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Characterization of fast-decaying PET radiotracers solely through LC-MS/MS of constituent radioactive and carrier isotopologues

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Abstract

Background: The characterization of fast-decaying radiotracers that are labeled with carbon-11 ($t_{1/2} = 20.38$ min), including critical measurement of specific radioactivity (activity per mole at a specific time) before release for use in positron-emission tomography (PET), has relied heavily on chromatographic plus radiometric measurements, each of which may be vulnerable to significant errors. Thus, we aimed to develop a mass-specific detection method using sensitive liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) for identifying ^{11}C -labeled tracers and for verifying their specific radioactivities.

Methods: The LC-MS/MS was tuned and set up with methods to generate and measure the product ions specific for carbon-11 species and $M + 1$ carrier (predominantly the carbon-13 isotopologue) in four ^{11}C -labeled tracers. These radiotracers were synthesized and then analyzed before extensive carbon-11 decay. The peak areas of carbon-11 species and $M + 1$ carrier from the LC-MS/MS measurement and the calculated abundances of carbon-12 carrier and $M + 1$ radioactive species gave the mole fraction of carbon-11 species in each sample. This value upon multiplication with the theoretical specific radioactivity of carbon-11 gave the specific radioactivity of the radiotracer.

Results: LC-MS/MS of each ^{11}C -labeled tracer generated the product ion peaks for carbon-11 species and $M + 1$ carrier at the expected LC retention time. The intensity of the radioactive peak diminished as time elapsed and was undetectable after six half-lives of carbon-11. Measurements of radiotracer-specific radioactivity determined solely by LC-MS/MS at timed intervals gave a half-life for carbon-11 (20.43 min) in excellent agreement with the value obtained radiometrically. Additionally, the LC-MS/MS measurement gave specific radioactivity values (83 to 505 GBq/ μmol) in good agreement with those from conventional radiometric methods.

Conclusions: ^{11}C -Labeled tracers were characterized at a fundamental level involving isolation and mass detection of extremely low-abundance carbon-11 species along with the $M + 1$ carrier counterpart. This LC-MS/MS method for characterizing fast-decaying radiotracers is valuable in both the development and production of PET radiopharmaceuticals.

Keywords: PET radiotracers, Carbon-11, Specific radioactivity, LC-MS/MS

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Background

Positron-emission tomography (PET), as an expanding biomedical research and diagnostic imaging technique, relies on the use of radiotracers labeled with a positron emitter, often either carbon-11 ($t_{1/2} = 20.38$ min) or fluorine-18 ($t_{1/2} = 109.7$ min) [1-3]. The short physical half-lives of these radiotracers demand rapid techniques for establishing the quality of each production batch before release. A suitable technique for this purpose must conclusively identify the radiotracer and also provide specific radioactivity (SA), the ratio of the amount of radioactive compound (GBq) to the amount of compound (mol) in radioactive and non-radioactive forms (isotopologues) at a specific measurement time. High-performance liquid chromatography (HPLC) is almost universally applied to assure radiotracer identity and to measure radiochemical purity and SA at the end of each radiotracer synthesis [4,5]. Typically, ^{11}C -labeled tracers are produced with only a low degree of ^{11}C enrichment (<0.1%) [1], representing a SA value on the order of 100 GBq/ μmol . Nevertheless, an accurate determination of SA is critical in many applications especially where the radiotracer is intended to bind to low-density protein targets, such as neurotransmitter receptors, enzymes or transporters, or in specific cases where the radiotracer is pharmacologically very potent, such as some radiotracers for dopamine, nicotinic, or opiate receptors [6-8].

The identification of a PET radiotracer with HPLC relies on comparison of its retention time (t_R) measured with a radioactivity detector with that of the reference non-radioactive compound measured with another detector, often an absorbance detector. In conventional measurement of the SA of a radiotracer, the detector giving the mass response is calibrated for compound mass, and the radioactivity associated with a particular carrier mass peak is measured in a calibrated detector, invariably a dose calibrator (an ionization detector). Surrogate radioisotopes (e.g., ^{137}Cs , ^{57}Co) are often used to calibrate ionization detectors for measuring positron emitters. The accuracy of measurements is highly vulnerable to errors in instrument settings, and to changes in sample containers and geometries, as demonstrated in several studies with fluorine-18 [9-14]. Similar issues will pertain to shorter-lived carbon-11, although this has not been well studied [15]. In addition, the presence of unknown inadvertent impurities in the radiotracer may compromise the mass measurements and hence the accuracy of derived SA. Hitherto, no non-radiometric method for verifying conventional HPLC-radiometric measurements of ^{11}C -labeled tracer SA has been reported.

We recognized the potential of a highly sensitive mass spectrometric technique for developing a reliable method to characterize fast-decaying PET radiotracers. Such a method seemed practical using triple quadrupole liquid

chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) because its specificity, sensitivity, and detection range were envisaged to be adequate for measuring both the radioactive species and the carrier counterpart. Additionally, the MS/MS can generate, isolate, and measure ions specifically for the positron-emitting species in the presence of a thousandfold excess carrier. Here, we describe the successful use of sensitive LC-MS/MS alone to identify and measure $t_{1/2}$ and the SA of ^{11}C -labeled tracers. The SA values obtained by this new method are compared with those from HPLC-radiometric methods. The LC-MS/MS method was found especially useful for verifying the validity of HPLC-radiometric methods.

Methods

Radiotracers

The PET radiotracers used were [^{11}C]PBR28 (radioligand for translocator protein (18 kDa)) [16,17], [^{11}C]dLop (radiotracer of *P*-glycoprotein function) [18,19], [^{11}C]MePPEP (cannabinoid subtype 1 receptor radioligand) [20,21], and [^{11}C](*R*)-rolipram (radioligand for cAMP phosphodiesterase IV) [22] (Figure 1A,B,C,D). Each radiotracer was prepared in high radiochemical purity (>99%) and formulated in sterile saline solution, as described previously [16-22].

HPLC-radiometric measurement of SA

Freshly prepared and formulated radiotracer solution (100 μL), contained in a syringe, was measured for radioactivity in a calibrated ionization chamber (Atomlab 300, Biodex, Shirley, NY, USA) and then injected onto HPLC (Beckman, Fullerton, CA, USA). The radiotracer was eluted isocratically (acetonitrile(aq) 10 to 100 mM HCO_2NH_4 or 0.1% $\text{CF}_3\text{CO}_2\text{H}$) on a reverse-phase column (Onyx, Prodigy, or Luna, Phenomenex, Torrance, CA, USA) equipped with radioactivity and absorbance detectors. The mass of the carrier in the injectate was determined from a linear calibration curve generated from injections of known amounts of authentic non-radioactive standards. The SA was calculated from the decay-corrected activity present in the same volume of injectate, as measured in the calibrated ionization chamber.

LC-MS/MS

Methods for radiotracer analyses were developed on an API 5000 LC-MS/MS system (AB Sciex, Foster City, CA, USA), consisting of Shimadzu LC (Columbia, MD, USA) interfaced via electrospray with triple quadrupole MS/MS operated in positive ionization mode. The technique principally involves quantitative measurement of two ions that differ by two mass-to-charge ratio (m/z) units, one for the ^{11}C -labeled species, denoted [^{11}C]_{species}, and another for the carrier, denoted [$M + 1$]_{carrier}. [$M + 1$]_{carrier} ions are generated

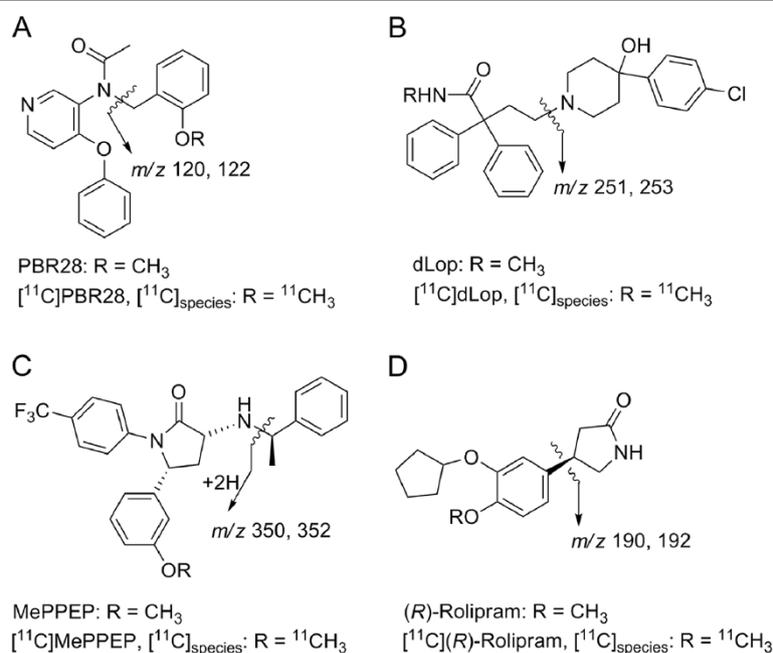


Figure 1 Chemical structures of compounds and their ¹¹C-labeled analogs as PET tracers (A-D). The bond cleavage site in generating product ion by collision-induced dissociation is shown for each molecule.

from isotopologues having predominantly one carbon-13 atom or another heavy stable isotope (²H, ¹⁵N, or ¹⁷O) of very low abundance. Thus, for example, a solution of PBR28 was infused into the MS/MS, and, upon recording the product ions, compound-dependent and gas parameters were optimized for the m/z 349 $[M + H]^+ \rightarrow 121$ transition for monitoring [¹²C]_{species}. An acquisition method for [¹¹C]PBR28 was set up using the same instrument parameters and with the multiple-reaction monitoring table being edited to perform transitions, m/z 348 \rightarrow 120 and m/z 350 \rightarrow 122 for measuring [¹¹C]_{species} and $[M + 1]_{\text{carrier}}$ respectively (Figure 1A).

Similarly, MS/MS was tuned with dLop, MePPEP, and (R)-rolipram involving transitions m/z 463 $[M + H]^+ \rightarrow$ 252, m/z 455 $[M + H]^+ \rightarrow$ 351, and m/z 276 $[M + H]^+ \rightarrow$ 191, respectively. Methods were set up to acquire product ions to measure [¹¹C]_{species} and $[M + 1]_{\text{carrier}}$ in these radiotracers. The dwell time in these methods ranged between 100 and 175 ms. In each method, the possible cross-talk between transitions was verified by inserting a dummy $[M + H]^+ \rightarrow$ product ion transition between those for [¹¹C]_{species} and $[M + 1]_{\text{carrier}}$. The masses for the dummy transition differed by 5 to 10 amu from those for target ions. Each compound's product ion (Figure 1) was further verified by LC-MSⁿ ($n = 2, 3$) analysis in an ion-trap mass analyzer (LCQ Deca, Thermo Scientific, San Jose, CA, USA).

Each radiotracer sample was diluted four- to tenfold, and 2 to 5 μ L of solution (<500 kBq) was injected with

an autosampler ($n = 6$ or 12) at about 5-min intervals onto LC-MS/MS. Samples of [¹¹C]PBR28, [¹¹C]dLop, and [¹¹C]MePPEP were chromatographed at 40°C on a C18 column (2 \times 20 mm, 3 μ m; Phenomenex) with a gradient of binary solvents (A/B; 400 μ L/min), where A was 10 mM MeCO₂NH₄ in water/acetonitrile (90:10 v/v) and B was 10 mM MeCO₂NH₄ in acetonitrile/water (90:10 v/v). In the analysis of [¹¹C]PBR28, for example, the pumps ran 70% A:30% B for 0.1 min and then a gradient reaching 20% A:80% B over 1 min. After 3.5 min, the mobile-phase composition was returned to the initial condition. [¹¹C](R)-Rolipram was chromatographed on the same column eluted with a gradient of water (A) and acetonitrile (B) (A/B; 0.2% acetic acid): 80% A:20% B for 0.1 min, reaching 20% A:80% B over 2 min.

The relative abundance of the M + 1 peak was measured by injecting a solution of PBR28 onto the LC-MS/MS setup to perform m/z 349, 350 $[M + H]^+ \rightarrow$ 121, 122 (¹²C, M + 1) transitions and thereby to provide m/z 122 to 121 peak area ratio (%). Other compounds were similarly analyzed using respective acquisition methods edited to monitor product ions of ¹²C and M + 1 species. The measured abundance (%) of M + 1 species relative to ¹²C was used for converting M + 1 into ¹²C peak area for radiotracer carrier. The peak area ratio (%) of M + 2 to M + 1 was measured by injecting 40 and 400 pg of PBR28 and acquiring transitions m/z 350, 351 \rightarrow 122, 123.

Calculations

The SA of the radiotracer (Bq/mol) was calculated as

$$\frac{A_{M,M+1}^*}{A_{M+1,M} + A_{M,M+1}^*} \times SA_{\text{Theoretical}} \quad (1)$$

where (1) $A_{M,M+1}^*$ is the sum of the measured $[^{11}\text{C}, M]_{\text{species}}$ peak area and $[^{11}\text{C}, M + 1]_{\text{species}}$ area calculated from the relative abundances [23] of ^{13}C , ^2H , ^{15}N , and ^{17}O in the product ion; (2) $A_{M+1,M}$ is the sum of the measured $[M + 1]_{\text{carrier}}$ peak area and $[^{12}\text{C}]_{\text{carrier}}$ area calculated from the measured $M + 1$ abundance in the non-radioactive tracer; and (3) $SA_{\text{Theoretical}}$ is the theoretical SA of carbon-11 (3.413×10^{20} Bq/mol), the product of $\ln 2/t_{1/2}$ and Avogadro's number. The SA of the radiotracer was decay-corrected to give the SA at the end of radiosynthesis (SA_0) as $SAe^{\lambda t}$, where t is the time between the end of synthesis and peak elution in LC-MS/MS and λ is 0.034 min^{-1} ($\ln 2/20.38$). A plot of $\ln SA$ ($n = 12$) versus clock time of peak elution in Prism 5.02 (GraphPad, La Jolla, CA, USA) gave the value for λ and thus $t_{1/2}$ ($t_{1/2} = \ln 2/\lambda$).

Results

In order to exemplify the non-radiometric LC-MS/MS method that we developed for identifying and measuring the SA of ^{11}C -labeled tracers, we report results on four radiotracers applied in human PET studies, namely $[^{11}\text{C}]$ PBR28, $[^{11}\text{C}]$ dLop, $[^{11}\text{C}]$ MePPEP, and $[^{11}\text{C}]$ (*R*)-rolipram (Figure 1). We illustrate the development of the procedure with the example of $[^{11}\text{C}]$ PBR28. In the MS/MS mode of a triple quadrupole analyzer, the $[M + H]^+$ ion of non-radioactive PBR28 was dissociated and then one of the transitions, m/z 349 $[M + H]^+ \rightarrow 121$, suitable for monitoring $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ in $[^{11}\text{C}]$ PBR28 was selected, and instrument parameters were optimized. The MS/MS of PBR28 in an ion-trap analyzer also yielded the product ion, m/z 121, in high abundance. In the same mass analyzer, another dissociation (MS^3) involving this target ion generated secondary product ions that were consistent with the genesis of the m/z 121 ion and thus the respective $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ ions by benzylic cleavage, as shown in Figure 1A.

LC-MS/MS of non-radioactive PBR28 showed transitions m/z 349 $[M + H]^+ \rightarrow 121$ (^{12}C) and 350 $[M + H]^+ \rightarrow 122$ ($M + 1$) associated with a peak eluting at 1.61 min on a short reverse-phase LC column. The measured relative abundance (8.88%) of the $m/z = 122$ for $M + 1$ species was close to the value (8.83%) calculated for the product ion formed by the benzylic cleavage of the $[M + H]^+$ ion of PBR28 (Figure 1A). Subsequently, the MS/MS acquisition method was edited to monitor the m/z 348 \rightarrow 120 transition for $[^{11}\text{C}]_{\text{species}}$ in $[^{11}\text{C}]$ PBR28. As expected, a similar LC-MS/MS analysis of non-

radioactive PBR28 showed no peak for an m/z 348 \rightarrow 120 transition. Furthermore, analysis of a fully decayed preparation of $[^{11}\text{C}]$ PBR28 showed no peak for this transition. Thus, carrier PBR28 did not generate a peak due to the m/z 120 ion or any other spurious peak when performing the m/z 348 \rightarrow 120 transition. Moreover, a dummy transition, entered between the transitions for $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ showed no cross-talk during the acquisition.

The possibility that coeluting carrier PBR28 might affect the ionization of $[^{11}\text{C}]$ PBR28 was tested by injecting high (400 pg) and low (40 pg) amounts of PBR28 into the LC-MS/MS and acquiring the product ions from $[M + H]^+$ of the $M + 1$ and $M + 2$ species. The peak area ratio (%; mean \pm standard deviation (SD); $n = 3$) of $M + 2$ to $M + 1$ was 6.64 ± 0.02 at 400 pg and 6.52 ± 0.09 at 40 pg of PBR28. These ratios agreed well with the calculated $M + 2$ to $M + 1$ ratio of 6.56%. Thus, excess $[^{12}\text{C}]_{\text{carrier}}$ did not cause either $[^{11}\text{C}]_{\text{species}}$ or $[M + 1]_{\text{carrier}}$ to ionize differently, and their ion currents were therefore expected to represent their respective concentrations.

MS/MS of a sample of freshly prepared $[^{11}\text{C}]$ PBR28 (approximately 400 kBq) detected an m/z 348 \rightarrow 120 transition specific for the $[^{11}\text{C}]_{\text{species}}$ and m/z 350 \rightarrow 122 for the constituent $[M + 1]_{\text{carrier}}$. The LC peaks (Figure 2A,B) due to the product ions, m/z 120 and 122 for $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ respectively, appeared in the ion chromatogram at the same t_R as reference PBR28. As shown with ion chromatogram overlays, the peak for the $[^{11}\text{C}]_{\text{species}}$ with $m/z = 120$ diminished in intensity and was undetectable after elapse of about six $t_{1/2}$ of carbon-11. The intensity of the peak for $[M + 1]_{\text{carrier}}$ remained constant throughout the analysis, as expected. Mass detection by MS/MS of $[^{11}\text{C}]_{\text{species}}$ along with $[M + 1]_{\text{carrier}}$ as LC peaks at the t_R of PBR28 confirmed the identity of the $[^{11}\text{C}]_{\text{species}}$ including the position of the carbon-11 atom as being in the methoxy group of the methoxybenzyl moiety (Figure 1A).

LC-MS/MS methods were similarly set up for the characterization of $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ in $[^{11}\text{C}]$ dLop, $[^{11}\text{C}]$ MePPEP, and $[^{11}\text{C}]$ (*R*)-rolipram preparations. Analysis of $[^{11}\text{C}]$ dLop showed LC peaks at $t_R = 2.02$ min due to the product ions m/z 251 and 253 of $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ respectively (Figure 1). $[^{11}\text{C}]$ MePPEP showed LC peaks at $t_R = 2.19$ min arising from the acquisition of product ions m/z 350 and 352 of $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ respectively. Similarly, analysis of $[^{11}\text{C}]$ (*R*)-rolipram showed LC peaks at $t_R = 1.96$ min upon acquiring the product ions m/z 190 and 192 of $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ respectively.

The LC-MS/MS measurements of $[^{11}\text{C}]_{\text{species}}$ and carrier gave the mole fraction of the $[^{11}\text{C}]_{\text{species}}$, which, upon multiplication with the theoretical SA of carbon-11, gave the SA of the radiotracer (Equation 1). In

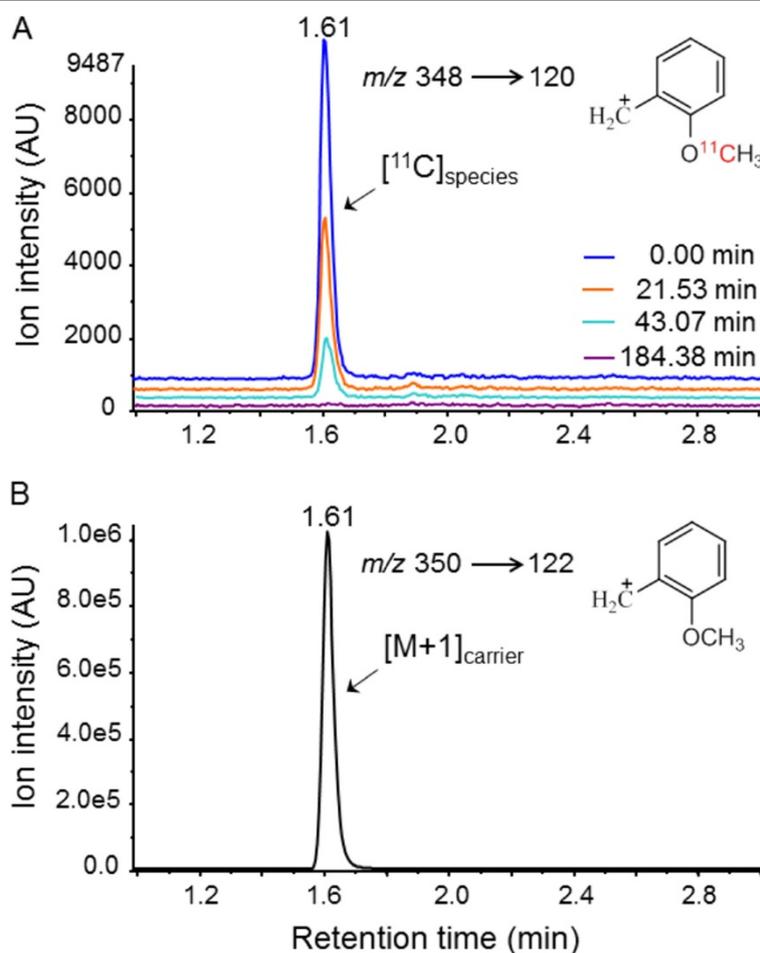


Figure 2 LC-MS/MS of the same [¹¹C]PBR28 preparation at four time-points of carbon-11 decay. The ion chromatogram overlay showing peaks for [¹¹C]_{species} as it decayed (A) and unchanging peak for [M + 1]_{carrier} (B) during the same analysis. Their respective product ions *m/z* 120 and 122 were monitored.

calculating the mole fraction of [¹¹C]_{species}, the measured peak area for [M + 1]_{carrier} was converted into that for [¹²C]_{carrier} using the previously measured [M + 1]¹²C ratio in PBR28, dLop, MePPEP, or (*R*)-rolipram. This indirect measurement had the benefit of neatly circumventing possible saturation of the detector by [¹²C]_{carrier} at the concentration of the radiotracer needed for measuring [¹¹C]_{species}. Also, in this method, the masses of acquired ions differed by two units, and any contribution of [¹¹C, M + 2]_{species} to the [M + 1]_{carrier} abundance would therefore have been negligible.

As examples, the measured peak areas of [¹¹C]_{species} and [M + 1]_{carrier} and the derived peak areas used for calculating SA are shown for [¹¹C]PBR28 and [¹¹C](*R*)-rolipram in Table 1. These data show that the peak area and area ratio for [¹¹C]_{species} and the SA were halved after elapse of one *t*_{1/2} of carbon-11 while the peak area for [M + 1]_{carrier} remained constant, as expected. The measurement of [¹¹C]_{species} and [M + 1]_{carrier} is

quantitative as reflected in the decay-corrected SA values. The area ratio 7.9240×10^{-4} for [¹¹C]PBR28 implies that 1 out of 1,262 molecules was labeled with carbon-11 at the time of MS/MS detection. This ratio is consistent with the low degree of radionuclide enrichment found in ¹¹C-labeled preparations. Also, in Table 1, the peak areas for [¹¹C](*R*)-rolipram are for a preparation injected onto LC-MS/MS after elapse of 30.88 and 52.38 min from the end of synthesis. The comparable decay-corrected SA values indicate good accuracy of the measurement even after elapse of two *t*_{1/2} of the radiotracer.

The accuracy of the LC-MS/MS method for measuring the SA of PET radiotracers was further supported through monitoring the loss of mass of [¹¹C]_{species} as the radionuclide decayed. Three preparations each of [¹¹C]PBR28 and [¹¹C](*R*)-rolipram and two of [¹¹C]dLop were analyzed by injecting each 12 times at known intervals of about 5 min. The SA of the radiotracer was calculated for

Table 1 Calculation of specific radioactivity from the peak areas of $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$ in radiotracers

Radiotracer	Elution time ^a (h:min:s)	Peak areas					SA ^e (GBq/ μmol)	SA ^f (GBq/ μmol)
		¹¹ C	¹¹ C	M + 1	¹² C	Ratio		
		$\times 10^{-4}$	$\times 10^{-3b}$	$\times 10^{-6}$	$\times 10^{-7c}$	$\times 10^{4d}$		
$[^{11}\text{C}]$ PBR28	10:21:23	2.3920	1.8531	2.6506	2.9849	7.9240	270.4	330.3
	10:42:55	1.2103	0.9376	2.7072	3.0486	3.9271	134.0	340.6
$[^{11}\text{C}]$ (R)-Rolipram	11:15:49	0.5745	0.7009	1.5974	1.1970	4.7490	162.1	463.3
	11:37:19	0.2827	0.3449	1.6280	1.2199	2.2936	78.3	464.9

^aElution time for coeluting LC peaks of $[^{11}\text{C}]_{\text{species}}$ and $[M + 1]_{\text{carrier}}$; ^bcalculated peak area for $[^{11}\text{C}, M + 1]_{\text{species}}$ (Equation 1); ^ccalculated peak area from measured M + 1/¹²C ratio in PBR28 and (R)-rolipram; ^dpeak area ratio of $[^{11}\text{C}]_{\text{species}}$ to the sum of carrier and $[^{11}\text{C}]_{\text{species}}$; ^especific radioactivity calculated from the peak area ratio; ^fdecay-corrected specific radioactivity.

each injection at the clock time of the peak elution in the ion chromatogram. Plots of the natural logarithm of SA versus measurement time gave straight lines ($r^2 > 0.99$) as shown, for example, for a $[^{11}\text{C}]$ PBR28 preparation (Figure 3). In each case, the decay constant λ for carbon-11 was obtained from the negative slope of the regression equation ($y = -\lambda x + b$). The $t_{1/2}$ ($t_{1/2} = \ln 2 / \lambda$) of carbon-11 was estimated from these decay constants to be 20.43 ± 0.24 min (mean \pm SD; $n = 8$), which is in excellent agreement with the accepted value of 20.38 min ascertained by measuring β^+ emission [24]. The accuracy of these $t_{1/2}$ measurements with LC-MS/MS implies that the measured peak areas of decaying $[^{11}\text{C}]_{\text{species}}$ and the stable $[M + 1]_{\text{carrier}}$ truly represent their concentrations.

Decay-corrected SA was measured with the LC-MS/MS method on three preparations of each of the four radiotracers (Table 2). Each preparation was measured six times over a period of 30 min. These measurements were

remarkably reproducible (<5% RSD). Before LC-MS/MS analysis, each radiotracer was also analyzed by the calibrated radiometric method (activity using an ionization chamber dose calibrator and mass using HPLC apparatus) regularly used in our laboratory for quality control. Each analysis required at least 10 min for completion and was performed only once because of the time constraint imposed by the short half-life. The SA measurements obtained with LC-MS/MS deviated by $\pm 7.4\%$ overall from the mean of both radiometric and LC-MS/MS methods (Table 2). The possible sources of error in the radiometric method include both the HPLC apparatus and the dose calibrator used for measuring carrier and radioactivity, respectively. The HPLC method used a linear calibration curve ($r^2 > 0.99$) generated with authentic non-radioactive compounds, such as, for example, PBR28 (>99% pure; RTI International, Research Triangle Park, NC, USA) or (R)-rolipram (>98%; Tocris, Ellisville, MO, USA). The quantification of the carrier may not always be

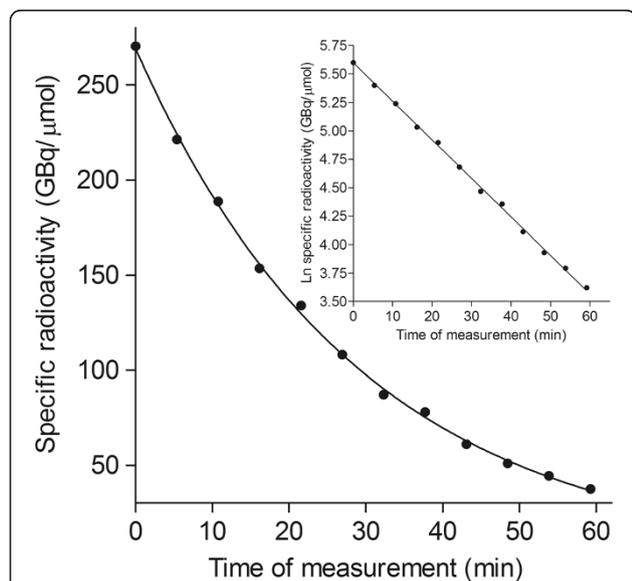


Figure 3 Plot of specific radioactivity of $[^{11}\text{C}]$ PBR28 versus time. It shows exponential loss of $[^{11}\text{C}]_{\text{species}}$ mass due to radionuclide decay. A semilog plot of the same data is shown in the inset.

Table 2 Specific radioactivity (decay-corrected) of PET radiotracers measured by LC-MS/MS and radiometric methods

Radiotracer	SA (GBq/ μmol)		Difference ^b (%)
	LC-MS/MS ^a	Radiometry	
$[^{11}\text{C}]$ PBR28	265.5 \pm 8.5	290.8	-9.1
	330.4 \pm 5.8	363.6	-9.6
	167.0 \pm 2.9	173.3	-3.7
$[^{11}\text{C}]$ dLop	234.1 \pm 3.2	237.9	-1.6
	227.1 \pm 6.1	253.2	-10.9
	241.4 \pm 7.0	264.0	-9.1
$[^{11}\text{C}]$ MePPEP	160.1 \pm 7.2	151.5	+5.5
	244.0 \pm 11.7	238.0	+2.5
	82.5 \pm 3.0	92.4	-11.3
$[^{11}\text{C}]$ (R)-Rolipram	473.9 \pm 4.8	459.9	+3.0
	505.0 \pm 9.5	450.5	+11.4
	459.0 \pm 5.8	411.1	+11.0

^aMean \pm SD specific radioactivity from six consecutive analyses; ^bDifference (%) = [(LC-MS/MS - radiometry) / mean of both methods] \times 100.

accurate due to lack of an internal standard and possible presence of a coeluting impurity. Measurements of the radioactivity of ^{11}C -labeled preparations ($n = 6$) in one ionization chamber were compared with measurements in another to assess their reliability. The two ionization chambers showed 10% to 12% differences in the radioactivity measurements even though both detectors had been identically calibrated. The lack of accuracy and reproducibility in measurements with ionization chambers [10-14] may at least partially explain the difference in SA measured by radiometric-HPLC and LC-MS/MS methods. Other radiochemical detectors likely suffer similar deficiencies, especially as surrogate isotopes are also used for their calibration.

Discussion

Here, we demonstrated the capability of electrospray ionization (ESI)-MS/MS to ionize and isolate $[\text{M} + \text{H}]^+$ of ^{11}C species and then generate and detect the product ion-bearing carbon-11. The mass-specific detection of ^{11}C species along with $[\text{M} + 1]_{\text{carrier}}$ at the expected t_R allowed unambiguous identification of the radiotracer. By monitoring $[\text{M} + 1]_{\text{carrier}}$ instead of the carbon-12 counterpart, we avoided saturation of the MS/MS detector during simultaneous measurement of trace concentrations of ^{11}C species. The measurement of both species gave the SA and $t_{1/2}$ of the radiotracer while also confirming radiotracer identity. The results from multiple analyses showed that the LC-MS/MS method is fast, accurate, and reproducible enough for characterizing low-enriched, fast-decaying radiotracers. This mass-specific detection method is suitable for validating the conventional method of characterization of PET radiotracers.

We verified that the excess carrier does not impact the ionization and detection of coeluting ^{11}C species in our LC-MS/MS system. The peak for ^{11}C species declined at an expected rate and then disappeared from the ion chromatogram as carbon-11 in the product ion decayed. Secondly, the ratio of $\text{M} + 2$ to $\text{M} + 1$ isotopologues was not altered when the radiotracer's non-labeled standard was injected at a concentration comparable to that of the carrier in the radiotracer. Also, the concentrations of ^{11}C species and $[\text{M} + 1]_{\text{carrier}}$ in radiotracers were within the dynamic range of detection of the triple quadrupole MS/MS system. Before being injected into LC-MS/MS, the radiotracer preparations were diluted adequately to avoid possible saturation of the ionization and detection systems. Furthermore, no cross-talk was observed between the $[\text{M} + \text{H}]^+ \rightarrow$ product ion transition for ^{11}C species and that for $[\text{M} + 1]_{\text{carrier}}$.

Carbon-11 decays to a stable nuclide, boron-11. This energetic decay event would instantly transform any molecule in which the carbon-11 had been incorporated. The product of carbon-11 decay would have a t_R and

mass different from those of the ^{11}C species or the $[\text{M} + 1]_{\text{carrier}}$. Accordingly, the LC-MS/MS acquisition detected no product or chemical impurity from the decay of any of these PET radiotracers. The measurement showed exponential loss of mass of ^{11}C species as the radionuclide decayed thereby gave the same expected $t_{1/2}$ value for each radiotracer.

The radiometric method of SA measurement involves two detection systems, and both are prone to errors. For example, we observed a significant difference between two dose calibrators in recording the radioactivity of the same radiotracer preparation. The sources of error in the HPLC measurement of mass may include the weighing of standard, preparation of the calibration curve solutions, injection, chromatography, and UV detection. In contrast, the LC-MS/MS technique measures the mole fraction of ^{11}C species in a radiotracer similar to the isotope ratio in a compound. Thus, SA was obtained simply by entering into a spreadsheet: (1) the measured LC-MS/MS peak areas of ^{11}C species and $[\text{M} + 1]_{\text{carrier}}$, (2) the abundance of $[\text{M} + 1]_{\text{carrier}}$ measured in the reference standard, (3) the area for the ^{11}C , $\text{M} + 1$ species ion calculated from the natural abundance of heavy stable isotopes in ^{11}C species, and (4) the theoretical maximum SA of a compound labeled with one carbon-11 atom, as detailed under the 'Calculations' subsection.

The rapid LC-MS/MS method described here enables definitive characterization of radiotracers used in human PET imaging. A mass-specific detection method such as this is not susceptible to errors commonly associated with the use of the radio-HPLC method, as discussed. It may not be feasible to use triple quadrupole MS/MS regularly; however, it provides a wholly independent non-radiometric means for confirming identity and verifying SA of a radiotracer when needed. A less expensive and more commonly used single quadrupole MS instrument may offer the sensitivity to measure ^{11}C species and $[\text{M} + 1]_{\text{carrier}}$ by selected ion monitoring of respective $[\text{M} + \text{H}]^+$ following ESI. However, such a less specific MS method can work only if no $[\text{M} - 1]^+$ ion is generated during ionization. Other mass analyzers such as time-of-flight and linear ion traps may also be suitable for the characterization of PET radiotracers. Finally, the LC-MS/MS method should be applicable to all ^{11}C -labeled tracers, except in instances where no ^{11}C -containing product ion is obtained or where interfering ions are generated. If a labeled product ion is not available, selected ion monitoring of a precursor ion ($[\text{M} + \text{H}]^+$) with a high-resolution MS can substitute the MS/MS method for measuring ^{11}C species and $[\text{M} + 1]_{\text{carrier}}$. Also, a high-resolution MS is likely to resolve any interfering ions of isobaric species from the product ion of ^{11}C species in ^{11}C -labeled tracers. In principle, LC-MS/MS may be used for characterizing PET radiotracers labeled

with other short-lived positron emitters. Although an isotope separator has been used to identify the position of ^{18}F in labeled 1,1,1,2-tetrafluoroethane [25], no parallel mass spectrometric method has yet been reported for measuring the SA of ^{18}F -labeled tracers. The LC-MS/MS method should also be suitable for this purpose, as shown with the analysis of an ^{18}F -labeled tracer in our preliminary report [26].

Conclusions

A simultaneous mass detection of $[^{11}\text{C}]_{\text{species}}$ and $[M+1]_{\text{carrier}}$ in ^{11}C -labeled PET tracers was achieved using the LC-MS/MS technique. A single rapid analysis can verify the identity including the position of label in a ^{11}C -labeled tracer preparation as well as provide a fully independent measure of SA. The LC-MS/MS method is a valuable adjunct to other techniques used in the quality control of PET radiopharmaceuticals.

Abbreviations

$[^{11}\text{C}]_{\text{species}}$: carbon-11 species; $[M+1]_{\text{carrier}}$: ^{13}C , ^2H , ^{15}N , or ^{17}O carrier isotopologue; ESI: electrospray ionization; HPLC: high-performance liquid chromatography; LC-MS/MS: liquid chromatography-mass spectrometry/mass spectrometry; PET: positron-emission tomography; SA: specific radioactivity; t_R : HPLC or LC retention time.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HUS and WVP designed the experiments and wrote the manuscript. HUS and CLM performed the LC-MS/MS and processed the data. CLM and YZ prepared the radiotracers and performed the HPLC measurements. HUS conceived the study and interpreted the data. All authors read and approved the final manuscript.

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References

1. Fowler JS, Wolf AP: Working against time: rapid radiotracer synthesis and imaging the human brain. *Acc Chem Res* 1997, **30**:181–188.
2. Miller PW, Long NJ, Vilar R, Gee AD: Synthesis of ^{11}C , ^{18}F , ^{15}O , and ^{13}N radiolabels for positron emission tomography. *Angew Chem Int Ed* 2008, **47**:8998–9033.
3. Cai L, Lu S, Pike VW: Chemistry with $[^{18}\text{F}]$ fluoride ion. *Eur J Org Chem* 2008, **2008**:2853–2873.
4. Meyer G-J: Some aspects of radioanalytical quality control of cyclotron-produced short-lived radiopharmaceuticals. *Radiochim Acta* 1982, **30**:175–184.
5. Passchier J: Fast high performance liquid chromatography in PET quality control and metabolite analysis. *Quart J Nucl Med Mol Imaging* 2009, **53**:411–416.
6. Kung M-P, Kung HF: Mass effect of injected dose in small rodent imaging by SPECT and PET. *Nucl Med Biol* 2005, **32**:673–678.
7. Mizrahi R, Houle S, Vitcu I, Ng A, Wilson AA: Side effects profile in humans of $[^{11}\text{C}]$ -(+)-PHNO, a dopamine $D_{2/3}$ agonist ligand for PET. *J Nucl Med* 2010, **51**:496–497.
8. Madsen K, Marner L, Haahr M, Gillings N, Knudsen GM: Mass dose effects and in vivo affinity in brain PET receptor studies - a study of cerebral 5-HT₄ receptor binding with $[^{11}\text{C}]$ SB207145. *Nucl Med Biol* 2011, **38**:1085–1091.
9. Zimmerman BE, Cessna JT: Development of a traceable calibration methodology for solid $^{68}\text{Ge}/^{68}\text{Ga}$ sources used as a calibration surrogate for ^{18}F in radionuclide activity calibrators. *J Nucl Med* 2010, **51**:448–453.
10. Mo L, Reinhard MI, Davies JB, Alexiev D, Baldock C: Calibration of the Capintec CRC-712M dose calibrator for ^{18}F . *Appl Radiat Isot* 2006, **64**:485–489.
11. Zimmerman BE, Kubicek GJ, Cessna JT, Plascjak PS, Eckelman WC: Radioassays and experimental evaluation of dose calibrator settings for ^{18}F . *Appl Radiat Isot* 2001, **54**:113–122.
12. Zimmerman B, Kinahan P, Galbraith W, Allberg K, Mawlawi O: Multicenter comparison of dose calibrator accuracy for PET imaging using a standardized source [abstract]. *J Nucl Med* 2009, **50**(Suppl 2):472P.
13. Tyler DK, Baker M, Woods MJ: NPL secondary standard radionuclide calibrator. Syringe calibration factors for radionuclides used in nuclear medicine. *Appl Radiat Isot* 2002, **56**:343–347.
14. Cessna JT, Schultz MK, Leslie T, Bores N: Radionuclide calibrator measurements of ^{18}F in a 3 ml plastic syringe. *Appl Radiat Isot* 2008, **66**:988–993.
15. Woods DH, Baker MI, Keightley JD, Keightley LJ, Makepeace JL, Pearce AK, Woodman AP, Woods MJ, Woods SA, Waters S: Standardisation of ^{11}C . *Appl Radiat Isot* 2002, **56**:327–330.
16. Briard E, Zoghbi SS, Imaizumi M, Gourley JP, Shetty HU, Hong J, Cropley V, Fujita M, Innis RB, Pike VW: Synthesis and evaluation in monkey of two sensitive ^{11}C -labeled aryloxyanilide ligands for imaging brain peripheral benzodiazepine receptors in vivo. *J Med Chem* 2008, **51**:17–30.
17. Fujita M, Imaizumi M, Zoghbi SS, Fujimora Y, Farris AG, Suhara T, Hong J, Pike VW, Innis RB: Kinetic analysis in healthy humans of a novel positron emission tomography radioligand to image the peripheral benzodiazepine receptor, a potential biomarker for inflammation. *Neuroimage* 2008, **40**:43–52.
18. Lazarova N, Zoghbi SS, Hong J, Seneca N, Tuan E, Gladding RL, Liow J-S, Taku A, Innis RB, Pike VW: Synthesis and evaluation of $[N\text{-methyl-}^{11}\text{C}]N\text{-desmethyl-loperamide}$ as a new and improved PET radiotracer for imaging P-gp function. *J Med Chem* 2008, **51**:6034–6043.
19. Kreis WC, Liow J-S, Kimura N, Seneca N, Zoghbi SS, Morse CL, Herscovitch P, Pike VW, Innis RB: P-glycoprotein function at the blood-brain barrier in humans can be quantified with the substrate radiotracer ^{11}C - $N\text{-desmethyl-loperamide}$. *J Nucl Med* 2010, **51**:559–566.
20. Donohue SR, Krushinski JH, Pike VW, Chernet E, Phebus L, Chesterfield AK, Felder CC, Halldin C, Schaus JM: Synthesis, ex vivo evaluation, and radiolabeling of potent 1,5-diphenylpyrrolidin-2-one cannabinoid subtype-1 receptor ligands as candidates for in vivo imaging. *J Med Chem* 2008, **51**:5833–5842.
21. Terry GE, Liow J-S, Zoghbi SS, Hirvonen J, Farris AG, Lerner A, Tauscher JT, Schaus JM, Phebus L, Felder CC, Morse CL, Hong JS, Pike VW, Halldin C, Innis RB: Quantitation of cannabinoid CB₁ receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand. *Neuroimage* 2009, **48**:362–370.
22. DaSilva JN, Lourenco CM, Meyer JH, Hussey D, Potter WZ, Houle S: Imaging cAMP-specific phosphodiesterase-4 in human brain with $R\text{-}[^{11}\text{C}]rolipram$ and positron emission tomography. *Eur J Nucl Med* 2002, **29**:1680–1683.
23. Lide DR: *CRC Handbook of Chemistry and Physics*. 83rd edition. Boca Raton: CRC; 2002.
24. Azuelos G, Kitching JE: Half-lives of some $T = \frac{1}{2}$ mirror decays. *Phys Rev C* 1975, **12**:563–566.
25. Pike VW, Waters SL, Aigbirhio FI, Makepeace J, Tanner RJN: Novel use of an isotope separator to determine the position of fluorine-18 in labelled 1,1,1,2-tetrafluoroethanes. *Org Mass Spectrom* 1994, **29**:499–504.
26. Shetty HU, Morse CL, Zhang Y, Pike VW: Characterization of PET radiotracers through LC-MS/MS measurement of the radiotracer and accompanying $[^{13}\text{C}]$ carrier [abstract]. In *Proceedings of the 58th ASMS Conference on Mass Spectrometry and Allied Topics: May 23–27 2010; Salt Lake City*. Santa Fe: ASMS; 2010. MP24:519.

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