

# Improved method for the quality assurance of [C-11]choline

E. Mishani\*, I. Ben-David, Y. Rozen

Hebrew University, Hadassah Hospital, Department of Medical Biophysics and Nuclear Medicine, Il-91120, Jerusalem, Israel

Received 9 September 2001; received in revised form 11 November 2001; accepted 19 November 2001

## Abstract

[C-11]choline is a very promising radiomarker for the diagnosis of various human tumors using Positron Emission Tomography (PET) [1]. The existing quality control process for [C-11]choline is complicated and combines two HPLC methods with limited separation and sensitivity which prevent the accurate determination of the specific activity. We have developed a new efficient single HPLC method for the detection of choline chloride and dimethylaminoethanol with high resolution and sensitivity using cation-exchange chromatography. © 2002 Elsevier Science Inc. All rights reserved.

*Keywords:* Choline chloride; Dimethylaminoethanol; Cation-exchange chromatography; Quality control; Carbon-11

## 1. Introduction

Choline labeled with Carbon-11 is a promising biomarker for tumor imaging using PET. It has been shown that there is an increased synthesis of membranal phosphatidylcholine in tumor cells that is correlated with high uptake of this radiopharmaceutical in malignant tissues. It is particularly effective in imaging tumors localized in the brain, lung, esophagus, rectum, prostate and urinary bladder [2,3]. In contrast to [F-18]fluorodeoxyglucose (FDG), the uptake of [C-11]choline in benign structures such as the normal brain, heart and urinary tract is negligible, resulting in a higher target to background signal ratio for tumors located near those benign structures [4].

[C-11]Choline is prepared by a [C-11]methylation reaction of dimethyl amino ethanol (DMAE) using labeled methyl iodide (MeI), and a simple sep-pak purification step [5,6]. It is therefore very important to determine accurately the quantity of DMAE in the final product. At the present time the most common method for the determination of the purity of [C-11]Choline in the final product solution is by two separate reverse phase high performance liquid chromatography (HPLC) methods. This quality control process is rather complex and the limited separation efficiency prevents an accurate determination of the specific activity. We report here a new method for the quality control of

[C-11]Choline and dimethylaminoethanol by cation-exchange chromatography on a single HPLC system with high sensitivity and resolution.

## 2. Materials and methods

### 2.1. HPLC equipment

This study was performed with the following HPLC equipment: *pump*, Varian solvent delivery system 9012Q; *radiodetector*, PMT/scintillator Bioscan flow count; *refractometer* (Refractive Index, RI), Varian star 9040; *UV detector*, Varian variable wavelength UV-VIS 9050 operated at 210 nm; *conductivity detector*, Alltach 550; *Columns*, Inertsil ODS-2 250\*6 mm (Alltec Associates Inc., Deerfield, IL 60015), Astec reversed-phase column 250\*4.6 mm (Astec Advanced Separation Technologies Inc., Whippany, NY 07981, USA), Cation exchange IC-PAK™ Cation M/D 150\*3.9 mm (Waters Corporation, Milford, Massachusetts).

### 2.2. Standard solutions

Choline chloride (Across, Geel, Belgium), and Dimethylaminoethanol (Aldrich, Milwaukee, WI) standards were prepared in saline solutions by serial dilution of 1.0 mg/mL stock solutions. Choline chloride and dimethylaminoethanol standards of 100, 50, 10, 5, 2, 1  $\mu$ g/mL (ppm) were used in this study.

\* Corresponding author.

E-mail address: mishani@md2.huji.ac.il (E. Mishani).

Table 1  
Cation exchange chromatography of standard solution of choline chloride and DMAE

Eluent	% CH <sub>3</sub> CN	Flow (mL/Min)	Rt <sub>0</sub> (min) NaCl	DMAE				Choline				Rs
				μg/mL	Rt min	W <sub>1/2</sub> (sec)	K'	μg/mL	Rt min	W <sub>1/2</sub> (sec)	K'	
2.6mM HNO <sub>3</sub>	—	1	6.4	10	10.6	37.5	0.65	9	17.7	162	1.76	2.5
3.5mM HNO <sub>3</sub>	—	1	4.9	10	8	26	0.62	9	13.3	62	1.7	4.3
<b>4mM HNO<sub>3</sub></b>	—	<b>1</b>	<b>4.5</b>	<b>10</b>	<b>7.3</b>	<b>25</b>	<b>0.62</b>	<b>9</b>	<b>12</b>	<b>57</b>	<b>1.64</b>	<b>4</b>
4.5mM HNO <sub>3</sub>	—	1	4.2	10	6.7	24	0.61	9	10.8	52	1.6	3.8
4.5mM HNO <sub>3</sub>	—	1.1	3.8	10	6	22	0.6	9	9.8	49	1.6	3.8
4.5mM HNO <sub>3</sub>	—	1.4	2.9	10	4.7	18	0.6	9	7.6	38	1.6	3.7
4.5mM HNO <sub>3</sub>	2	0.9	4.6	10	6	16.1	0.3	61	8.8	37	0.9	3.6
4.5mM HNO <sub>3</sub>	2	0.9	—	—	—	—	—	30	8.8	36	0.9	3.6
5mM HNO <sub>3</sub>	—	1	3.8	10	6.1	19.7	0.6	9	9.9	50.8	1.6	3.8
<b>5mM HCl</b>	—	<b>1</b>	<b>3.8</b>	<b>10</b>	<b>5.6</b>	<b>16.3</b>	<b>0.5</b>	<b>9</b>	<b>9.1</b>	<b>40</b>	<b>1.4</b>	<b>4.4</b>
5mM HCl	—	1.1	3.5	10	5.4	16.6	0.5	9	8.4	38	1.42	4
5mM HCl	—	1.1	—	—	—	—	—	2	8.4	36	1.42	4
5mM HCl	—	1.1	—	—	—	—	—	1	8.4	36	1.42	4

### 2.3. HPLC eluents

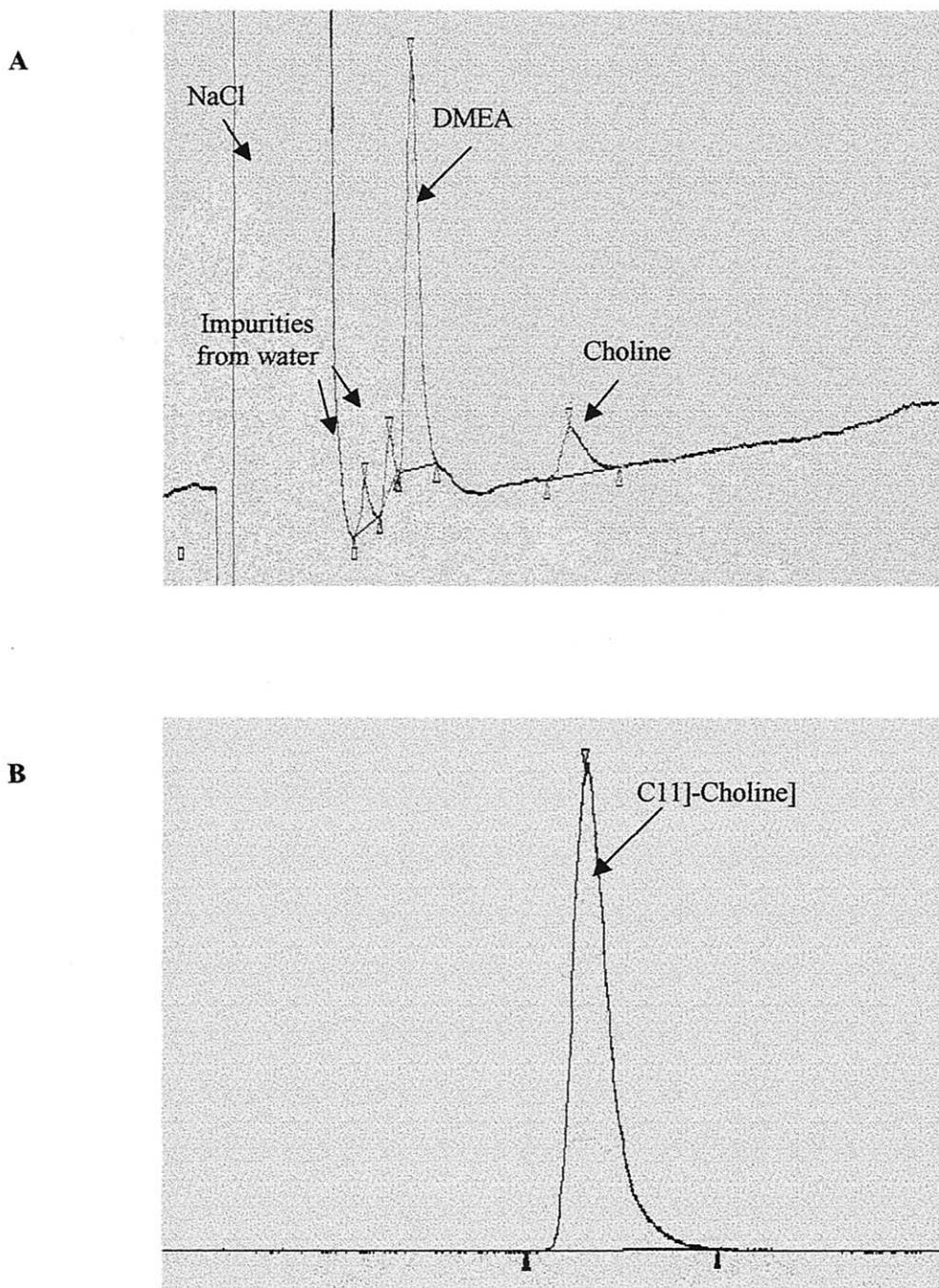
For the analysis of choline chloride (Inertsil ODS-2 column, RI detector) and DMAE (Astec reversed-phase column, UV detector) by the previously known method, the following eluents were used: **A** - 1 mM naphthalene-2-sulfonic acid/0.05 M 85% H<sub>3</sub>PO<sub>4</sub>. **B** - 0.05 M sodium borate/0.1 M NaOH 45/55, pH = 12. For the analysis of choline chloride and DMAE using the cation exchange IC-PAK<sup>TM</sup> column (conductivity detector), the following eluents were used: **C** - mixtures of nitric acid at different concentrations (pH = 2.5), and acetonitrile (Table 1). **D** - 100% 5 mM HCl (pH = 2.5).

### 3. Results

As a control method, we used the existing quality control process for [C-11]choline, eluting an Inertsil ODS-2 column with solvent system A, at a flow rate of 1 ml/min. The mass was detected by a refractometer (RI), and radioactivity was determined using a flow counts detector. The results of the injection of the final solution of [C-11]choline using this method showed a single peak at the radiochromatogram with retention time of 9.0 minutes corresponding to [C-11]choline. The cold mass peaks at the RI chromatogram at 8.2 minutes and 8.0 minutes, corresponded to choline chloride and dimethylaminoethanol respectively. The poor separation between the mass peaks interfered with the accurate calculation of the quantities of each component, and the specific activity of [C-11]choline could only be estimated. As suggested by the group from Groningen University Hospital, in order to determine accurately the quantity of DMAE in the final product a second HPLC method was performed as follows: Astec Reversed-phase column (250\*4.6 mm) was eluted with solvent system B (pH 12), at flow rate of 0.8 ml/min. The peak of dimethylaminoethanol was observed using a UV detector operated at 210 nm with

a retention time of 8.8 minutes. Choline chloride, emerging with the solvent front, could not be detected by the UV detector.

The results of the injections of the standards solutions of choline and DMAE using the cation exchange column and the conductivity detector are summarized in Table 1. The characteristics of different eluents at different concentration of the competing ion and content of organic solvent additives at different flow rates are shown. The separation of choline chloride and dimethylaminoethanol peaks was expressed in terms of the resolution (Rs). When HPLC separations are carried out correctly, individual bands will closely resemble a Gaussian curve. Average baseline peakwidth (W), can be measured by drawing a line parallel to the baseline between points on the curve with values that are 13.4% of the peak height. Since a band closely resemble a Gaussian curve, bandwidth at half height W<sub>1/2</sub> equals 0.588W. Resolution (Rs) is equal to the distance between the peak centers divided by the average baseline peakwidth (W),  $Rs = 2(Rt_1 - Rt_2)/(W_1 + W_2)$  (Rt<sub>1</sub> and Rt<sub>2</sub> are the retention times of the first and second adjacent bands and W<sub>1</sub> and W<sub>2</sub> are their baseline bandwidths). It can also be calculated from the retention time and bandwidth at half height of each peak ( $Rs = 1.18 \times (Rt_2 - Rt_1) / (W_{1/2,1} + W_{1/2,2})$ ). To increase resolution, the two bands must either be moved farther apart, or bandwidth reduced. It should be noted that these relationships are principally valid for Gaussian peaks, but can practically be applied also for non-symmetrical peaks. The retention of each component was also expressed in terms of its capacity factor K' [ $K' = (Rt - R_0)/R_0$ ; R<sub>0</sub> = solvent of the sample (NaCl)]. The sensitivity (detection limit of choline) for each eluent was also determined. Since the best results were obtained with HCl as eluent, injections of the final solution of [C-11]choline was performed with this mobile phase. The chromatograms are presented in Scheme 1. A single peak was observed in the radiochromatogram with Rt of 9.2 minutes which corresponded to [C-11]choline. The peaks of DMAE and choline chloride were clearly observed



Scheme 1. Chromatogram of [C-11]choline solution using cation exchange column and 100% 5mM HCl as mobile phase (A, Mass; B, Radioactivity)

in the conductivity chromatogram and concentrations of 7 ppm and 3.2 ppm respectively (based on calibration curves) and specific activity of 2.5 Ci/ $\mu$ mol (EOB) were easily calculated.

#### 4. Discussion

The existing quality control process of [C-11]choline is complex and combines two HPLC methods with limited

separation and low sensitivity (RI detector), which prevent the accurate determination of the specific activity. As part of our current research interest, we were looking for a better and faster quality assurance process for [C-11]choline, based on a single HPLC method, with higher resolution and sensitivity, which would commensurate with the time limitation imposed by the use of short-lived radioisotope. Since the final solution of [C-11]choline contains only two major mass components both in the form of ammonium salts (choline chloride, and hydrochloride salt of DMAE) we

turned our attention to ion exchange chromatography. Two mobile phases were tested under different conditions i.e. concentration of the competing ion and content of organic solvent additives, flow rate, pH, and concentration of standards. Optimal results were examined in terms of high capacity factor ( $K'$ ), high resolution ( $R_s$ ), low retention time, and high detection limit. Starting with 100% 2.6mM  $\text{HNO}_3$  as mobile phase at flow rate of 1 mL/min the retention time of choline was too long (17.7 min) with broad peak shape ( $W_{1/2} = 162$  sec) (Table 1). Increasing the concentration of  $\text{HNO}_3$  to 3 mM dramatically decreased the width of the choline peak to  $W_{1/2} = 69$  sec., however, the retention time was still long. Increasing further the concentration of  $\text{HNO}_3$  to 4.5 mM and increasing the flow to 1.4 mL/min reduced the retention time and  $W_{1/2}$  of the peak of choline to 7.6 min. and 38 sec. respectively. In that system,  $K'$  of DMAE was too low (0.6) and the resolution dropped down from 4.5 to 3.7. Addition of acetonitrile at concentration of 2% gave low capacity factor for DMAE, lower resolution of 3.6 and low sensitivity (30 ppm limit of detection for choline). The best results with  $\text{HNO}_3$  as mobile phase were obtained at concentration of 4mM at flow rate of 1 mL/min. (see Table 1). However, the peakwidth of choline was still large and limited the detection of choline to 10 ppm. The detection limit of DMAE was sufficiently high (< 1 ppm) since the average quantity of this component in the final solution of [C-11]choline is 7 ppm. Best results were obtained with 5mM HCl as mobile phase. The optimal retention times of the solvent and the two components lead to good resolution of 4.4 with high detection limit for choline of 1 ppm. The final solution of [C-11]choline was injected under these conditions and, under the regular conditions that were used in the past (using two HPLC systems). The specific activity, which could not be determine under the pervious method could be calculated from the results of the new method and found to be  $2.5\text{Ci}/\mu\text{mol}$ . The quantity of DMAE that was found with the new method was

6.9 ppm with excellent agreement with the results from the previous method.

## 5. Conclusion

We have developed a simple and reliable quality assurance process for [C-11]choline based on a cation exchange chromatography. This single HPLC method affords higher resolution and sensitivity and commensurate with the time limitation imposed by the use of short-lived radioisotope. Specific activity of [C-11]choline and DMAE quantities could accurately be determined after establishing the appropriate calibration curves.

## Acknowledgments

The authors thank Professor W. Vaalburg for helping us to initiate the research with [C-11]choline in our institution.

## References

- [1] T.H. Que, J. Pruijm, H.M. Zweers, H.J. Mensink, W. Vaalburg, Imaging of bladder cancer using C-11 choline Positron Emission Tomography, *J. Nuc. Med.* 41 (Suppl) (2000) 74.
- [2] T.H. Que, J.T.M. Plukker, W. Vaalburg, Staging of gastroesophageal cancer with PET using C-11 choline and F-18 FDG, *J. Nucl. Med.* 41 (Suppl) (2000) 141.
- [3] T. Hara, N. Kosaka, N. Shinoura, T. Kondo, PET imaging of brain tumor with [methyl-11C]choline, *J. Nucl. Med.* 38 (1997a) 842.
- [4] T. Hara, N. Kosaka, T. Kondo, H. Kishi, O. Kobori, Imaging of brain tumor, lung cancer, esophagus cancer, colon cancer, prostate cancer and bladder cancer with [methyl-11C]choline, *J. Nucl. Med.* 38 (1997b) 250.
- [5] N. Shinoura, et al. Brain tumors: detection with C-11 choline PET, *Radiology* 202 (2) (1997) 497–503.
- [6] E. Mishani, M. Bocher, I. Ben-David, Y. Rozen, D. Laky, R. Chisin, [C-11]Choline-Automated preparation and clinical utilization. *J. Labelled Cpd. Radiopharm.* 44 (Suppl 1) (2001) S379.