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RESEARCH ARTICLE

A highly reproducible method for the measurement of [6-*O*-methyl-¹¹C]diprenorphine and its radio-metabolites based on solid-phase extraction and radio-high-pressure liquid chromatography

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Described here is a method for the measurement of the radio-metabolites of the positron emission tomography radiotracer [6-*O*-methyl-¹¹C]diprenorphine ([¹¹C]diprenorphine) using in-line solid-phase extraction (SPE) combined with radio-high-pressure liquid chromatography analysis. We believe that this method offers a reliable and reproducible approach to [¹¹C]diprenorphine metabolite analysis. In addition, different SPE stationary phases are assessed for their efficiency for loading, retention and elution of the parent molecule and its metabolites. Having assessed C4, phenyl and C18 stationary phase, we concluded that a C18 SPE was optimal for our method. Finally, *in silico* predictions of diprenorphine metabolism were compared with *in vivo* metabolism of [¹¹C]diprenorphine induced by hepatic microsomal digestion and analysed by matrix-assisted laser desorption/ionisation mass spectrometry. It was found that there was a high degree of agreement between the two methods and in particular the formation of the diprenorphine-3-glucuronide metabolite.

KEYWORDS

C-11, HPLC, metabolites, PET, SPE, [¹¹C]Diprenorphine

1 | INTRODUCTION

[6-*O*-methyl-¹¹C]diprenorphine ([¹¹C]diprenorphine) is an important positron emission tomography (PET) radio-ligand that binds to multiple subtypes of opioid receptor (μ , δ and κ sites) with high affinities (K_i values of approximately 0.2 nM).¹ Diprenorphine acts as an antagonist at μ and δ opioid receptors and as a weak agonist at the κ receptors.² Opioid receptors are associated with a wide variety of physiological processes including analgesia, epilepsy,³ central poststroke pain,⁴ dysphoria and euphoria.⁵ [¹¹C]Diprenorphine PET is a powerful tool to study the role of the endogenous opioid system in these biological processes, allowing quantitative imaging of opioid

receptor density and displacement by endogenous opioids.^{2,5–13} However, [¹¹C]diprenorphine will undergo metabolism *in vivo*, and this is significant for PET imaging.^{6,9,14} If radiolabelled metabolites are produced on the timescale of a clinical PET scan, then a method is required to determine the radio-metabolite concentrations as a function of time.

Having the ability to identify ¹¹C-labelled metabolites is also important in order to estimate their influence on the scan data. For example, knowing whether radio-metabolites cross the blood–brain barrier and their affinity for opioid receptors is of high importance to accurately model [¹¹C]diprenorphine binding in the brain. At various time points during the PET scanning session, the

ratio of parent [^{11}C]diprenorphine to each radio-metabolite needs to be established. An accurate correction for arterial plasma radio-metabolite concentrations is necessary to derive a correct plasma input function. This in turn is needed to accurately model quantitative binding of [^{11}C]diprenorphine to available opioid receptors in the brain.⁶

A variety of methods are available for the extraction and measurement of radiotracer metabolites. The measurement of radio-metabolites in human plasma is very challenging. The radio-metabolite concentrations are low and diminish with time, requiring that relatively large volumes of plasma (5 ml) be analysed. Such volumes of plasma cannot be analysed directly using analytical HPLC (<4.6 mm i.d. columns) without overloading the column, yielding very poor chromatographic resolution and dramatically shortening high-pressure liquid chromatography (HPLC) column life. Chromatographic detection normally employs flow-through detectors, giving an immediate, real-time measurement of chromatographic peak height and area; however, the short sample residence time in the detector leads to poor detection limits. This can be improved by collecting fractions of eluent and measuring each fraction off-line in a radio-detector, thus extending measurement time significantly, limited only by the availability of radio-detectors for the number of samples generated. Large plasma samples can be injected onto preparative scale HPLC columns; however, these have correspondingly higher mobile phase flow rates and generate larger volume fractions for off-line detection.¹⁵ Widely available multiwell detectors such as the Perkin Elmer 10-well automatic gamma counter (AGC) are limited in sample capacity, and the

chromatography is best performed using an analytical scale column to allow eluted fractions to be collected in the 1- to 3-ml volume range.

The alternative to direct analysis (HPLC column injections) is to remove the protein fraction from the sample and preconcentrate the metabolites into smaller volume samples. This has been achieved in a number of ways: (1) protein precipitation, (2) liquid-liquid extraction, and (3) metabolite trapping by solid-phase extraction (SPE). The efficacy of these methods will depend on the lipophilicity of the parent tracer and metabolites and the extent to which these species are coprecipitated with plasma proteins. However, these methods have inherent problems associated with them. Performing protein precipitation/extraction can lead to the loss of metabolites, which may be bound to the plasma proteins. Furthermore, using a vacuum manifold with SPE cartridge extraction may lead to the loss of volatile metabolites present in the sample. We believe that the use of in-line SPE to clean up and concentrate the sample followed by HPLC analysis is the optimum method of metabolite analysis. With this method, it is possible to start with a 5-ml blood sample that can be processed and worked up so that metabolite measurements can be performed on an analytical HPLC column. Figures 1–3 depict the configuration of in-line SPE followed by HPLC for [^{11}C]diprenorphine metabolite analysis.

Diprenorphine has a cLogP value calculated as 2.57, so it is important to select the appropriate SPE and HPLC stationary phases, which will retain [^{11}C]diprenorphine as well as the less lipophilic radio-metabolites such as [^{11}C]diprenorphine-3-glucuronide (calculated cLogP value of 0.69). The metabolism of diprenorphine has been

FIGURE 1 Schematic of in-line SPE and HPLC system. Valves are shown in sample inject position

FIGURE 2 Schematic of in-line SPE and HPLC system. Valves are shown in SPE load position

FIGURE 3 Schematic of in-line SPE and HPLC system. Valves are shown in SPE elution position

studied previously with different radiolabelled analogues of the molecule (Figure 4). Diprenorphine has been labelled with ^{11}C at the C17 position of the molecule¹⁶ (*N*-[^{11}C]diprenorphine); however, it has been suggested that diprenorphine is rapidly metabolised via *N*-dealkylation¹⁷ resulting in the loss of the radiolabelled moiety. More recently, an automated synthesis of an ^{18}F fluorinated analogue, 6-*O*-[2-[^{18}F]fluoroethyl]-6-*O*-desmethyldiprenorphine ([^{18}F] fluoroethyl-diprenorphine), was described.¹⁸ [^{18}F] fluoroethyl-diprenorphine was shown to have similarities in the pharmacokinetics and receptor binding properties to [6-*O*-methyl- ^{11}C]diprenorphine. However, [^{18}F]fluoroethyl-diprenorphine showed lower brain

uptake and rapid metabolism, with the radio-metabolites accounting for 20% of the radioactive signal in mouse brain 30 min after the administration of the tracer.¹⁸ The position of the ^{11}C radiolabelled at the C6 position means that it is less susceptible to metabolism (compared with *N*-[^{11}C]diprenorphine) and the molecule is less rapidly metabolised compared with [^{18}F]fluoroethyl-diprenorphine.¹⁸

Predicting radiotracer stability and metabolism in vivo can be challenging; however, experiments using pooled human liver microsomes to 'digest' the radiotracer can be used to give an insight into what metabolites of the radiotracer might be expected in vivo. Biotransformations are evoked by exposing [^{11}C]diprenorphine to

FIGURE 4 Radiolabelled analogues of diprenorphine

pooled human liver microsomes, a source of the cytochrome P450 enzyme, and uridine glucuronosyl transferase (UGT) enzymes; both of which are important enzymes involved in the expulsion of foreign chemicals/drugs in the body. In addition, computational programs can be used to predict the most likely chemical transformations that occur on a molecule during phase 1 or 2 metabolism. These predictions are made using specialised computer software such as Meteor Nexus metabolite prediction software.

A method to accurately and reproducibly measure [¹¹C]diprenorphine metabolites using in-line SPE and HPLC analysis has been developed. The metabolites of [¹¹C]diprenorphine from clinical scans have been compared with in silico predictions, as well as simulated metabolism of [¹¹C]diprenorphine using pooled human liver microsomes. The major radio-metabolite of [¹¹C]diprenorphine has also been identified by matrix-assisted laser desorption/ionisation mass spectroscopy (MALDI-MS) as [¹¹C]diprenorphine-3-glucuronide.

2 | MATERIALS AND EQUIPMENT

Diprenorphine standards were purchased from Tocris Bioscience (Oxfordshire, UK). Pooled human liver microsomes, Bovine serum albumin, UGT reaction solution A and UGT reaction solution B were all purchased from BD Biosciences (USA). Sodium dihydrogen phosphate buffer, HPLC grade methanol and sodium hydroxide were purchased from Sigma Aldrich Company LTD (Dorset, UK). Acrodisc CR 4-mm syringe filters with a 0.45-μm PTFE membrane (Part number: 4472) were purchased from Pall Life Sciences (Portsmouth, UK).

HPLC analysis was performed on a Shimadzu Prominence HPLC system running Laura 3 software from LabLogic (South Yorkshire, UK) and LC solutions from Shimadzu LTD, UK. The HPLC system was run with a CBM-20A controller, a LC-20AB solvent delivery system,

an SPD-20A absorbance detector and an SIL-20A auto sampler. A dual BGO coincidence detection and a flow-count radio HPLC detection system were both purchased from Bioscan (Washington DC, USA). An ACE 5 C18 HL 150 mm × 4.6 mm HPLC column (Product code: ACE-121-1546) was used and purchased from Hichrom (Berkshire, UK).

A PK121R centrifuge was purchased from Thermo Fisher Scientific LTD (Massachusetts, USA), and a 1470 AGC was purchased from PerkinElmer (Massachusetts, USA).

Shim-Pack MAYI-C4 SPE cartridges (Product code: 099-01586-15) were purchased from Shimadzu LTD, UK; Isolute PH SPE packing material (Product code: 9360-0025) was purchased from Biotage (Uppsala, Sweden) and C18 SPE packing material (Product code: WAT020594) was purchased from Waters LTD (Hertfordshire, UK). Empty SPE column assemblies and fittings (30 mm L × 4.6 mm ID, Product code: AQ0-7262) were purchased from Phenomenex (Macclesfield, UK).

3 | METHODS

3.1 | [6-O-methyl-¹¹C]diprenorphine ([¹¹C]diprenorphine) production

[¹¹C]Diprenorphine was synthesised according to a previously reported method¹⁰ with some adaptations made to the radiochemistry and automated on a GE TRACERlab FX_{FE} radiochemistry system.¹⁹ The product was formulated for human administration and produced in line with good manufacturing practice guidelines.

3.2 | [¹¹C]Diprenorphine metabolite analysis for PET scans

[¹¹C]Diprenorphine metabolites were analysed in discrete blood samples by in-line SPE followed by HPLC analysis.

Discrete blood samples were spun down in a centrifuge at 4000 g for 2 min at 20°C. Following this, 2 ml of the plasma layer was extracted and added to 3 ml of sodium dihydrogen phosphate buffer (NaH_2PO_4 , 5 mM), which was prespiked with stable diprenorphine (15 μl of 1-mg/ml solution). The sample was then loaded onto a 5-ml stainless steel loop with the injection valve in the load position (Figure 1). Next, the injection valve was switched to the inject position and the SPE mobile phase (NaH_2PO_4 , 5 mM at a flow rate of 1 ml/min) swept the sample onto a prepacked stainless steel SPE column (3×0.48 cm) containing a C18 bulk packing material over a period of 5 min (Figure 2). It should be noted that SPE columns packed with Phenyl and with C4 stationary phases were also used for different experiments. The SPE column was then washed for a further 3.5 min with NaH_2PO_4 at 1 ml/min. The liquid from the SPE load and SPE washing steps containing plasma proteins and the most polar metabolites were collected in a preweighed vial containing sodium hydroxide (NaOH , 5 M, 50 μl). Five millilitres of aliquots of the SPE breakthrough was then taken weighed and counted in a well counter. The SPE column was then eluted in the reverse direction onto an ACE-5 C18 HL HPLC column with methanol: NaH_2PO_4 (60:40) changing to 80:20 over the first 2.5 min. The HPLC eluent was monitored for UV absorbance at 254 nm and collected into fractions (1.6 and 2.4 ml), which were counted in a 10-well AGC in which one position was left empty for background corrections.

In order to assess the suitability of the SPE column being used, radioactive recovery measurements of the whole system (SPE and HPLC) were performed. This was done by injecting a sample of [^{11}C]diprenorphine on to the whole system and measuring the radioactivity from the SPE breakthrough (measured in the well counter) and HPLC eluent from the column (collected into fractions and measured in the AGC). Radioactivity measurements from the AGC and the well counter were processed, and recoveries were calculated by comparing the radioactivity of the [^{11}C]diprenorphine injected onto the system to the decay corrected radioactivity in the SPE breakthrough aliquots and HPLC eluent from the column.

The research study was approved by Stockport NHS Research Ethics Committee (reference 09/H1012/44) and permission granted by ARSAC (radiation protection agency).

3.3 | Production of [^{11}C]diprenorphine metabolites by means of digest, using pooled human liver microsomes

[^{11}C]diprenorphine metabolites were generated by performing a digest using pooled human liver microsomes.

To a centrifuge tube containing pooled human liver microsomes (25 μl , approximately 0.5 mg), UGT reaction mixture solution A (200 μl , containing 25-mM UDPGA) and UGT reaction mixture solution B (82 μl , containing 5X-UGT assay buffer with alamethicin) were added. [^{11}C]diprenorphine and stable diprenorphine (10 μl of 1 mg/ml) were added to the assay followed by water (175 μl) and bovine serum albumin (175 μl). The digestion was then incubated in a water bath (37°C) for 45 min to 1 h, after which the digestion was terminated with the addition of acetonitrile (500 μl). The reaction mixture was then spun at 16,060 g for 5 min at 4°C, after which the reaction mixture was separated from the microsome pellets. Next the reaction mixture was filtered through an Acrodisc CR 4-mm syringe filter with a 0.45- μm PTFE membrane and then analysed via in-line SPE and HPLC.

4 | RESULTS

4.1 | In silico analysis of [^{11}C]diprenorphine metabolites

A computational analysis of the metabolism of [^{11}C]diprenorphine was performed using Meteor Nexus software (Lhasa LTD, Leeds, UK). Meteor Nexus is knowledge-based computational software used to predict molecular metabolism.²⁰ A chemical structure is submitted to the software that uses a knowledge base and takes information about the lipophilicity, functional groups and the likelihood of competing biotransformations to predict the molecules metabolic fate. The software predicted that diprenorphine would undergo oxidative *N*-dealkylation via the family of CYP450 enzymes as part of phase 1 metabolism. The Nexus software also predicted oxidative *O*-dealkylation of diprenorphine, again by means of the enzyme family CYP450 and as part of phase 1 metabolism. Another predicted metabolite of diprenorphine was formed from the glucuronidation of the aromatic alcohol via a uridine 5'-diphospho-glucuronosyltransferase (UGT) enzyme as a result of phase 2 metabolism to diprenorphine-3-glucuronide. The metabolites predicted by Meteor are depicted in Figure 5.

4.2 | [^{11}C]Diprenorphine metabolite analysis for PET

Arterial blood samples were taken from patients at 5, 10, 20, 30, 40, and 60 min post-[^{11}C]diprenorphine injection. The blood samples were processed as detailed in the methods section and analysed by in-line C18 SPE and

FIGURE 5 Diprenorphine and its metabolites (including calculated cLogP values) predicted by Meteor Nexus

HPLC analysis. Figure 6 shows the profile of [^{11}C]diprenorphine, the major metabolite [^{11}C]diprenorphine-3-glucuronide and SPE breakthrough at various time points post injection. The data show pooled averages for each time point for 55 [^{11}C]diprenorphine PET scans.

4.3 | [^{11}C]Diprenorphine recoveries with various SPE stationary phases

Various SPE stationary phases were investigated to determine which would be optimal for trapping and eluting both parent and metabolite molecules with minimal loss. Three stationary phases were considered, a C4 phase (Shim-Pack MAYI-C4), a C18 phase and a phenyl stationary phase. The C18 and the phenyl stationary phases

were packed into stainless steel column assemblies, whereas the MAYI C4 material was prepacked. Recoveries on the whole system (SPE and HPLC) were performed as described in the methods section, and the results are summarised in Table 1.

5 | DISCUSSION

5.1 | In silico analysis of [^{11}C] diprenorphine metabolites

The metabolites of diprenorphine that were predicted by Meteor Nexus metabolite prediction software can be seen, along with their cLogP values, in Figure 5. It is clear to see that the cLogP values for the metabolites are

FIGURE 6 Metabolite profiles of [^{11}C] diprenorphine, [^{11}C]diprenorphine-3-glucuronide and SPE breakthrough (polar metabolites). Data are based on pooled averages of 55 [^{11}C]diprenorphine PET scans

TABLE 1 [^{11}C]diprenorphine recoveries for different SPE column stationary phases

SPE packing material	Average [^{11}C]diprenorphine recovery (%)	Average SPE breakthrough (%)	<i>n</i>
Phenyl (Isolute Ph)	76.2 $\pm$ 7.4	0.6 $\pm$ 0.3	8
C4 (Shimadzu MAYI)	98.7 $\pm$ 2.9	3.9 $\pm$ 2.6	6
C18 (Waters)	93.9 $\pm$ 4.5	1.4 $\pm$ 0.5	86

all lower than that of the parent diprenorphine molecule (cLogP = 2.57). We would therefore expect that the metabolites of diprenorphine would elute before the parent molecule on a C18 phase HPLC column, and this is what we do observe (HPLC chromatograms available in Supporting Information). Meteor Nexus predicts the loss of the cyclopropyl moiety from the molecule, which has long been suspected as a major metabolite of diprenorphine.¹⁷ The software also predicted a loss of a methyl group from the 6-methoxy position of diprenorphine. If this was to happen with [^{11}C]diprenorphine, we would lose the ^{11}C radiolabel. Finally, glucuronidation of diprenorphine at the 3-hydroxy

position to produce [^{11}C]diprenorphine-3-glucuronide is predicted. We would anticipate that glucuronidation would occur at this position as it is the more acidic and reactive hydroxyl group.

[^{11}C]diprenorphine metabolites were generated using a digestion method with hepatic microsomes and isolated as described in the methods section, and the sample was analysed by MALDI-MS. The analyte (0.5 μl) was spotted onto a plate along with a matrix of α -cyano-4-hydroxycinnamic acid in 1:1:0.05 (water:acetonitrile:trifluoroacetic acid). The results showed three peaks of note; the major peak in the analysis at m/z 426 represents [diprenorphine + H] $^{+}$, and the peak at m/z 849 is the

FIGURE 7 MALDI-MS spectrum of diprenorphine digest showing peaks for parent diprenorphine (m/z 426), diprenorphine dimer (m/z 849) and diprenorphine glucuronide (m/z 602)

diprenorphine dimer. The peak at m/z 602 is diprenorphine glucuronide, thought to be the major metabolite Figure 7.

We have also shown good agreement between the predicted *in silico* metabolism of diprenorphine and the *in vitro* metabolism of [^{11}C]diprenorphine produced by hepatic microsome digestion. The Meteor software predicted the formation of diprenorphine-3-glucuronide via phase 2 metabolism and the UGT enzyme. We confirmed this was the case by performing a digestion of [^{11}C]diprenorphine with hepatic microsomes and confirmed the formation of [^{11}C]diprenorphine-3-glucuronide by MALDI-MS analysis. By identifying the major metabolite of [^{11}C]diprenorphine, we can start to answer important questions such as will [^{11}C]diprenorphine-3-glucuronide cross the blood–brain barrier, and if it does, will it compete with [^{11}C]diprenorphine for available opioid receptor sites? The cLogP of [^{11}C]diprenorphine-3-glucuronide ($\text{cLogP} = 0.69$) suggests that its passage across the blood–brain barrier is unlikely not taking into account transporter proteins. The pharmacology of [^{11}C]diprenorphine-3-glucuronide is unclear; however, we can compare how other glucuronides of opioids act on opioid receptors. For example, morphine-3-glucuronide has been shown to have a low affinity at μ -receptors,²¹ whereas buprenorphine-3-glucuronide was reported to have some biological activities that could contribute to the total pharmacology of buprenorphine.²² More work is required to analyse the blood–brain barrier permeability and affinity diprenorphine-3-glucuronide may have on opioid receptors.

5.2 | [^{11}C]Diprenorphine metabolite analysis for PET

Figure 6 shows the profile of [^{11}C]diprenorphine, the major metabolite [^{11}C]diprenorphine-3-glucuronide and of the SPE breakthrough at various time points post-[^{11}C]diprenorphine intravenous injection in human volunteers. From Figure 6, we can see that the percentage of [^{11}C]diprenorphine at 5 min postinjection is around 70% and [^{11}C]diprenorphine-3-glucuronide accounts for 25% of the radioactivity at this timepoint. As we would expect, the percentage of [^{11}C]diprenorphine falls at later timepoints finally reaching 34% at 60 min, and the percentage of [^{11}C]diprenorphine-3-glucuronide increases until a plateau of 52% is reached at 30 min. The amount of [^{11}C]diprenorphine-3-glucuronide at 60 min postinjection remains at 52%. The SPE breakthrough accounts for 4% of the activity at 5 min postinjection, which increases to 13% at 60 min. This fraction contains the most polar metabolites, which are not retained by the

SPE and this polar radio metabolite could be [^{11}C]MeOH, produced from *O*-dealkylation of [^{11}C]diprenorphine as predicted by Meteor Nexus software.

Our metabolite data from [^{11}C]diprenorphine PET scans is in agreement with that reported by Sadzot et al. and Jones et al.^{6,14} in which we also see a decrease of the parent [^{11}C]diprenorphine fraction over the 90-min period of the scan corresponding with the ^{11}C radio-labelled metabolites increasing over that same time period. Similarly, we also observe a broad ^{11}C radio-labelled metabolite peak eluting prior to [^{11}C]diprenorphine in the HPLC analysis (Figure S1), which can be interpreted as a more polar substance and possibly two ^{11}C radiolabelled metabolites.

5.3 | [^{11}C]Diprenorphine recoveries with various SPE stationary phases

Table 1 shows [^{11}C]diprenorphine recovery measurements for the SPE stationary phases tested. We began by investigating the C4 (MAYI) prepacked SPE column, which is designed for biological sample pretreatment. The MAYI SPE cartridges have a packing material in which the interior of the pore is modified with C4 groups and the outer silica surface is coated with a hydrophilic polymer. Plasma proteins should be unable to access the pore and do not adhere to the polymer coating making them easy to elute, whereas low-molecular weight molecules (such as diprenorphine) will enter the pores and be retained. From Table 1, we can see that on average, we saw good [^{11}C]diprenorphine recoveries, 98.7% ($n = 6$). However we found that a significant amount of the parent was breaking through ($3.9\% \pm 2.6\%$). The next SPE stationary phase was the phenyl reverse phase packed into stainless steel SPE column assemblies, a set-up that has been used previously for [^{11}C]diprenorphine PET studies. A lower average percentage of [^{11}C]diprenorphine recovery was observed with this phase ($76.2\% \pm 6.8\%$, $n = 8$). Interestingly, this SPE stationary phase has been used previously for a [^{11}C]diprenorphine PET study where it was reported that $>98\%$ of total plasma activity was retained by the SPE.⁶ However, when we used the phenyl stationary phase, it was discovered that the recoveries were low for a similar in-line SPE HPLC set-up. [^{11}C]Diprenorphine was retained strongly by the phenyl phase likely because of the π - π interactions between the analyte and the phenyl phase. However [^{11}C]diprenorphine was not completely removed from the SPE column during the elution step as evidenced by the low recovery ($76.2\% \pm 7.4$). The phenyl reverse phase also had a lower average percentage of SPE

breakthrough with $0.6\% \pm 0.3\%$ compared with the C4 and C18 phases. Finally, we investigated a C18 stationary phase packed in-house. The C18 stationary phase showed good recoveries with an average of $93.9\% \pm 4.5\%$ ($n = 86$) of the radioactivity recovered and only $1.4\% \pm 0.5\%$ of radioactivity breaking through the SPE. If we compare the percentage of SPE breakthrough of [^{11}C]diprenorphine in plasma for the C18 SPE column with the MAYI C4 SPE column, we can see that much more of the radioactivity is retained by the C18 material. This may suggest that the parent ([^{11}C]diprenorphine) could be breaking through the C4 cartridge possibly in a plasma protein bound form.

6 | CONCLUSION

A method is described to measure the metabolites of the PET radiotracer [^{11}C]diprenorphine via an in-line SPE method followed by HPLC analysis. After assessing various stationary phases of C4, phenyl and C18, we concluded that the C18 phase packed into a stainless column assembly (30 mm L $\times$ 4.6 mm ID) was the most optimal of the conditions investigated. This conclusion was made by investigating the loading, retention and subsequent elution of [^{11}C]diprenorphine from the SPE as well as performing experiments to measure the recovery of [^{11}C]diprenorphine.

We believe that our method of [^{11}C]diprenorphine metabolite analysis should be the method of choice for [^{11}C]diprenorphine PET scanning, offering a reliable and robust approach. ^{11}C radio-metabolites can be successfully quantified with minimal loss to the system, using SPE to clean up and concentrate the sample before analysis by analytical HPLC.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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