Automated radiosynthesis of [^{18}F]CETO, a PET radiotracer for imaging adrenal glands, on Synthra RNplus

Matthew Hird[1], Joseph J. Russell[2], Lei Li Corrigan[2], Istvan Boros[2], Patrik Nordeman[4], Gunnar Antoni[4], Mark Gurnell[3], Franklin I. Aigbirhio[1].

1: Molecular Imaging Chemistry Laboratory, Wolfson Brain Imaging Centre, Department of Clinical Neuroscience, University of Cambridge, West Forvie Building, Forvie Site, Cambridge, UK

2: Radiopharmaceutical Unit, Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, Cambridge, UK

3: Welcome-MRC Institute of Metabolic Science & Department of Medicine, University of Cambridge, & Addenbrooke's Hospital, Cambridge Biomedical Campus, Cambridge, UK.

4: Uppsala Biomedicinska Centrum, BMC, Husarg, 3

Short title: Automated radiosynthesis of the adrenal PET radiotracer [^{18}F]CETO

Word count: Tables: 1; Figures: 7.

Keywords: [^{18}F]CETO, fluorine-18 radiosynthesis, Synthra RNplus, adrenal, PET imaging, primary aldosteronism.

Disclosures: none of the authors have any relevant disclosures

Funding: This work was supported by a grant awarded by the Medical Research Council and by the NIHR Cambridge Biomedical Research Centre.

Correspondence:

Matthew Hird, Radiopharmaceutical Unit, Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, Cambridge Biomedical Campus, CB2 0QQ Cambridge, UK.

Email: mh910@cam.ac.uk

Abstract

Primary aldosteronism (PA) is the leading secondary cause of hypertension. Determining whether one (unilateral) or both (bilateral) adrenal glands are the source of PA in a patient remains challenging, and yet it is a critical step in the decision whether to recommend potentially curative surgery (adrenalectomy) or lifelong medical therapy (typically requiring multiple drugs).

Recently, we have developed a fluorine-18 radiopharmaceutical [^{18}F]CETO to permit greater access to PA molecular imaging. Herein, we report an automated synthesis of this radiotracer.

To manufacture the radiopharmaceutical routinely for clinical PET studies we implemented an automated radiosynthesis method on a Synthra RNplus© synthesizer for which Cl-tosyletomidate was used as the precursor for radiolabelling via nucleophilic [^{18}F]fluorination.

[^{18}F]CETO was produced with 35 ± 1% (n = 7), decay corrected and 25 ± 4% (n = 7) non-decay corrected radiochemical yield with molar activities ranging from 150 to 400 GBq/μmol. The GMP compliant manufacturing process produces a sterile formulated [^{18}F]CETO injectable solution for human use as demonstrated by the results of quality control. Automation of the radiosynthesis of [^{18}F]CETO should facilitate uptake by other adrenal centres and increase access to molecular imaging in PA.

Introduction

High blood pressure (hypertension) is one of the most important risk factors for death and disability worldwide, affecting >1 billion individuals [1] [2]. In the UK, more than a quarter of all adults have hypertension, many of whom remain undiagnosed [3]. Left untreated, hypertension predisposes to premature cardiovascular (cerebrovascular disease, heart attack, heart failure) and kidney (renal failure) disease and is an important risk factor for early onset cognitive decline and premature death [4]. Primary aldosteronism (PA) is the leading secondary cause of hypertension, estimated to account for 5–14% of all cases and 20–25% of patients with treatment resistant hypertension [i.e. uncontrolled blood pressure (BP) despite taking ≥3 antihypertensive agents][5]PA is characterised by autonomous secretion of the endogenous mineralocorticoid aldosterone, with resultant unregulated sodium and water retention, and consequent hypertension ± hypokalaemia[6]. In approximately half of all cases, the source of aldosterone excess can be attributed to one adrenal gland, known as a unilateral PA, e.g. due to an aldosterone-producing adenoma (APA), nodule (APN) or micronodule (APM), whilst the remainder of affected individuals have bilateral disease, traditionally referred to as bilateral adrenal hyperplasia (BAH)[7].

Demonstration of a unilateral cause of PA opens the door to potentially curative surgery; in contrast, patients with bilateral PA are traditionally managed with lifelong targeted medical therapy, including mineralocorticoid receptor antagonists MRA, e.g., spironolactone or eplerenone[6,7]. Although anatomical imaging such as computed tomography (CT) and magnetic resonance imaging (MRI) is readily available in most centres, it is unable to reliably distinguish unilateral from bilateral PA in a significant proportion of patients and may even result in inappropriate surgery such as removal of an incidental non-functioning adrenal adenoma[7]. Therefore, guidelines and expert consensus groups have traditionally recommended adrenal vein sampling (AVS) to distinguish unilateral and bilateral PA. However, AVS is invasive and technically challenging, and is only reliably available in a small number of specialist centres in most countries. Accordingly, we and others have proposed molecular imaging with positron emission tomography (PET) computed tomography (PET/CT) as an alternative method for distinguishing unilateral and bilateral causes of PA due to their advantage of being able to image the actual biological function of the adrenals, as well as being able to distinguish between both incidentalomas and APA, which is very advantageous . Although several radiotracers have been proposed[8], the strongest evidence to date supporting a role for molecular imaging in

subtyping PA comes from studies with the radiotracer [^{11}C]methyl-1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylate ([^{11}C]Metomidate)[9,10]. Importantly, in a recent large-scale prospective, within patient trial, dexamethasone-suppressed [^{11}C]Metomidate PET/CT was shown to be non-inferior to AVS in identifying surgically curable PA[11].

Etomidate and the related 1-[(1R)-1-phenylethyl]-1H-imidazole-5-carboxylic acid esters [^{11}C]Metomidate and para-chloro-2-[^{18}F] fluoroethyl-etomidate ([^{18}F]CETO) (Figure 1) selectively bind to the highly homologous cytochrome P-450 enzymes CYP11B1 (11β-hydroxylase) and CYP11B2 (aldosterone synthase), which are expressed in the zona fasciculata (ZF) and zona glomerulosa (ZG) of the adrenal cortex respectively. Pre-treatment with the exogenous glucocorticoid dexamethasone (e.g., for 72 h prior to PET scanning) facilitates ligand selectivity for CYP11B2 in vivo due to suppression of CYP11B1 expression[9]

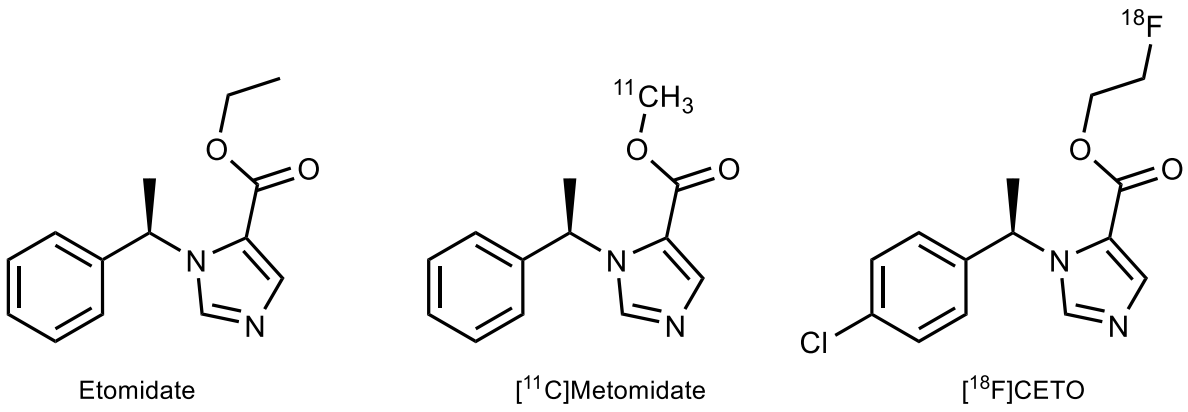


Fig. 1: (L-R) Etomidate, [^{11}C]Metomidate and [^{18}F]CETO.

Although [^{11}C]Metomidate is now established as an alternative to AVS for not only lateralising, but also localising the source of aldosterone excess in unilateral PA, the short half-life of carbon-11 (t ½ = 20.4 min) means that it can only be made available at PET centres with an on-site cyclotron facility. In addition, in some patients liver accumulation of the radiotracer partially obscures visualisation of the right adrenal gland [12] a radiotracer with similar kinetic properties, but potentially lower liver uptake than [^{11}C]metomidate, and based on the long lived fluorine-18 radionuclide ($t_{1/2}$ = 110 min) is preferable for wider application of this PET imaging technology.

Erlandsson *et al* previously reported the development of several fluorine-18 -labelled analogues of metomidate, including [^{18}F]CETO (para-chloro-2-[^{18}F]fluoroethyletomidate (Fig. 1), which

shows specific high binding affinity to the adrenal glands (Kd = 0.66 nM) [13] [14]. In addition, preclinical studies [15] have established that it has appropriate properties for clinical PET application for imaging adrenal glands in vivo [14].

In order to undertake clinical PET studies, we have established a radiochemistry method on a commercially available module for manufacturing [^{18}F]CETO under GMP conditions based on a previously reported radiosynthesis of [^{18}F]CETO (Fig. 2) on an in-house module [14].

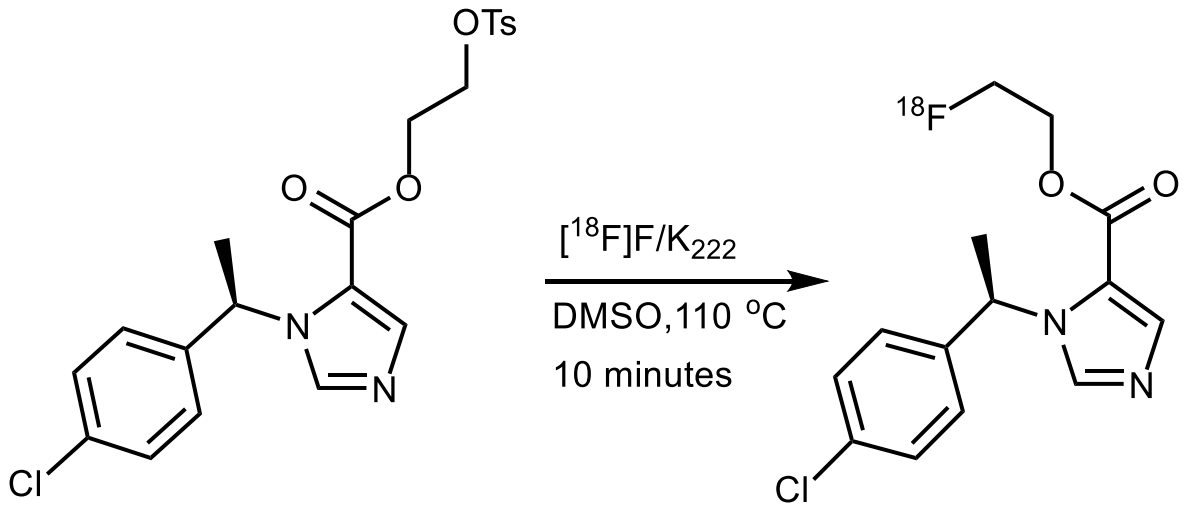


Fig. 2: Radiosynthesis of [^{18}F]CETO using Cl-tosyletomidate as the precursor.

Herein, we report development of the fully automated and GMP compliant radiosynthesis of [^{18}F]CETO, using a commercially available Synthra RNplus automated radiosynthesis module (Fig 3).

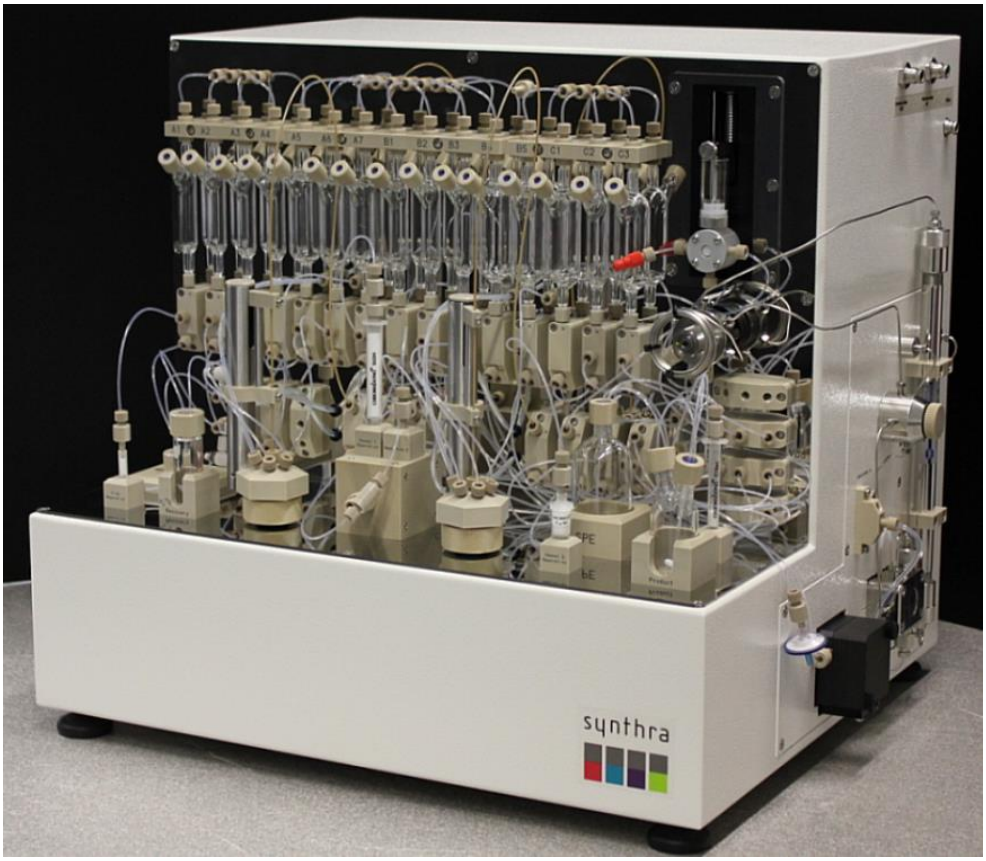


Fig. 3: Picture of Synthra RNplus©, reproduced with kind permission from Synthra.

Methods and materials

All anhydrous solvents were sourced from Merck and Acros and were used as received. Water for injection was purchased from Severn biotech and PBS was purchased from ABX. Milli-Q system was used to manufacture purified water (18.2 MΩxcm) for cleaning of the synthesises module. The radiolabelling precursor and the reference material were obtained from Pharmasynth, Estonia. The Sep-Pak C18 light (2 mL EtOH, 10 mL water to activate), QMA carbonate (46 mg, no conditioning) and alumina (no conditioning) were purchased from Waters. Ethanol for injection was purchased from Merck.

Kryptofix solution was made before each production and consists of K222 (7.5 ± 0.1 mg in 400 µL acetonitrile) and potassium carbonate (1.1 ± 0.1 mg in 400µL BP water) with additional 700 µL of acetonitrile to make up 1.5 mL of solution. To ensure homogeneity and proper complexation of Kryptofix 2.2.2 and K2CO3, sonicate further the prepared mixture at ambient temperature for approx. 10 min.

The module used for GMP production was Synthra RNplus©, a commercially available fluorine-18 synthesiser module.

Radiochemical yields for the product (based on estimated [^{18}F]fluoride produced by cyclotron beam conditions) were based on [^{18}F]CETO and are non-decay corrected unless otherwise stated.

Radiosynthesis of [^{18}F]CETO on a Synthra RNplus module

Prior to each production, the automated synthesizer was cleaned using purified water, absolute ethanol, and acetone. The cleaning method was a routine, validated cleaning programme that removes any residual materials/impurities which may have remained on the module from previous radiochemical synthesis and prepares the module for use. This involved cleaning of the module and then sample collection and HPLC analysis from the final product vial which is checked via HPLC. As the module is used for several different tracer productions, each radiotracer and cleaning method is validated to ensure no cross contamination through tubing etc.

To ensure sterilisation of the final compound, bulk material is delivered to a talia hot cell and dispensed through a 0.22 µM filter using a clio dispensing module in a grade A environemnt.

The performance of the sterilisation step and bioburden studies were performed during validation process *via* an external contract research organisation who undertook a complete analysis of microbial counts after full dispensing without filter. The validation was designed to test for both the absence of any bacteriostatic or fungistatic effect on the product.

The automated Synthra RNplus visualisation is as follows (see Figure 4):

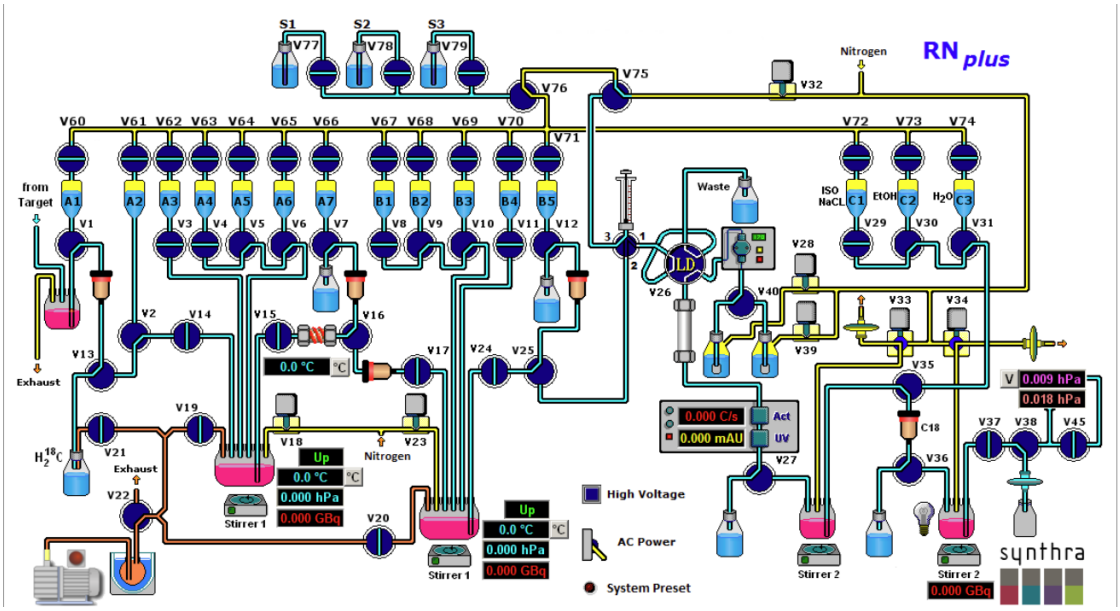


Fig. 4: Graphical user interface of Synthra RNplus. Image reproduced with kind permission from Synthra.

The automated Synthra RNplus set-up was as follows:

- **A1**: with 1.0 ml Kryptofix eluent solution (K222./K_2CO_3 solution).
- Fill **vial A2** with 2.0 ml of acetonitrile.
- Fill **vial A3** with 2.0-3.0 mg of Cl-tosyletomidate precursor dissolved in 0.5 ml of anhydrous DMSO.
- Fill **vial A4** with 2.0 ml of water (Water for injection).
- Fill **vial C1** with 8.2 ml of Phosphate buffered saline.
- Fill **vial C2** with 0.8 ml of ethanol absolute.
- Fill **vial C3** with 10 ml of water (Water for injection).

- Fill **SPE flask** with 30 ml of water (Water for injection).
- Fill the **product collection vial** with 7.2 ml of phosphate buffered saline.
- QMA carbonate cartridge.
- **C18** light cartridge, conditioned with 2 mL Ethanol, 10 ml Water then dried with 2 ml air.
- Sep-Pak Alumina Light cartridge, no conditioning.

Preparative HPLC method: Semipreparative column Gemini-NX C18 250x10 mm, 5 μm, 100 Å, eluting with Acetonitrile/50mM Ammonium formate (50:50, pH 3.5) flow 5 ml/min, λ = 254 nm.

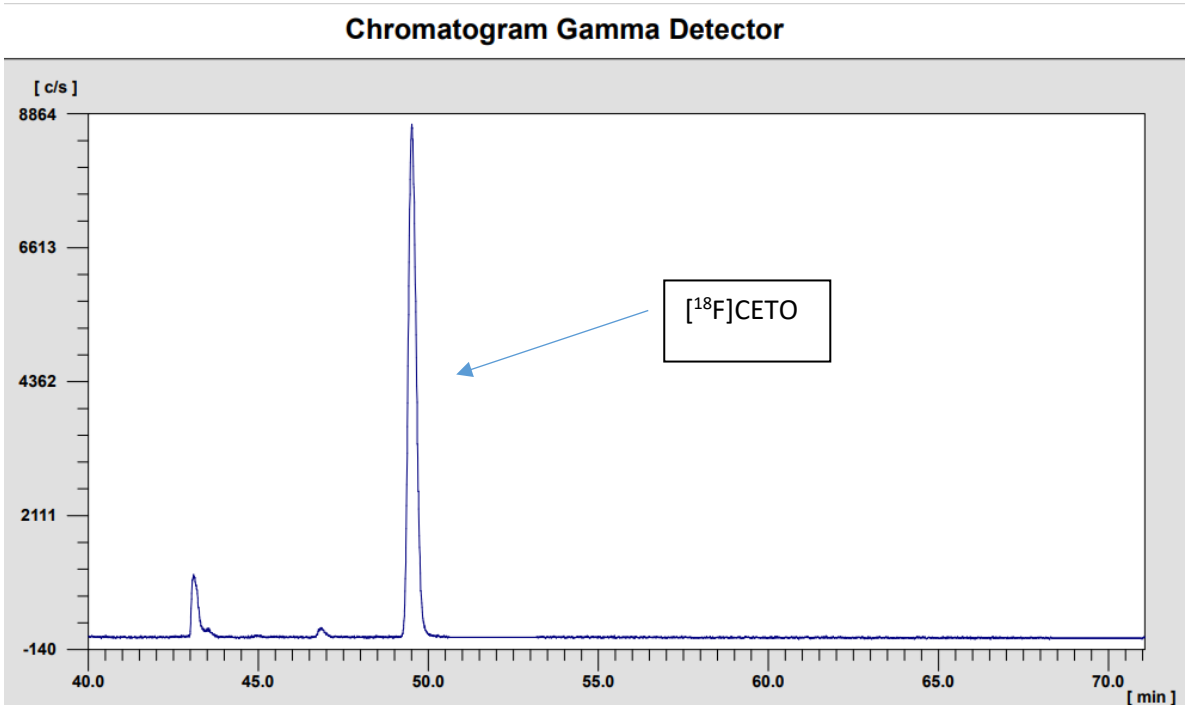
This method is based on a previously reported method [13]. [^{18}F]Fluoride is produced in the cyclotron target via the $^{18}O(p,n)^{18}F$ nuclear reaction using GE PETtrace cyclotron applying 16.5 MeV protons onto ~2.4 mL [^{18}O]H_2O enriched water. The produced [^{18}F]fluoride is delivered from the cyclotron target into the target vial of the Synthra RNplus.

Typically, bombardment was performed at 35 μA to produce ~30 GBq of [^{18}F]fluoride. The produced [^{18}F]fluoride was trapped onto a QMA carbonate cartridge and eluted using Kryptofix®/K_2CO_3 solution into the reactor vessel of the synthesiser. [^{18}F]Fluoride azeotropic drying is then conducted at 100°C for 10 min. Once drying is completed, S_N2 substitution labelling reaction of the precursor (figure 2) in anhydrous DMSO takes place in the reactor at 110 °C for 10 min.

Once reaction complete, the mixture was cooled to 30°C using compressed air (cooled using liquid nitrogen) and quenched with water for injection. The reaction solution was then passed through an alumina light cartridge (Waters) to remove any excess [^{18}F]fluoride. Due to the need to keep the configuration of the module, fixed since used for other radiotracer productions, this was transferred into the module's second reactor vessel and then loaded onto a semi-preparative high-pressure liquid chromatography (HPLC) injection loop, via an injection syringe. The semi-preparative HPLC purification method (as described above) isolates the desired [^{18}F]CETO {peak retention time (RT) between 9 & 10 minutes} (Fig 5) which was collected in a SPE flask containing 30 ml of water for injection. Fig 5 shows the timeline as being approx. 49 minutes, this is due to the semi

preparative HPLC conditioning starting at the beginning of the time list, thus is not representative of the actual time [^{18}F]CETO spends on the semi preparative column.

Once the required peak was collected and solution thoroughly mixed, the entire solution (approx. 35 ml) was passed through a conditioned Waters C18 light SPE cartridge with the desired [^{18}F]CETO product being retained on the cartridge. The [^{18}F]CETO product was washed with water for injection (10 ml) and then eluted with ethanol and formulated as a PBS solution and finally dispensed in a Grade A isolator by passing the mixture through a sterile filter (Millex durapore PVDF, membrane filter, 0.22 µm). The product can be split into as many different doses as required, using the Comecer Clio apparatus to complete this activity.



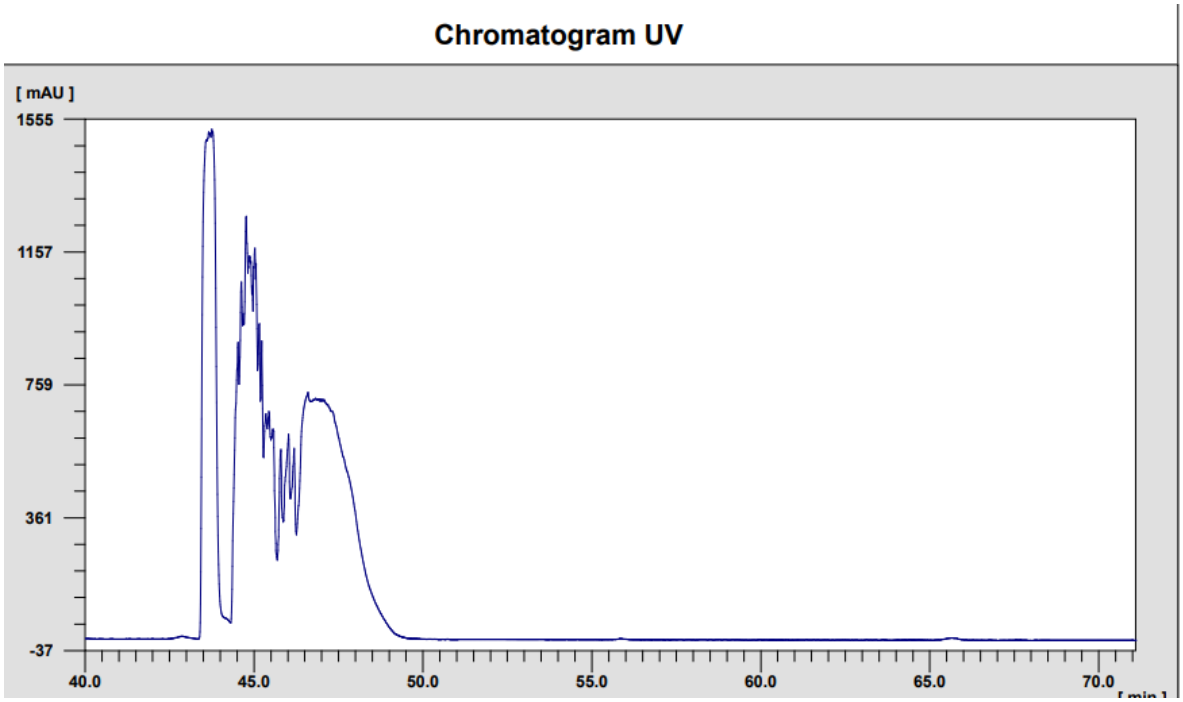


Fig. 5: UV and gamma detector semi-prep chromatogram profile in the synthesis of [^{18}F]CETO.

Quality Control analysis procedures

Pre-release quality control procedures for [^{18}F]CETO were completed in approximately 30 minutes. The pH of the product was determined using a Seven Excellence pH meter with a visual check being performed. Chemical and radiochemical identity and purity were determined by analytical radio-HPLC on the ThermoFisher© Ultimate 3000 HPLC system using Gemini NX 5µm C18 100Å, 100 × 4.6 mm) eluting with a gradient of 40% - 80% acetonitrile with 50 mM ammonium formate (pH 3.5) at 1.5 ml/min over 10 minutes. Injection volume of 25µl. The HPLC system was equipped with an UV detector (λ = 254 nm) and EG&G Ortec Amplifier 0.70 kV radioactivity detector.

The radiochemical purity (RCP) was determined as the percentage of the [^{18}F]CETO peak on the radio-chromatogram (figure 6). Radiochemical identity was confirmed and assessed by comparison of the retention times of the [^{18}F]CETO radioactive peak to the unlabelled CETO reference standard peak in the co-injection sample. The chemical mass of [^{18}F]CETO and other cold impurities were determined by quantification and relative comparison of the corresponding UV absorbance peaks of the QC sample and reference standards of known concentrations in a calibration curve (figure 7).

The acceptance limit of 10 µg for CETO in the injection dose was established based on the selected animal toxicity studies were completed by Charles Rivers laboratories following ICH guidelines. The maximal allowable injection volume (V_{max}) of radiotracers produced at the Wolfson Brain Imaging Centre (WBIC) is by default limited to 10 mL and was calculated based on the chemical mass of [^{18}F]CETO and non-radioactive impurities relative to their specification (<1.5 µg/dose for individual unidentified impurity, <3.0 µg/dose for total unidentified impurity) V_{max} = 10 µg/specification of [^{18}F]CETO found in a QC sample (µg/mL). Molar activity (A_m) was calculated as the ratio of radioactivity in GBq against the amount of CETO only (µmol) at the time of application (15-20 min after the end of synthesis).

The integrity of the Millex durapore PVDF, membrane filter, 0.22 µm) was evaluated using the filter integrity (i.e., bubble point) test after dispensing from a grade A dispensing cell using a Comecer Clio dispenser with disposable kit. Bacterial endotoxins and residual solvent analyses are a pre-release test. Radionuclidic identity and sterility of the products were assessed as part of the post release tests.

Endotoxins content in [^{18}F]CETO doses was measured with the Charles River Laboratories Endosafe Portable Test System. Residual solvent analysis was performed on a Thermo Focus Gas Chromatograph equipped with the Thermo column TG-624, 30m x 0.32mm x 1.8um) and a flame ionization detector.

Radionuclidic identity was determined by measuring [^{18}F]CETO activities in triplicate at different time points (20-30 min intervals) using a Veenstra VDC-505 radioisotope dose calibrator. The half-life was calculated as per the following: $t_{1/2} = -\ln 2 \cdot ((t_2 - t_1)/\ln(A_2/A_1))$, whereby t_1 and t_2 are two separate times and A_1 and A_2 are radioactivity levels measured at times t_1 and t_2, respectively.

Stability studies on the final [^{18}F]CETO product was conducted and it was shown that the radiotracer is stable for up to 10 hours (98.76% to 98% RCP over that time frame) which indicates that the amount of radiolysis observed is minimal. This testing is repeated every 2 years.

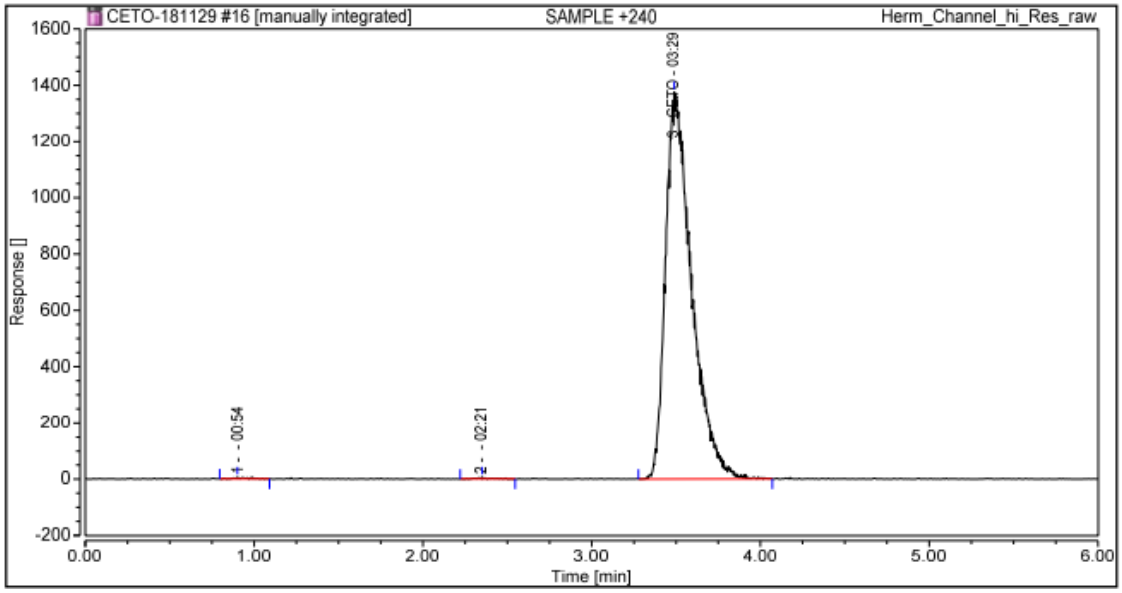


Fig. 6: Radiochemical analytical HPLC result for [^{18}F]CETO.

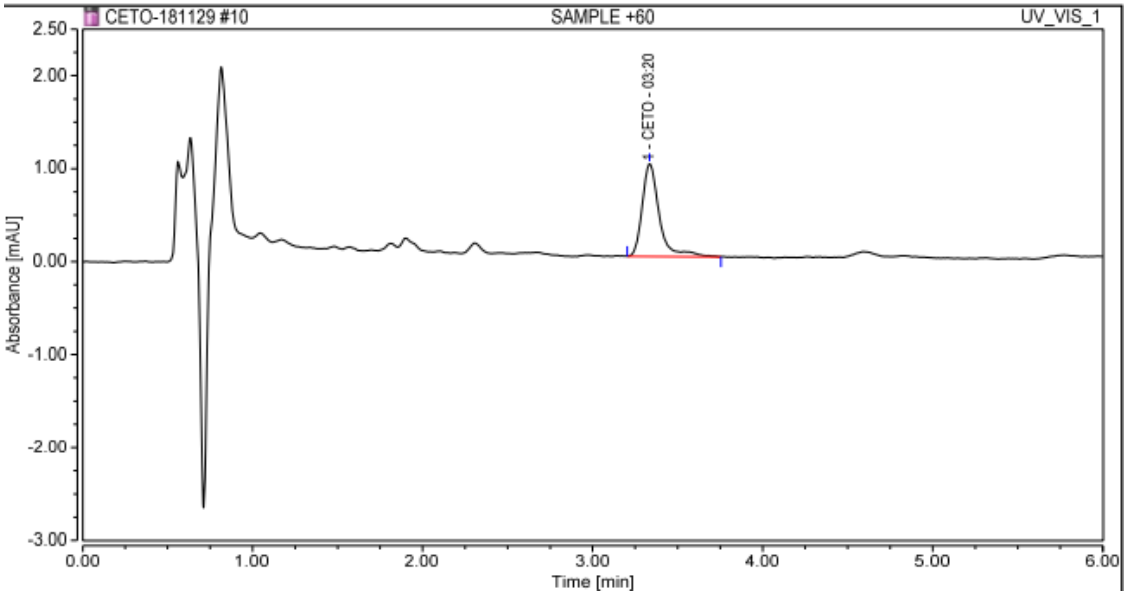


Fig. 7: UV chromatogram for [^{18}F]CETO analytical run.

Results and discussion

The radiosynthesis of [^{18}F]CETO is based on the nucleophilic [^{18}F]fluorination of the Cl-tosyletomidate precursor (Fig. 2).

One of the key advantages in this user's eye, of the Synthra RNplus module, was the ease of modification and programming of the time-list, to ensure a prompt, reliable and efficient synthesis. The size of the reactor vessel allows for larger volumes, which was particularly

helpful for the injection of the quenched crude reaction mixture onto the semi-preparative HPLC column.

Following established GMP guidelines and regulations, release criteria were established, based on toxicological and pre-clinical studies. These pre-release tests and their specification and frequency are shown below in Table 1:

Pre-Release Test	**Specification**	**Method**
Appearance	Clear colourless solution	Visual
pH	4.0-8.0	pH paper or pH meter
HPLC system suitability test	Retention time difference less than 8 seconds in two injections of reference solution.	HPLC
Radiochemical identity	Retention time of [^{18}F]CETO corresponds to that of reference CETO standard, corrected for the dead-volume between UV and radio-detector.	HPLC
Chemical content CETO	< 10 μg/dose.	HPLC
Individual unidentified impurity	< 1.5 μg/dose.	HPLC
Total unidentified Impurity	< 3.0 μg/dose.	HPLC
Radiochemical Purity [^{18}F]CETO	≥ 95%.	HPLC
Residual solvent DMSO	(≤ 5000 ppm) ≤ 50 mg/dose	GC
Residual solvent acetonitrile	≤ 410 ppm, 4.1 mg/dose	GC
Residual solvent ethanol	≤ 10 %	GC
Bacterial endotoxins	< 175 EU/dose	Ph. Eur. 2.6.14
Filter integrity test	Bubble point ≥ 50 psi (3.45 bar)	Clio dispenser
Post-release test	**Specifications**	

Sterility	Pass Test	Ph. Eur. 2.6.1
Chemical purity Kryptofix 2.2.2	< 2.20 mg/dose	Spot test

Table 1: Criteria for release of [^{18}F]CETO for first-in-man studies.

The total [^{18}F]CETO radiosynthesis time from the end of [^{18}F]fluoride production was ~50 minutes. The irradiation and the [^{18}F]fluoride typically required ~30 minutes. Starting with 20–30 GBq of [^{18}F]Fluoride, between 4.5 and 7 GBq of [^{18}F]CETO was produced in the final product vial at end-of-radiosynthesis, as a 5 % EtOH solution in PBS, with molar activities of 180 to 300 GBq/μmol. The non-decay corrected radiochemical yield was 25 ± 1% (n = 7) and if decay corrected 35 ± 4% (n = 7). The QC analysis of the produced [^{18}F]CETO indicated that the radiochemical product with >95% radiochemical purity (RCP) conforms to GMP release criteria.

Conclusions

A reproducible and robust radiosynthesis of [^{18}F]CETO using the Synthra RNplus module has been established with good radiochemical yield and radiochemical purity at end-of-synthesis, in compliance with GMP regulations. This time-list/protocol allows for the wider implementation of the described production protocol in other clinical PET centres for routine manufacturing of [^{18}F]CETO for adrenal imaging, with application in the investigation of patients with PA.

Acknowledgements

The authors would particularly like to thank Dr Selena Milicevic-Sephton for her initial training on the use of the Synthra RNPlus module as well as Molecular Imaging Chemistry Laboratory (MICL) team members for their helpful insights at various points during the project.

Funding

This work was supported by the Medical Research Council (MR/P01710X/1), and the NIHR Cambridge Biomedical Research Centre (Nutrition, Obesity, Metabolism & Endocrinology

theme). The views expressed are those of the authors and not necessarily those of the MRC, the NIHR or the Department of Health.

Bibliography

1. Reddy, K. S. Global Burden of Disease Study 2015 provides GPS for global health 2030. *The Lancet* **388**, 1448–1449 (2016).
2. Ettehad, D. *et al.* Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *The Lancet* **387**, 957–967 (2016).
3. Hypertension in adults: diagnosis and management. *Nice Guideline (NG136)* (2022).
4. Fuchs, F. D. & Whelton, P. K. High Blood Pressure and Cardiovascular Disease. *Hypertension* **75**, 285–292 (2020).
5. Young Jr, W. F. Diagnosis and treatment of primary aldosteronism: practical clinical perspectives. *J Intern Med* **285**, 126–148 (2019).
6. Mullen, N. *et al.* Treating Primary Aldosteronism-Induced Hypertension: Novel Approaches and Future Outlooks. *Endocr Rev* bnad026 (2023) doi:10.1210/endrev/bnad026.
7. Funder, J. W. *et al.* The management of primary aldosteronism: Case detection, diagnosis, and treatment: An endocrine society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* **101**, (2016).
8. Werner, R. A., Schirbel, A., Buck, A. K., Fassnacht, M. & Hahner, S. Adrenal functional imaging. *Presse Med* **51**, 104114 (2022).
9. Burton, T. J. *et al.* Evaluation of the sensitivity and specificity of 11C-metomidate positron emission tomography (PET)-CT for lateralizing aldosterone secretion by Conn's adenomas. *Journal of Clinical Endocrinology and Metabolism* **97**, (2012).
10. O'Shea, P. M. *et al.* 11C-Metomidate PET/CT is a useful adjunct for lateralization of primary aldosteronism in routine clinical practice. *Clin Endocrinol (Oxf)* **90**, 670–679 (2019).
11. Wu, X. *et al.* [11C]metomidate PET-CT versus adrenal vein sampling for diagnosing surgically curable primary aldosteronism: a prospective, within-patient trial. *Nat Med* **29**, 190–202 (2023).
12. Mendichovszky, I. A. *et al.* Targeted Molecular Imaging in Adrenal Disease-An Emerging Role for Metomidate PET-CT. *Diagnostics (Basel)* **6**, (2016).
13. Erlandsson, M., Karimi, F., Lindhe, Ö. & Långström, B. 18F-Labelled metomidate analogues as adrenocortical imaging agents. *Nucl Med Biol* **36**, 435–445 (2009).
14. Silins, I. *et al.* Para-chloro-2-[^{18}F]fluoroethyl-etomidate: A promising new PET radiotracer for adrenocortical imaging. *Int J Med Sci* **18**, 2187–2196 (2021).
15. Silins, I. *et al.* First-in-human evaluation of [18F]CETO: a novel tracer for adrenocortical tumours. *Eur J Nucl Med Mol Imaging* **50**, 398–409 (2023).