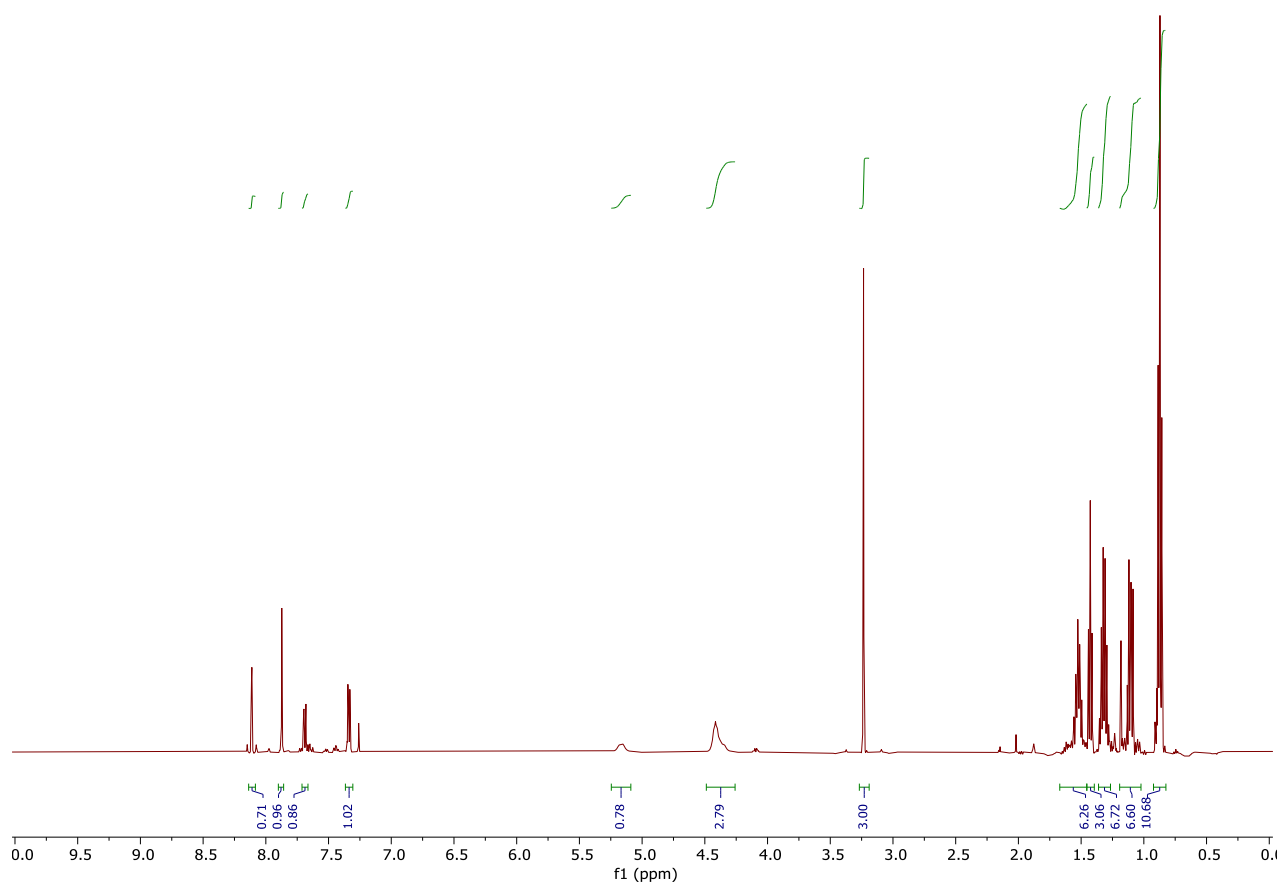


Effective preparation of [^{18}F]Flumazenil using copper mediated late stage radiofluorination of a stannyl precursor

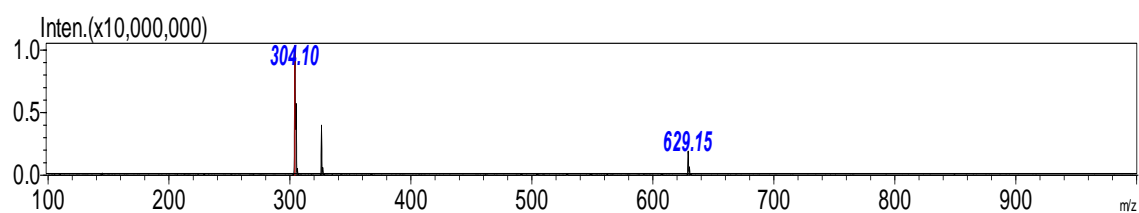
Mohammad B. Haskali ^{1,2*}, Peter D. Roselt ², Terence J O'Brien³, Craig A. Hutton ^{4,5}, Idrish Ali ³, Lucy Vivash ³, Bianca Jupp ³

- 1 Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC 3010, Australia
 - 2 The Radiopharmaceutical Research Laboratory, The Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia; peter.roselt@petermac.org
 - 3 Department of Neuroscience, Central Clinical School, Monash University, Melbourne, VIC 3004, Australia; Terence.O'Brien@monash.edu (T.J.O.); idrish.ali@monash.edu (I.A.); lucy.vivash@monash.edu (L.V.); bianca.jupp@monash.edu (B.J.)
 - 4 School of Chemistry, The University of Melbourne, Melbourne, VIC 3010, Australia; chutton@unimelb.edu.au
 - 5 Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC 3010, Australia
- * Correspondence: mo.haskali@petermac.org; Tel.: +61-(3)-8559-6913

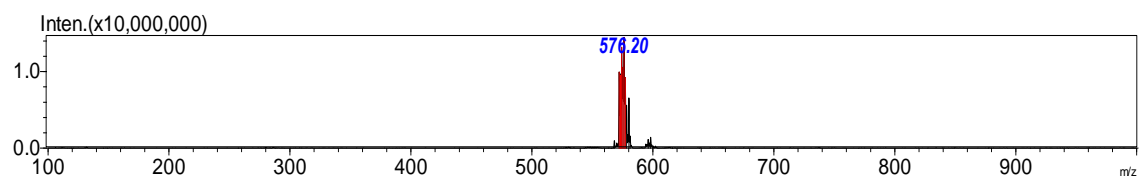
¹H NMR Spectrum of stannyl-mazenil 3



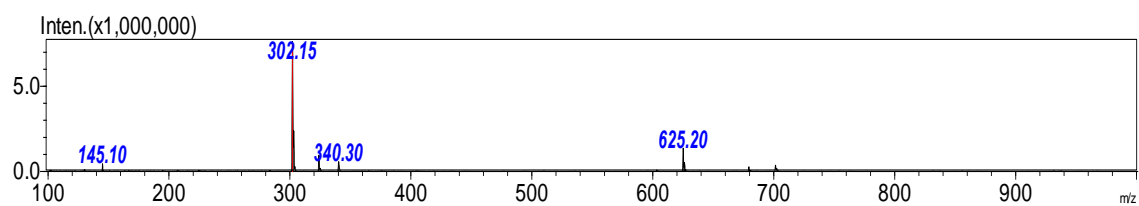
MS Analysis of flumazenil 1



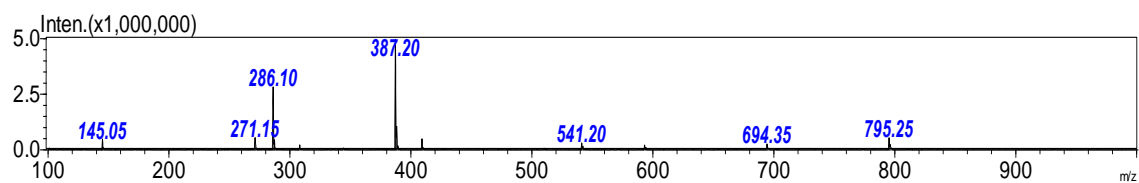
MS Analysis of stannyl-mazenil 3



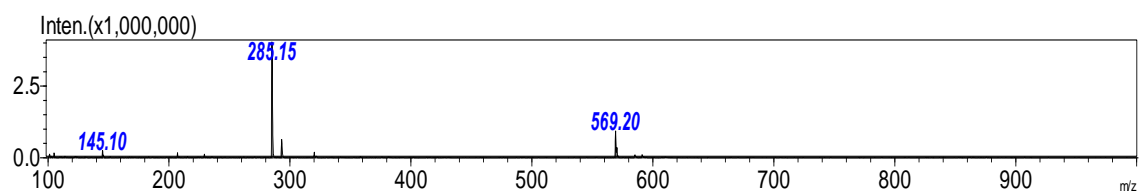
MS Analysis of hydroxy-mazenil 4



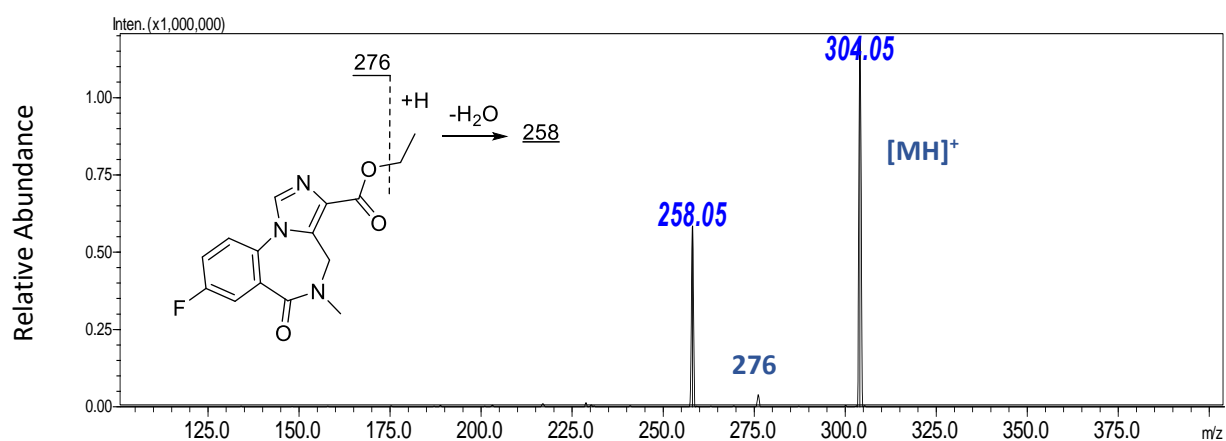
MS Analysis of des-fluoro-flumazenil 5



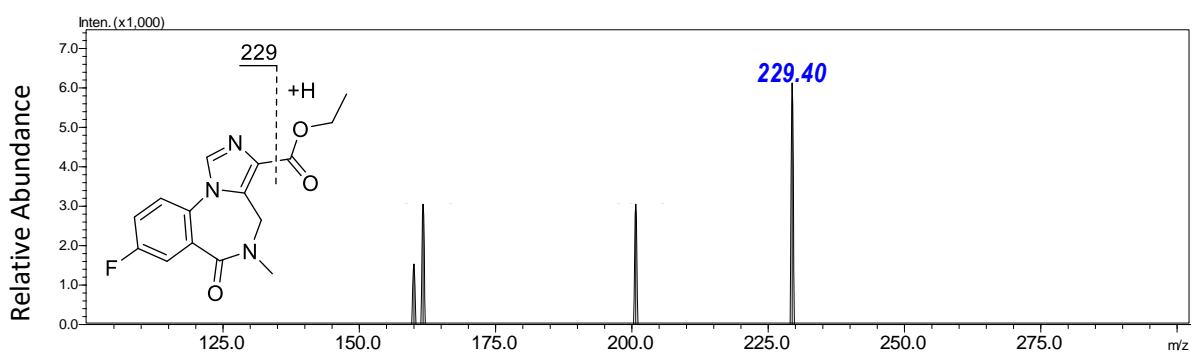
MS Analysis of dimeric-mazenil 6



MS-MS of Flumazenil 1; fragmentation of M+H ion at m/z 304

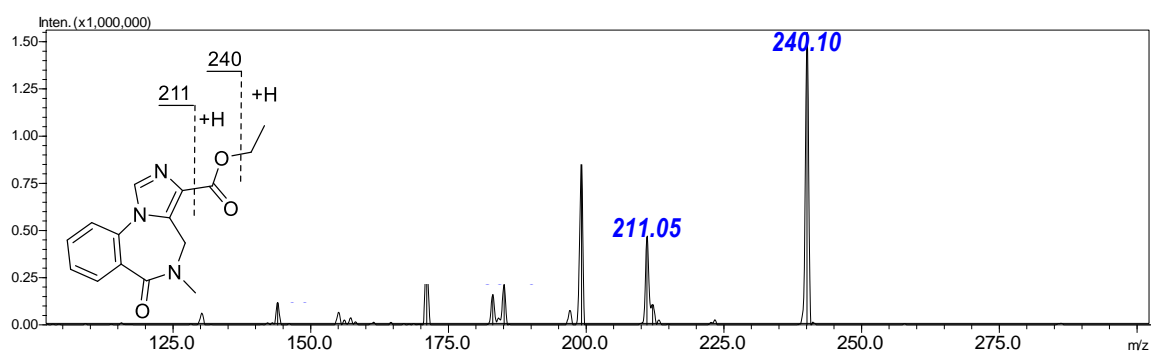


Fragmentation profile at collision energy 10 eV



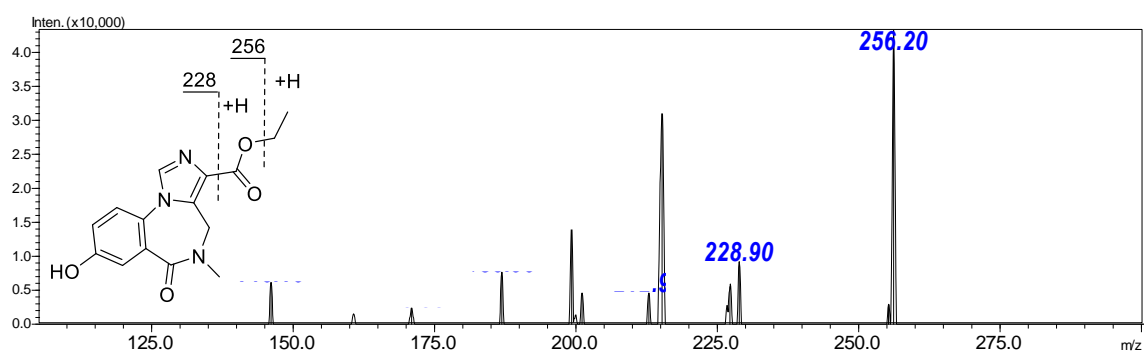
Fragmentation profile at collision energy 35 eV

MS-MS of desfluoro-flumazenil 5; fragmentation of M+H ion at m/z 286



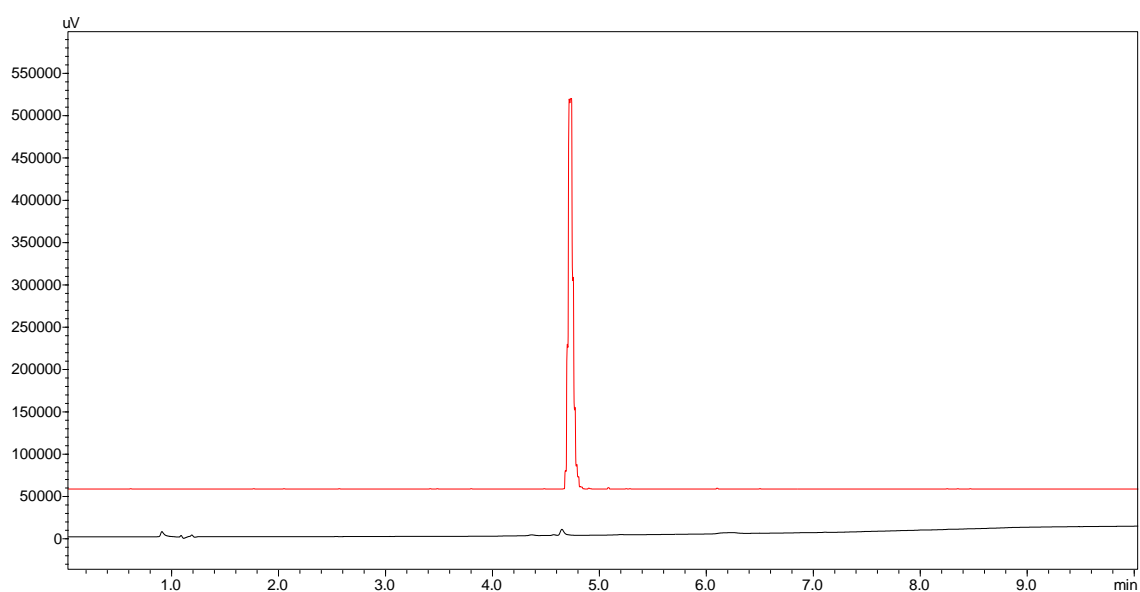
Fragmentation profile at collision energy 10 eV

MS-MS of hydroxyl-mazenil 4; fragmentation of M+H ion at m/z 302



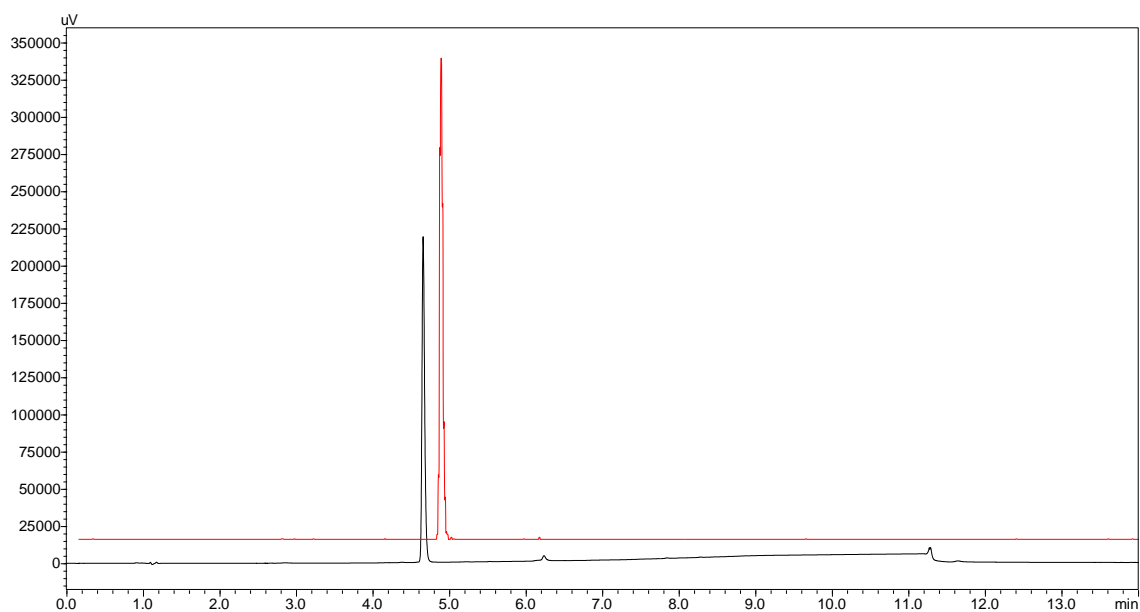
Fragmentation profile at collision energy 10 ev

HPLC chromatogram of purified [^{18}F]FMZ 1



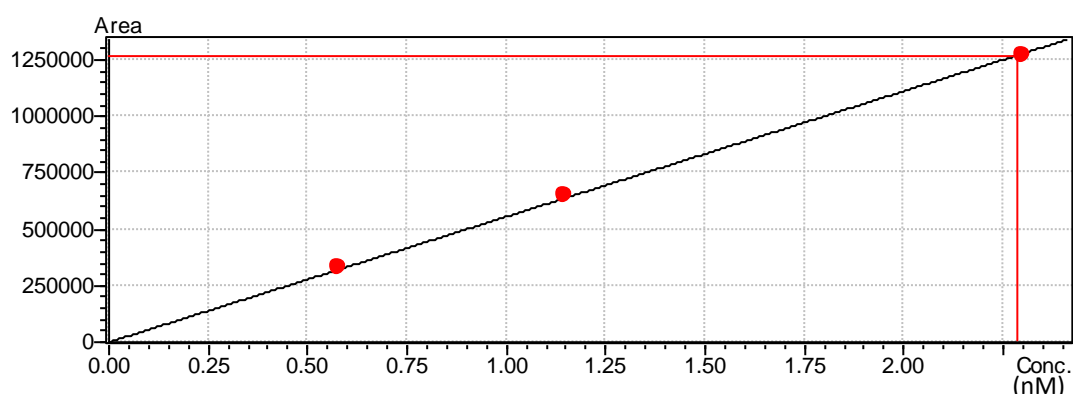
HPLC analysis performed on Kinetex 5 μm XB-C18 4.6 x 150 mm column, 0.1% TFA in 15–90% MeCN:H₂O over 7 min.

[^{18}F]FMZ 1 co-injected with reference standard



HPLC analysis performed on Kinetex 5 μm XB-C18 4.6 x 150 mm column, 0.1% TFA in 15–90% MeCN:H₂O over 7 min.

FMZ 1 Calibration curve



Calibration Information - Compound ID# 1

Compound ID#: 1

Final Column Display: ☒ Area/Height ☐ File Name

Curve Fit Type: Linear

R = 0.9999384

R² = 0.9998767

RSS = 2.412432e+008

%RSD = 1.492423

Y = (556684)X + (0)

Date Processed: 10/02/2021 1:25:22 PM

Level	Cal. Point	Average	# of Reps.	Area 1
1	<input checked="" type="checkbox"/>	328255	1	328255
2	<input checked="" type="checkbox"/>	651778	1	651778
3	<input checked="" type="checkbox"/>	1272810	1	1272810

[¹⁸F]FMZ Automated Production Recipe

Step	Step Message	Step Condition	Step Time (seconds)	V01 (0=off, 1=on)	V02 (0=off, 1=on)	V03 (0=off, 1=on)	V04 (0=off, 1=on)	V05 (0=off, 1=on)	V06 (0=off, 1=on)	V07 (0=off, 1=on)	V08 (0=off, 1=on)	V09 (0=off, 1=on)	V10 (0=off, 1=on)	V11 (0=off, 1=on)	V12 (0=off, 1=on)	V13 (0=off, 1=on)	V14 (0=off, 1=on)
1	Load KHCO3(2 mg in 0.1mL Water), MeCN (0.9mL), K222	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Load FMZ precursor and Pyridine/Cu in DMA (1mL) into vial 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Load 0.1% TFA in H2O:MeCN (60:40, 3mL) into vial 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Load Saline (5mL) into vial 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Load Ethanol (1mL) into vial 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Load saline (10mL) into vial 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Load Water (30ml) into HPLC Flask 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Pressurize Reactor 1	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Waiting for pressure leak in Reactor 1	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Pressure testing Reactor 1	20	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	Install conditioned QMA cartridge at position QMA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	Install conditioned C-18 cartridge at position SPE B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	Check HPLC Eluent A = 0.1% TFA in water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	Check HPLC Eluent B = 0.1% TFA in MeCN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	Connect final product collection vial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Pressurize reagent vials	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	Waiting for pressure leak in reagent vials	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	Pressure testing reagent vials	22	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Ready for F- from cyclotron - Press NEXT when transferred	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	Trapping F- onto QMA cartridge	0	60	0	0	0	0	0	0	0	0	0	0	0	0	1	1
21	Elution of QMA cartridge into Reactor 1	0	60	1	0	0	0	0	0	0	0	0	0	0	0	0	0
22	Recording activity in Reactor for RCY	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
24	MeCN for azeotropic drying	0	60	0	1	0	0	0	0	0	0	0	0	0	0	0	0
24	Drying fluoride (gas + vacuum)	0	340	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	Drying fluoride (vacuum only)	0	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	Cooling Reactor 1	12	400	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	Addition of precursor in tBuOH-MeCN (vial 3) to Reactor 1	0	25	0	0	0	1	0	0	0	0	0	0	0	0	0	0
28	Reaction at 140C for 20min	0	600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	Cooling Reactor 1	12	400	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	Transferring Reactor 1 to Intermediate Vial 1	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	Addition of 0.1%TFA water (vial 6) to Reactor 1	0	30	0	0	0	0	1	0	0	0	0	0	0	0	0	0
32	Pressurize gas lines	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	Transferring Reactor 1 to Intermediate Vial 1	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	Injecting into HPLC loop - Waiting for fluid detector 1 ON	30	60	0	0	0	0	0	0	0	0	1	0	0	0	0	0
35	Injecting into HPLC loop - Waiting for fluid detector 1 OFF	32	60	0	0	0	0	0	0	0	0	1	0	0	0	0	0
36	HPLC purification of [18F]FBR (40min gradient to 5%A 95%B)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	Collecting product peak into HPLC Flask 1 - Click NEXT to	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	Trapping purified [18F]FBR onto C-18 at SPE-B	0	200	0	0	0	0	0	0	0	0	0	0	1	0	0	0
39	Washing FMZ with Saline	0	60	0	0	0	0	0	0	0	0	0	1	0	0	0	0
40	Elution of FMZ into dose vial	0	40	0	0	0	0	0	0	0	1	0	0	0	0	0	0
41	Dilution with 10 ml saline	0	100	0	0	0	0	0	0	1	0	0	0	0	0	0	0
42	Recording activity in Final Product Vial for RCY	6	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
43	Transfer of final product vial out	0	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	Stopping HPLC 2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	End of synthesis - Click ABORT to finish	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[¹⁸F]FMZ Automated Production Recipe Continued

[illegible]

