JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 271–280.
DOI: 10.1002/jlcr.557

Research Article

A combined loop-SPE method for the automated preparation of [^{11}C]doxepin

R. Iwata[1,2,*], C. Pascali[3], A. Bogni[3], K. Yanai[4], M. Kato[4], T. Ido[2] and K. Ishiwata[5]

[1] *Graduate School of Engineering Tohoku University, Sendai 980-8579, Japan*
[2] *CYRIC Tohoku University, Sendai 980-8578, Japan*
[3] *National Cancer Institute, 20133 Milan, Italy*
[4] *Graduate School of Medicine Tohoku University, Sendai 980-8575, Japan*
[5] *Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0022, Japan*

Summary

A simple and versatile loop-solid phase extraction (SPE) method was developed for the automated preparation of [^{11}C]doxepin, a histamine H_1 receptor antagonist, from [^{11}C]methyl triflate ([^{11}C]MeOTf). This labeling agent was passed through a Teflon or Tefzel loop coated internally with a film of the precursor solution. The reaction products were then flushed from the loop to a short SPE column, where they were concentrated and then injected onto a semi-preparative HPLC column simply by switching an injection valve. By applying this combined loop-SPE technique the whole procedure turned out to be easily automated. The formation of [^{11}C]methylated doxepin ([^{11}C]methyldoxepin) was observed and the ratio of doxepin to methyldoxepin was found to be clearly correlated with the mass ratio of nordoxepin to MeOTf. This observation highlights the importance of [^{11}C]MeOTf specific activity in the [^{11}C]methylation of secondary amines. Using this method, [^{11}C]Doxepin was prepared in over 40% radiochemical yield from high specific activity [^{11}C]MeOTf. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: automated preparation; loop-SPE method; doxepin; methyl triflate; carbon-11

*Correspondence to: R. Iwata, Department of Quantum Science and Energy Engineering, Graduate School of Tohoku University, Aramaki, Aoba-KU, Sendai 980-8579, Japan.
E-mail: ren.iwata@qse.tohoku.ac.jp

Received 3 October 2001
Revised 12 November 2001
Accepted 21 November 2001

Introduction

Due to the rapid expansion of positron emission tomography (PET) as a powerful advanced tool for clinical studies in fields such as tumour diagnosis and receptor mapping, automated synthesis of radiopharmaceuticals labeled with positron-emitting radionuclides is becoming increasingly important. Automation, especially when applied to routine preparations, requires simple and reliable synthetic procedures. Among those developed so far, the loop method is thought to be very simple and hence suitable for this purpose.[1–3] This technique is based on the use of a length of coiled small diameter tubing, 'coated' on the inner surface with a small portion of precursor solution. This setup serves as a reaction vessel on which a flowing labeling agent is efficiently trapped and rapidly reacted. Reaction products are then transferred to a subsequent purification step simply by elution with an appropriate solvent. Thanks to its simplicity the loop method has been successfully used in reactions with [^{11}C]cyclohexanecarbonyl chloride,[1] [^{11}C]methyl iodide ([^{11}C]MeI)[2] and [^{11}C]methyl triflate ([^{11}C]MeOTf[3]).

As demonstrated in the preparation of [^{11}C]raclopride. [3] [^{11}C]MeOTf is considered to be more suitable for the loop method than [^{11}C]MeI in terms of volatility and reactivity since, in general, it requires neither cooling during trapping nor heating for reacting. Furthermore, its higher reactivity allows the use of smaller volumes of precursor solution.

Doxepin (**2**: [3-(6*H*-dibenzo[*b*,*e*]oxepin-11-ylidene)-propyl]-dimethylamine) is a tricyclic antidepressant and histamine H_1 receptor antagonist. ^{11}C-Labeled doxepin ([^{11}C]**2**) was first prepared by *N*-[^{11}C]methylation of free base nordoxepin (**1**) from [^{11}C]MeI[4] and since then it has been extensively used for measuring histamine H_1 receptor occupancy of antihistamines by PET.[5–7]

In this work we applied a slightly modified loop method for the preparation of [^{11}C]**2** from [^{11}C]MeOTf and introduced SPE to simplify the injection of the reaction solution onto the preparative HPLC column. The role played by the specific activity of [^{11}C]MeOTf is also discussed.

Results and discussion

Efficient injection of a reaction solution onto an HPLC column is one of the most difficult procedures to automate. In the previously reported

loop method for the preparation of [^{11}C]raclopride, the eluate from the loop was first collected in a reservoir and then transferred into an injection loop by suction with a syringe.[3] To avoid co-injecting air this method required the volume of eluate to be equal or larger than the volume taken up by the syringe, which in turn had to be smaller than the volume of the injection loop. Careful and troublesome volume adjustments were thus needed.

In a recently described similar loop method,[2] the authors opted for a more straightforward quantitative injection of the whole reaction mixture and, inevitably, the co-injection of a large volume of air. This practice is not generally recommended since it would lead to the progressive deterioration of expensive HPLC columns. It should be noted that a similar method in which the injection loop was replaced by a short column packed with a precursor adsorbed on an inert support was also developed.[8] With this setup the whole reaction mixture could be injected onto an HPLC column by switching an injection valve.

Enrichment by SPE is a commonly used technique for the on-line injection of biological samples such as dialysates or metabolites onto HPLC columns. This approach has been automated and applied for instance to the analysis of plasma samples taken following the administration of ^{11}C-labeled radioligands.[9] Our present work is the successful example of the application of the same approach to an automated radiosynthesis.[10] By removing the need for careful calibration and by allowing the use of larger volumes of solvent in the loading phase, the SPE method supplies a much more convenient and efficient process than the conventional procedure described above. However, the solvent has to be carefully selected since it must cope with two conflicting requirements. Elution from the loop needs a less polar solvent, while extraction by the SPE (ODS) column requires a more polar solvent. Data for the efficiency of [^{11}C]doxepin recovery and SPE obtained using several solvent compositions are listed in Table 1. Among all the solvents used, the results afforded by water showed the greatest variation, possibly due to the low solubility of [^{11}C]**2** in this solvent. Addition of 10% MeCN to water in order to increase the solubility of [^{11}C]**2** considerably improved the efficiency of eluting from the loop. However, this was to the detriment of the efficiency of the SPE step. Although a reduction in the MeCN content did bring some improvement, the conditions were still far from optimal. Better results were obtained by adding acetic acid to the water to decrease the pH. By

Table 1. Solvent effects on [^{11}C]doxepin preparation

Solvent	Efficiency		
	Elution from the loop (%)	Extraction by the SPE column (%)	Overall efficiency (%)
Water	44–98	89–99	42–92
10% MeCN	98	15	15
5% MeCN	99	22	22
2% MeCN	98	33	32
0.5% MeCN	83	72	60
0.2–0.5% AcOH	90–98	>99	90–98

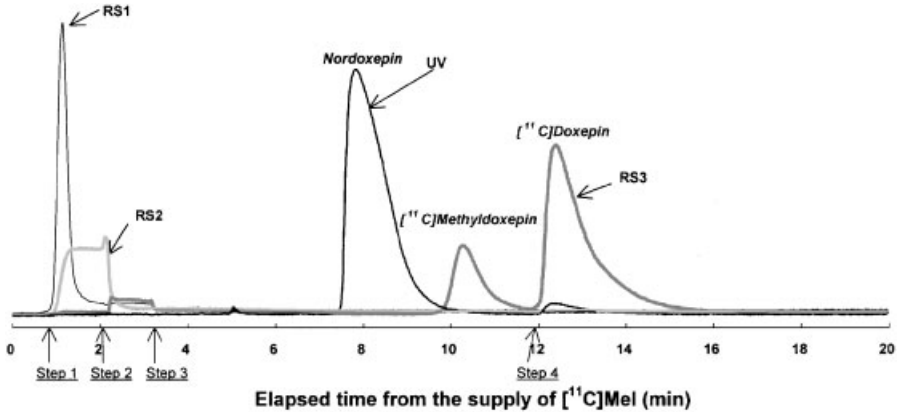
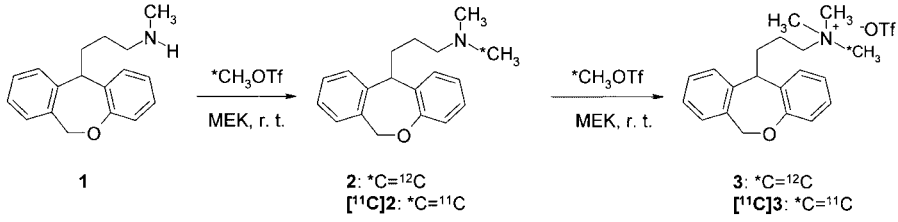


Figure 1. A typical profile (line RS1 and loop RS2) and semi-preparative HPLC trace (UV and radioactivity) from the automated preparation of [^{11}C]doxepin

this procedure we were able to automatically inject [^{11}C]**2** in the overall efficiencies of over 90% onto the HPLC column.

Nevertheless, radiochemical yields of [^{11}C]**2** were variable. A typical semi-preparative HPLC trace of the reaction mixture is shown in Figure 1. A significantly large radioactive peak was always observed to precede that of [^{11}C]**2** when [^{11}C]MeOTf was used as methylating agent, and this more polar product was identified as [^{11}C]methyldoxepin ([^{11}C]**3**: [3-(6*H*-dibenzo[*b*,*e*]oxepin-11- ylidene)-propyl]-[^{11}C]trimethyl-ammonium), the dimethylated product of nordoxepin, produced by the reaction shown in Scheme 1. Methylation of nordoxepin was also examined using [^{11}C]MeI. The results listed in Table 2 clearly indicate that dimethylation does not take place when [^{11}C]MeI was used instead of [^{11}C]MeOTf, possibly reflecting the difference in reactivity between



Scheme 1. The reaction of [^{11}C]MeOTf with nordoxepin

Table 2. [^{11}C]Doxepin and [^{11}C]methyldoxepin radiochemical yields

Labeling agent	Precursor	Solvent[a]	[^{11}C]Doxepin yield[b] (%)	[^{11}C]Doxepin/ [^{11}C]methyldoxepin
[^{11}C]MeI	Nordoxepin HCl	MEK	3	100/0
[^{11}C]MeI	Nordoxepin HCl	DMF	23	100/0
[^{11}C]MeOTf	Doxepin[c]	CHO	0	0/100
[^{11}C]MeOTf	Nordoxepin HCl	MEK	20–82	Varies according to nordoxepin/MeOTf mass ratio (see Figure 2)
[^{11}C]MeOTf	Nordoxepin HOTf	MEK		

[a] MEK: methylethylketone, DMF: dimethylformamide, CHO: cyclohexanone.
[b] Decay-corrected radiochemical yields of [^{11}C]doxepin based on [^{11}C]MeI or [^{11}C]MeOTf.
[c] This run was made to confirm the formation of [^{11}C]methyldoxepin.

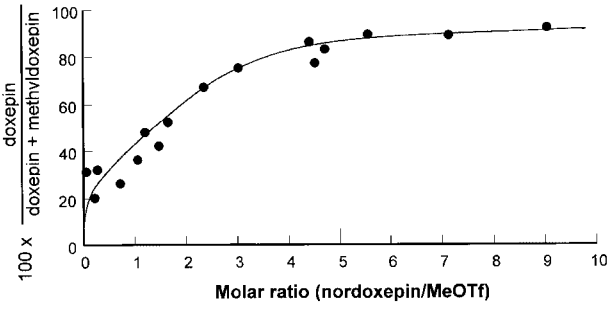


Figure 2. Influences of [^{11}C]MeOTf specific activity on [^{11}C]doxepin yield

these two agents. In addition, since the order of the S_N2 methylation rate with amines is normally tertiary amine > secondary amine > primary amine, it is not surprising that the effect of the specific activity of [^{11}C]MeOTf is more evident and important in reactions with secondary amines, such as nordoxepin, than that with primary amines. Thus, as can be seen in Figure 2, higher radiochemical yield of [^{11}C]**2** can be achieved only using high specific activity [^{11}C]MeOTf (at least 200 GBq/

μmol at EOB) or, as a less desirable alternative, by increasing the amount of precursor.

Effects of the salt form of nordoxepin on radiochemical yields were also investigated. As seen in Table 2, there was no distinct difference in radiochemical yield between the two salt forms of nordoxepin. This result, the complete opposite of what was observed for [^{11}C]raclopride,[3] is probably due to the higher reactivity shown by [^{11}C]MeOTf towards nordoxepin than demethylraclopride, so that its side reaction with chloride to produce volatile [^{11}C]methyl chloride was less relevant.

Experimental

[^{11}C]Carbon dioxide ([^{11}C]CO_2) was produced by the $^{14}N(p,\alpha)^{11}C$ reaction on an N_2 target containing 0.5% O_2 with 12 MeV protons from a Cypris HM12 cyclotron (SHI). It was then converted to [^{11}C]MeI by the gas phase iodination via [^{11}C]CH_4 with an MeI MicroLab system (GE).

Nordoxepin (**1**) hydrochloride was purchased from Sigma. The conversion of its salt form from chloride to triflate was carried out as previously described.[3] Methylethylketone (MEK) was obtained from Wako Pure Chem. and was used as reaction solvent without further purification. Silver triflate (AgOTf) was purchased from Aldrich and coated on Graphpac GC (80–100 mesh, Alltech) as previously described.[3] This fine powder was then mixed with quartz sands (10–30 mesh) at a nearly 1/1 ratio and the mixture was packed in a glass column (4×50 mm). The column was used for more than 20 runs without changing the packing material.

HPLC analysis was performed on a TSKgel ODS-80 column (4.6×150 mm, Tosoh) with a solvent system of MeCN-0.05 M $HCOONH_4$ (40/60) at a flow rate of 1.5 ml/min.

Automated system

An original automated system (Ligand synthesis system) was purchased from SHI and modified for the present method (Figure 3). The automated system was controlled with a PC through the Cupid program (SHI) working on Windows NT. The AgOTf column to convert [^{11}C]MeI to [^{11}C]MeOTf[11] was heated in advance with a furnace at 200°C under a He flow at 50 ml/min.

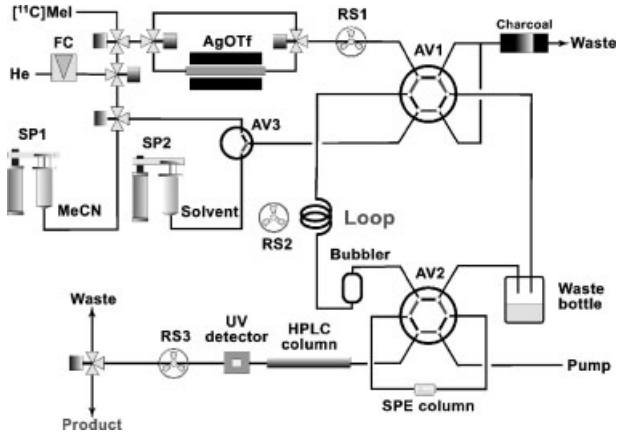


Figure 3. A flow chart of the automated system; FC: automatic flow controller, RS: radiation sensor, AV: air-actuated valve, SP: syringe pump

The loop (approx. 3 cm diameter) was made of 5 cm long Teflon (PTFE) or Tefzel (ETFE) tubing (*o.d.* 1.6 mm × *i.d.* 0.75 mm). An empty short column (4 × 40 mm) was connected to the outlet of the loop as a bubbler so that it could retain any excess precursor solution from the loop and allow [^{11}C]MeOTf to bubble through it during the trapping step.

One of the 6-way valves (AV2, Valco) was used as an HPLC injector, to which a short SPE column (4.6 × 5.8 mm, Prelute ODS cartridge, Gilson) was connected as a substitute for a sample loop. This column was carefully selected among commercially available guard columns so as to minimize back pressure and maximize trapping efficiency of the product when a solvent flowed through it. Since back pressure in the solvent flowing line was still too high, an air-actuated 3-way valve (AV3, Valco) was preferred to an ordinary 3-way solenoid valve (Burkert). One of the syringe pumps (SP2) was used for passing the solvent through the loop and SPE column. The other (SP1) was used, after synthesis, for cleaning up the loop and lines with MeCN followed by drying with a flow of He.

Three radiation sensors (RS1–3) traced the movement of ^{11}C-radioactivity. The passage of [^{11}C]MeOTf through the line from the AgOTf column to the loop was monitored by RS1, the accumulation of ^{11}C-radioactivity on the loop by RS2, and the eluting ^{11}C-radioactivity from the HPLC column by RS3. This eluate from the column was also monitored by a UV (254 nm) detector and all these data were displayed in real time on a PC screen (see Figure 2).

Radiosynthesis of [^{11}C]doxepin

Nordoxepin hydrochloride or triflate (1 mg) was dissolved in 50–60 μl of methylethylketone and 2 μl of 1.2 M NaOH added to the solution. The mixture was injected onto the loop with a glass syringe and the loop was then connected to the system. This preparation was generally done 10 min before the end of the irradiation (EOB).

Immediately after EOB, [^{11}C]CO_2 was transferred to the MeI MicroLab system and converted to [^{11}C]MeI. After the conversion process, *ca* 11 min, [^{11}C]MeI was passed with He at a flow rate of 35 ml/min to the AgOTf column. By switching the 6-way valve AV1 the converted [^{11}C]MeOTf coming out from the column was directed to the loop (Step 1).

A rapid increase in signal from RS2 was observed. Once it reached a plateau AV1 was switched back and the injector AV2 switched in turn. The solvent (6 ml) held in the syringe (SP2) was then pushed through the loop and SPE column (Step 2). Activity measurements of the solvent after elution from loop and SPE column were used to determine the extraction efficiencies of these two components.

After having emptied SP2, AV2 was switched back and the products retained by the SPE column were flushed back onto a semi-preparative HPLC column (Tosoh TSGgel ODS-80T$_M$, $5.5 \times 300\,mm^2$) using an MeCN-33 mM $HCOONH_4$ solvent system (40/60) at a flow rate of 8.5 ml/min (Step 3).

The [^{11}C]**2** fraction was collected into a rotary evaporator flask (Step 4) and after taking up a sample for determining the specific activity, 0.2 ml of 25% ascorbic acid solution was added to prevent radiolysis and the mixture was evaporated to dryness. The residue was dissolved in saline and the solution collected into a vial through a membrane filter. No radiolytically decomposed product was found in the final solution and radiochemical purity was always more than 99%.

The formation of [^{11}C]**3** was confirmed by the reaction of [^{11}C]MeOTf with doxepin, by which [^{11}C]**3** was almost quantitatively produced see Table 2).

Conclusion

In the present study, the preparation of [^{11}C]doxepin from [^{11}C]MeOTf could be greatly simplified by the loop-SPE method. Using the automated system, the described [^{11}C]doxepin was prepared in radio-

chemical purities of over 99% within 40 min from EOB after the optimization of the solvent used for transferring [^{11}C]doxepin from the loop to the SPE column. However, a marked effect of [^{11}C]MeOTf specific activity on [^{11}C]doxepin yield was observed. High specific activities or, alternatively, large amounts of precursor were needed to achieve higher radiochemical yields of [^{11}C]doxepin (over 40% decay corrected, based on [^{11}C]MeOTf) and thus higher ratios of [^{11}C]doxepin to [^{11}C]methyldoxepin. The loop-SPE method herein applied to the preparation of [^{11}C]doxepin is simple and versatile enough to be used for the automated preparation of various ^{11}C-radiopharmaceuticals.

Acknowledgements

The authors wish to thank Dr F. Brady (Imaging Research Solutions Limited, Cyclotron Building, Hammersmith Hospital, London) for helpful and critical discussion. This study was supported by a Grant-in-Aid (No. 13470177) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. McCarron JA, Turton DR, Pike VW, Poole KG. *J Label Compd Radiopharm* 1996; **38**: 941–953.
2. Wilson AA, Garcia A, Jin L, Houle S. *Nucl Med Biol* 2000; **27**: 529–532.
3. Iwata R, Pascali C, Bogni A, Miyake Y, Yanai K, Ido T. *Appl Radiat Isot* 2001; **55**: 17–22.
4. Ravert HT, Dannals RR, Wilson AA, Wagner HN Jr. *J Label Compd Radiopharm* 1992; **31**: 403–407.
5. Yanai K, Watanabe T, Yokoyama H, *et al*., *Neurosci Lett* 1992; **137**: 145–148.
6. Yanai K, Watanabe T, Yokoyama H, Hatazawa J, Iwata R, Ishiwata K, Meguro K, Itoh M, Takahashi T, Ido T, Matsuzawa T. *J Neurochem* 1992; **59**: 128–136.
7. Higuchi M, Yanai K, Okamura N, *et al*., *Neuroscience* 2000; **99**: 7215–7229.
8. Iwata R., Pascali C, Yuasa M, Yanai K, Takahashi T, Ido T. *Appl Radiat Isot* 1992; **43**: 1083.

9. Luthra SK, Osman S, Turton DR, Vaja V, Dowsett K, Brady F. *J Label Compd Radiopharm* 1993; **32**: 518–520.
10. Luthra SK, Brady F, Turton DR, *et al*., *Appl Radiat Isot* 1994; **45**: 853–873.
11. Jewett DM. *Appl Radiat Isot* 1992; **43**: 1383–1385.

