

METHODOLOGY

Open Access



# Improving the stability of $^{11}\text{C}$ -labeled L-methionine with ascorbate

Michael Woods<sup>1</sup>, Leo Leung<sup>1</sup>, Kari Frantzen<sup>1</sup>, Jennifer G. Garrick<sup>1</sup>, Zhengxing Zhang<sup>2</sup>, Chengcheng Zhang<sup>2</sup>, Wade English<sup>1</sup>, Don Wilson<sup>1</sup>, François Bénard<sup>1,2,3</sup> and Kuo-Shyan Lin<sup>1,2,3\*</sup> 

\* Correspondence: klin@bccrc.ca

<sup>1</sup>Department of Functional Imaging, BC Cancer Agency, Vancouver, BC, Canada

<sup>2</sup>Department of Molecular Oncology, BC Cancer Agency, Vancouver, BC, Canada  
Full list of author information is available at the end of the article

## Abstract

**Background:** Carbon-11 labeled L-methionine ( $^{11}\text{C}$ -MET) is a popular tracer used in the clinic for imaging brain tumors with positron emission tomography. However, the stability of  $^{11}\text{C}$ -MET in its final formulation is not well documented in literature. Recently, we observed fast degradation of HPLC-purified  $^{11}\text{C}$ -MET over time, and systematic investigation was conducted to identify the cause.

**Results:** In this study, we verified the degraded product as  $^{11}\text{C}$ -labeled methionine sulfoxide ( $^{11}\text{C}$ -METSO). To minimize oxidation, ascorbate (100 ppm) was added to the HPLC eluant, and the resulting HPLC-purified  $^{11}\text{C}$ -MET was stable in the final formulation solution without noticeable degradation for up to 1 h after the end of synthesis.

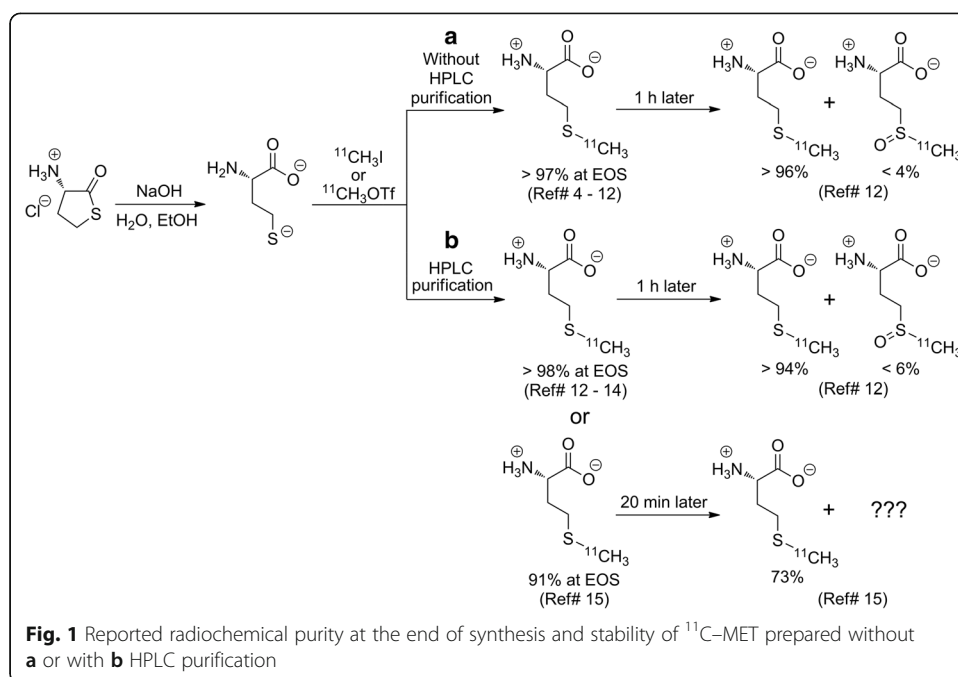
**Conclusions:** Our data suggest that to minimize degradation, ascorbate can be added to the  $^{11}\text{C}$ -MET formulation solution especially if it is not administered into patients soon after the end of synthesis.

**Keywords:** C-11 L-methionine, Stability, Homocysteine, Methionine sulfoxide, Oxidation, Ascorbate, Positron emission tomography

## Background

Carbon-11 labeled L-methionine ( $^{11}\text{C}$ -MET) is a promising tracer for imaging brain tumors with positron emission tomography (PET) (Watanabe et al. 2016; Maffione et al. 2009; Glaudemans et al. 2013). Its synthesis has been previously optimized by  $^{11}\text{C}$ -methylation of L-homocysteine in solution or on a C-18 Sep-Pak cartridge with or without HPLC purification (Pascali et al. 1999; Tang et al. 2004; Lodia et al. 2007; Lodi et al. 2008; Boschi et al. 2009; Cheung et al. 2009; Quincoces et al. 2010; Pascali et al. 2011; Bogni et al. 2003; Nagren et al. 1998; Oh et al. 1998; Fukumura et al. 2004). While most groups reported > 97% radiochemical purity of  $^{11}\text{C}$ -MET at the end of synthesis (EOS) even without HPLC purification, the stability of  $^{11}\text{C}$ -MET in the final formulation solution has not been well documented in literature (Pascali et al. 1999; Tang et al. 2004; Lodia et al. 2007; Lodi et al. 2008; Boschi et al. 2009; Cheung et al. 2009; Quincoces et al. 2010; Pascali et al. 2011; Bogni et al. 2003; Nagren et al. 1998; Oh et al. 1998).

Bogni et al. reported the formation of a minor amount (< 6%) of C-11 L-methionine sulfoxide ( $^{11}\text{C}$ -METSO) at 1 h after EOS in  $^{11}\text{C}$ -MET solution prepared either with or without HPLC purification (Fig. 1) (Bogni et al. 2003). The degradation of  $^{11}\text{C}$ -MET



was attributed to radiolysis as the degradation rate was affected by total radioactivity and chemical composition (ethanol and L-homocysteine) of the  $^{11}\text{C}$ -MET solutions. Fukumura et al. observed fast degradation of HPLC-purified  $^{11}\text{C}$ -MET solution with radiochemical purity at 91.2 and 72.9% for samples assayed at EOS and 20 min after EOS, respectively (Fig. 1b) (Fukumura et al. 2004). The instability of  $^{11}\text{C}$ -MET was likely caused by radiolysis as samples with higher specific activity also showed significantly faster degradation rates. The degraded radioactive product was not identified in this report. However, it was shown that adding ethanol (EtOH, 1.5%) and Tween 80 (3%) into the final formulation solution (in saline) or using saline containing ascorbate (1000 ppm) as the eluant improved the radiochemical purity of HPLC-purified  $^{11}\text{C}$ -MET to 99.9%, and no significant degradation was observed over 1 h after EOS (Fukumura et al. 2004).

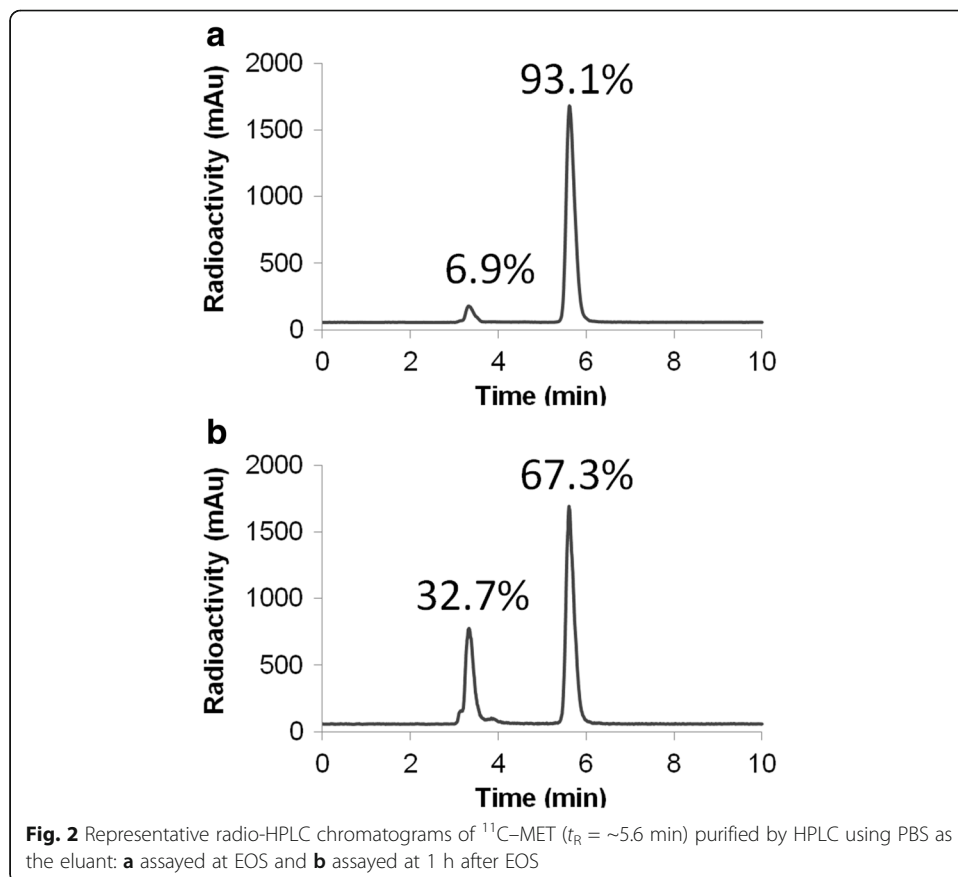
In this methodology article, we communicate our experience on the preparation of HPLC-purified  $^{11}\text{C}$ -MET. We report here the instability of HPLC-purified  $^{11}\text{C}$ -MET, our systematic investigation to find out the cause of rapid degradation, and the strategy to improve the stability of  $^{11}\text{C}$ -MET in the final formulation solution.

## Results and discussion

To set up  $^{11}\text{C}$ -MET production at our institution, we used the simplest method, Sep-Pak cartridge without HPLC purification (Gomzina et al. 2011), for our initial attempt. However, we obtained much lower radiochemical purity (< 90%, data not shown) as compared to > 97% reported by others (Pascali et al. 1999; Tang et al. 2004; Lodia et al. 2007; Lodi et al. 2008; Boschi et al. 2009; Cheung et al. 2009; Quincoces et al. 2010; Pascali et al. 2011; Bogni et al. 2003). For the preparation of  $^{11}\text{C}$ -MET using Sep-Pak cartridge without HPLC purification, L-homocysteine and unhydrolyzed L-homocysteine thiolactone end up in the final formulation solution as well. In order to achieve higher chemical and radiochemical purities, we decided to use HPLC purification for subsequent preparations of  $^{11}\text{C}$ -MET.

Using phosphate-buffered saline (PBS, 3.93 mM, 3.0 mL/min) as the HPLC eluant and a C18 column (Luna C18 semi-preparative column, 5  $\mu$ , 250  $\times$  10 mm)  $^{11}\text{C}$ -MET was obtained in  $19 \pm 4\%$  ( $n = 4$ ) decay-corrected radiochemical yield from  $^{11}\text{C}$ -MET with  $93.5 \pm 0.6\%$  ( $n = 4$ ) radiochemical purity and  $21.0 \pm 5.1$  GBq/ $\mu\text{mol}$  specific activity at the end of synthesis (EOS). The analytical HPLC column was a Luna C18 column (5  $\mu$ , 250  $\times$  4.6 mm), the eluant was phosphate buffer (1 mM, pH 3), and the flow rate was 1.0 mL/min. A minor radioactive by-product was observed at  $t_{\text{R}} = \sim 3.3$  min (Fig. 2a). No residual L-homocysteine or L-homocysteine thiolactone was detected in the final  $^{11}\text{C}$ -MET solution as monitored by UV detector (set at 220 nm). The radiochemical purity of  $^{11}\text{C}$ -MET quickly dropped to  $75 \pm 6\%$  ( $n = 4$ ) at 1 h after EOS with concurrent increase of the radioactive by-product (Fig. 2b). Our radiochemical purity data (93.5% at EOS and 75% at 1 h after EOS) were lower than the data (>99% at EOS and >96% at 1 h after EOS) reported by Bogni et al. (Bogni et al. 2003), but were comparable to the data (91.2% at EOS and 72.9% at 20 min after EOS) reported by Fukumura et al. (Fukumura et al. 2004).

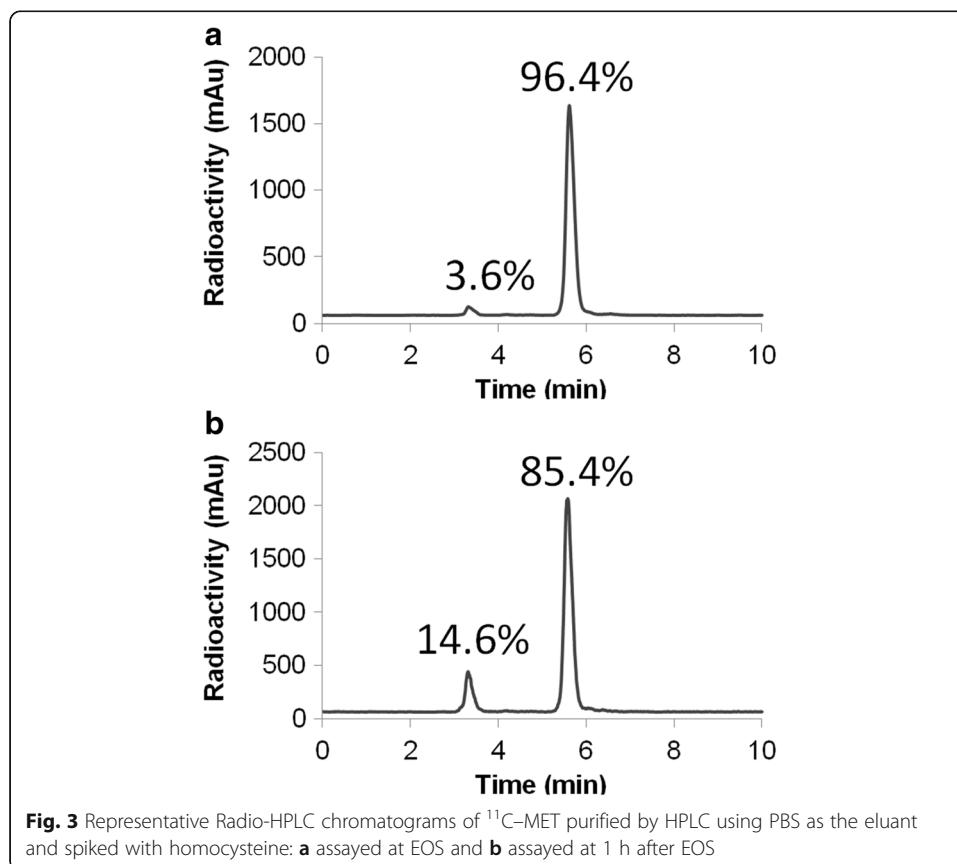
To our knowledge, there was only one report on degradation of  $^{11}\text{C}$ -MET prepared using Sep-Pak cartridge without HPLC purification (Bogni et al. 2003). A minor amount (<4%) of  $^{11}\text{C}$ -METS0 was formed over a 1-h period after EOS (Bogni et al. 2003). Presumably, the remaining L-homocysteine ending up in the final formulation solution could serve as a free radical scavenger, and prevent degradation of  $^{11}\text{C}$ -MET. To verify this hypothesis, we added 0.5 mg of L-homocysteine in the product collection

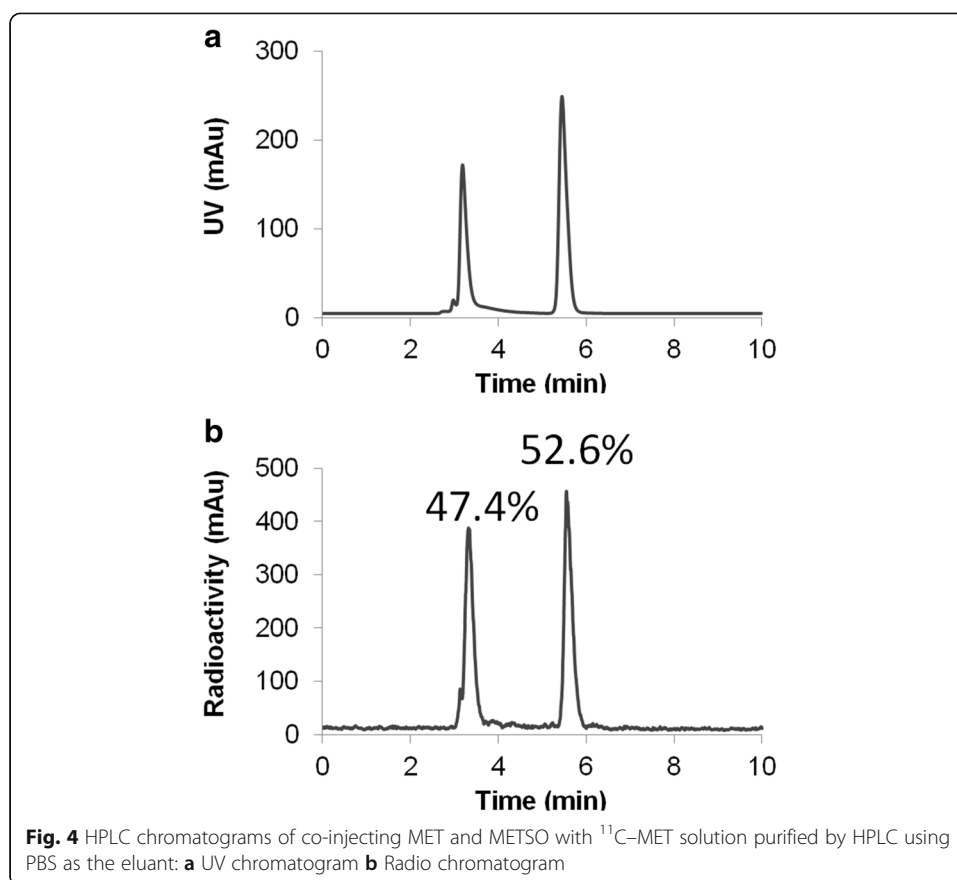


vial of the synthesis module. After mixing with the HPLC-purified  $^{11}\text{C}$ -MET eluate fraction, the mixed solution was passed through a sterile filter and checked by HPLC. As shown in Fig. 3a, the radiochemical purity of  $^{11}\text{C}$ -MET increased to  $97.5 \pm 1.5\%$  ( $n = 2$ ) at EOS with a minor radioactive by-product eluted at the same retention ( $t_R = \sim 3.3$  min) of the degraded product as shown in Fig. 2b. However, the content of this radioactive by-product still slowly increased over time even in the presence of L-homocysteine (radiochemical purity of  $^{11}\text{C}$ -MET:  $91.1 \pm 8.3\%$  at 1 h after EOS, Fig. 3b).

Next, we tried to identify the radioactive degradation product in our HPLC-purified  $^{11}\text{C}$ -MET solution. Previously,  $^{11}\text{C}$ -METSO was reported to be the degradation product of  $^{11}\text{C}$ -MET prepared with or without HPLC purification (Bogni et al. 2003). To verify this, we co-injected the degraded  $^{11}\text{C}$ -MET solution (1 h after EOS) with MET and METSO into HPLC. As shown in Fig. 4, the UV peak of METSO co-eluted with the radio peak of the radioactive by-product ( $t_R = \sim 3.3$  min), confirming  $^{11}\text{C}$ -METSO was indeed the degradation by-product of our HPLC-purified  $^{11}\text{C}$ -MET. In addition, we also conducted mass analysis for the decayed product solution, and confirmed the presence of METSO (see Additional file 1: Figure S1).

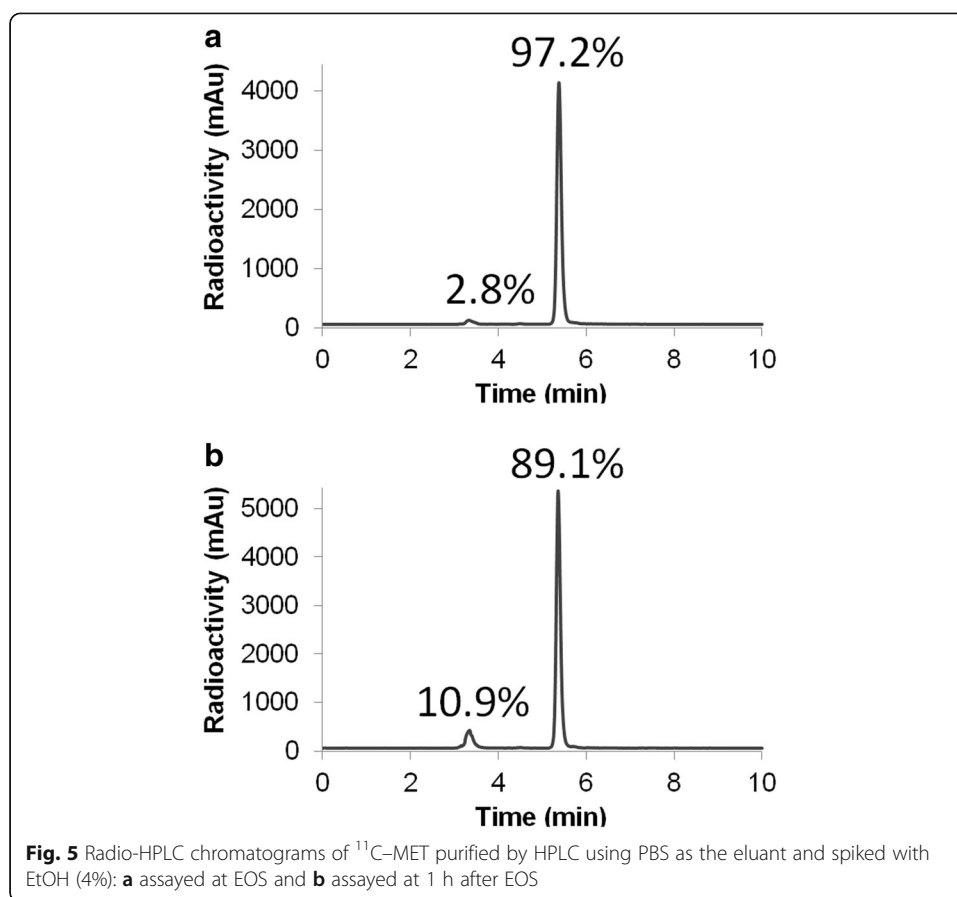
Previously, Bogni et al. suggested that  $^{11}\text{C}$ -MET prepared without HPLC purification could be stabilized by the remaining EtOH and L-homocysteine in the final product solution (Bogni et al. 2003). Fukumura et al. also demonstrated that HPLC-purified  $^{11}\text{C}$ -MET could be stabilized by the addition of EtOH (1.5%) and Tween 80 (3%) to the final product solution (Fukumura et al. 2004). Since EtOH is relatively nontoxic and readily available, we tested if EtOH alone could be used to stabilize the HPLC-purified  $^{11}\text{C}$ -MET solution.





We added EtOH (4%) immediately after the  $^{11}\text{C}$ -MET-containing HPLC eluate fraction was collected in the final product vial, and checked radiochemical purity of  $^{11}\text{C}$ -MET over time. The radiochemical purities of EtOH-containing  $^{11}\text{C}$ -MET solution were  $94.7 \pm 3.3\%$  and  $77.7 \pm 16.1\%$  ( $n = 2$ ) at EOS and 1 h after EOS, respectively. Representative HPLC chromatograms are shown in Fig. 5. These data suggest that EtOH alone is not very effective to prevent degradation of HPLC-purified  $^{11}\text{C}$ -MET solution. The previous findings by others might be due to the combination of EtOH with L-homocysteine or Tween 80 (Bogni et al. 2003; Fukumura et al. 2004).

Interestingly,  $^{11}\text{C}$ -METSO was observed in Figs. 2, 3 and 5 even at EOS, suggesting  $^{11}\text{C}$ -METSO was formed quickly after  $^{11}\text{C}$ -MET was separated from L-homocysteine during HPLC purification. To stabilize  $^{11}\text{C}$ -MET final formulation solution and minimize the formation of  $^{11}\text{C}$ -METSO even during the HPLC purification process, we added ascorbate directly into HPLC solvent. Instead of 1000 ppm tested by Fukumura et al. (Fukumura et al. 2004), we used only 100 ppm of ascorbate. The eluate fraction containing  $^{11}\text{C}$ -MET was collected and passed through a sterile filter.  $^{11}\text{C}$ -MET was obtained in  $22 \pm 3\%$  ( $n = 8$ ) decay-corrected radiochemical yield from  $^{11}\text{CH}_3\text{I}$  with a  $99.2 \pm 0.9\%$  ( $n = 8$ ) radiochemical purity at EOS. No significant degradation of  $^{11}\text{C}$ -MET solution was observed as the radiochemical purity was  $98.2 \pm 1.7\%$  ( $n = 8$ ) at 1 h after EOS (Fig. 6). These data are consistent with the observation of Fukumura et al. (Fukumura et al. 2004), and suggest that 100 ppm of ascorbate is sufficient to minimize the formation of  $^{11}\text{C}$ -METSO.



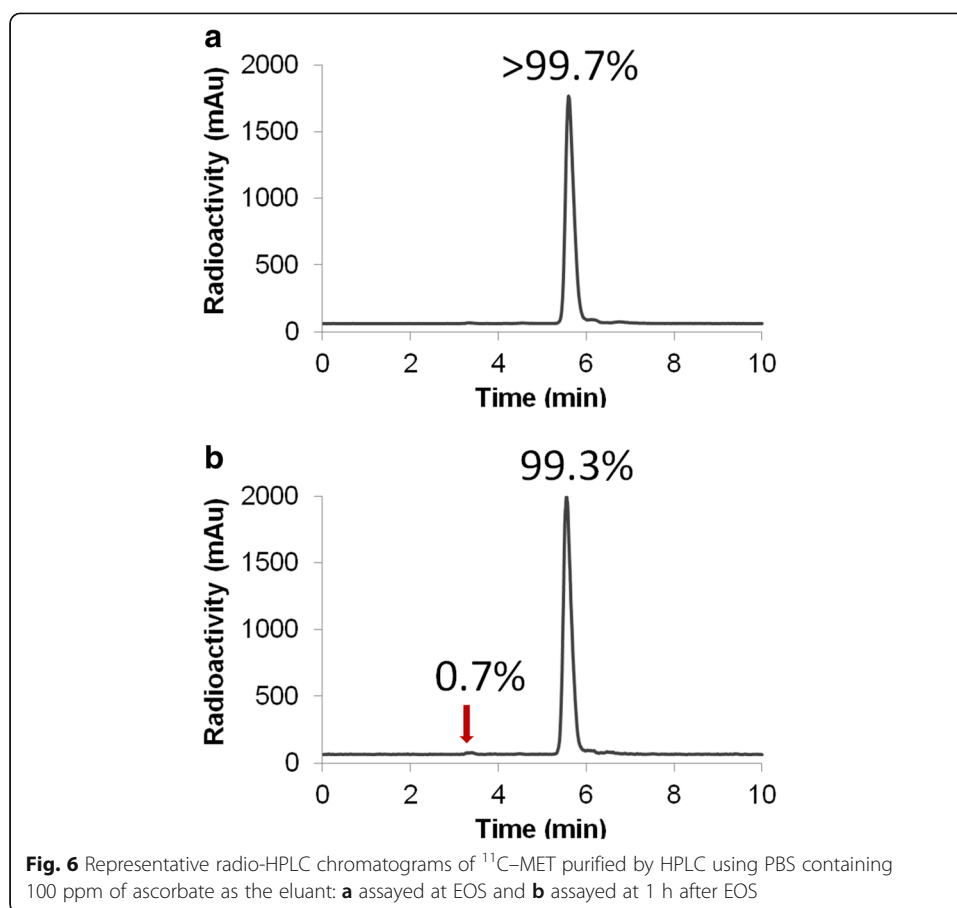
## Conclusions

We successfully verified the degradation of HPLC-purified  $^{11}\text{C}$ -MET was due to the formation of  $^{11}\text{C}$ -METS0. Presence of L-homocysteine or EtOH in the final  $^{11}\text{C}$ -MET formulation solution could slow down the degradation of  $^{11}\text{C}$ -MET. Adding ascorbate to the HPLC solvent greatly improved the radiochemical purity and stability of HPLC-purified  $^{11}\text{C}$ -MET solution. This could be very useful especially if  $^{11}\text{C}$ -MET is not used immediately after EOS. The tested concentration (100 ppm) contains only ~1.4 mg of ascorbate in the entire dose (~13.5 mL). It is safe for administration as this mass of ascorbate is much lower than the usual therapeutic parenteral dose (100–250 mg).

## Methods

### General methods

METS0 and sodium phosphate monobasic were purchased from Sigma-Aldrich (Oakville, Canada). For preparation of the QC HPLC solvent and the phosphate buffer used to elute the reaction mixture off the cartridge, sodium phosphate monobasic was diluted with water to the specified concentrations without adjusting pH. Vitamin C injection (ascorbic acid, 250 mg/mL) and sodium phosphate injection (3 mmol/mL) were purchased from Sandoz (Boucherville, Canada). Saline injection (0.9% NaCl) was purchased from Baxter (Mississauga, Canada). The semi-preparative HPLC solvents were prepared by mixing sodium phosphate injection and saline injection (with or



without vitamin C injection) to the specified concentrations. All other chemicals and solvents were obtained from commercial sources, and used without further purification. Sep-Pak tC18 Plus Short cartridges (400 mg) were obtained from Waters (Milford, MA). C-11 methane was produced by 18-MeV proton bombardment of an  $\text{N}_2/\text{H}_2$  (10%  $\text{H}_2$  in  $\text{N}_2$ ) target using an Advanced Cyclotron Systems Inc. (Richmond, Canada) TR19 cyclotron. C-11 methane was converted to C-11 methyl iodide ( $^{11}\text{CH}_3\text{I}$ ) in gas phase using a GE (Chicago, IL) TRACER FX C Pro module. Purification of  $^{11}\text{C}$ -MET was conducted using the HPLC component of the synthesis module on a Phenomenex (Torrance, CA) Aqua C18 semi-preparative column ( $5\ \mu$ ,  $250 \times 10\ \text{mm}$ ). The HPLC solvent was phosphate-buffered saline (PBS, 3.93 mM) or PBS containing 100 ppm of ascorbate, and the flow rate was 3.0 mL/min. Millex-GS 0.22  $\mu\text{m}$  sterile filter was purchased from EMD Millipore (Billerica, MA). Radioactivity was measured using a Capintec (Ramsey, NJ) CRC<sup>®</sup>-Ultra R dose calibrator. Mass analysis was performed using an AB SCIEX (Framingham, MA, USA) 4000 QTRAP mass spectrometer system with an ESI ion source.

#### Synthesis and purification of $^{11}\text{C}$ -MET

The tC18 Sep-Pak cartridge was preconditioned with EtOH (5 mL) and sterile water (10 mL). The remaining water in the cartridge was pushed out with air (10 mL). Five minutes before EOS, 85  $\mu\text{L}$  of L-homocysteine thiolactone hydrochloride aqueous

solution (25 mg in 600  $\mu$ L water) was mixed with 200  $\mu$ L of NaOH solution (0.7 mL of 10 N NaOH aqueous solution diluted with 4.3 mL water and 5.0 mL EtOH). From this, 200  $\mu$ L of the mixed solution was loaded to the tC18 Sep-Pak cartridge. After passing  $^{11}\text{CH}_3\text{I}$  by helium (15 mL/min) through the tC18 Sep-Pak cartridge, the reaction mixture was eluted off the cartridge with phosphate buffer (50 mM, 2 mL) and purified by HPLC. The eluate fraction ( $\sim$  1.5 mL) containing  $^{11}\text{C}$ -MET was collected, diluted with HPLC eluant (12 mL), and passed through a Millex-GS sterile filter into a final product vial.

### Quality control of $^{11}\text{C}$ -MET

Chemical purity, radiochemical purity and radiochemical identity of  $^{11}\text{C}$ -MET and by-products were determined using an Agilent (Santa Clara, CA) HPLC system equipped with a model 1200 quaternary pump, a model 1200 UV absorbance detector (set at 220 nm), and a Bioscan (Washington, DC) NaI scintillation detector. The operation of the Agilent HPLC system was controlled using the Agilent ChemStation software. The HPLC column used was a Phenomenex Luna C18 analytical column (5  $\mu$ , 250  $\times$  4.6 mm). The HPLC solvent was phosphate buffer (1 mM, pH 3), and the flow rate was 1.0 mL/min.

### Additional file

**Additional file 1: Figure S1.** Identification of METSO from decayed  $^{11}\text{C}$ -MET solution. (DOCX 260 kb)

### Abbreviations

$^{11}\text{CH}_3\text{I}$ : Carbon-11 labeled methyl iodide;  $^{11}\text{C}$ -MET: Carbon-11 labeled L-methionine;  $^{11}\text{C}$ -METSO: Carbon-11 labeled methionine sulfoxide; EOS: End of synthesis; EtOH: Ethanol; HPLC: High performance liquid chromatography; NaOH: Sodium hydroxide; PBS: Phosphate-buffered saline; PET: Positron emission tomography; ppm: Part per million;  $t_R$ : Retention time; UV: Ultraviolet light

### Acknowledgments

This work was supported by the Canadian Institutes of Health Research (FDN-148465) and the Leading Edge Endowment Fund.

### Authors' contributions

MW, DW, FB and KSL conceived and designed the complete study. MW, LL, KF, JGG, ZZ, CZ and WE conducted the experiments. MW, LL, ZZ, CZ and KSL summarized and interpreted the data. The manuscript was drafted by KSL with critical revisions from MW, LL, KF, JGG, ZZ, CZ, and WE. All authors read and approved the final manuscript.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

### Author details

<sup>1</sup>Department of Functional Imaging, BC Cancer Agency, Vancouver, BC, Canada. <sup>2</sup>Department of Molecular Oncology, BC Cancer Agency, Vancouver, BC, Canada. <sup>3</sup>Department of Radiology, University of British Columbia, Vancouver, BC, Canada.

Received: 30 July 2017 Accepted: 11 September 2017

Published online: 04 October 2017

### References

- Bogni A, Bombardieri E, Iwata R, Cadini L, Pascali C. Stability of L-[S-methyl- $^{11}\text{C}$ ]methionine solutions. *J Radioanal Nucl Chem.* 2003;256:199–203.
- Boschi S, Lodi F, Cicoria G, Ledesma JR, Knopp R, Rizzello A, Di Pierro D, Trespidi S, Marengo M. Development of a modular system for the synthesis of PET [ $^{11}\text{C}$ ]labelled radiopharmaceuticals. *Appl Radiat Isot.* 2009;67:1869–73.
- Cheung M-K, Ho C-L. A simple, versatile, low-cost and remotely operated apparatus for [ $^{11}\text{C}$ ]acetate, [ $^{11}\text{C}$ ]choline, [ $^{11}\text{C}$ ]methionine and [ $^{11}\text{C}$ ]PIB synthesis. *Appl Radiat Isot.* 2009;67:581–9.



- Fukumura T, Nakao R, Yamaguchi M, Suzuki K. Stability of  $^{11}\text{C}$ -labeled PET radiopharmaceuticals. *Appl Radiat Isot.* 2004; 61:1279–87.
- Glaudemans AWJM, Enting RH, Heesters MAAM, Dierckx RAJO, van Rheenen RWJ, Walenkamp AME, Slart RHJA. Value of  $^{11}\text{C}$ -methionine PET in imaging brain tumours and metastases. *Eur J Nucl Med Mol Imaging.* 2013;40:615–35.
- Gomzina NA, Kuznetsova OF. *L*-[Methyl- $^{11}\text{C}$ ]-methionine of high enantiomeric purity production via *online*- $^{11}\text{C}$ -methylation of *L*-homocysteine thiolactone hydrochloride. *Russ J Bioorg Chem.* 2011;37:191–7.
- Lodi F, Rizzello A, Trespidi S, Di Pierro D, Marengo M, Farsad M, Fanti S, Al-Nahhas A, Rubello D, Boschi S. Reliability and reproducibility of *N*-[ $^{11}\text{C}$ ]methyl-choline and *L*-[*S*-methyl- $^{11}\text{C}$ ]methionine solid-phase synthesis: a useful and suitable method in clinical practice. *Nucl Med Commun.* 2008;29:736–40.
- Lodi F, Trespidi S, Di Pierro D, Marengo M, Farsad M, Fanti S, Franchi R, Boschi S. A simple Tracerlab module modification for automated on-column [ $^{11}\text{C}$ ]methylation and [ $^{11}\text{C}$ ]carboxylation. *Appl Radiat Isot.* 2007;65:691–5.
- Maffione AM, Nanni C, Ambrosini V, Trespidi S, Lopci E, Allegri V, Castellucci P, Montini G, Boschi S, Fanti S.  $^{11}\text{C}$ -Methionine PET/CT in central nervous system tumours: a review. *Curr Radiopharma.* 2009;2:160–4.
- Nagren K, Halldin C. Methylation of amide and thiol functions with [ $^{11}\text{C}$ ]methyl triflate, as exemplified by [ $^{11}\text{C}$ ]NMSP, [ $^{11}\text{C}$ ]flumazenil and [ $^{11}\text{C}$ ]methionine. *J Label Compd Radiopharm.* 1998;41:831–41.
- Oh S-J, Choe YS, Kim YS, Choi Y, Kim SE, Lee KH, Ha H-J, Kim B-T. Development of an automated system for the routine preparation of carbon-11 labeled radiopharmaceuticals. *Bull Kor Chem Soc.* 1998;19:952–6.
- Pascali C, Bogni A, Cucchi C, Laera L, Crispu O, Maiocchi G, Crippa F, Bombardieri E. Detection of additional impurities in the UV-chromatogram of *L*-[*S*-methyl- $^{11}\text{C}$ ]methionine. *J Radioanal Nucl Chem.* 2011;288:405–9.
- Pascali C, Bogni A, Iwata R, Decise D, Crippa F, Bombardieri E. High efficiency preparation of *L*-[*S*-methyl- $^{11}\text{C}$ ]methionine by on-column [ $^{11}\text{C}$ ]methylation on C18 sep-Pak. *J Label Compd Radiopharm.* 1999;42:715–24.
- Quincoces G, Lopez-Sanchez L, Sanchez-Martinez M, Rodriguez-Fraile M, Penuelas I. Design and performance evaluation of single-use whole-sterile “plug & play” kits for routine automated production of [ $^{11}\text{C}$ ]choline and [ $^{11}\text{C}$ ]methionine with radiopharmaceutical quality. *Appl Radiat Isot.* 2010;68:2298–301.
- Tang G-H, Wang M-F, Tang X-L, Luo L, Gan M-Q. Automated synthesis of (*S*-[ $^{11}\text{C}$ -methyl]-*L*-methionine and (*S*-[ $^{11}\text{C}$ -methyl]-*L*-cysteine by on-column [ $^{11}\text{C}$ ]methylation. *J Nucl Radiochem.* 2004;26:77–83.
- Watanabe A, Muragaki Y, Maruyama T, Shinoda J, Okada Y. Usefulness of  $^{11}\text{C}$ -methionine positron emission tomography for treatment-decision making in cases of non-enhancing glioma-like brain lesions. *J Neuro-Oncol.* 2016;126:577–83.

Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](http://springeropen.com)

---