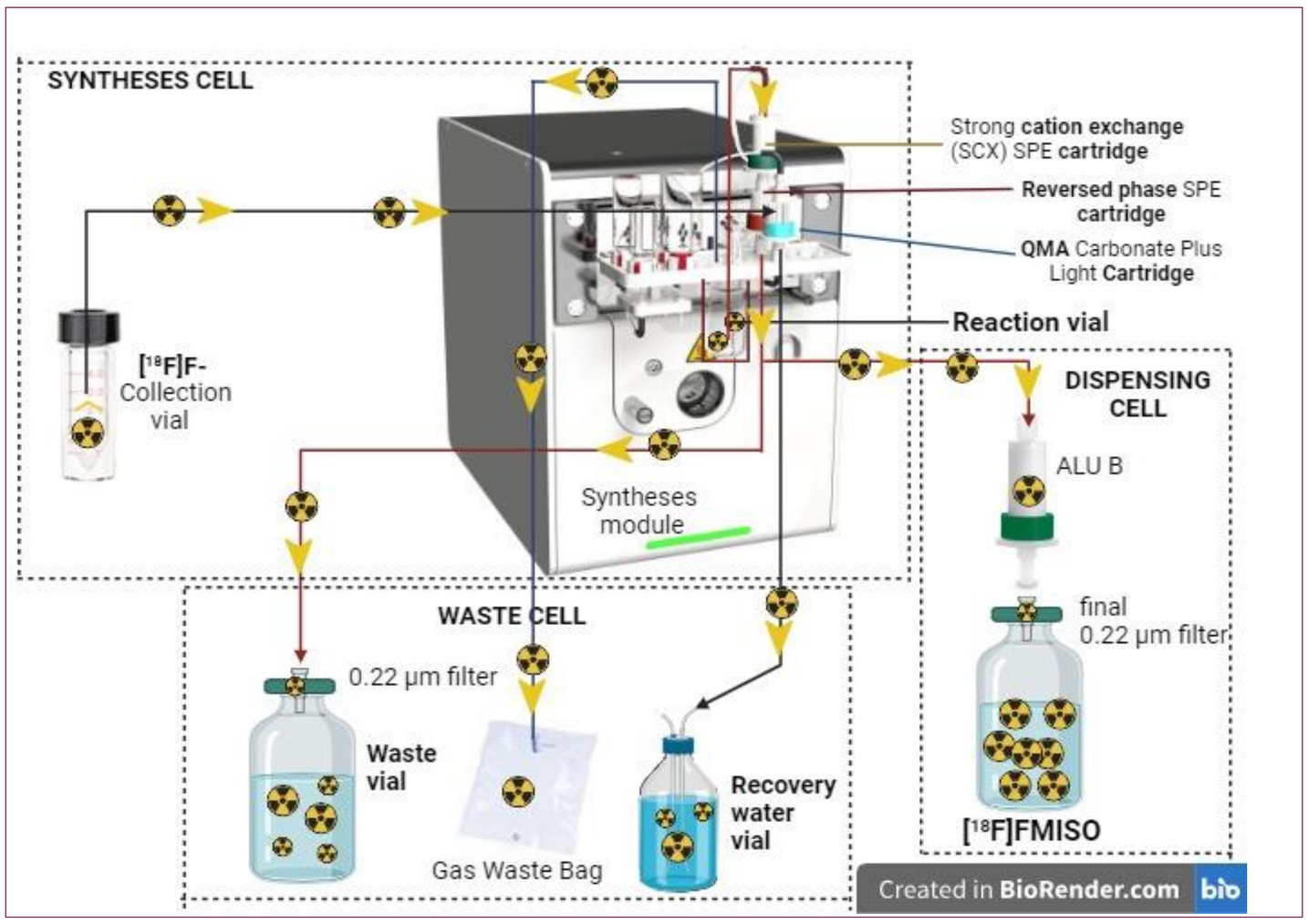
Materials and methods

- Commercial NITTP precursor (1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol).
- Acidic hydrolysis of the tetrahydropyranyl-protection group.
- SPE purification optimization with six different RP extraction cartridge (HLB light, HLB plus, tC18, C18 environmental, PS-RP and HLB light + HLB light) in combination of cation exchange cartridge.

Results

[18F]FMISO radiochemical yield, decay corrected (mean ± SD %) of the product in relation with the fluorination reaction parameters (5 mg NITTP)

Time (min)	Temperature (°C)	Yield (d.c.) ($\bar{X}$ ± SD %)
3	100	5.32 ± 0.56
5	100	14.45 ± 1.14
7	100	32.33 ± 2.46
10	100	37.61 ± 2.46
10	110	42.79 ± 1.99
7	120	37.93 ± 1.59
10	120	46.77 ± 3.19
10	130	53.18 ± 3.44
7	140	30.07 ± 1.24



Conditions under which different syntheses were carried out

	Radio-fluorination	Hydrolysis	Cartridge
S-1	120°C 10 min	4 mL 0,05 M HCl; 90°C 2 min	SCX + 30 mg HLB
S-2	120°C 10 min	2 mL 0,1 M HCl; 90°C 2 min	SCX + 30 mg HLB
S-3	120°C 10 min	4 mL 0,1 M HCl; 90°C 2 min	SCX + 30 mg HLB
S-4	120°C 10 min	4 mL 0,1 M HCl; 100°C 2 min	SCX + 30 mg HLB
S-5	120°C 10 min	4 mL 0,1 M HCl; 110°C 2 min	SCX + 30 mg HLB
S-6	120°C 10 min	4 mL 0,1 M HCl; 110°C 4 min	IC-H+ + 30 mg HLB
S-7	120°C 10 min	4 mL 0,1 M HCl; 120°C 3 min	IC-H+ + C18 Environmental
S-8	130°C 10 min	4 mL 0,1 M HCl; 120°C 3 min	IC-H+ + C18 Environmental
S-9	130°C 10 min	4 mL 0,1 M HCl; 120°C 3 min	SCX + C18 Environmental

To evaluate the influences of time and temperature on the hydrolysis, we observed the reaction at different temperatures (90-120 ºC) and reaction times (2-4 min).

Unhydrolyzed [18F]FMISO intermediates were never detected in the final product confirming that complete hydrolysis was achieved in all runs, but at temperature lower than 110 ºC more chemical unknown peaks were detected.

Results of HPLC analyses of samples from conducted syntheses C1-C9

Peak	S-1 R/T (s) Area (mAU*s)	S-2 R/T (s) Area (mAU*s)	S-3 R/T (s) Area (mAU*s)	S-4 R/T (s) Area (mAU*s)	S-5 R/T (s) Area (mAU*s)	S-6 R/T (s) Area (mAU*s)	S-7 R/T (s) Area (mAU*s)	S-8 R/T (s) Area (mAU*s)	S-9 R/T (s) Area (mAU*s)
Standard solution – UV detector									
DMM (10 mg/mL)	03,38 272,6	03,35 309,8	03,40 344,96	03,36 334,8	03,37 323,9	03,38 344,6	03,40 305,6	03,36 333,9	03,39 348,4
FMISO (10 mg/mL)	05,32 356,3	05,27 400,2	05,36 443,63	05,28 433,2	05,30 444,3	05,33 444,6	05,36 397,7	05,39 392,5	05,36 451,66
Radiochromatogram peak results of the final sample									
[18F]FMISO*	05,29 100%	05,29 100%	05,31 100%	05,29 100%	05,27 100%	05,27 100%	05,34 100%	05,40 100%	05,34 100%
UV chromatogram peak results of final sample									
Peak 1 DMM	03,38 475,1	03,36 336,1	03,36 198,6	03,40 269,70	03,37 155,1	03,37 118,1	03,40 150,1	03,40 164,1	03,44 462,1
Peak 2	04,08 81,75	04,27 416,2	03,57 300,5	04,40 108,1	04,13 149,8	04,05 154,2	04,00 220,2	04,15 231,2	/
Peak 3 FMISO	05,32 10,71	05,32 50,8	05,34 44,6	05,46 8.35	05,30 12,3	05,29 22,2	05,36 23,8	05,39 50,8	05,37 24,8
Peak 4	06,03 183,7	06,03 119,0	06,06 113,0	06,04 181,0	06,00 57,2	06,01 81,2	06,07 90,0	06,03 41,0	/
Peak 5	07,39 83,8	07,39 21,1	07,44 67,3	07,41 152,1	07,33 4,28	07,29 87,2	07,47 34,1	07,39 21,1	
Peak 6	09,43 24,2	09,44 33,3	09,52 10,52	09,46 4.5	09,39 6,3	09,35 12,3	09,55 4,6	09,56 9,0	/

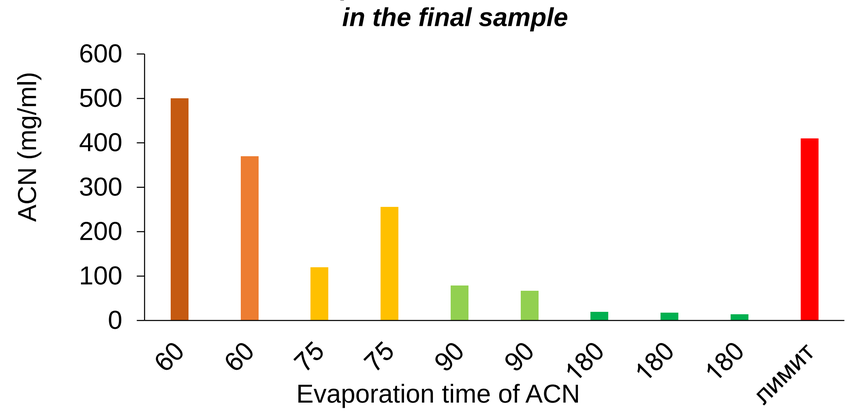
In our study, the cartridges should retain most of the product, while most of impurities pass through the cartridges and are collected in waste vial. In general, polarity of the impurities and final product, type of cartridge, particle size and amount of sorbent, could influence the purification efficiency.

Trapping and elution behavior of [18F]FMISO and by-product DMM with different reversed-phase cartridges

RP cartridge # (weight of sorbent and type)	[18F]FMISO eluted in waste (mean % d.c. ± SD)	[18F]FMISO retained on RP cartridge (mean % d.c. ± SD)	By-product DMM eluted in waste (mg/mL, mean ± SD)	By-product DMM in final product (mg/mL , mean ± SD)
30 mg HLB light	14.44% ± 3.88%	0.48% ± 0.15%	40.19 ± 7.83	6.26 ± 0.82
175 mg PS-RP	34.42% ± 4.58%	0.89% ± 0.20%	37.12 ± 6.66	9.2 ± 0.85
225 mg HLB plus	0%	29.95% ± 4.67%	3.80 ± 1.33	16.24 ± 4,9
820 mg C18 Environmental	1.65% ± 0.48%	1.71% ± 0.61%	1.60 ± 0.31	6.70 ± 0.55
400 mg tC18	20.78% ± 2.31%	12.02% ± 1.34%	32.34 ± 5.71	8.27 ± 0.67
(30 mg HLB light) x 2	0%	10.23% ± 2.50	17.50 ± 2.23	6.50 ± 0.56

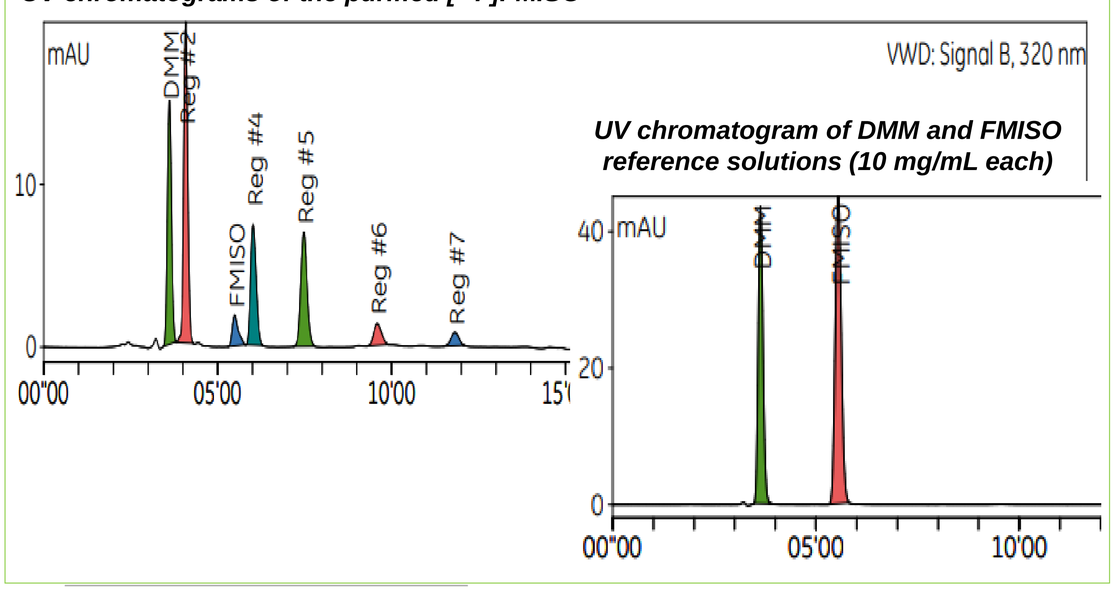
The most effective purification cartridges: Oasis HLB Light, Maxi-Clean SCX, Alumina B Plus Light.

Effect of acetonitrile evaporation time on acetonitrile concentration in the final sample



Considering that when the time is long, the competitive elimination reactions or probability of radiolysis of non-hydrolyzed [18F]FMISO intermediates is obvious, further experiments were performed in order to check whether such a reaction is possible at that time.
Also, our goal was to be able to choose the optimal time for evaporation of acetonitrile through this approach.

UV chromatograms of the purified [18F]FMISO



Conclusion

In this study, a synthesis of [18F]Fluoromisonidazole with SPE purification was successfully developed that can be carried out under aseptic conditions, producing a radiopharmaceutical of quality that meets all the criteria defined in Ph. Eur. Monograph for Fluoromisonidazole (18F) injection.