Development, validation and implementation of radio-HPLC methods for the P2X7-receptor-targeted [^{11}C]GSK1482160 radiopharmaceutical

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Abstract

A radio-analytical RP-HPLC method was developed and validated to support production of the P2X7-receptor-targeted [^{11}C]GSK1482160 radiopharmaceutical. Method validation included characterization of retention times, peak shapes, linearity, accuracy, precision, selectivity, limits of detection and quantitation (UV signal), radiochemical stability, as well as analytical method range and robustness. The validated radio-HPLC method is suitable for the definition of [^{11}C]GSK1482160 radiochemical identity, radiochemical purity as well as molar activity, and is being employed in support of human studies with [^{11}C]GSK1482160.

1. Introduction

The P2X7 receptor is expressed on cells of hematopoietic origin, such as activated macrophages, monocytes, and microglia (Adinolfi et al., 2018; Barlett et al., 2014; Collo et al., 1997; Di Virgilio et al., 2017; Domercq et al., 2013; Sanz et al., 2009; Skaper et al., 2010; Volonte et al., 2012), and is rapidly up-regulated by inflammatory stimuli. Thus, the P2X7 receptor appears attractive as a possible molecular target for development of diagnostic imaging agents to evaluate and monitor tissue inflammation.

GSK1482160 is a negative allosteric modulator of P2X7 function (Abdi et al., 2010; Ali et al., 2012) with documented bioavailability, pharmacokinetics, pharmacodynamics, safety, and tolerability after oral administration to humans (Ali et al., 2012). The high affinity of GSK1482160 (K_d = 1.1 nM) for the human P2X7 receptor (Territo et al., 2017), coupled with its established human pharmacology (Ali et al., 2012), make [^{11}C]GSK1482160 (Gao et al. 2015; Territo et al., 2017), (Figure 1), an attractive candidate radiopharmaceutical for assessing whether P2X7 receptor expression can serve as a marker for PET detection of regional inflammation.

We report here the development and validation of a reversed-phase high performance liquid chromatography (RP-HPLC) radio-analytical method suitable for pre-release quality control testing of [^{11}C]GSK1482160 radiopharmaceutical intended for administration to human research subjects.

2. Materials and Methods

[^{11}C]GSK1482160 was synthesized following the methods described previously (Gao et al. 2015). A Waters 1524 HPLC Binary System, equipped with both a Waters 2487 UV Detector and a Carroll & Ramsey single channel high sensitivity radiation detector, was used to provide a qualitative and quantitative determination of [^{11}C]GSK1482160 in radiopharmaceutical samples. The validated radio-HPLC method employed a C18 Prodigy column, 5 μm ODS – 3 V 100 Å (250 x 4.6 mm), eluted isocratically with mobile phase, containing 35.6% acetonitrile : 64.4% 20 mM aqueous phosphoric acid at a flow rate of 1.80 mL/min (20 μL HPLC sample injections). Non-radioactive GSK1482160 and desmethyl-GSK1482160 precursor (Gao et al., 2015) were detected and analyzed based on their UV absorbance at 275 nm (the measured λ_{max} value of both GSK1482160 and desmethyl-GSK1482160), while [^{11}C]GSK1482160 and radiochemical impurities were detected with the downstream scintillation detector. Desmethyl-GSK1482160 precursor and GSK1482160 reference standard solutions were prepared at various concentration levels ranging from 0.3 nmol/mL to 15.0 nmol/mL (in the expected concentration range of the radiopharmaceutical sample synthesized) to demonstrate analytical HPLC method performance.

Reversed phase high performance liquid chromatography (RP-HPLC) radio-analytical method validation included characterization of retention times (t_R), peak shapes, linearity, accuracy, precision-repeatability, selectivity, system suitability, chemical and radiochemical stability, as well as limits of detection and quantitation (UV signal) for both desmethyl-GSK1482160 precursor and GSK1482160. Data obtained from linearity, accuracy and precision studies was used to assess method range and robustness. Molar activity was calculated from the quantity of radioactivity injected and the

GSK1482160 carrier mass quantified with the UV detector. The method established with the non-radioactive standards was validated in three production runs of [^{11}C]GSK1482160 radiopharmaceutical product, demonstrating tracer identity, chemical and radiochemical purity and radiochemical stability.

In addition, we report the findings from our subsequent implementation of the method in support of PET studies with [^{11}C]GSK1482160 in ten normal human volunteers.

3. Results and Discussion

A robust reverse phase (C_{18}) HPLC method for [^{11}C]GSK1482160 was developed and validated, employing isocratic elution with a mobile phase of 35.6% acetonitrile : 64.4% 20 mM aqueous phosphoric acid. In accordance with *USP <1225> Validation of compendial procedures* (2018), typical analytical performance characteristics along with methods by which it may be measured were assessed to demonstrate the suitability of the HPLC analytical procedure. In characterizing the suitability of the HPLC method, we took into account the synthetic route to the [^{11}C]GSK1482160 radiopharmaceutical, the suitability of the chromatographic conditions, and the HPLC column along with the UV detector signal responses.

USP <1226> Verification of compendial procedures (2018) does not provide specific acceptance criteria for the performance characteristics of the radio-analytical HPLC methods due to the fact that the verification and validation requirements are based on the complexity of the procedure and the materials to which the procedure is applied to. Therefore, our acceptance criteria for the performance characteristics of the radio-HPLC analytical method was based on laboratory studies, demonstrating the identity, strength, quality and purity of [^{11}C]GSK1482160 radiopharmaceutical produced. Our implemented specifications for the product quality include: radiochemical purity ≥ 95%; desmethyl-GSK1482160 present at ≤ 5% of the UV-detected GSK1482160 mass; radiochemical identity established by HPLC retention time within 10% of the reference standard. These specifications are

consistent with former USP Monographs for other ^{11}C-labeled PET radiopharmaceuticals(*e.g.,* [^{11}C]raclopride). Our specification for molar activity was ≥ 0.50 mCi/nmol (≥18.5 MBq/nmol) at expiration. At this molar activity limit, the administered mass of GSK1482160 will remain below 20-µg, even at the expected maximum administered [^{11}C]GSK1482160 dose of 30 mCi (1.1 GBq). This maximal mass dose of GSK1482160 can be regarded as pharmacologically benign, based on the reported clinical trial oral dosing of GSK1482160, the associated blood levels of GSK1482160, and the reported minimum anticipated biological effect level of 4 ng/mL blood (Ali et al, 2012).

As such, system suitability testing was performed to verify that the accuracy and precision of the HPLC system was adequate for analysis. System parameters such as: resolution (R), tailing factor (T), plate count (N) and relative standard deviation (RSD) for ten replicate standard injections of the solution containing both analytes (desmethyl-GSK1482160 and GSK1482160) at 100% of the targeted concentration (target/nominal concentration of 10.0 nmol/mL) were determined. Selectivity (specificity) was demonstrated for both analyte peaks. Desmethyl-GSK1482160 precursor and GSK1482160 peaks were found to be well separated, with retention times (t_R) of 5.6 and 7.2 minutes, respectively (Figure 2), and without interference from other chemical entities. The resolution (R) between the two components was $R > 5$. The tailing factor (T) for both desmethyl-GSK1482160 and GSK1482160 standard was $1 < T < 2$. The plate count or theoretical plates (N) were $N > 6000$.

Standard samples solutions were prepared at five concentration levels (in the expected radiopharmaceutical sample concentration range) with triplicate sample preparation for each concentration, in order to establish calibration curves for both components, desmethyl-GSK1482160 and GSK1482160 respectively (see Appendix A. Supplementary material).

The minimum level at which both components can be reliably detected, as expressed by Limit of Detection (LOD), was calculated based on the signal-to-noise ratio. Measured signals of known low concentration standard solutions were compared to saline blank sample solutions, and the minimum concentration at which the analytes could be reliably detected was established. A signal-to-noise ratio

of 7:1 was considered to be acceptable for establishing LOD (the lowest standard concentration for six consecutive standard injections, with an RSD < 10%, was considered to provide reliable detection). The Limits of Detection (LOD) were 0.30 nmol/mL for both the desmethyl-GSK1482160 precursor and GSK1482160. A signal-to-noise ratio of 13:1 was considered acceptable for establishing the Limits of Quantitation (LOQ). The Limit of Quantitation was established according to *USP <1225> Validation of Compendial Procedures* (2018), as the lowest sample concentration that gives a signal-to-noise ratio of at least 10:1 (the minimum concentration at which the sample can be reliably quantified), or a peak height at least ten times the baseline noise level. Based on experimental data (Appendix A. Supplementary Material), the Limits of Quantitation (LOQ) were 0.60 nmol/mL for both components, desmethyl-GSK1482160 precursor and GSK1482160 respectively.

Method accuracy was demonstrated for desmethyl-GSK1482160 and GSK1482160 by preparing three replicate standard sample solutions at three concentration levels over the range of 50% to 150% of the nominal sample concentration (of 10.0 nmol/mL). The acceptance criteria as represented by the Relative Standard Deviation (RSD) for the three replicate samples was established as less than 5% and the mean sample recovery was deemed acceptable within the range of 90% to 110% of the sample concentration theoretical value. The RSD for both components was RSD < 5% and the % Average Sample Recovery was within the acceptable range (of 90% - 110% of theoretical value) for both components (experimental data is included in the Appendix A. Supplementary material).

The precision-repeatability was demonstrated by performing ten replicate HPLC injections of a standard sample solution at the nominal concentration (of 10 nmol/mL) and according to the HPLC method procedure. The precision-repeatability acceptance criteria was established by the Relative Standard Deviation, as RSD < 5.0% for the retention times, peak heights and peak area responses for both components (see Appendix A. Supplementary material).

Linearity was demonstrated in the sample concentration range of 1 nmol/mL to 15 nmol/mL (for both desmethyl-GSK1482160 and GSK1482160). The linearity acceptance criteria was established by the

correlation coefficient (R^2), as $R^2 \geq 0.980$ and the y-intercept, as y-intercept < 2% of the target concentration area response. The correlation coefficient (R^2) calculated at five standard concentration levels, was $R^2 > 0.999$, while the y-intercept % of target concentration response was < 2 % for both components (see Appendix A. Supplementary material).

Data obtained during linearity, accuracy and precision determinations were used to assess the range of the radio-HPLC analytical method. The analytical method has a suitable level of precision, accuracy and linearity in the sample concentration range of 1 nmol/mL to 15 nmol/mL. The HPLC analytical method proved to be robust and remained unaffected by small deliberate variations in the procedural parameters, or small changes in the chromatographic conditions (*e.g.,* unchanged across various batches of the HPLC columns, different HPLC systems, different analysts, minor changes in the ambient temperature of ± 2°C, minor mobile phase pH variations of ± 0.2, minor flow rate variations of ± 0.1 mL/min).

In addition, radiotracer identity for the [^{11}C]GSK1482160 radiopharmaceutical was established based on demonstrating an HPLC retention time identical to the non-radioactive GSK1482160 standard (Figure 2, Figure 3). The [^{11}C]GSK1482160, with a retention time of 7.2 minutes, was well resolved from an unidentified minor radiochemical impurity (< 5.0 %) with the retention time of 5.2 minutes (Figure 3). We have been unable to establish the chemical identity of this radiochemical impurity, as it generates no corresponding UV signal on the UV detector.

The stability of the [^{11}C]GSK1482160 product was demonstrated by repeating the radio-HPLC analysis at, or beyond, the radiopharmaceutical's expiration time (set as no more than one hour from the terminal sterilizing filtration). There was no evidence of the degradation of the [^{11}C]GSK1482160 product at room temperature, with the radiochemical purity always exceeding 95% at the expiration time for the validation batches (Table 1). The final formulation of the radiopharmaceutical drug product was isotonic sterile saline (0.9% sodium chloride) containing ~5% ethanol.

Radio-HPLC method validation involving the analysis of the [^{11}C]GSK1482160 from three production

runs consistently showed the the desmethyl-GSK1482160 precursor to be absent in the final product (Figure 4).

The undesired ^{11}C - isomeric side-product, which arises in small amounts by competitive ^{11}C-methylation at the side chain amide N position of the desmethyl-GSK1482160 precursor (Figure 1), was always effectively removed in the semi-preparative HPLC separation of the production process that precedes final [^{11}C]GSK1482160 formulation.

Radio-HPLC of the isomeric impurity produced (Figure 1) as a minor side-product and isolated as a separate fraction after collecting the [^{11}C]GSK1482160 fraction in semi-preparative HPLC shows a small quantity of [^{11}C]GSK1482160 at t_R = 7.2 minutes, in addition to the isomeric side-product with t_R = 7.9 minutes (Figure 5). Using the validated analytical HPLC method for [^{11}C]GSK1482160 analysis, we determined that the ^{11}C-side-product (t_R = 7.9 minutes; Figure 5) is well separated from the [^{11}C]GSK1482160 product (t_R = 7.2 minutes; Figure 3). The final formulated [^{11}C]GSK1482160 radiopharmaceutical product never showed evidence of radiochemical contamination by the undesired alkylation isomer.

Repeated chromatographic analyses show no evidence of the GSK1482160 reference standard degradation over one year, as well as no evidence of [^{11}C]GSK1482160 degradation over the one-hour period prior to its labeled expiration.

This radio-HPLC method has been fully implemented as part of the quality control procedures for the [^{11}C]GSK1482160 synthesized for use in PET imaging with human subjects. In production runs for the first ten subjects, [^{11}C]GSK1482160 radiochemical purity averaged 96.25 ± 1.25%, with a molar activity average of 0.51 ± 0.1 mCi/nmol (18.9 ± 3.7 MBq/nmol) at the labeled dose expiration time (Table 2). At the time of administration, molar activity averaged 1.03 ± 0.28 mCi/nmol (38.1 ± 10.4 MBq/nmol), with doses averaging 26 ± 2 mCi (962 ± 74 MBq).

4. Conclusions

A reversed phase high performance liquid chromatography (RP-HPLC) radio-analytical method was developed, and its performance validated to meet USP guidelines for linearity, accuracy, precision, selectivity, limit of detection, limit of quantitation (UV signal), range, and robustness. The analytical method is robust and unaffected by small changes in chromatographic conditions. The performance of this radio-analytical method is suitable for pre-release assessment of [^{11}C]GSK1482160 radiopharmaceutical, defining product radiochemical purity, molar activity, as well as the absence of contamination by the desmethyl-GSK1482160 precursor. This radio-HPLC method supports delivery of the [^{11}C]GSK1482160 radiopharmaceutical in accordance with the requirements for identity, strength, quality and purity outlined in *USP <823> Positron emission tomography drugs for compounding, investigational, and research uses* (2018).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at:

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Figures

Figure 1. Synthetic route to the [^{11}C]GSK1482160 radiopharmaceutical (Gao et al., 2015).

Figure 2. HPLC–UV: Example of the separation of desmethyl-GSK1482160 precursor and GSK1482160 (t_R Desmethyl-GSK1482160 = 5.6 minutes, t_R GSK1482160 = 7.2 minutes).

Figure 3. Radio-HPLC results for a typical [^{11}C]GSK1482160 radiopharmaceutical preparation (t_R Radiochemical impurity = 5.2 minutes, t_R [^{11}C]GSK1482160 = 7.2 minutes).

Figure 4. HPLC-UV results for a typical [^{11}C]GSK1482160 final product sample (t_R GSK1482160 = 7.1

minutes, sample concentration = 10 nmol/mL).

Figure 5. Radio-HPLC of the isomeric impurity fraction (t_R [^{11}C]GSK1482160 = 7.2 minutes, t_R Isomeric impurity = 7.9 minutes).

Table 1. Characteristics of three [^{11}C]GSK1482160 validation batches analyzed using the validated radio-HPLC analytical method.

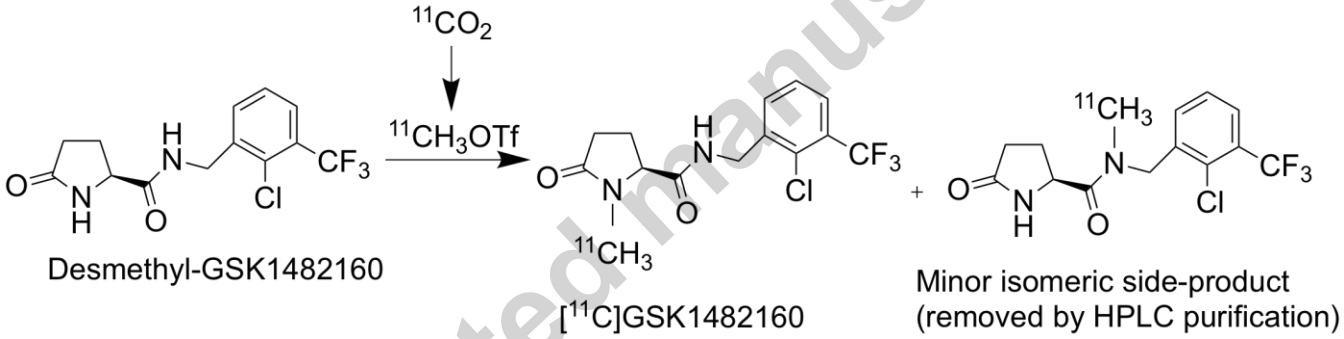
Sample ID	[^{11}C]GSK1482160 t_R (minutes)		[^{11}C]GSK1482160 Radiochemical purity (%)		GSK1482160 reference standard t_R (minutes)	[^{11}C]GSK1482160 Molar activity at expiration time	
	Initial analysis	Repeat analysis after expiration time	Initial analysis	Repeat analysis after expiration time		mCi/nmol	MBq/nmol
110416 GSK-A	7.128	7.126	98.80	98.04	7.097	0.50	18.50
110716 GSK-A	6.955	7.133	97.00	96.00	7.137	0.52	19.24
110716 GSK-B	7.137	7.118	96.50	95.74	7.130	0.49	18.13
Mean	7.073	7.126	97.43	96.59	7.121	0.50	18.62
STDEV	0.103	0.008	1.210	1.260	0.021	0.015	0.565

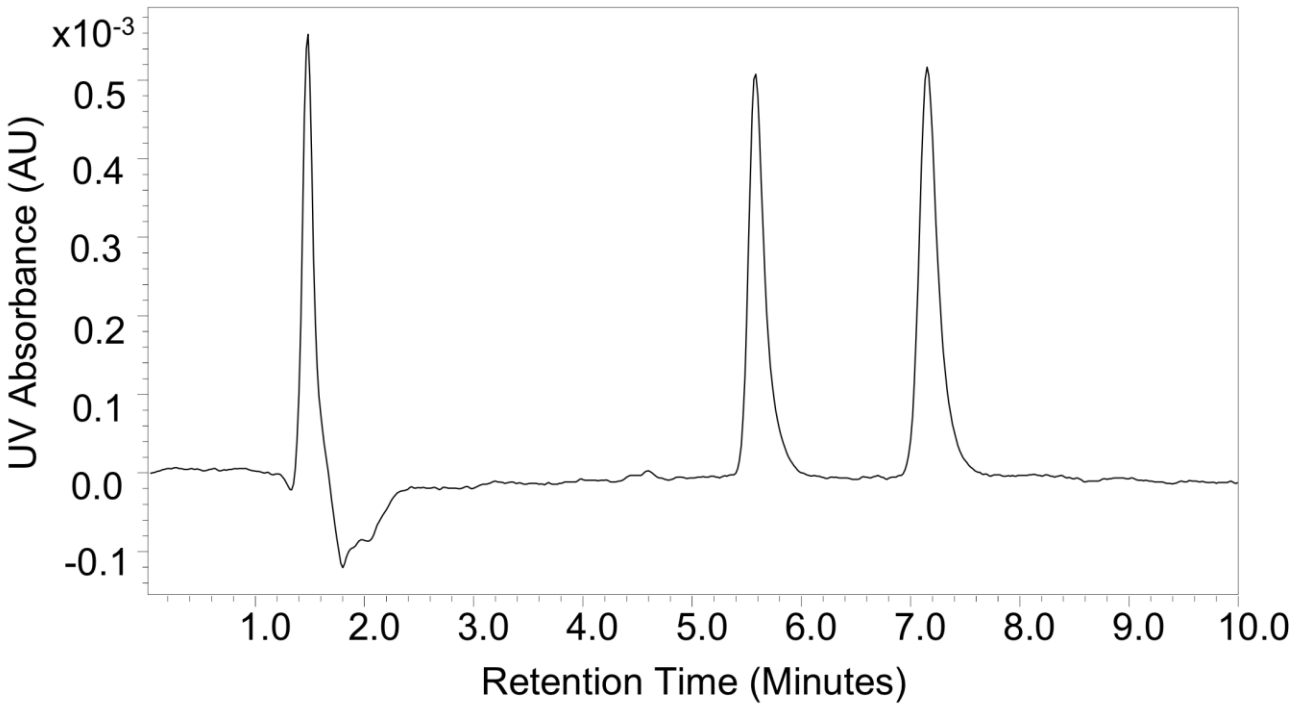
Table 2. [^{11}C]GSK1482160 study in ten volunteers used for PET imaging.

Sample ID	Radiochemical purity (%)	GSK mass (nmol/mL)	Sample volume (mL)	Activity at EOS (mCi)	Activity at expiration (mCi/mL)	Molar activity at expiration (mCi/nmol)
081817 GSK-A	97.40	3.90	11.30	131.00	1.95	0.50
083017 GSK-A	96.80	3.56	10.90	125.50	1.81	0.51
091517 GSK-A	95.30	3.70	10.80	167.30	1.85	0.50
092817 GSK-A	96.40	5.25	11.20	175.70	2.64	0.50
100217 GSK-A	97.40	4.47	10.60	121.80	2.30	0.51
100317 GSK-A	96.30	5.36	11.20	187.60	2.73	0.51
102717 GSK-A	95.00	4.45	10.30	241.00	2.28	0.51
110917 GSK-A	97.20	4.36	10.30	159.00	2.27	0.52
111017 GSK-A	97.50	4.30	10.00	121.90	2.20	0.51
112017 GSK-A	96.60	10.00	10.50	214.00	5.18	0.52
Mean	96.59	4.93	10.71	164.48	2.52	0.51
STDEV	0.87	1.87	0.44	41.21	0.98	0.01

Highlights

- A radio-analytical method was validated for the [^{11}C]GSK1482160 radiopharmaceutical.
- The method proved to be robust and unaffected by small changes in the chromatographic conditions.
- The [^{11}C]GSK1482160 radiopharmaceutical exhibits high radiochemical stability.
- [^{11}C]GSK1482160 radiopharmaceutical can be delivered in accordance with *USP* <*823*> requirements.
- The P2X7-receptor-targeted [^{11}C]GSK1482160 meets release criteria for human use.

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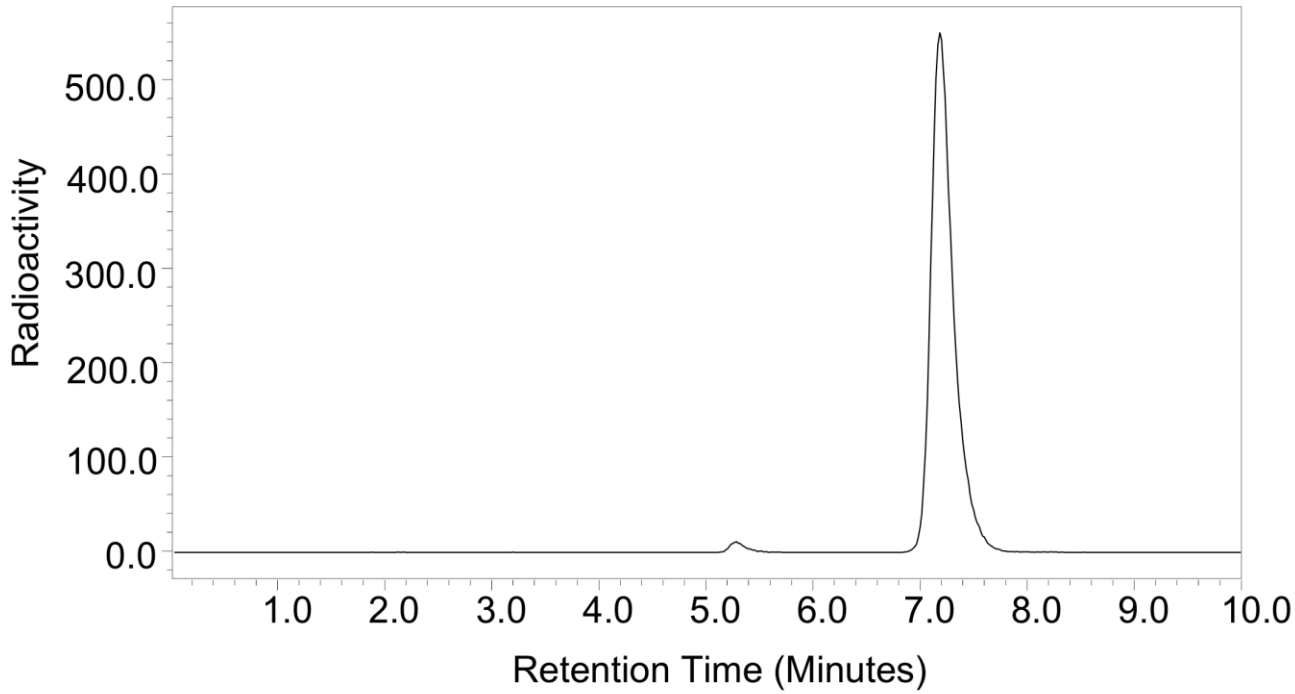
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