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Stability assessment of PET radiopharmaceuticals under extreme conditions: an ICH-compliant study on radiochemical and enantiomeric purity

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Abstract

Background The stability testing of radiopharmaceuticals is a critical aspect of drug development and regulatory approval. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) provides comprehensive guidelines for stability testing, as outlined in the ICH Harmonised Tripartite Guideline Q1A(R2). In this study, the stability of α -(2-[¹⁸F]fluoroethyl)-L-tyrosine ([¹⁸F]FET) and methyl-[¹¹C]-L-methionine ([¹¹C]MET) was evaluated under GMP conditions to ensure their quality and safety. These radiopharmaceuticals are widely used in brain tumor imaging, yet their stability remains insufficiently studied. Given the short half-life of [¹¹C]MET (20 min), rapid preparation and administration are required, whereas the longer half-life of [¹⁸F]FET (110 min) allows for transportation, making stability considerations crucial due to potential environmental effects on the injection solution.

Results Samples from each batch were tested at different temperatures and pH values across the entire expiration period. The radiochemical purity of [¹⁸F]FET remained between 98.04 and 100% by TLC, while UPLC values ranged from 95.93 to 99.59%. Differences between these methods stem from their sensitivity and operational principles. While UPLC provides precise separation, it may trap free fluoride, leading to overestimated purity values. In contrast, TLC allows for complete sample evaluation but has lower separation efficiency. Enantiomeric purity assessments confirmed that only the L-form was present, with no detectable D-enantiomer, in the case of [¹¹C]MET a maximum of 1.7% D-enantiomer was also detected. Stability remained above the 95% threshold between -20 and 50 °C, with slight reductions at basic pH values. Even under stressed conditions no decomposition products were detected, and enantiomeric purity exceeded 90%, confirming the robustness of [¹⁸F]FET and [¹¹C]MET stability.

Conclusion In this report, the stability of the [¹⁸F]FET and [¹¹C]MET radiopharmaceuticals was studied within the expiration time. [¹⁸F]FET remained stable until the end of the 12-h expiration time. [¹¹C]MET samples stored even under stressed conditions did not decrease under the acceptable limit during the shelf life

of the radiopharmaceutical. These findings confirm that both radiopharmaceuticals maintain their stability within the defined shelf life, ensuring their reliability for clinical use. Further studies could explore additional environmental stress factors to enhance stability assessments and optimize storage conditions.

Keywords [^{18}F]FET, [^{11}C]MET, Stability, Radiopharmaceuticals, Quality control, Quality assurance, ICH

Background

The labelled biologically active molecules used for positron emission tomography (PET) can be of different nature. Carbohydrates (glucose), amino acids, peptides and other small molecules can all be used to trace the biochemical processes taking place in the body. *o*-(2- ^{18}F fluoroethyl)-L-tyrosine (^{18}F FET) and methyl- ^{11}C -L-methionine (^{11}C MET) are mainly used for the diagnosis of oncological cases, including for the detection of brain tumors and gliomas, due to their, that amino acid metabolism is higher in tumor tissue than in healthy tissue (Joshi et al. 2021; Langen et al. 2017; Wester et al. 1999; Glaudemans et al. 2013; Stegmayr et al. 2021; Långström et al. 1987; Vävere and Snyder 2012). ^{18}F FET received Orphan Drug Designation (ODD) from the FDA on October 6, 2020, for its use as a PET imaging agent for glioma, a type of brain tumor (<https://telixpharma.com/news-views/telix-granted-fda-orphan-drug-designation-for-glioma-imaging-agent>; <https://www.cancernetwork.com/view/pet-imaging-agent-earns-fda-fast-track-designation-in-recurrent-glioma>). In the quality control of radiopharmaceuticals, the European Pharmacopoeia (Ph. Eur.) requires, among other things, the determination of pH, radiochemical and enantiomeric purity (Fluoroethyl-L-Tyrosine 2015; European Pharmacopoeia 2008). Liquid and paper chromatography methods are used to determine the latter two parameters. HPLC provides efficient separation of all components, however, free fluoride can be adsorbed onto the column, leading to incorrect results. On the other hand, the components do not evaporate from the TLC plates, i.e. their total amount can be tested, but the separation with this method is less efficient. By comparing the results of the two test methods, we can already obtain a reliable value for radiochemical purity. With the HPLC system, we can not only separate the radioactive components, but we can also separate the L- and D-forms, so the enantiomeric purity of the preparation can be determined. Although the synthesis employs the L-form of the precursor, chirality inversion may still occur (Siddiq et al. 2018; Mueller et al. 2011; Giglio et al. 2018; Pascali et al. 1999; Gomzina and Kuznetsova 2011).

The shelf life of preparation is particularly important for radiopharmaceuticals. The stability program is an all-encompassing documented procedure that is in effect on an ongoing basis. ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) (ICH Q1A (R2)) guidelines also includes a list of specifications, minimum and maximum permissible values of parameters, storage conditions and stability test reports. In the case of all radiopharmaceuticals, one of the main goals of the stability program is to identify and determine the degradation products and their ratio compared to the active ingredient (Elsinga et al. 2010; Gillings et al. 2020; Holler et al. 2022). The stability of radiopharmaceuticals is primarily determined by the concentration of radioactivity, as well as the formulation and overall stability of the molecule. In addition to isotopic decay, the phenomenon of autoradiolysis must also be taken into account (Saha 2018; Scott et al. 2009). As a result of radiolysis, in the case

of the [^{18}F]FET, free radicals are generated during the interaction between radiation and water molecules, which trigger the decay of the radioactive active ingredient and mainly free fluoride (Kuchar and Mamat 2015; Búriová et al. 2005). In addition to free fluoride, other smaller molecular fragments containing fluoride are also formed, which further decompose and whose presence helps to reduce the proportion of the active ingredient. These molecules are detected as pollutants during the tests. Our laboratory has already investigated how activity concentration is related to the amount of ethanol content. Based on literature reports, auxiliary substances—such as ethyl alcohol—neutralize the free radicals that cause decomposition, which causes a change in the radiochemical purity (Wang et al. 2009). The results of the measurements showed that ethanol has no particular effect on the active ingredient of [^{18}F]FET, because it is stable on its own, i.e. without a stabilizer. In the case of [^{11}C]MET, stability studies showed that the rate of decomposition was influenced by the total activity and chemical composition of the solutions (Bogni et al. 2003; Woods et al. 2017). Gomez et al. also investigated the thermal stability at 121 °C, with the aim of determining the autoclavability of the sample. The final product proved to be unstable, as the radiochemical purity dropped to 85% after 20 min. Depending on this, they had to discard the option of autoclaving (Gómez et al. 2008).

Although the literature does not really address this, it is believed that temperature and pH can also affect the radioactive purity. We expect that as the temperature increases, the decomposition process will be faster, i.e. the radiochemical purity will decrease, and the shift of the pH to the alkaline range will presumably promote the epimerization processes. Based on these, the goal of our work was to examine the radioactive and enantiomeric purity of the radiopharmaceuticals under different conditions, thereby making sure that the drug is safe for the patient within its shelf life, even in case of environmental changes occurring during transport or storage.

Materials and methods

Chemicals and reagents

All reagents and solvents were obtained from commercial suppliers and used without further purification. For the HPLC and UPLC systems HPLC–MS grade acetonitrile (ACN) and methanol (MeOH) (VWR, Debrecen, Hungary) and deionized water (Milli-Q, 18.2 MW cm Ω^{-1} , Merck, Budapest, Hungary) were used. Sodium hydroxide, sodium acetate (NaAc), sodium hydroxide (50%), potassium dihydrogen phosphate, phosphoric acid, acetic acid, N-isobutyryl-L-cysteine (IBLC), *o*-phthaldialdehyde (OPA), L-proline, copper(II) acetate and silica gel 60 TLC plastic sheets were obtained from Sigma Aldrich (Budapest, Hungary). D,L-Fluoroethyl-tyrosine and L-Tyrosine (TET), D,L-Methionine and L-Homocysteine were purchased from ABX (Radeberg, Germany).

[^{18}F]FET and [^{11}C]MET were prepared based on the previously reported methods (Fig. 1) (Búriová et al. 2005; Wang et al. 2009). The environmental parameters during

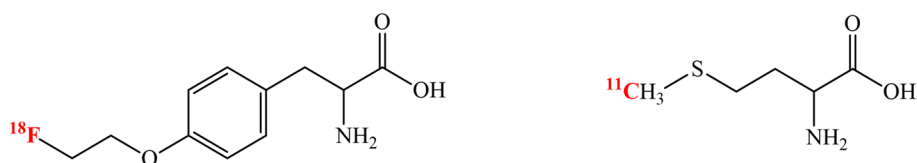


Fig. 1 Chemical structures of [^{18}F]FET and [^{11}C]MET

the syntheses was $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$; $60\% \text{ RH} \pm 5\% \text{ RH}$. The average radioactivity concentration of the tested batches was 1.32 GBq/mL EOS for [^{18}F]FET and 316.71 MBq/mL EOS for [^{11}C]MET.

UPLC and HPLC systems

In the case of [^{18}F]FET, chromatographic experiments were performed on a Waters Acquity UPLC I-Class System equipped with PDA detector, Binary Solvent Manager, a Sample Manager (FTN Injector with 100 μL loop) and a Column Manager. A photomultiplier tube (Hamamatsu Photonics), equipped with a plastic scintillator was used as radioactivity detector. The data were evaluated by Empower 3 chromatography software. The radiochemical purity measurement was achieved with a Acquity UPLC BEH C18 1.7 μm 2.1 \times 50 mm (Waters) column, with a gradient program. 0.1% phosphoric acid (A) and acetonitrile (B) were used as eluents, 100:0 (v/v) for 1.0 min, then increased to 87.5:12.5 (v/v) for 3 min, then 0:100 (v/v) for 4 min, and finally followed by an isocratic regime for 1 min. The chromatographic method lasted for 10 min. Experiments were carried out at a flow rate of 0.6 mL/min, volume of injection 10 μL and the column temperature of 40 $^{\circ}\text{C}$. During the evaluation, the retention times of the impurities were as follows: 3.8 min for [^{18}F]tBu-FET, while 4.6 min for [^{18}F]Tr-tBu-FET.

The enantiomeric purity measurement was achieved with a Acquity UPLC BEH C18 1.7 μm 2.1 \times 100 mm (Waters) column, with a gradient program. 0.1% phosphoric acid (A) and acetonitrile (B) were used as eluents which was increased from the initial ratio 100:0 (v/v) to 40:60 (v/v) in 18 min, then 1 min later it was reduced back to 100:0 (v/v). As derivatizer, 10 mg/mL N-isobutyryl-L-cysteine (IBLC) in 0.25 M, pH10.4 borate buffer and 3.5 mg/mL ortho-phthalaldehyde (OPA) dioxane solution were used. For pH adjustment Mettler Toledo MP220 pH meter with an InLab 413/IP67 combined pH electrode was used. The chromatographic method lasted for 30 min. Experiments were carried out at a flow rate of 0.6 mL/min, volume of injection 10 μL . During the evaluation, the retention times of the enantiomeric pair were as follows: 14.4 min for [^{18}F]-L-FET, while 14.6 min for [^{18}F]-D-FET (SI Fig. S1).

Jasco HPLC system was used for [^{11}C]MET measurements. The equipment consists of the following units: degasser, pump system, low-pressure gradient generator, automatic injector, manual column changer, column, radioactivity detector, ChromNav evaluation software. Merck Lichrospher 100 RP-18.5 μm , 250 \times 4 mm columns were used for the examinations, with an isocratic program. 1.4 g/L, pH3.3 KH_2PO_4 was used as eluent. The chromatographic method lasted for 6 min. Experiments were carried out at a flow rate of 1.0 mL/min, volume of injection 20 μL . During the evaluation, the retention times of the L-[methyl- ^{11}C]methionine was 4.1 min (SI Fig. S2).

To determine the enantiomeric purity, 30 mM NaAc, 17 mM L-proline and 8 mM Cu(II)Ac were used as eluents. The chromatographic method lasted for 15 min. Experiments were carried out at a flow rate of 2.0 mL/min, volume of injection 20 μL . The retention times of the L-[methyl- ^{11}C]methionine was 5.5 min and the D-[methyl- ^{11}C]methionine was 8.0 min (SI Fig. S3). The validation of the liquid chromatographic methods revealed that the reproducibility of the %Area values was not more than 2% RSD.

MiniGita TLC scanner

In the case of [^{18}F]FET, the determination of the radiochemical purity was also investigated using the thin-layer chromatography method using a Raytest miniGita Star Radio-TLC scanner (Beta Detector GMC) by GINA Star TLC software using silica gel 60 type chromatography thin layer with methanol-acetic acid mobile phase in the ratio of 90:10 (v/v). Development path length is 11 cm. Radioactivity value of the applied sample was not greater than 2.5 MBq. The scanning range was set to 0–140 mm, and the test time was set to 3 min. During the procedure, R/F = 0 for fluoride ions and R/F = 0.7 for [^{18}F]FET were detected (SI Fig. S4). The RSD of the %Area values obtained by the radio-TLC method was not more than 3%.

Results

After the synthesis, the Quality Control (QC) Laboratory receives a sample of the radiopharmaceuticals, from which it was determined whether the drug was suitable for the patient using quality control tests (SI Fig. S5 and S6). It was important that the product was monitored from production time to the end of the expiration time to establish stability, so during the test the 12-h expiration period generally defined for [^{18}F]FET had to be covered, while in the case of L-[methyl- ^{11}C]methionine the expiration time was only two hours. It was necessary to carry out the stability test at different temperatures since the [^{18}F]FET radiopharmaceutical was transported by road to other PET centrum, so taking the environmental conditions into account, we tested the radiochemical and enantiomeric purity of the preparation at the specified temperatures until the end of the expiration time. The temperature range chosen in our study (–20 to 50 °C) was in accordance with ICH Q1A(R2) stress testing principles (ICH Q1A (R2)). Radiopharmaceuticals are generally characterized by short half-lives and used in a controlled temperature environment. This suggests that extreme conditions may lead to potential changes in the molecule. Storage at –20 °C reflects delayed use, 25 °C corresponds to typical room handling, whereas 40–50 °C represents accelerated stress conditions that may occur during preparation or transport (European Medicines Agency (EMA) 2018). In general, it can be stated that radioactive decay is not related to temperature or pH, but it can affect radiochemical purity or enantiomeric integrity, potentially leading to radiolysis or racemization. This susceptibility is primarily due to the amino acid-based nature of these molecules, such as [^{11}C]methionine (Friedman 1999; Yamada et al. 1983). Furthermore, the stability of the solutions was analyzed at the following pH values: 2; 4.5; 7; 8.5; 10. The acceptable pH range given by the pharmacopoeia was between 4.5 and 8.5. The pH of the investigated solutions was adjusted using a 50 w/w% sodium hydroxide solution or 85 w/w% phosphoric acid applying a pH meter with microelectrode. Chemical effects outside the permissible pH value, as well as temperatures of –20 °C, 50 °C and 90 °C can be considered stress conditions. At the same time, based on experience, the chemical effect of production batches is usually 7.1. In addition, samples with a pH of 2 and 10, which were outside the permissible extreme values, were also examined, to obtain information about the stability of the [^{18}F]FET even under extreme conditions. Thus, as part of the stability program, each production batch was measured at 4 times at different temperatures and pH. The standard deviation of the radiochemical purity values did not exceed 0.1%.

$$RCP = \frac{A_{tracer}}{A_{tracer} + A_{others}}$$

$$EP = \frac{A_{L-tracer}}{A_{L-tracer} + A_{D-tracer}}$$

RCP: radiochemical purity
 A_{tracer} : radioactivity of the radiotracer
 A_{others} : radioactivity of the other radiolabeled components

EP: enantiomeric purity
 $A_{L-tracer}$: radioactivity of the L-form of the radiotracer
 $A_{D-tracer}$: radioactivity of the D-form of the radiotracer

Fig. 2 Formulas of RCP% and EP%

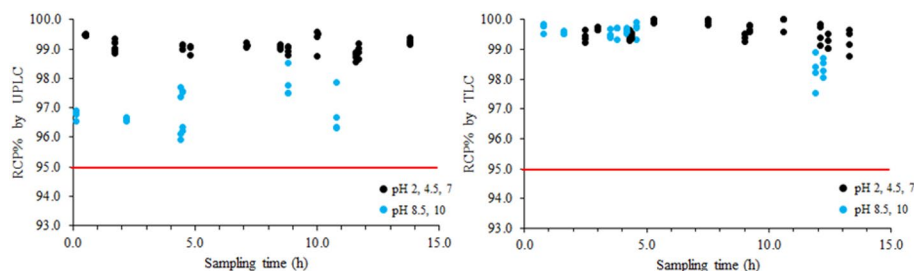


Fig. 3 RCP% of [¹⁸F]FET was determined by UPLC and TLC at -20, 2, 25 and 50 °C

Stability of [¹⁸F]FET

The pH of the batches was between 7.1 and 7.4, so only the storage temperature was controlled during the first measurement. -20; 2; 25; and stored the samples at 50 °C during the entire shelf-life expiration time, for which a freezer, a refrigerator, a cabinet dryer and calibrated thermometers were used to monitor the temperature. The active ingredient was well separated from the impurities by TLC and UPLC measurements. During the 12-h test period, we did not experience any new, previously unidentified peaks, which suggests that changing the temperature does not greatly affect the stability. The % value of purity was determined by comparing the activity of the active ingredient with the activity of the impurities (Fig. 2).

These values were quite high, over 99% during the TLC test and only reduced to 98.55% during the UPLC test. (Fig. 3.) This is much higher than the minimum defined in the Ph. Eur. monograph. 95% value. The graph also shows that the deviations were low at the individual temperature points at a given time, so they were not marked separately in the figure. During both the UPLC and TLC evaluations, the determination of the peak area was performed by manual integration, as a result of which, as well as due to the very small concentration of the samples, inaccuracies may have occurred. Previously tested parameters were examined how change with temperature, if the pH is set to the lowest value 4.5 allowed by the authority. This was achieved with 85% phosphoric acid. During the procedure with the TLC scanner, the purity values were between 99.03 and 99.67% (SI Table 2), while when measured with the UPLC system, values were between 98.65 and 99.18% (SI Table 1). In both cases, the specified minimum of 95% was exceeded. Even at the minimum allowable pH value, no decreasing trend was observed in the purity, the stability of the [¹⁸F]FET is not changed by a decrease in pH to 4.5.

At a pH of 2, in the TLC test, a purity value of 100% was observed, which was due to the decrease in the radioactivity of the impurities below the limit of quantitation (LOQ). During measurement with UPLC, the value of radiochemical purity was between 98.76% and 99.59%, which is also higher than the average values so far. Despite this, the

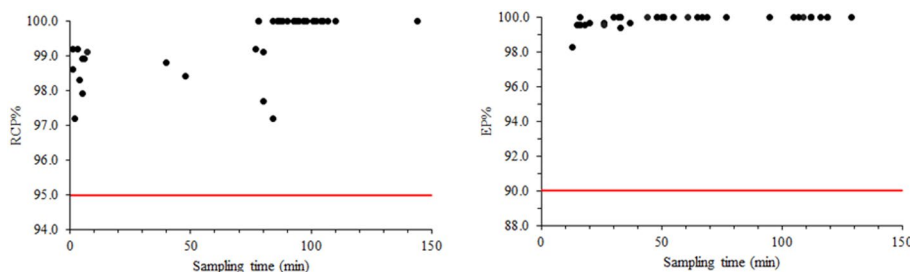


Fig. 4 RCP% and EP% of [^{11}C]MET was determined at 25 and 90 °C (pH10)

preparation was considered stable during the entire test period and no new, unidentified peaks appeared on the chromatogram, which would indicate the progress of decomposition. The permissible upper limit of the alkaline range (pH 8.5) was reached with 0.1 mol NaOH, while the temperature and stability test parameters and the course of the evaluation were not changed. During the analysis of the radiochemical purity measured with a TLC scanner, the percentage of the active substance from the areas below the integrated peak fell between 99.85 and 97.52%. It is important to note that these values have greatly decreased in the measurement results of 11.9 h, because at the previous three times we obtained values above 99% for all four temperature parameters. During the UPLC tests, the entire expiration time was covered in three points so far. In contrast to the 99% results experienced during the TLC tests, low values were detected between 96.10 and 97.76% with UPLC. The main reason for this was that due to the higher efficiency of UPLC, more polluting components could be detected. At a pH value of 10, which was outside the permissible limit, values between 98.04 and 99.92% were found during the RCP test with TLC. In this case as well, it only dropped below 99% at the final measurement point, but this was still well above the permissible 95%, so the radiopharmaceutical can be said to be stable even under stressful conditions. Compared with TLC, UPLC gave lower values, which may be due to the sensitivity of the detector, however—since purity was obtained over 95% during the entire test—the UPLC results only confirm that the radiopharmaceutical is stable.

During the enantiomeric purity test, nothing other than the L-enantiomer peak of the active substance appeared in the chromatogram, so the enantiomeric purity was 100% at all pH and at all temperatures (SI Table 3).

Stability of [^{11}C]MET

In the case of L-[methyl- ^{11}C]methionine, a pH value of 7 could be measured in most cases. Thus, in the first step, stability of the active ingredient was examined based on this chemistry. The initial RCP% was over 97% at both temperatures (Fig. 4). From the values, it can be seen that in the case of measurements taken at a given time, a decrease and then an increase can be observed in the case of RCP% (SI Table 4). Over time, a value of 100% was observed, due to the fact that the intensity of the impurity peaks fell below the detection limit during the decay of the ^{11}C isotope. From this, we can conclude that the chemical decomposition of the active substance and the appearance of radiochemical decomposition products during the expiration period are not significant. RCP% showed an appropriate value during the stability test, as it was exceeded the 95% limit both at room temperature and at 90 °C, and the use of a stabilizer was not necessary. The initial RCP% was over 98% at both temperatures at the lowest (4.5) and highest (8.5) acceptable

pH values defined by the pharmacopoeia. As time progressed, value of 100% was measured, which was attributable to the fact that the intensity of the contamination peaks decreased below the detection limit. pH2 and pH10 were stress conditions, outside the permissible pH range. In the case of RCP%, it can also be said that some contaminant peaks appeared, but the radiochemical purity still did not fall below the 95% threshold. This value did not decrease with time. This means that there were no new contaminant peaks on the chromatogram, which predicts the decay of the radioactive material. Trend was also valid for the sample stored at 90 °C. Therefore, no change in the purity of the radiopharmaceutical can be observed even with extreme alkaline solutions.

During the investigation of the enantiomeric purity at 25 °C and 90 °C, the appearance of the D-form was observed only in the initial measurements, in the other cases only the L-form was detected. The latter was due to the fact that during the rapid decay of the ^{11}C isotope, the intensity of the contaminant D-form decreased below the detection limit. Based on the results obtained, it can be said that the initial EP% was above 98% at both temperatures, which increased to 100% as time progressed (Fig. 4). So, even at higher temperatures, the epimerization of the L-form does not take place between pH2 and pH10 (SI Table 5).

In conclusion, it can be stated that [^{11}C]MET preserves the initial radiochemical and enantiomeric purity at all tested pH values, even at 90 °C. The injection solution shows adequate stability during the shelf life of the radiopharmaceutical.

Discussion

The stability assessment of [^{18}F]FET and [^{11}C]MET PET radiopharmaceuticals under ICH-compliant stress conditions provides essential insight into the robustness and reliability of these tracers in real-world clinical and logistical settings. Given the pharmacopoeial requirements for radiochemical and enantiomeric purity in parenteral radiopharmaceuticals, our findings offer compelling evidence supporting their safe use throughout their respective shelf lives. For this purpose, samples from each batch were measured at different temperatures (−20; 2; 25; 50; 90 °C) and different pH values (2; 4.5; 7.1; 8.5; 10) at several time points, covering the whole shelf life.

[^{18}F]FET demonstrated a robust radiochemical stability between a wide range of temperature settings (from −20 to 50 °C) and pH values, including those well beyond their pharmacopoeial limits (pH2 and 10). Consistent with our findings, Wester et al. reported a similarly high radiochemical purity of 97–99% in their study, confirming the tracer's chemical robustness (Wester et al. 1999). These broad testing conditions allowed us to comprehensively evaluate the tracer's stability profile, beginning with the well-studied [^{18}F]FET. Radiochemical purities between 98.04 and 100% were obtained by TLC, while UPLC gave values between 95.93 and 99.59%. The results obtained during the experiment show a slight difference compared to those reported by our research group in a previous study. Similar radiochemical purity values were obtained using the UPLC methods and thin layer chromatography performed according to the recommendations of the Ph. Eur. Monographs (Långström et al. 1987; Józszai et al. 2022). The deviation between the two measurement techniques is due to the sensitivity and operational variation of the systems. Although the UPLC is effective in separating and detecting all components, free fluoride may be trapped on the column, so that the amount of the active substance is not compared to the total amount, resulting to incorrect values. By

contrast, with TLC plates, the components are unable to evaporate, meaning the entire quantity can be examined. However, the method is associated with lower separation efficiency (Rezes et al. 2020). According to Hooijman et al. results while radio-TLC offers a rapid and straightforward method for quality assessment, radio-HPLC provides a superior ability to detect degradation products such as radiolysis impurities. These degradation products often remain undetected by radio-TLC, underscoring the importance of radio-HPLC in ensuring the efficacy and safety of the radiopharmaceutical. The findings suggest that combining both techniques can enhance the robustness of quality control in radiopharmaceutical production (Hooijman et al. 2022). These observations are consistent with the results of our own investigations, further confirming the complementary roles of radio-TLC and radio-HPLC in comprehensive radiopharmaceutical quality control. Taking the test results into consideration, it can be stated that between pH2 and pH10, as well as between -20 and 50 °C, no changes occurred that would cause us to fall below the prescribed minimum purity of 95%. A lower RCP% could be measured at a basic pH value, but radiochemical purity ($> 98.04\%$) under all test conditions exceeds the Ph. Eur. acceptance criteria, reinforcing the PET tracer's safety and efficacy. In the study published by Holler et al., pH and temperature were also identified as critical factors influencing the stability and quality of [^{18}F]FDG. The authors emphasize that precise control of these parameters during synthesis is essential to ensure optimal radiochemical yield and purity. Their findings highlight the importance of pH and temperature optimization to minimize degradation and maintain product integrity, consistent with other reports in radiopharmaceutical manufacturing (Holler et al. 2022).

The enantiomeric purity remained at 100% throughout all test scenarios, confirming the stereochemical integrity of the L-form. The absence of new or unidentified degradation peaks in both UPLC and TLC chromatograms, even under extreme temperatures and pH conditions, suggests that [^{18}F]FET possesses a robust stability profile. These findings are further supported by Reddy et al., who reported similar profile of enantiomeric purity for no-carrier-added 6- ^{18}F fluoro-L-dopa under rigorous quality control conditions. Their study employed chiral HPLC methods with stringent control of chromatographic parameters, including mobile phase composition and temperature, to accurately quantify enantiomeric character (Reddy et al. 1993). This is particularly relevant for export-optimized PET production facilities, where transport to distant PET examination sites may result in variable and unpredictable storage conditions.

In the case of [^{11}C]MET, although its half-life imposes a narrower window for clinical application, the radiopharmaceutical also exhibited a commendable degree of stability (Debrezzeni-Máté et al. 2024). Enantiomeric purity also remained consistently high, and no significant epimerization to the D-form was observed. Furthermore, significant epimerization of the L-isomer was not observed, even under alkaline conditions. Hence, the enantiomeric purity was higher than the 90% limit. These observations are strongly supported by the methodology of Giglio et al., who developed an enhanced chiral HPLC protocol for assessing enantiomeric purity of [^{11}C]MET. Employing a chiral Astec Chirobiotic T column ($10\text{ cm} \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$), with water/methanol (20:80) as mobile phase at 1 mL/min , the authors achieved complete resolution of D- and L-methionine in under 7 min, effectively replacing slower chiral TLC. The method consistently demonstrated enantiomeric purity exceeding 90%, with no detectable D-epimer peaks even when assessed under mildly alkaline sample preparation conditions. This confirms that the

chiral integrity of [^{11}C]MET (L-isoform) remains intact throughout routine radiosynthesis, in alignment with European Pharmacopeia standards. However, interpretation of radiochemical purity over time requires caution, as factors such as isotope decay can influence apparent stability metrics (Giglio et al. 2018). Notably, the observed increase in radiochemical purity over time, particularly at high temperatures, may reflect the decay-related disappearance of short-lived impurity signals rather than actual improvement in chemical stability. This highlights the need for nuanced interpretation of purity data in short-lived isotopes like carbon-11. This phenomenon is well illustrated by the findings of Woods et al. (2017), who investigated the post-synthetic stability of [^{11}C]methionine and observed a gradual increase in radiochemical purity during the first 60 min following end-of-synthesis. Notably, RCP values increased from approximately 94% to over 97%, despite no corresponding chemical purification or processing steps occurring during this interval. The authors attributed this apparent improvement not to enhanced molecular stability, but rather to the physical decay of short-lived radiolabeled impurities, which were initially co-eluting or unresolved in the chromatographic analysis. These results underscore the importance of carefully interpreting radiochemical purity data for short-lived positron emitters such as carbon-11, where decay kinetics of minor by-products can significantly distort perceived stability. Consequently, they recommend incorporating decay-corrected impurity profiling and time-point standardization in QC protocols to avoid misleading conclusions regarding chemical robustness under thermal stress conditions (Woods et al. 2017). Nevertheless, even under extreme thermal and pH conditions, no substantial degradation was detected, and radiochemical purity remained above the critical 95% limit. Despite these complexities, our data consistently indicate no substantial degradation across tested conditions, supporting the overall stability and suitability of both tracers for clinical application.

In addition, systematic evaluation of further stress factors—including light exposure, oxygen and moisture content, mechanical agitation, and repeated freeze–thaw cycles—would provide a more comprehensive understanding of potential degradation pathways, thereby supporting robust shelf-life assignments and facilitating large-scale clinical applications.

Conclusion

The stability data presented in this study demonstrate that both [^{18}F]FET and [^{11}C]MET maintain high levels of chemical and stereochemical integrity well beyond the conditions typically encountered during routine clinical use and transport. These findings are consistent with pharmacopeial standards and supported by robust analytical methods, underscoring the reliability and safety of these PET tracers throughout their shelf lives. From a regulatory and practical perspective, the demonstrated robustness of these tracers suggests increased flexibility in transportation and storage logistics, which may help reduce batch rejection rates due to marginal purity decreases and facilitate broader clinical adoption. Specifically, for [^{11}C]MET, although its short half-life limits prolonged storage/transport, our results indicate that local use remains feasible without significant impact from external factors on radiochemical or enantiomeric purity. It is critical to interpret radiochemical purity data carefully, especially for short-lived isotopes like carbon-11, as observed increases in purity over time may reflect decay-related reduction of short-lived impurities rather than actual chemical stabilization. This

phenomenon, well documented in the literature, highlights the importance of incorporating decay-corrected impurity profiling and standardized sampling times in quality control protocols. Looking ahead, future studies should aim to evaluate tracer stability under real-world transport conditions with fluctuating temperatures, as well as investigate long-term storage strategies such as cryopreservation or multi-dose vial use. These efforts would further enhance the clinical utility of [^{18}F]FET and [^{11}C]MET, particularly in high-throughput PET centers requiring flexible and reliable radiopharmaceutical supply chains.

Based on the experiments conducted, [^{18}F]FET and [^{11}C]MET have a suitable stability profile in the time window beyond the expiration date. However, the selection of an ideal tracer in a larger scale clinical trial requires a different perspective that takes additional factors into consideration. These include the feasibility of a robust synthesis, dosimetry, and regulatory acceptance. For example, a tracer with a longer half-life or higher yield, a readily available quality control method, may facilitate the spread of a tracer in multi-center studies where logistics are simple, and results are consistent. Moreover, clinical indications and target specificity should guide selection to maximize diagnostic or therapeutic utility.

Abbreviations

ACN	Acetonitrile
[^{11}C]MET	Methyl-[^{11}C]-L-methionine
[^{18}F]FET	<i>o</i> -(2-[^{18}F]fluoroethyl)-L-tyrosine
EOS	End of Synthesis
EP	Enantiomeric Purity
FTN	Flow Through Needle
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
IBLC	N-isobutryl-L-cysteine
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
MeOH	Methanol
MS	Mass Spectrometry
NaAc	Sodium acetate
NaOH	Sodium hydroxide
OPA	<i>o</i> -Phthalaldehyde
PDA	Photodiode Array Detector
PET	Positron Emission Tomography
Ph. Eur.	European Pharmacopoeia
QC	Quality Control
RCP	Radiochemical Purity
R/F	Retardation Factor
RH	Relative Humidity
TET	L-Tyrosine
TLC	Thin Layer Chromatography
UPLC	Ultra Performance Liquid Chromatography

Supplementary Information

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Supplementary Material 1

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Author contributions

VF, LV, AF and IJ performed to the conception of the work, the acquisition, analysis, and interpretation of data with additions from GF. VF, GF and IJ drafted and revised the manuscript. All authors read and approved the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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