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Rapid and sensitive measurement of PET radioligands in plasma by fast liquid chromatography/radiometric detection

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ABSTRACT

A fast and sensitive liquid chromatographic (fast-LC) method with radiometric detection was developed and validated to analyze positron emission tomography (PET) radioligands in plasma during PET studies. The plasma samples were deproteinized with acetonitrile and the extracts were injected into the fast-LC system coupled to an on-line radioactivity detector. Under the optimum conditions, complete separation of target PET radioligands from their radioactive metabolites was achieved within the short run time of only 3.5-min. The limits of detection were 1.0–1.2 Becquerel (Bq) for ^{11}C and ^{18}F-labeled compounds. This method can successfully be applied to study the metabolism of a wide variety of PET radioligands in human and monkey plasma with higher numbers of samples to be analyzed compared to the traditional LC method.

1. Introduction

To quantify physiological processes measured with positron emission tomography (PET), the time course of parent radioligand concentration in arterial plasma is usually required as an input function if a PET pharmacokinetic model is employed [1–3]. In such instances, since the majority of ligands are metabolized within the body, total radioactivity in arterial plasma needs to be corrected for the presence of radioactive metabolites. This is particularly important when dealing with radioligands that bind to targets for which there is no reference region in the brain as well as to investigate metabolic profiles of new PET radioligands whose in vivo behavior has not yet been fully characterized. The particular importance is to identify potential metabolites of lipophilic character which may enter the brain [4].

The most widely used method to measure the concentration of PET radioligands and their radioactive metabolites in plasma is liquid chromatography (LC) coupled with on-line [5–7] or off-line [8,9] radioactivity detector (radio-LC). However, the sufficient separation of a target PET radioligand from its radioactive metabolites by conventional radio-LC, i.e. using a semi-preparative column (150–300 mm length, 7–10 mm I.D., 5–10 μm particles), takes a relatively long time (10–20 min) and therefore only a limited number of plasma samples from a PET imaging study (typically ~6 samples for ^{11}C-labeled ligands) can be analyzed. Extensive metabolism and rapid clearance from the circulation, combined with the short half-lives of the positron emitters (e.g. ^{11}C: $t_{1/2}$ = 20.4 min, ^{18}F: $t_{1/2}$ = 109.8 min), can yield samples containing a diminishingly low concentration of radioactivity at late time points of a PET study. This may yield plasma data of poor accuracy and can have a significant impact on quantitative PET pharmacokinetic analysis. To reduce statistical errors in radioactive counting and determine the parent fraction accurately, a large sample volume injection (~2 mL plasma) is often required especially in later plasma samples from a PET experiment. However, it is a considerable challenge to accommodate such large volumes in a conventional semi-preparative radio-LC. Off-line radioactivity detection, often performed by collecting fractions of the LC effluent followed by counting with a well counter, is commonly used for this purpose since it is superior in sensitivity compared to on-line flow-through radiometric detection [8,9]. Nevertheless off-line procedures are laborious, time-consuming and only a limited number of samples/fractions can be processed during a PET study. In addition, some PET radioligands are known to metabolize to numerous substances in the body, making their separation from one another difficult.

In the last 5 years, ultra-high pressure LC or fast-LC techniques have received much attention due to their competitive advantages, especially in regards to reduced analysis time and increased resolution and sensitivity [10–13]. These LC techniques utilize columns packed with small particles (typically < 3 μm), resulting in simultaneous improvement of efficiency, optimum velocity and mass transfer. Therefore they are used in many disciplines, e.g. clinical studies, environmental analyses, new drug development, proteomics, genomics and metabonomics studies [10–13] where

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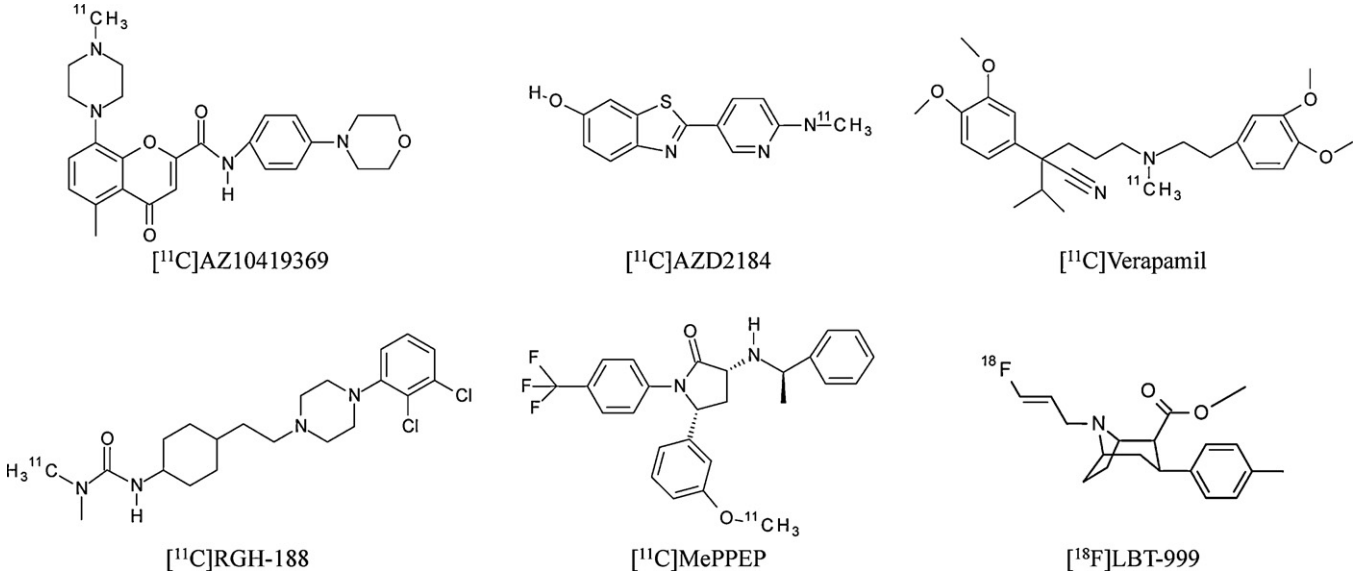

Fig. 1. PET radioligands examined in this study.

high sample analysis rate, superior peak capacity and ultra-high sensitivity are required. The advantages offered by these techniques can be beneficial in metabolism studies of short-lived PET radioligands where analysis time and sensitivity are of great importance.

The aim of the present work was to develop a general radio-LC method employing fast-LC and on-line radiometric detection for the metabolite analysis of a wide array of PET radioligands. The applicability of this method was demonstrated for the metabolite analysis of [^{11}C]AZ10419369 (a serotonin 5-HT_{1B} receptor radioligand) [14], [^{11}C]AZD2184 (an Aβ amyloid radioligand) [15,16], [^{11}C]MePPEP (a cannabinoid CB_1 receptor radioligand) [17], [^{11}C]Verapamil (a P-glycoprotein ligand) [18], [^{11}C]RGH-188 (a dopamine D_3/D_2 receptor radioligand) and [^{18}F]LBT-999 (a dopamine transporter radioligand) [19] (Fig. 1) in human and monkey plasma samples taken during PET studies.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (HPLC grade) was obtained from Fischer Scientific. Ammonium formate and ammonium hydroxide were purchased from Sigma–Aldrich, sodium dihydrogen phosphate monohydrate and di-sodium hydrogen phosphate dihydrate were acquired from Fluka. Authentic PET ligand standards (Fig. 1) were obtained from Astra-Zeneca (AZ10419369, AZD2184), National Institute of Mental Health (MePPEP), Sigma–Aldrich (Verapamil), Gideon Richter (RGH-188) and Tours University (LBT-999) and used without further purification. [^{11}C]AZ10419369, [^{11}C]AZD2184, [^{11}C]MePPEP, [^{11}C]Verapamil, [^{11}C]RGH-188 and [^{18}F]LBT-999 radiopharmaceuticals were prepared according to the published procedures [14–19].

2.2. Radio-LC system

LC analysis was carried out using a quaternary LC pump (L-7100; Hitachi), coupled to a manual injection valve (7125; Rheodyne) with a 5.0 mL loop. Chromatographic separation was performed on a reversed phase column (XBridge OST C_{18}, Waters, 50 mm × 10 mm I.D., 2.5 μm) placed after a guard column (10 mm × 10 mm I.D., 5 μm), using a mixture of CH_3CN and 50 mM ammonium formate buffer (pH 9.3) or 20 mM sodium phosphate buffer (pH 7.2) at a flow rate of 8 mL/min. The mobile phase compositions and gradient profiles were as described in Table 1. The effluent from the column was monitored by a UV absorption detector (L-7400; Hitachi) and an on-line dual bismuth germanium oxide coincidence (BGO) radiation detector (S-2493Z; Oyokoken) [6,7] housed in a shield of 50 mm thick lead. The γ energy window was set between 380 keV and 640 keV. The accumulation time of radiation detector was 3.3 s, and the flow cell volume was 400 μL (capillary PEEK, 0.51 mm I.D., 0.79 mm O.D. × 2000 mm). Data collection and control of the LC system were performed using chromatographic software (D-7000; Hitachi).

2.3. Metabolite analysis of PET radioligands in human and monkey plasma

The human and monkey PET studies were approved by the Regional Ethics Committee and by the Animal Research Ethical Committee in Stockholm. After intravenous administration of radioligands into humans or monkeys, whole blood samples were collected in heparini-treated syringes at pre-specified time points. The blood samples were centrifuged at 2000 × *g* for 2–4 min at room temperature to separate plasma. The supernatant plasma samples (0.2–0.5 mL) were mixed with 1.4 times volume of acetonitrile. The resulting denatured protein emulsion was stirred with a vortex mixer and centrifuged at 2000 × *g* at room temperature for 2–4 min. After addition of water (~4 mL) to the supernatant plasma–acetonitrile mixture, the mixture (approximately 5 mL) was subsequently injected into the radio-LC system. The amounts of unchanged parent radioligand were expressed as a percentage of the sum of areas of all the detected radioactive peaks (decay-corrected to the time of injection on the LC).

2.4. Method comparison

Analysis was performed by conventional radio-LC as well as fast-LC in order to compare the outcomes. Chromatographic separation was achieved at a flow rate of 6.0 mL/min on a semi-preparative LC column (μBondapak C_{18}, Waters, 300 mm × 7.8 mm I.D., 10 μm). The operating parameters of the conventional radio-LC method were as described earlier [5,14,16,19]. The mobile phase and gradient profiles were as described in Table 1.

Table 1
Chromatographic separation conditions for the metabolite analysis of PET radioligands by fast radio-LC and conventional radio-LC.

PET radioligands	Fast radio-LC				Conventional radio-LC		
	Mobile phase	Gradient profile (acetonitrile %, v/v)	UV wavelength (nm)	Retention time of radioligands (min)	Mobile phase	Gradient profile (acetonitrile %, v/v)	Retention time of radioligands (min)
[^{11}C]AZ10419369	Acetonitrile/50 mM ammonium formate buffer (pH 9.3)	25 → 55% (0–2 min), 55% (2–2.5 min), 55 → 25% (2.5–2.6 min), 25% (2.6–3.5 min)	254	2.40	Acetonitrile/100 mM ammonium formate	30 → 52% (0–11 min), 52 → 90% (11–12.5 min), 90% (12.5–13 min), 90 → 30% (13–13.5 min), 30% (13.5–15 min)	12.3
[^{11}C]AZD2184	Acetonitrile/20 mM sodium phosphate buffer (pH 7.2)	20 → 50% (0–2 min), 50% (2–2.5 min), 50 → 20% (2.5–2.6 min), 20% (2.6–3.5 min)	340	2.29	Acetonitrile/100 mM ammonium formate	22 → 47% (0–10 min), 47 → 80% (10–11 min), 80 → 22% (11–12 min)	9.0
[^{11}C]Verapamil	Acetonitrile/50 mM ammonium formate buffer (pH 9.3)	45 → 85% (0–2 min), 85% (2–2.5 min), 85 → 45% (2.5–2.6 min), 45% (2.6–3.5 min)	280	2.36	Acetonitrile/100 mM ammonium formate	35 → 80% (0–7 min), 80% (7–9 min), 80 → 35% (9–9.5 min), 35% (9.5–10 min)	7.5
[^{11}C]RGH-188	Acetonitrile/50 mM ammonium formate buffer (pH 9.3)	45 → 85% (0–2 min), 85% (2–2.5 min), 85 → 45% (2.5–2.6 min), 45% (2.6–3.5 min)	254	2.37	Acetonitrile/100 mM ammonium formate	65% (isocratic elution)	7.0
[^{11}C]MePPEP	Acetonitrile/50 mM ammonium formate buffer (pH 9.3)	55 → 90% (0–2 min), 90% (2–2.5 min), 90 → 55% (2.5–2.6 min), 55% (2.6–3.5 min)	254	2.45	Acetonitrile/100 mM ammonium formate	50 → 90% (0–8 min), 90% (8–10 min), 90 → 50% (10–11 min), 50% (11–12 min)	7.8
[^{18}F]LBT-999	Acetonitrile/50 mM ammonium formate buffer (pH 9.3)	50 → 90% (0–2 min), 90% (2–2.5 min), 90 → 50% (2.5–2.6 min), 50% (2.6–3.5 min)	254	2.54	Acetonitrile/100 mM ammonium formate	20% (0–2 min), 20 → 80% (2–10 min), 80% (10–12 min), 80 → 20% (12–13 min), 20% (13–15 min)	10.5

3. Results and discussion

3.1. Optimization of fast-LC conditions

Preliminary experiments were carried out using non-radioactive references or radiopharmaceutical samples of PET radioligands, since standards were not commercially available for most of the radioactive metabolites and the half-lives of radioactive metabolites were extremely short, in order to establish the optimum conditions for separation of ^{11}C and ^{18}F-labeled radioligands from their radioactive metabolites in plasma.

The fast-LC method presented in this study utilizes a short (50 mm-length plus 10 mm guard) semi-preparative (10 mm I.D.) reversed phase column packed with small particles (2.5 μm). One of the drawbacks, however, is the small loading capacity of this column, which limits large sample injection onto the LC. The common procedure in PET radiometabolite analysis requires removal of proteins prior to LC injection, mostly done by protein precipitation using either organic solvents or strong acids followed by centrifugation. However, the high volume of organic solvents used for deproteinization is problematic especially when analyzing hydrophilic radioligands. The higher content of organic phase in the sample than that in the starting LC mobile phase, often causes insufficient separation even when using conventional semi-preparative reversed phase LC. In this study, acetonitrile was selected as the deproteinization reagent due to the simplicity, speed of this procedure and the fact that none of the six radioligands investigated were significantly bound to the plasma protein precipitate. The extraction recoveries of these radioligands were more than 98%. The separation conditions were optimized in terms of large sample applicability as well as rapidity and high resolution. To achieve sufficient separation of the radio-compounds present in the acetonitrile rich sample, the mobile phase used had a relatively high organic content. For this evaluation, initially we used 50 mM sodium phosphate (pH 2.1 and 7.2), ammonium acetate (pH 4.7) and ammonium formate (pH 9.3) as the ionic mobile phase modifier that would provide sufficient pH buffering capacity. Due to the limited concentration of sodium phosphate to be dissolved in acetonitrile at neutral pH, a lower concentration (20 mM) was selected for pH 7.2.

All of the investigated compounds have aliphatic or aromatic amine functional groups and therefore their chromatographic behavior would be influenced by the pH of the mobile phase. For example, the effect of the mobile phase pH and acetonitrile concentration on the retention factor (k) of [^{11}C]AZ10419639 is demonstrated in Fig. 2A. As expected, k value of [^{11}C]AZ10419639 increased as the pH of the mobile phase increased. This compound contains a tertiary aliphatic and two aromatic amines, thus is most lipophilic at high pH where it exists in its unprotonated form and higher acetonitrile content is needed to elute this radioligand. The retention factor of [^{11}C]AZ10419639 remained unchanged (k = 5) when 20% acetonitrile at pH 2.1 was employed as the mobile phase instead of 40% acetonitrile at pH 9.3. However, the injection volume of plasma extract dramatically influenced the peak shape of [^{11}C]AZ10419639 when the separation was performed with 20% acetonitrile at pH 2.1 (Fig. 2B). The analyte peak eluted at the solvent front and was split into two or three peaks with fronting and broadening as the sample volume increased (>1.0 mL plasma). The maximum plasma volume that could be injected with a 20% acetonitrile pH 2.1 mobile phase was only 0.5 mL, in contrast, elution

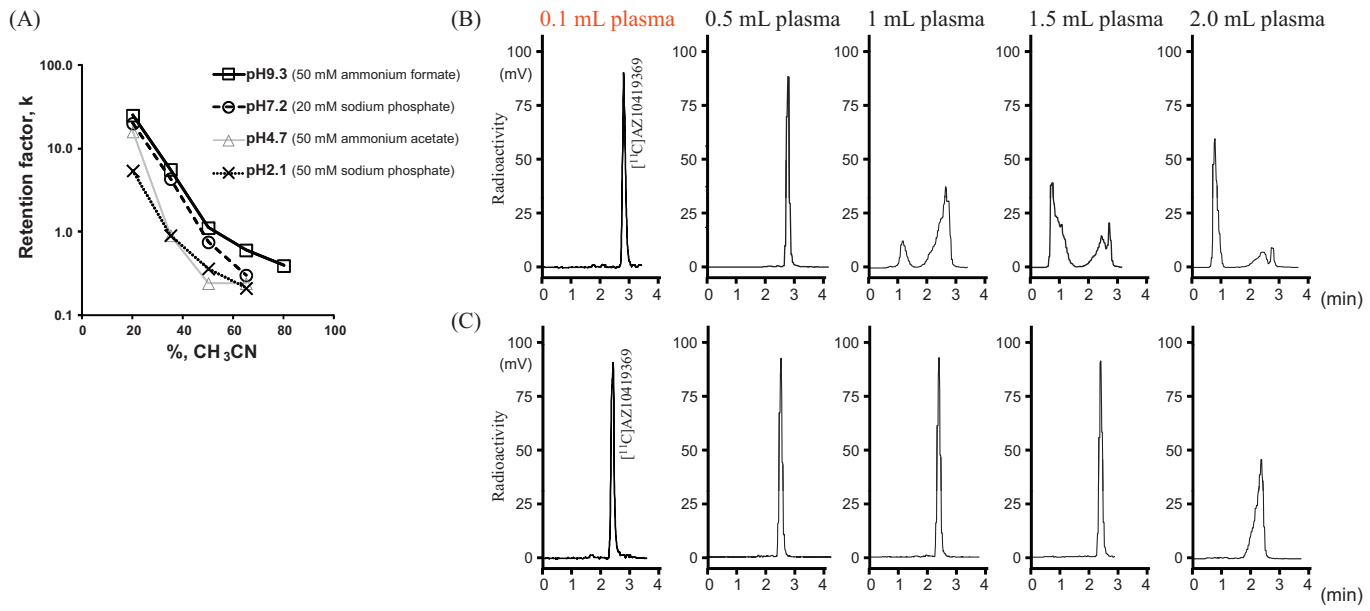

Fig. 2. (A) Effect of acetonitrile concentration on the retention factor (k) of [^{11}C]AZ10419369 eluted at different pH values. (B) Influence of plasma injection volume on the peak shape of [^{11}C]AZ10419369 eluted with acetonitrile/50 mM sodium phosphate pH 2.1, 20/80 (v/v) and (C) acetonitrile/50 mM ammonium formate pH 9.3, 40/60 (v/v). [^{11}C]AZ10419369 added to human plasma (0.1–2.0 mL) were deproteinized with acetonitrile as described in Section 2.3 and injected into the fast radio-LC system. Radioactivity injected: 1084 ± 23 Bq.

with a pH 9.3 mobile phase containing 40% acetonitrile provided improved sample loadability; up to 1.5 mL of treated plasma could be applied without any compromises on the [^{11}C]AZ10419639 peak shape and peak height (Fig. 2C).

Several other parameters affecting the chromatographic separation were investigated. LC columns packed with relatively smaller particles can maintain good peak capacity even at high flow rates, however high back pressure becomes a problem at such flow rates. The maximum column efficiency was attained at flow rates of 4–8 mL/min where 7000–9000 theoretical plates could be obtained for all the six compounds examined. To reduce the separation time and increase the resolution, the flow rate was adopted to 8 mL/min and gradient elution was used. Gradient elution was superior in sensitivity compared to isocratic mode where at least 2 times higher peaks could be obtained. Despite using smaller size particles, the back pressure was 18–25 MPa under optimized LC conditions, which is acceptable in conventional LC apparatuses.

For the efficient separation of PET radioligands from their radioactive metabolites, it is preferable that the k values of the PET radioligands are high enough for the separation, while at the same time the retention time of the PET radioligand should be as short as possible for the rapid analysis of short-lived radioligands. Therefore, the chromatographic conditions were selected as shown in Table 1, by which the target PET radioligands were eluted at ca. 2.5 min having narrow peak widths in the range of 0.15–0.20 min. The analysis was completed within 3.5 min and injection of a relatively large volume of plasma was tolerated (1.5–2.0 mL plasma) following this procedure. The flow cell volume of the radioactivity detector was evaluated in the range of 200–600 μL using PEEK tubing (0.51 mm I.D., 0.79 mm O.D.) and a 400 μL flow cell was optimal in both higher plate count and narrower peak width.

3.2. Method validation

Under the optimum conditions described above, reproducibility, linearity and detection limit were evaluated for each of the following radioligands: [^{11}C]AZ10419369, [^{11}C]AZD2184 and [^{11}C]MePPEP. A standard solution of radioligand (ca. 30,000 Becquerel (Bq)/mL) was prepared from radiopharmaceutical sample, and then further diluted with the starting mobile phase to the desired concentrations. A known volume (0.02–1 mL) of these solutions was injected into the radio-LC system. The injected radioactivity was calculated from the measured radioactivity, injection volume, dilution factor and decay-correction to the time of injection.

The relative standard deviations of the retention time and peak area were within 2.0% (n = 6) and 2.9% (n = 6), respectively obtained from repetitive injection at 1000 Bq level. The detector exhibited a linear response in the range of 1–20,000 Bq with coefficient of determination (r^2) of more than 0.999. The limits of detection (LOD) based on the standard deviation (σ) of background (BG) were in the range of 1.0–1.2 Bq (BG + 3.3σ), these LODs were at least 5-folds lower than those obtained by conventional semi-preparative LC procedures. These values were sufficient to perform metabolite studies in human and monkey plasma taken during PET studies, which usually contains 30–50,000 Bq/mL of parent radioligand in plasma. However, this radio-LC method is limited in the upper linear range to 20,000 Bq due to the very narrow analyte peaks provided by the fast-LC. Therefore, it should be confirmed that the samples remain within the linear range in particular for the early samples from PET experiments.

3.3. Metabolite analysis of PET radioligands in human and monkey plasma

The performance of the developed method was investigated by the radio-metabolite measurement of [^{11}C]AZD2184 in human arterial plasma taken during PET measurement (Fig. 3A). The results were compared to those obtained from using a conventional semi-preparative LC column with 7.8 mm I.D, 300 mm-length and 10 μm particles (Fig. 3B). The fast-LC method improved the speed of separation without any losses in chromatographic performance compared to the conventional LC. High column efficiency provided by the fast-LC resulted in 70% shorter retention time of [^{11}C]AZD2184 parent radioligand than that achieved by the conventional LC procedure. The metabolism rate of [^{11}C]AZD2184

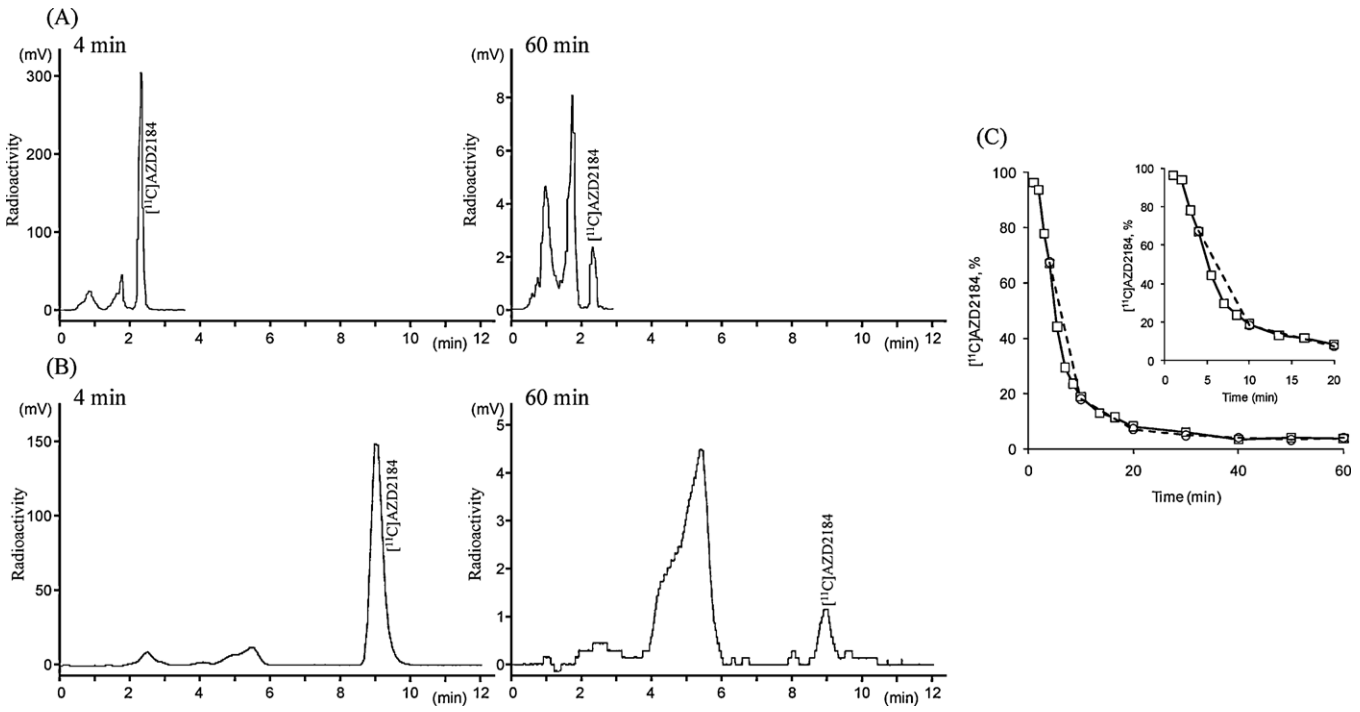

Fig. 3. (A) Typical radio-chromatograms of human plasma taken at 4 and 60 min after intravenous injection of [^{11}C]AZD2184 obtained by fast-LC and (B) conventional preparative LC. (C) Time course of percentage of unchanged [^{11}C]AZD2184 obtained by fast-LC (□) and conventional LC methods (○). Subject: healthy human volunteer (female), Administered radioactivity: 502 MBq. Arterial blood samples (2–4 mL) were drawn at 1, 2, 3, 4, 5.5, 7, 8.5, 10, 13.5, 16.5, 20, 30, 40, 50 and 60 min. All the 15 samples were analyzed by fast-LC (plasma sample volume: 0.5 mL) and 7 samples (at 4, 10, 20, 30, 40, 50 and 60 min) were analyzed by conventional-LC (plasma sample volume: 1.5 mL). Plasma radioactivity: 4 min; 12,000 Bq/mL, 60 min; 920 Bq/mL at the time of sample preparation.

in humans and monkeys is reported to be fast, forming several hydrophilic radioactive metabolites [15,16]. A similar radio-chromatographic profile was attained with the fast-LC method, where two major polar radioactive metabolites were observed at retention times of 0.8 min and 1.8 min (Fig. 3A) and their fractions increased time-dependently. The percentages of unchanged [^{11}C]AZD2184 obtained by this method were in good agreement with those achieved by the conventional method (Fig. 3C). The radioactivity of the parent radioligand in plasma samples was in the range of 19–4300 Bq/0.5 mL, which was within the linear range of the developed radio-LC method.

One of the special features of PET metabolite analyses is that they have to be performed within a strict time limit. This is particularly important for the late samples in which the parent radioligands are present in extremely low radioactive concentrations and this can have a significant impact on quantitative pharmacokinetic analysis of the PET radioligands. Problems associated with the metabolite analysis procedures currently used often need to be overcome to facilitate the use of a large sample volume and/or reduce number of samples. Indeed, the long run time of the conventional LC for [^{11}C]AZD2184 disturbs sensitive radiometric analysis especially for the late sample points due to radioactive decay (^{11}C: $t_{1/2}$ = 20.4 min). The run time of the conventional radio-LC analysis was 12 min and 7 samples (at 4, 10, 20, 30, 40, 50 and 60 min after administration) were analyzed during a 60 min PET study. The LC injection of the last sample was delayed for 30 min after sample preparation and 60% of the radioactivity was declined at the time of injection, thus a relatively large sample volume (1.5 mL) was needed for the accurate determination. In contrast, a metabolite analysis was completed after only 3.5 min using the fast-LC method, and as a result, all of the samples could be analyzed without any time-lag after sample preparation. The sensitivity of the radioactivity detection was five times better than the conventional-LC and at least 10-times higher for the late sample taken at 60 min and therefore only 0.5 mL of plasma was required for the efficient radiometric determination of this radioligand. Moreover, by using this rapid method it is possible to analyze not only 7 but all the 15 arterial plasma samples taken during a PET study (Fig. 3C) for the determination of PET radioligands as well as the measurement of whole blood/plasma radioactivity concentration for the input function within 75 min after administration of [^{11}C]AZD2184.

This method was successfully applied to the radio-metabolite analysis of [^{11}C]AZ10419369, [^{11}C]MePPEP, [^{11}C]Verapamil, [^{11}C]RGH-188 and [^{18}F]LBT-999 in human and monkey plasma. Fig. 4 shows radio-chromatograms of plasma taken at two different time points (early and late samples) during PET studies. As with published reports, the metabolism rate of [^{11}C]AZ10419369 was slow in human plasma [20], in contrast, other three radioligands were rapidly metabolized to form several radioactive metabolites in monkey plasma [17–19]. The improved sensitivity provided by the fast radio-LC procedure made it possible to accurately determine parent-radioligands present in the later samples even in a small injection volume (0.3–0.5 mL plasma), which contained only 12–200 Bq of the intact radioligands, and up to 24 plasma samples could be analyzed in one PET study of [^{18}F]LBT-999 (Fig. 5B). For all the samples (n = 116) tested significant concordance ($y = 0.985x + 0.426$, $r^2 = 0.9969$) was obtained between the fast and conventional LC method in the quantification of unchanged PET radioligands (Fig. 5C). For the six compounds evaluated in this study, all of their radioactive metabolites were eluted during the gradient conditions described in Table 1 with LC recoveries of 100.1 ± 3.5% calculated based on the amount of sample radioactivity injected onto the LC column and that recovered from effluent fraction after LC analysis. Although large sample volumes can be injected into this system (>1.5 mL plasma), the high sensitivity provided by it omitted such a need for the mentioned samples, however this feature can be beneficial when e.g. the metabolism and plasma clearance of the target radioligand

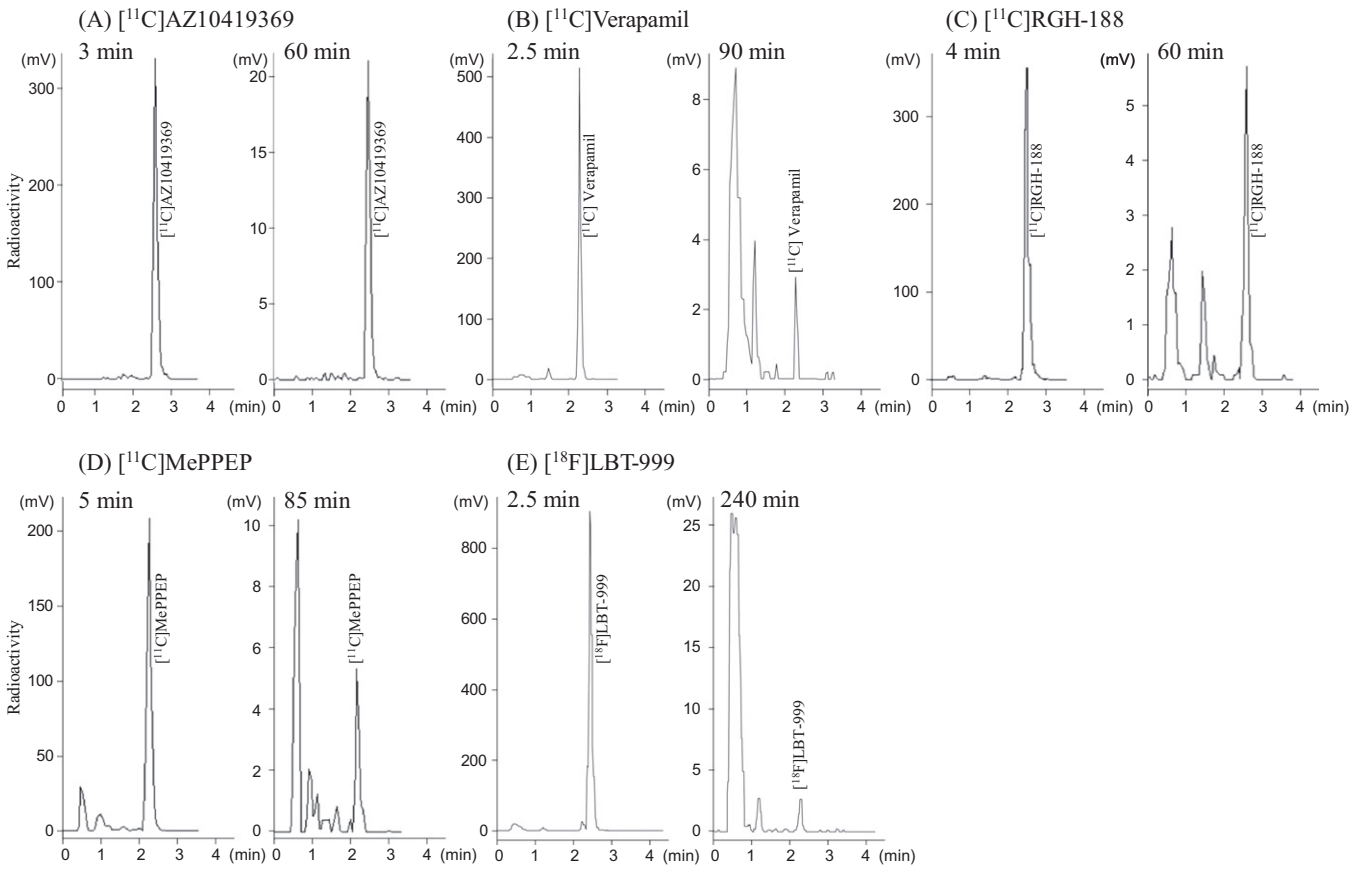

Fig. 4. (A) Fast LC radio-chromatograms of 0.5 mL human and (B–E) 0.3 mL monkey plasma taken at two different time points after administration of (A) [11C]AZ10419369, (B) [11C]Verapamil, (C) [11C]RGH-188, (D) [11C]MePPEP and (E) [18F]LBT-999. [11C]AZ10419369: healthy human volunteer (male), Administered radioactivity; 415 MBq, plasma sample volume; 0.5 mL, Plasma radioactivity; 4000 Bq (3 min), 220 Bq (60 min), [11C]Verapamil: Rhesus monkey (female), administered radioactivity; 205 MBq, plasma sample volume; 0.3 mL, plasma radioactivity; 5100 Bq (2.5 min), 130 Bq (90 min), [11C]RGH-188: Cynomolgus monkey (female), administered radioactivity; 129 MBq, plasma sample volume; 0.3 mL, plasma radioactivity; 4600 Bq (4 min), 60 min (200 Bq), [11C]MePPEP: Cynomolgus monkey (female), administered radioactivity; 139 MBq, plasma sample volume; 0.3 mL, plasma radioactivity; 4500 Bq (5 min), 220 Bq (85 min), [18F]LBT-999: Rhesus monkey (female), administered radioactivity; 191 MBq, plasma sample volume; 0.3 mL, plasma radioactivity; 13,000 Bq (2.5 min), 790 Bq (240 min).

is very fast and/or the determination of much later plasma sample is required for PET pharmacokinetic analysis (e.g. 120 min after administration of 11C-labeled radioligands).

In the present study we focused only on the determination of unchanged radioligands. For several radioligands, however, circulating radio-metabolite(s) can cross the blood brain barrier and their PET measurements are confounded by the accumulation of the radioactive metabolite(s) in brain [21–22]. Thus the collection of the PET data for the presence of brain-penetrant metabolite(s) is required to improve statistical fit to the pharmacokinetic model. In such cases, the LC analysis conditions should be optimized in a way to separate target metabolite(s) and parent radioligand from other non brain-penetrate radioactive metabolites even though the run-time might become slightly longer.

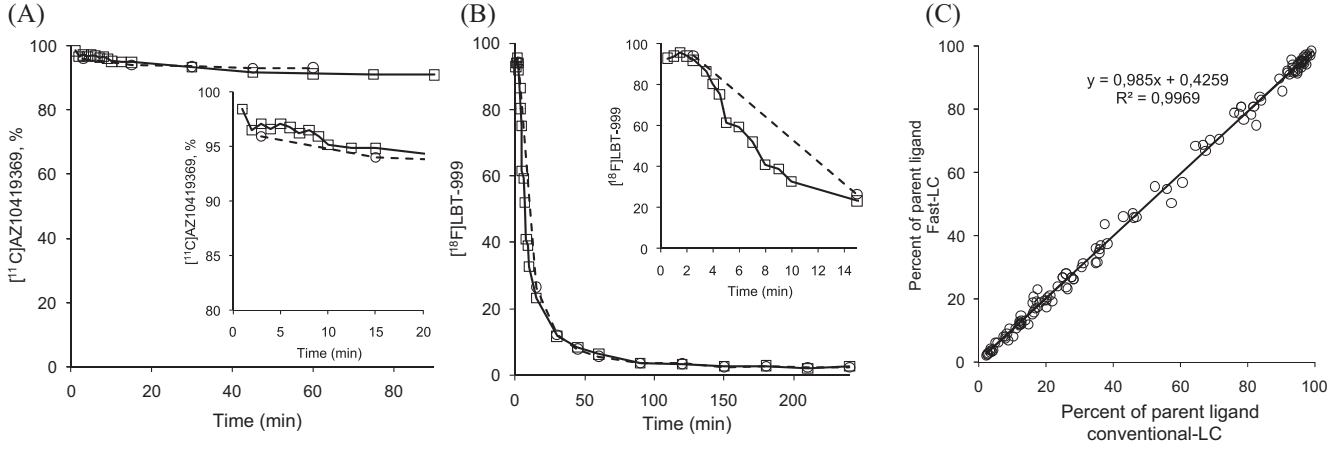

Fig. 5. (A) Comparison of unchanged parent [11C]AZ10419369 (human) and (B) [18F]LBT-999 (monkey) fraction in plasma obtained by fast-LC (□) and conventional LC (○) methods. (C) The correlation of parent PET radioligands fraction obtained by fast-LC and conventional-LC methods from all the samples tested ($n = 116$).

4. Conclusion

We developed a rapid on-line radio-LC method for the measurements of radioligands in plasma. This method benefits from high speed and sensitivity in radiometric analysis, allowing higher numbers of samples (up to 24 samples per PET measurement) with lower volumes to be analyzed compared to conventional radio-LC methods and results in a more accurate estimation of the metabolite corrected input function. This method can easily be adopted for the determination of a variety of PET radioligands in plasma.

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