Syntheses and preclinical evaluations of ^{11}C-labeled radioligands for imaging brain orexin-1 and orexin-2 receptors with positron emission tomography

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Radio-HPLC chromatograms

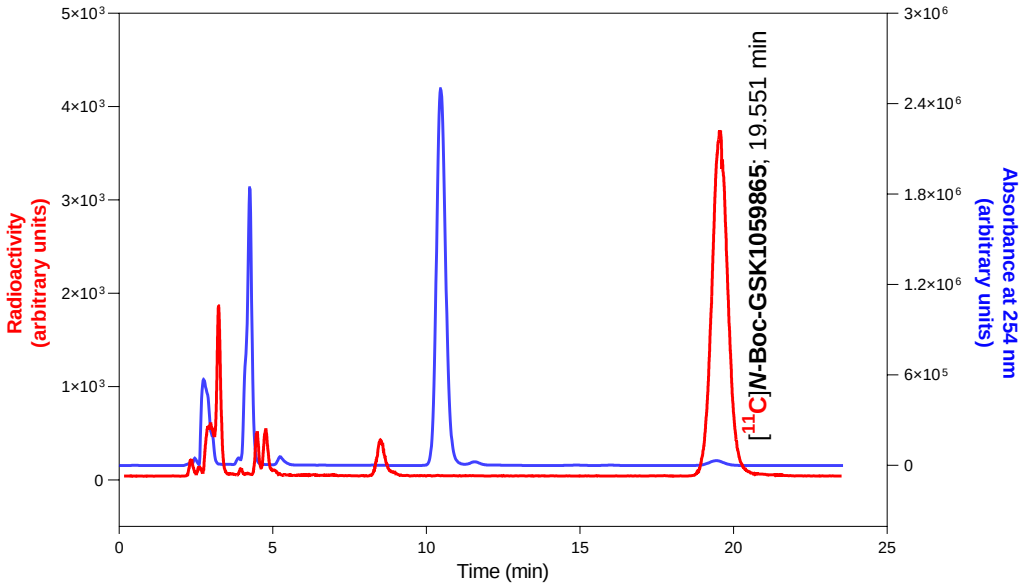


Figure S1. Semi-preparative HPLC chromatogram for crude [^{11}C]*N*-Boc-GSK1059865.

Method: Reversed phase column (Luna C18, 10 µm, 100 Å, 250 mm × 10 mm i.d.; Phenomenex) eluted isocratically at 6.0 mL/min with 0.1% TFA in H_2O (A)–MeCN (B) (32% A–68% B) and with the eluate monitored for radioactivity and for absorbance at 254 nm.

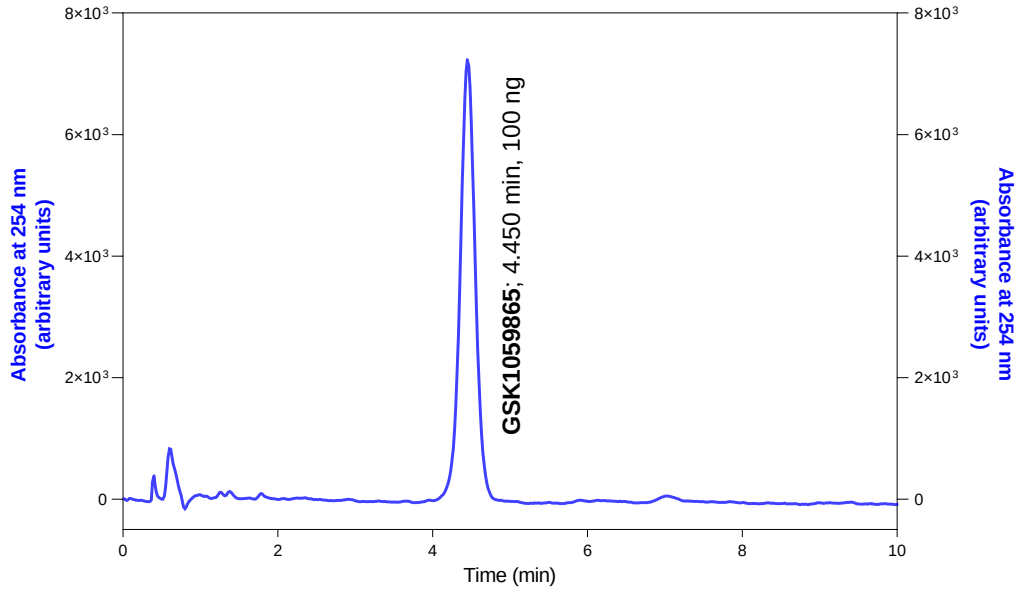
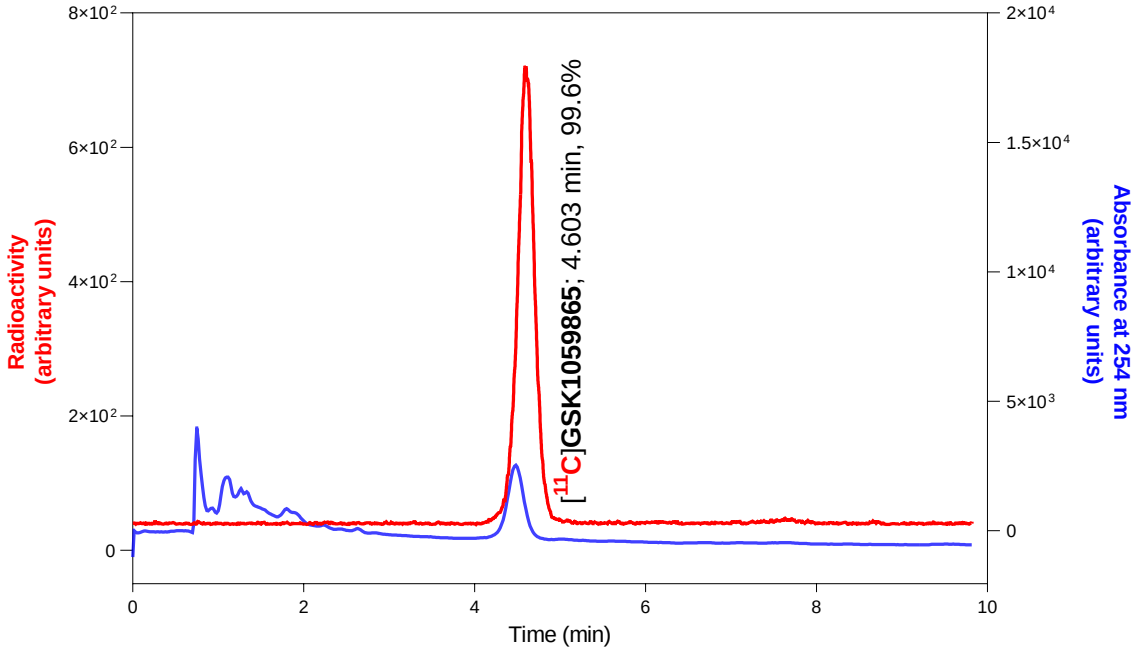
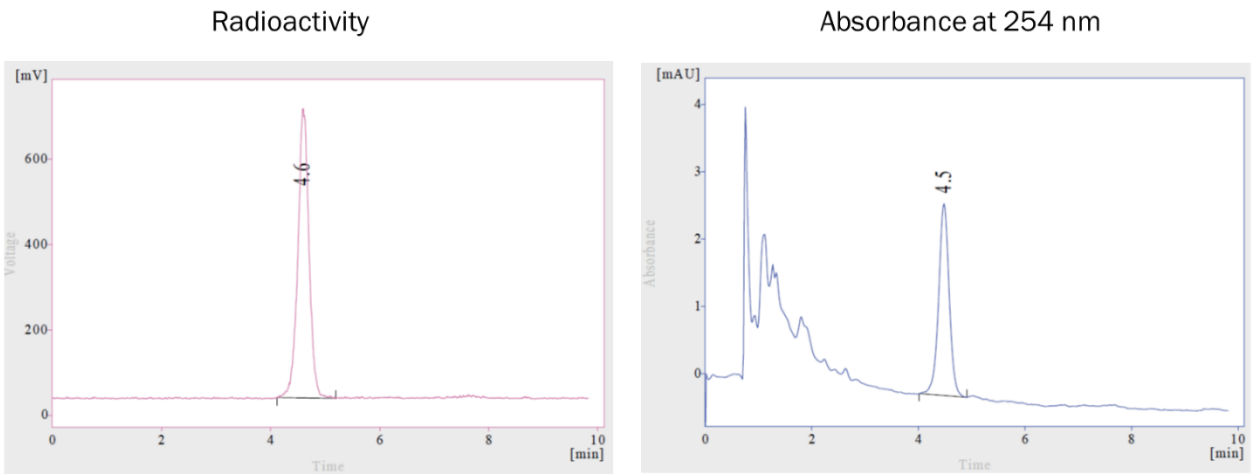


Figure S2. Analytical HPLC chromatogram for reference compound GSK1059865 (**1**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 3.0 mL/min with H_2O (A)–MeCN (B) (40% A–60% B) and with the eluate monitored for absorbance at 254 nm.





Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
4.589	9650.074	678.138	100.0	100.0
Total	9650.074	678.138	100.0	100.0

Reten. Time [min]	Area [µAU.s]	Height [µAU]	Area [%]	Height [%]
4.483	39320.000	2847.556	100.0	100.0
Total	39320.000	2847.556	100.0	100.0

Figure S3. Analytical HPLC chromatogram of formulated [^{11}C]GSK1059865 ([^{11}C]**1**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 3.0 mL/min with H_2O (A)–MeCN (B) (40% A–60% B) and with the eluate monitored for radioactivity and absorbance at 254 nm. Radioactivity injected = 0.0225 GBq.

Weight (ng)	AUC
20	16817
40	37015
60	56776
80	75995
100	98182
MW of **1** (g/mol):	436.33
QC area under the curve (AUC):	39320.00
Injected activity (μCi)	609.00
Mass (ng) = AUC / slope	40.95
Molar mass (nmol) = mass (ng) / MW	0.094
Molar activity (A_m) (mCi/μmol)	6488.30
Molar activity (A_m) (GBq/μmol)	240.07

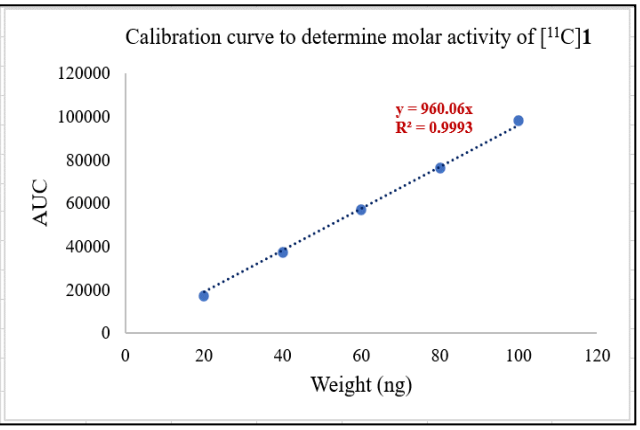


Figure S4. Calibration curve for the determination of molar activity (A_m) of [^{11}C]GSK1059865 ([^{11}C]**1**).

Procedure: 1.00 mg of reference GSK1059865 (**1**) was dissolved in 10 mL MeCN (Stock # 1: 0.1 mg/mL); 100 µL (10 µg) of stock # 1 was diluted to 10 mL with MeCN/H_2O (1: 1, v/v) to obtain stock # 2 with the concentration of **1** as 1.0 µg/mL (or 1 ng/µL). Samples of stock #2 [20 µL (20 ng), 40 µL (40 ng), 60 µL (60 ng), 80 µL (80 ng) and 100 µL (100 ng)] were injected successively onto a reversed phase column (Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 3.0 mL/min with H_2O (A)–MeCN (B) (40% A–60% B) and with the eluate monitored for absorbance at 254 nm. Retention time (t_R) of **1** = 4.45 min.

$$\textbf{Mass of carrier} = \frac{\left(\frac{39320}{960}\right)\textbf{ ng}}{\textbf{436.33 g/mol}} = \textbf{0.094 nmol;}\quad \textbf{Molar Activity } (A_m) = \frac{\textbf{0.0225 GBq}}{\textbf{0.094 nmol}} = \textbf{240 GBq/µmol at EOS}$$

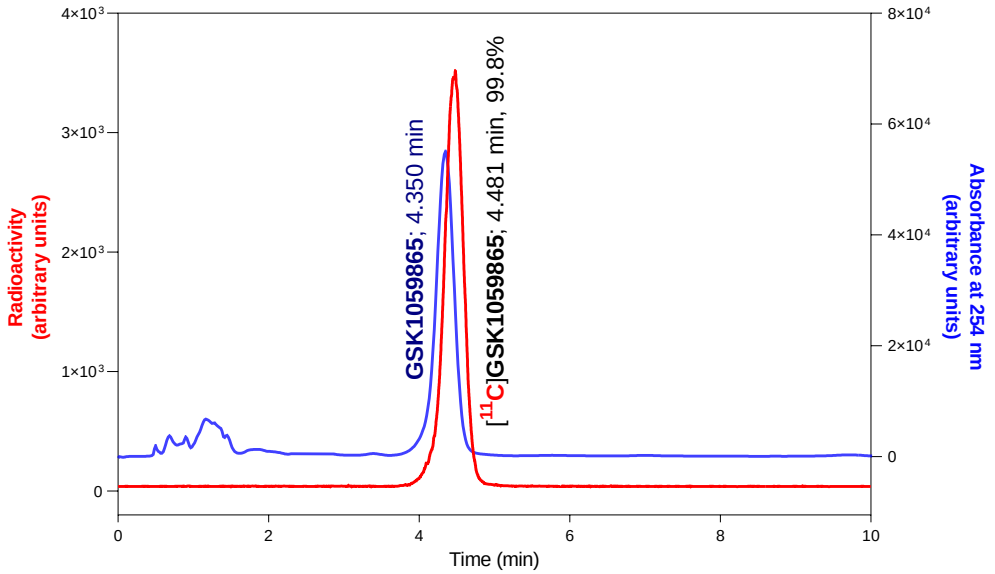


Figure S5. HPLC chromatogram from co-injection of formulated [^{11}C]GSK1059865 ([^{11}C]**1**) and GSK1059865 (**1**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 3.0 mL/min with H_2O (A)–MeCN (B) (40% A–60% B) and with the eluate monitored for radioactivity and absorbance at 254 nm.

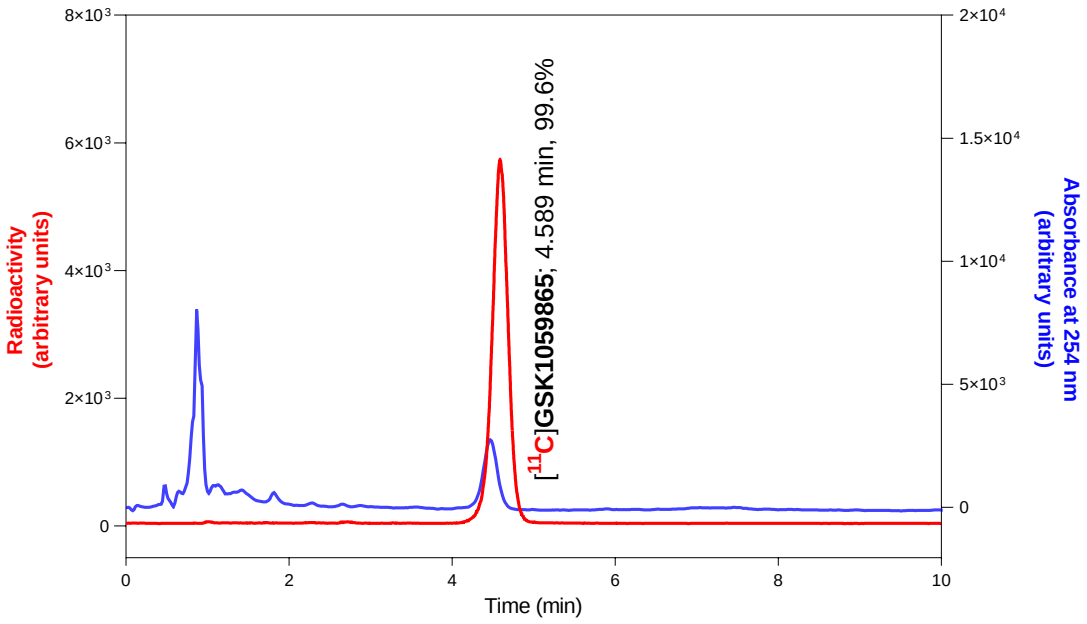


Figure S6. Determination of 1-hour time stability of formulated [^{11}C]GSK1059865 ([^{11}C]**1**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 3.0 mL/min with H_2O (A)–MeCN (B) (40% A–60% B) and with the eluate monitored for radioactivity and absorbance at 254 nm.

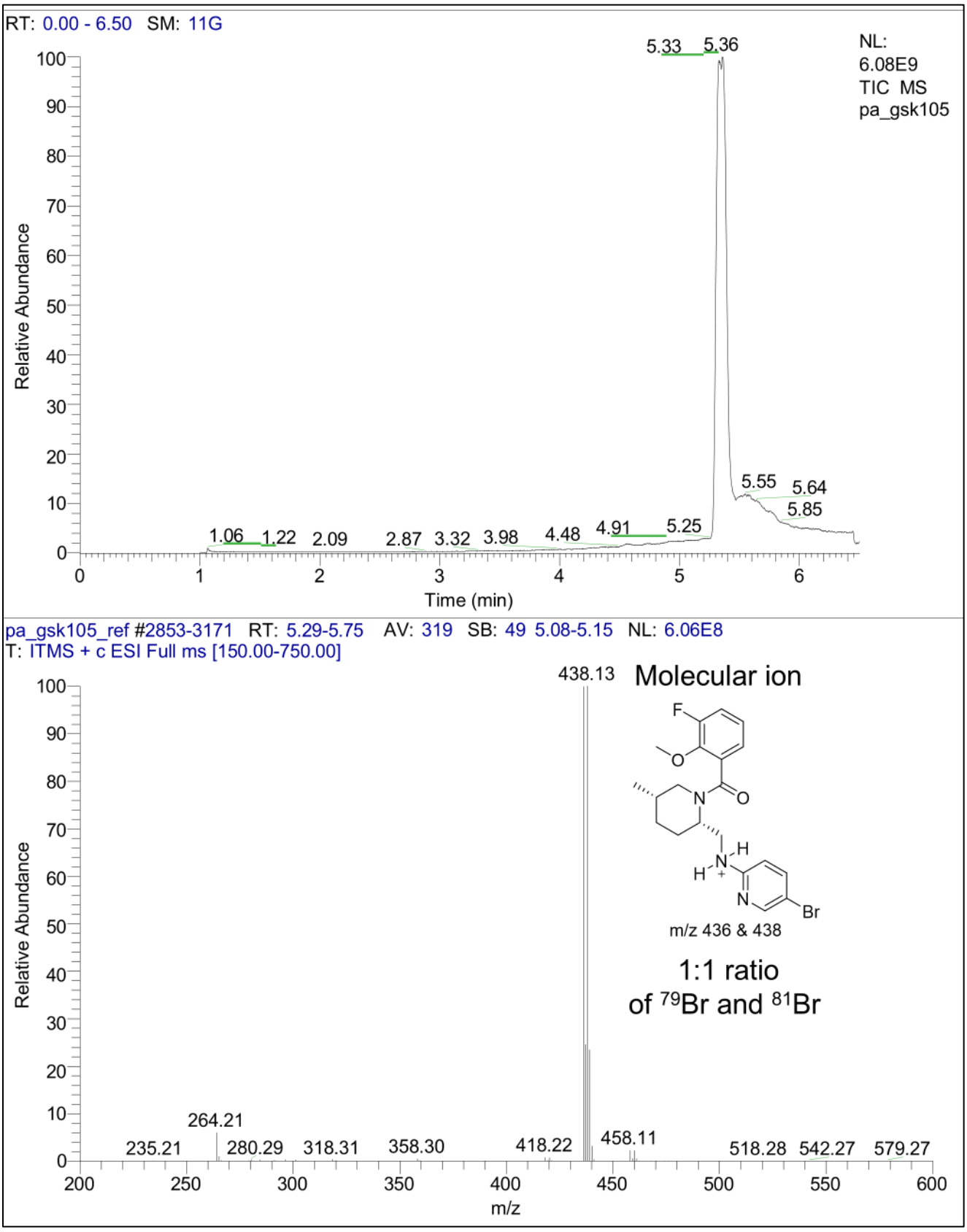


Figure S7. LC–MS spectrum of formulated [^{11}C]GSK1059865 ([^{11}C]**1**).

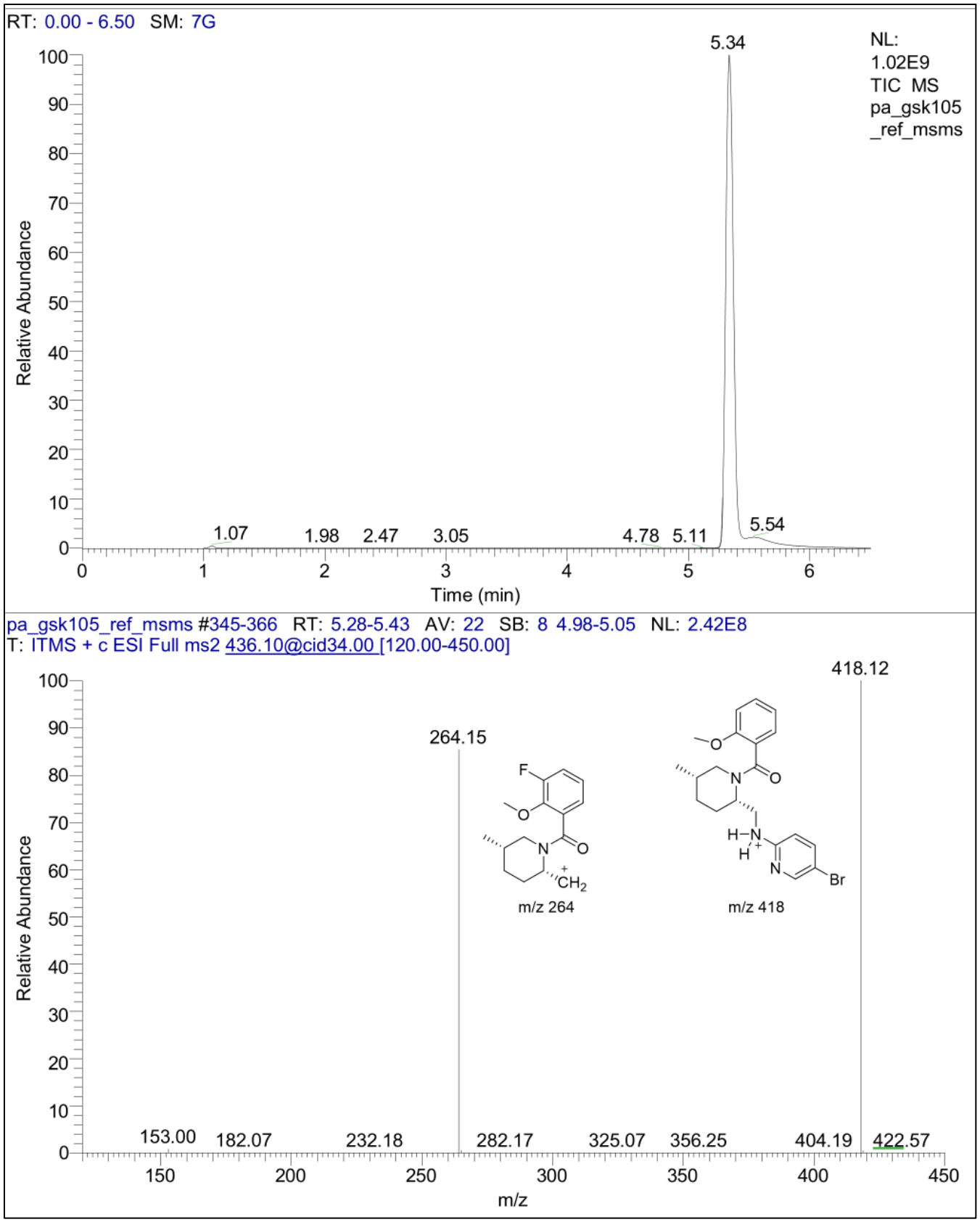


Figure S8. LC–MS/MS spectrum of formulated [^{11}C]GSK1059865 ([^{11}C]**1**).

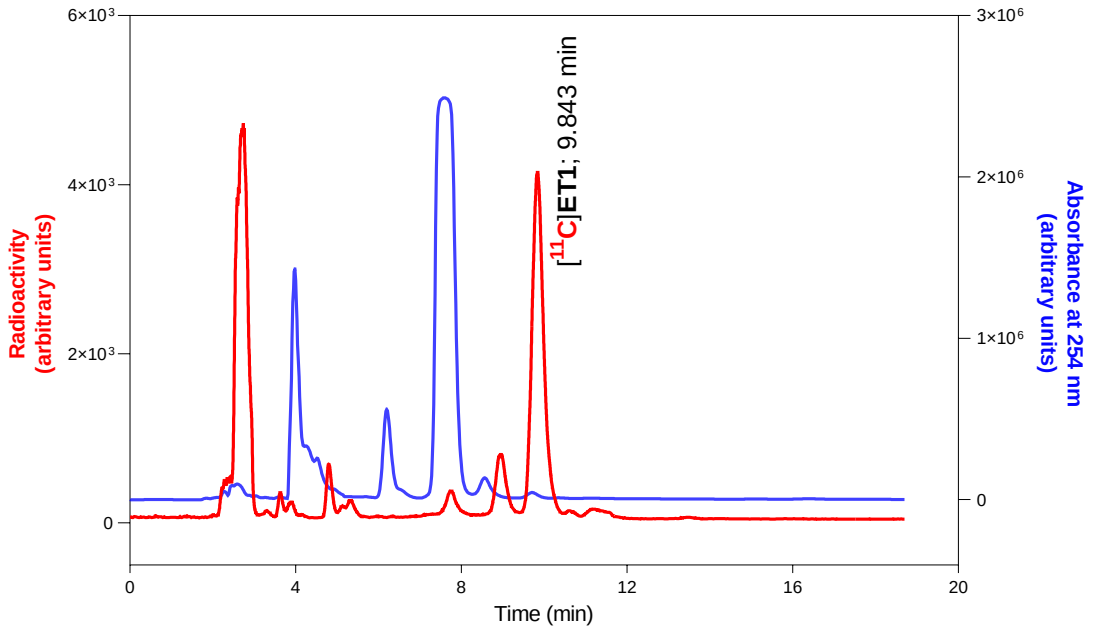


Figure S9. Semi-preparative HPLC chromatogram for crude [^{11}C]ET1 ([^{11}C]**2**).

Method: Reversed phase column (Luna C18, 10 µm, 100 Å, 250 mm × 10 mm i.d.; Phenomenex) eluted isocratically at 6.0 mL/min with 0.1 M aq. $HCOONH_4$ (A)–MeCN (B) (43% A–57% B) and with the eluate monitored for radioactivity and absorbance at 254 nm.

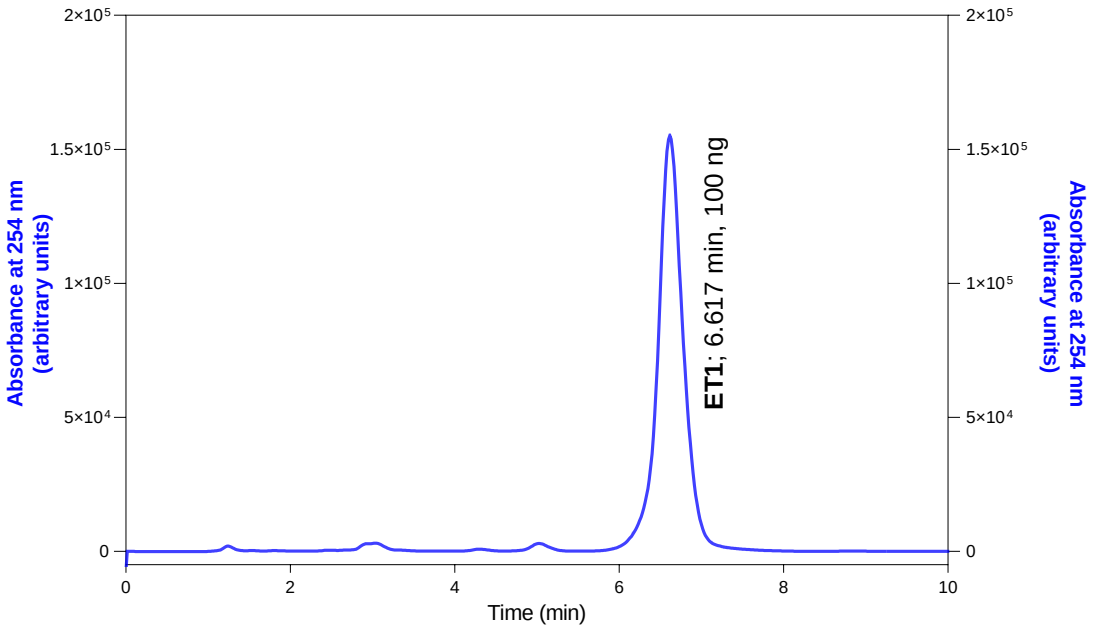
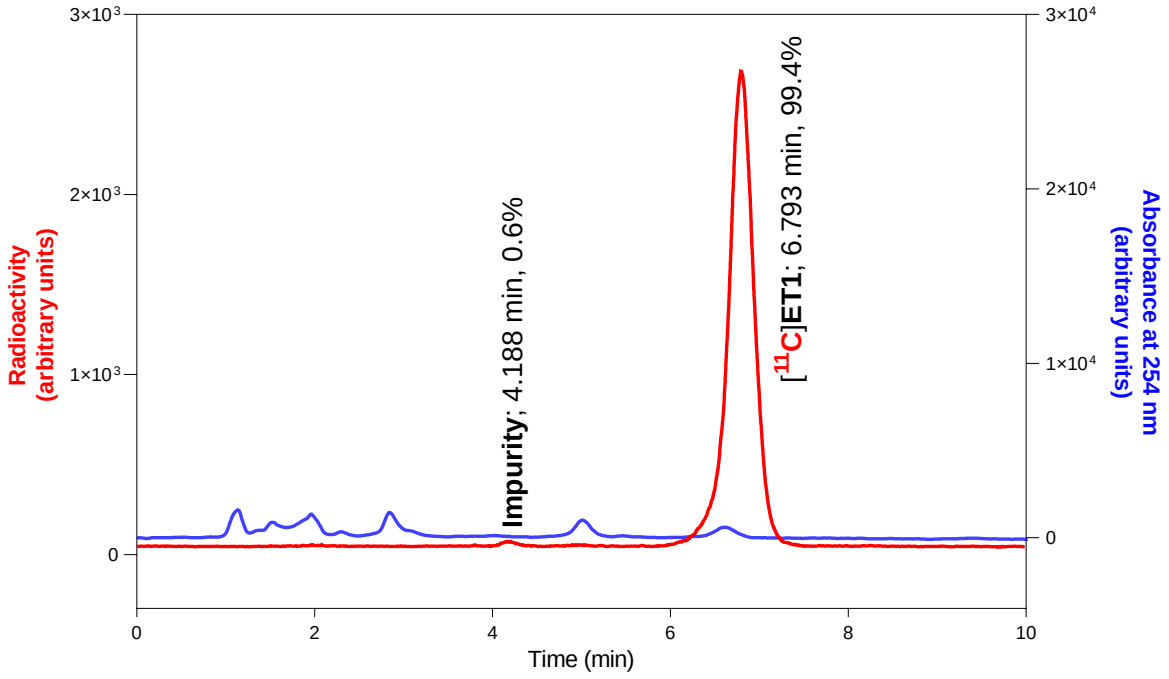
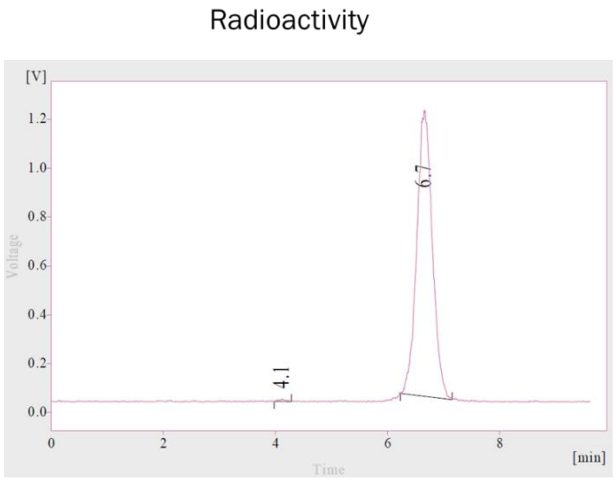


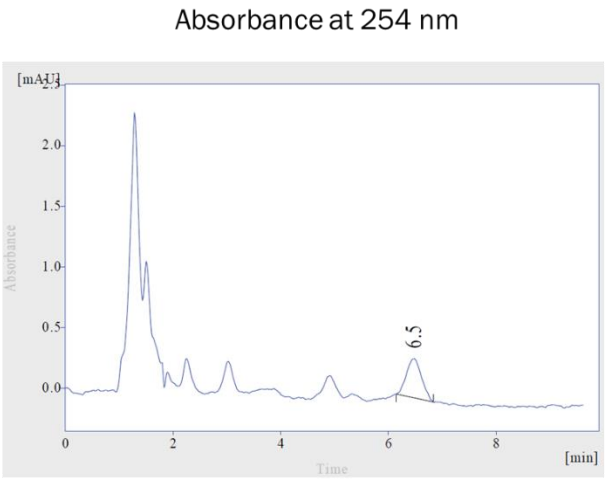
Figure S10. Analytical HPLC chromatogram for reference compound ET1 (**2**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 2.0 mL/min with 0.1 M aq. $HCOONH_4$ (A)–MeCN (B) (45% A–55% B) and with the eluate monitored for absorbance at 254 nm.





Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
4.119	93.829	11.044	0.4	0.9
6.653	22529.457	1169.999	99.6	99.1
Total	22623.286	1181.043	100.0	100.0



Reten. Time [min]	Area [µAU.s]	Height [µAU]	Area [%]	Height [%]
6.483	6324.500	323.707	100.0	100.0
Total	6324.500	323.707	100.0	100.0

Figure S11. Analytical HPLC chromatogram of formulated [^{11}C]ET1 ([^{11}C]**2**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 2.0 mL/min with 0.1 M aq. $HCOONH_4$ (A)–MeCN (B) (45% A–55% B) and with the eluate monitored for radioactivity and absorbance at 254 nm. Radioactivity injected = 0.00645 GBq.

Weight (ng)	AUC
20	15134.0
40	28298.0
60	44277.0
80	56775.0
100	70967.0
MW of **2** (g/mol):	464.56
QC area under the curve (AUC):	6324.50
Injected activity (μCi)	175.00
Mass (ng) = AUC / slope	8.85
Molar mass (nmol) = mass (ng) / MW	0.019
Molar activity (A_m) (mCi/μmol)	9190.94
Molar activity (A_m) (GBq/μmol)	340.06

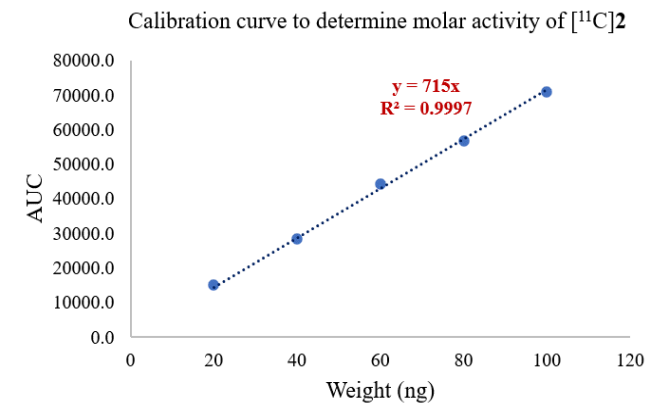


Figure S12. Calibration curve for the determination of molar activity (A_m) of [^{11}C]ET1 ([^{11}C]**2**).

Procedure: 1.00 mg of reference ET1 (**2**) was dissolved in 10 mL MeCN (Stock # 1: 0.1 mg/mL); 100 µL (10 µg) of stock # 1 was diluted to 10 mL with MeCN/H_2O (1: 1, v/v) to obtain stock # 2: with a concentration of **2** at 1.0 µg/mL (1 ng/µL). Samples of stock#2 [20 µL (20 ng), 40 µL (40 ng), 60 µL (60 ng), 80 µL (80 ng) and 100 µL (100 ng)] were injected successively onto a reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 2.0 mL/min with 0.1 M aq. $HCOONH_4$ (A)–MeCN (B) (45% A–55% B) and with the eluate monitored for absorbance at 254 nm. Retention time (t_R) of **2** = 6.62 min.

$$\textbf{Mass of carrier} = \frac{\left(\frac{6324.5}{715}\right)\textbf{ ng}}{\textbf{464.56 g/mol}} = \textbf{0.019 nmol;}\quad \textbf{Molar Activity } (A_m) = \frac{\textbf{0.00645 GBq}}{\textbf{0.019 nmol}} = \textbf{340 GBq/µmol at EOS}$$

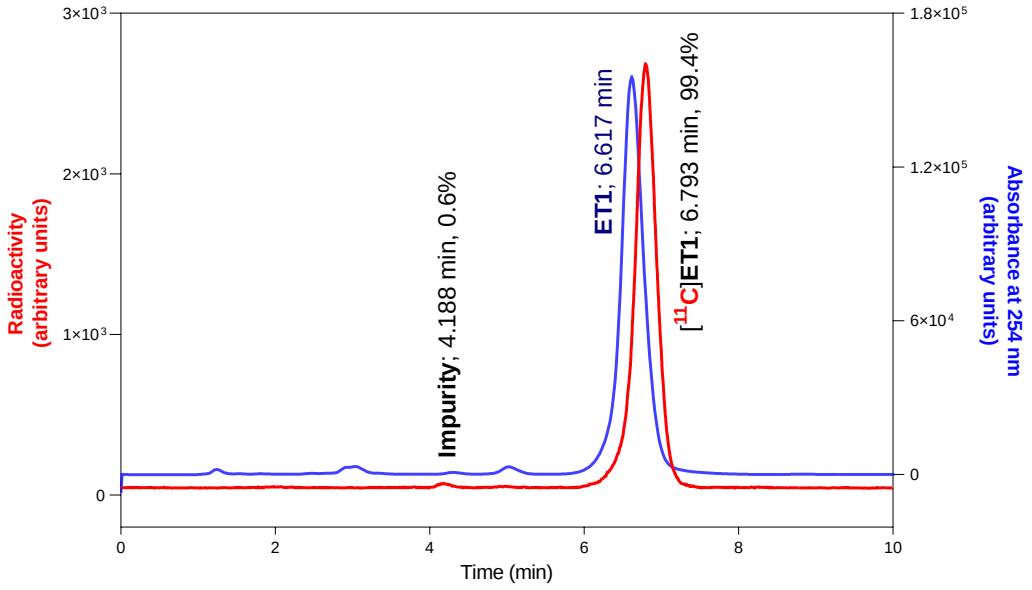


Figure S13. HPLC chromatogram from co-injection of formulated [^{11}C]ET1 ([^{11}C]**2**) and ET1 (**2**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 2.0 mL/min with 0.1 M aq. $HCOONH_4$ (A)–MeCN (B) (45% A–55% B) and with the eluate monitored for radioactivity and absorbance at 254 nm.

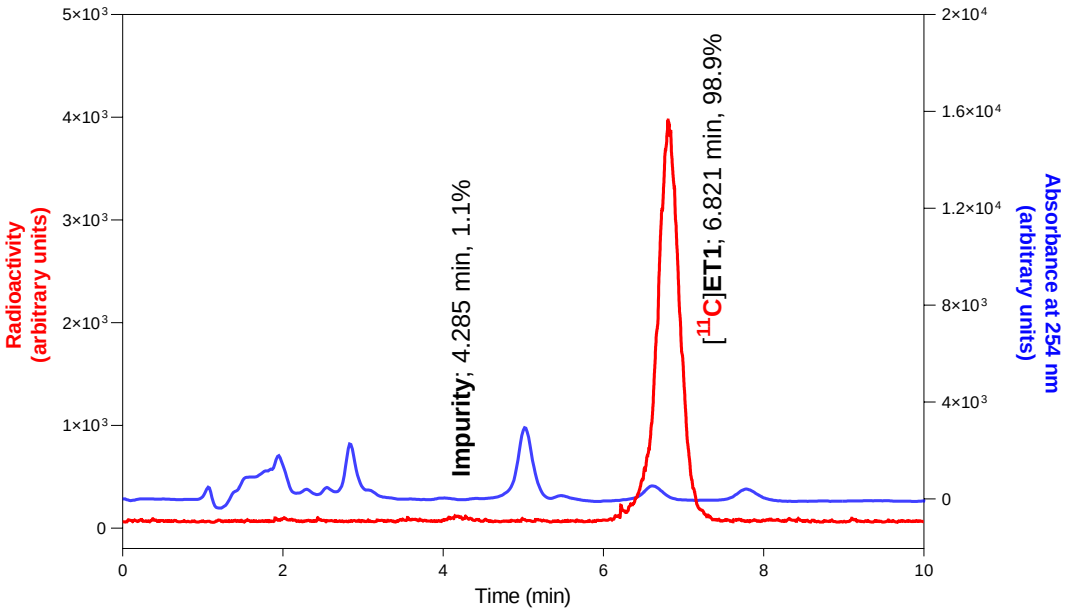


Figure S14. Determination of 1-hour time stability of formulated [^{11}C]ET1 ([^{11}C]**2**).

Method: Reversed phase column **(**Luna C18, 10 µm, 100 Å, 250 mm × 4.6 mm i.d.; Phenomenex) eluted isocratically at 2.0 mL/min with 0.1 M aq. $HCOONH_4$ (A)–MeCN (B) (45% A–55% B) and with the eluate monitored for radioactivity and absorbance at 254 nm.

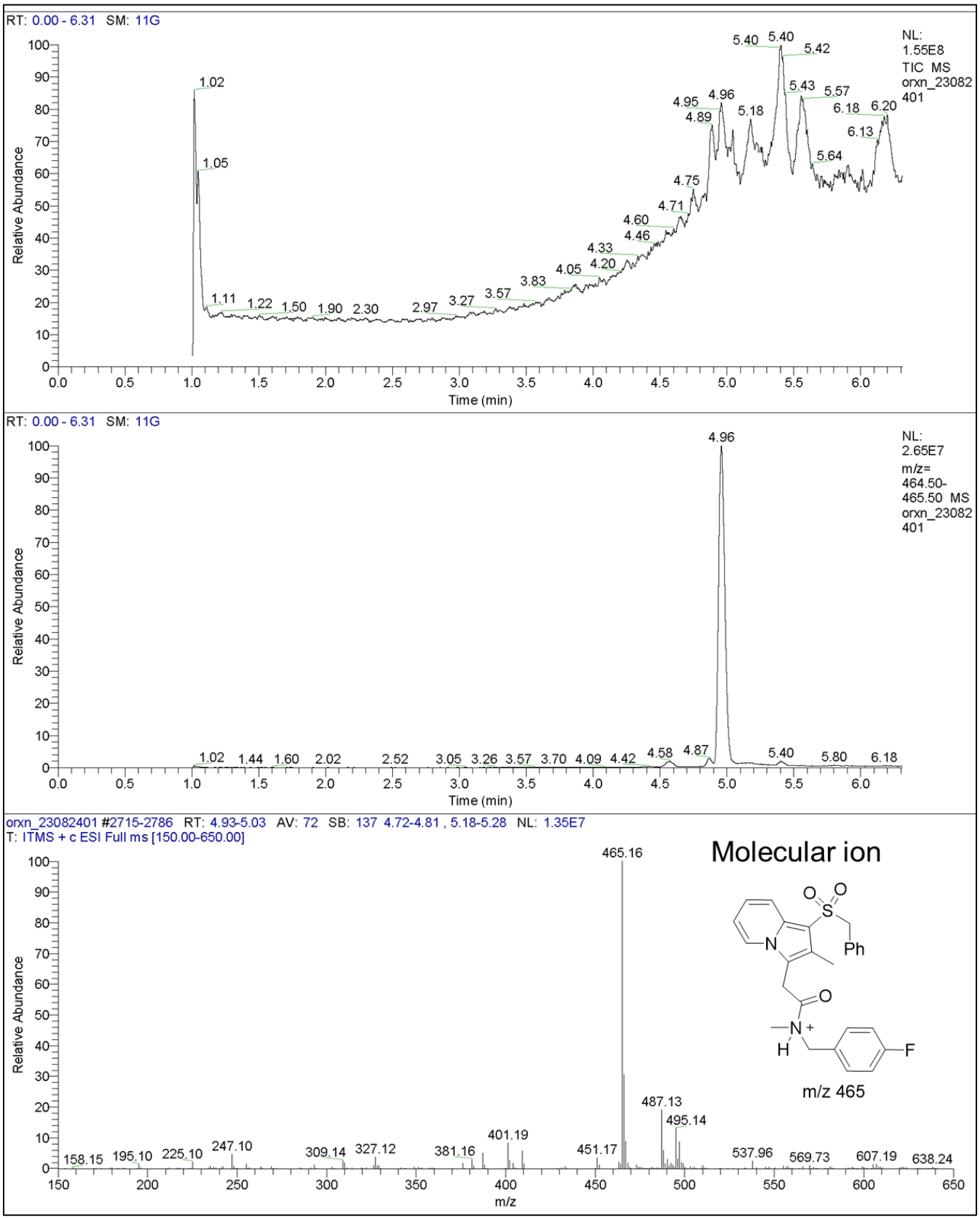


Figure S15. LC–MS spectrum of formulated [^{11}C]ET1 ([^{11}C]**2**).

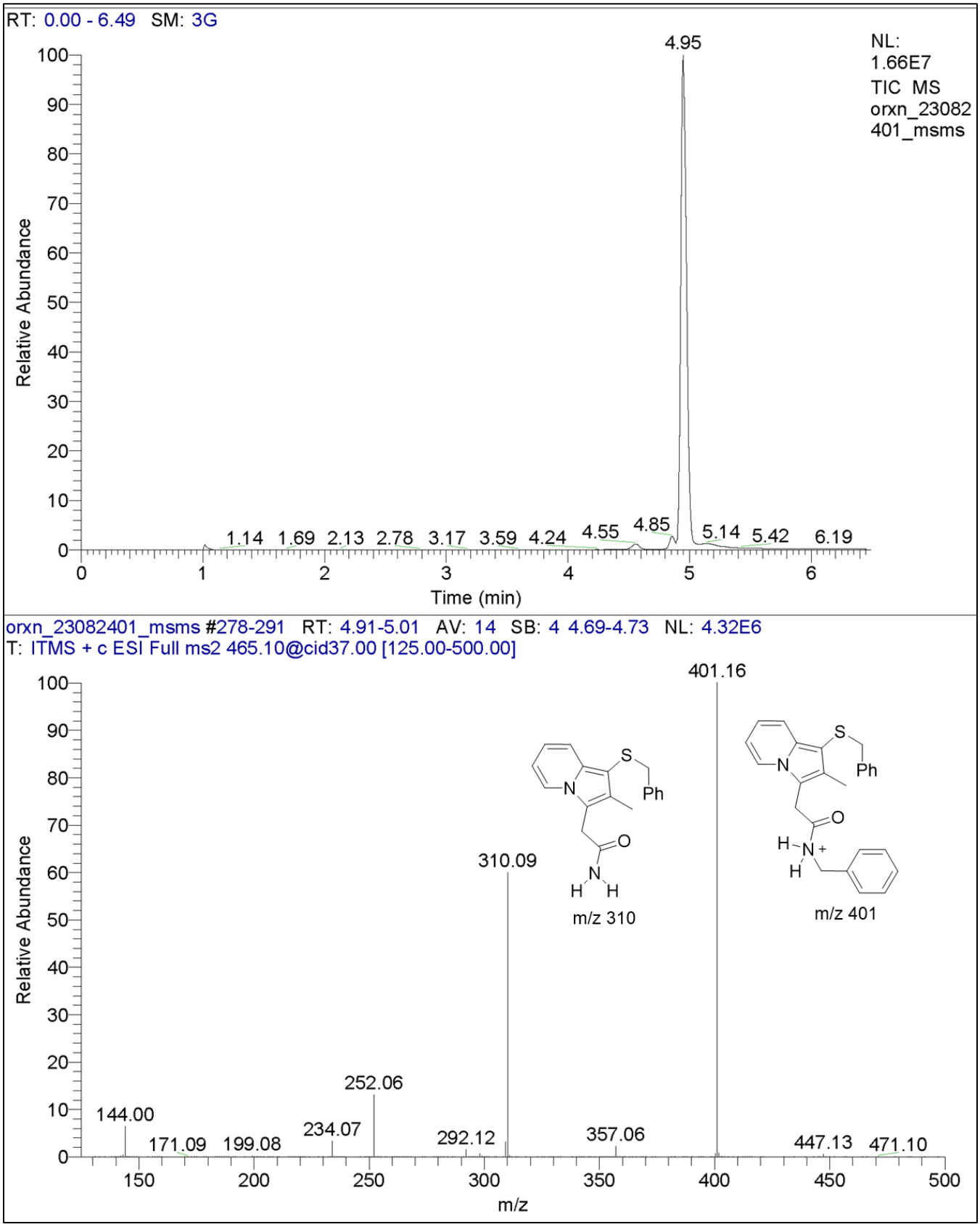


Figure S16. LC–MS/MS spectrum of formulated [^{11}C]ET1 ([^{11}C]**2**).

Table S1. V_T values for all monkey brain regions under baseline (BL) and preblock (BLK) conditions with [^{11}C]**1**.

Brain region	V_T [mL/cm^3] BL	V_T [mL/cm^3] BLK (suvorexant, 0.5 mg/kg)	Increase (%)
Whole brain (WB)	0.56	0.83	48
Frontal cortex (FC)	0.55	0.83	51
Cingulate cortex (AC)	0.58	0.85	47
Striatum (ST)	0.57	0.84	47
Insula (Insul)	0.55	0.83	51
Temporal cortex (TE)	0.55	0.82	49
Amygdala (Amy)	0.56	0.81	45
Hippocampus (HP)	0.58	0.84	45
Thalamus (TH)	0.58	0.87	50
Parietal cortex (PA)	0.58	0.82	41
Occipital cortex (OC)	0.59	0.84	42
Cerebellum (CE)	0.57	0.84	47
Hypothalamus (HY)	0.58	0.81	40
Pons (Pons)	0.61	0.89	46
Midbrain (MB)	0.62	0.87	40
Average			46

Table S2. V_T values for all monkey brain regions under baseline (BL) and preblock (BLK) conditions with [^{11}C]**2**.

Brain regions	V_T [mL/cm^{-3}] BL	V_T [mL/cm^{-3}] BLK (suvorexant, 0.5 mg/kg)	Increase (%)
Whole brain (WB)	1.25	1.45	16
Frontal cortex (FC)	1.29	1.50	16
Cingulate cortex (AC)	1.27	1.38	9
Striatum (ST)	1.22	1.40	14
Insula (Insul)	1.21	1.42	18
Temporal cortex (TE)	1.27	1.52	20
Amygdala (Amy)	1.23	1.43	17
Hippocampus (HP)	1.16	1.36	17
Thalamus (TH)	1.16	1.33	15
Parietal cortex (PA)	1.24	1.43	15
Occipital cortex (OC)	1.26	1.49	19
Cerebellum (CE)	1.22	1.35	11
Average			15