Nat. Volatiles & Essent. Oils, 2021; 8(4): 7309-7319

Manufacture Of Full Automatic Synthesizer To Prepare ^{18}f-Fch Radio Pharmaceutical

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Abstract:

Introduction: ^{18}F-Fluoromethylcholine ([^{18}F]FCH) Pharmaceuticals is used in PET/CT scans to diagnose prostate cancer and hepatocellular carcinoma. Due to lack of automatic synthesizer then the [^{18}F]FCH production is not yet performed in Vietnam. This study aims to prepare [^{18}F]FCH radiopharmaceuticals to fulfill the demand of pre-clinical studies and routine clinical practice. The objective of the study is to design and manufacture a full automatic synthesizer to produce [^{18}F]FCH.

Method: Automatic synthesizers are designed and manufactured to produce [^{18}F]FCH in 8 steps with the general principle as follows: Dibromomethane 10% in Acetonitrile is fluorinated to produce Fluorobromomethane which reacts with precursor Dimethylaminethanol 10% in Dimethyl Sulfoxide preloaded on Sep-Pak C18 Plus Short cartridge to produce ^{18}F-FCH. The obtained ^{18}F-FCH was purified by solid-phase extraction (SPE) using Sep-Pak CM Plus Short cartridge. The final product is the [^{18}F]FCH in 5 ml Saline.

Results: The automatic synthesizer is easy to use and stable in operation. All commands control the 8 steps of the synthesis process are performed accuracy 100%. Test for [^{18}F]FCH synthesis using this synthesizer confirmed that total time of the synthesis of a [^{18}F]FCH batch is 50 ± 5 min; synthesis yield is 11.7± 0.2% with no decay correction and radiochemical purity is more than 99,9% that meet the requirements of European Pharmacopoeia 2017.

Conclusion: The full automatic synthesizer could supply a sufficient amount of [^{18}F]FCH for both of pre-clinical studies and clinical practice, especially for early detection of prostate cancer and hepatocellular carcinoma in Vietnam.

Key words: Automatic synthesizer,[^{18}F]FCH, PET/CT, Prostate cancer, hepatocellular carcinoma

1. Introduction

Choline is a quaternary ammonium alkali belonging to group of vitamin B, as a precursor for the biosynthesis of phospholipids, which are essential components of all membranes. Choline enters

most cells using specific low affinity, sodium-independent transporters. The uptake of choline would reflect the proliferative activity of membrane lipid synthesis. Tumor cells with high proliferation rate, will have high uptake of choline to keep up with increased demands for the synthesis of phospholipids[1]. The radionuclide labeled choline analogs such as ^{18}F-Fluoroethylcholine ([^{18}F]FEC) and ^{18}F-Fluoromethylcholine ([^{18}F]FCH) were synthesized and successfully applied as Positron Emission Tomography (PET) imaging agents for detecting various types of tumors[2,3,4,5,6,7]. Compared to other tumor imaging agents, [^{18}F] FCH has been shown to be better than [^{18}F] FDG for prostate cancer (PC)[8,9,10] and hepatocellular carcinoma detections[4,11].

In Vietnam, we have many patients suffer from HCC and PC which leads to make a high demand of [^{18}F]FCH/ PET imaging to diagnose these diseases. In order to fulfill the demand of [^{18}F]FCH for detecting HCC, PC and other tumors, it is necessary to have a simple, reliable and yet high yield production method available for the synthesis of [^{18}F]FCH.

At present, Vietnam has four of cyclotron centre producing [^{18}F]FDG but not any among them produce [^{18}F]FCH because the lack of automatic synthesizer. Therefore, [^{18}F]FCH has not been produced in Vietnam. This study introduces the research on designing and manufacturing a full automatic synthesizer to produce [^{18}F]FCH for PET / CT diagnosis; especially for early detection of prostate cancer and hepatocellular carcinoma.

[^{18}F]FCH was first synthesized manually by DeGrado et al. 2001. Up to now, [^{18}F]FCH have been synthesized automatically using [^{18}F] FDG commercial modules modified such as TracerLab MX-FDG, TracerLab FX-FN, Synthera…[12,13,14,15,16]. At present, automated synthesis of [^{18}F] FCH using on-column reaction of [^{18}F] FBM with DMAE is the popular, simple, convenient method[14]. Thus, we also use this method with a home-made full automatic synthesizer for the synthesis of [^{18}F] FCH. The synthesizer to produce [^{18}F] FCH includes programming software to control hardware through RS232-COM communication standard.

1. Material and methods

1.1. Chemicals and equipments

Chemicals and cassette for automatic synthesis of [^{18}F] FCH consist of a [^{18}F]Choline Reagent Kit for GE TracerLab MX Synthesizer with Product No. K-623TM, and a [^{18}F]Choline Cassette with Product No. K-628TM from ABX Advanced Biochemical Compounds. Choline Chloride, Fluoromethylcholine for quality control of [^{18}F] FCH were also purchased from ABX (Germany). Naphthalene-2-sulfonic

acid >99% and Phosphoric acid >99,99% (HPLC grade) from Merck. $H_2{}^{18}O$ with enriched >98% Oxygen-18 were purchased from Rotem Industries (Israel).

Equipments for quality control of [^{18}F]FCH include: The HPLC system 1200 (Agilent) was equipped with a reflex index detector (RI) and flow count radioactivity detector and Inertil ODS-2 6 x 250mm column, 5µm Particle size (GL Sciences). The GC system 6850 (Agilent); the radio-TLC and Gabi Half Time system (Raytest, Germany).

1.2. Manufacture of full automatic synthesizer

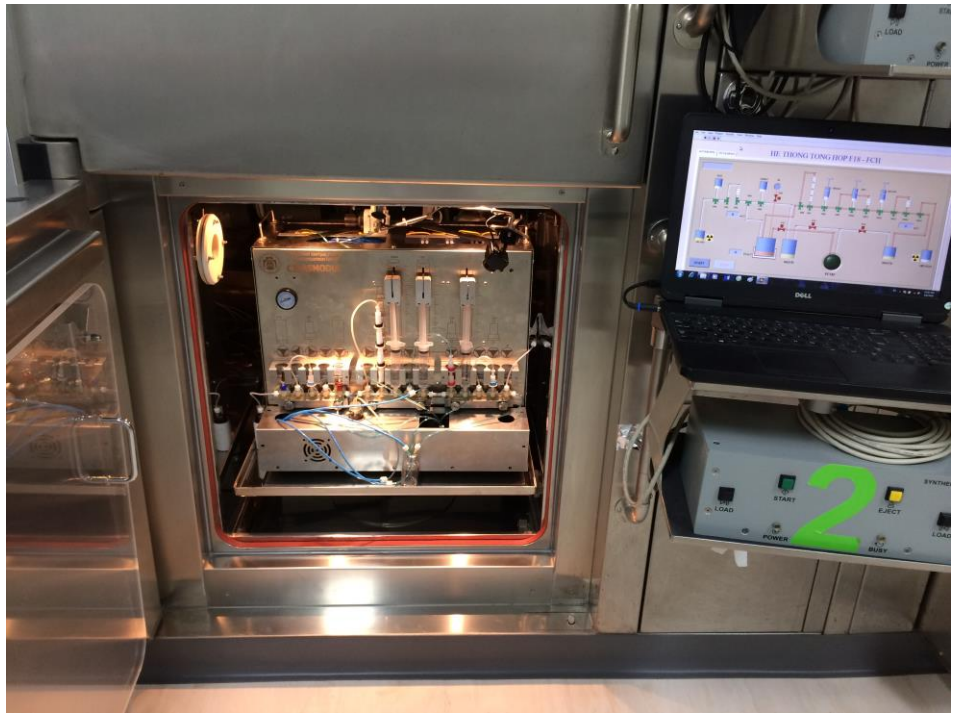

Figure 1. Home-made full automatic synthesizer for [^{18}F]FCH production

We design the synthesizer which could install with available hotcell in our facility (Fig.1). The synthesizer is manufactured to include a communication circuit board connected to computer, a circuit board controlling 19 motors to close - open 19 vales; a circuit to control a vacuum pump; a temperature control, monitoring circuit of the reaction process, a helium flow control circuit, and a radiation measurement circuit. The personal computer placed outside of the hotcell is installed with programming software using Labview tool to control and monitor the synthesizer through RS232-COM communication standard. The control software interface of the [^{18}F]FCH automatic synthesis is shown in figure 2.

1.3. Steps of automatic synthesis of [^{18}F]FCH

The automatic synthesis of the [^{18}F]FCH to include 8 steps is carried out through open-close control of the 19-valves on the synthesizer with control interface as shown in figure 2. The synthesis is continuously recorded using temperature and radiation sensors. The recorded data is presented graphically in figure 3.

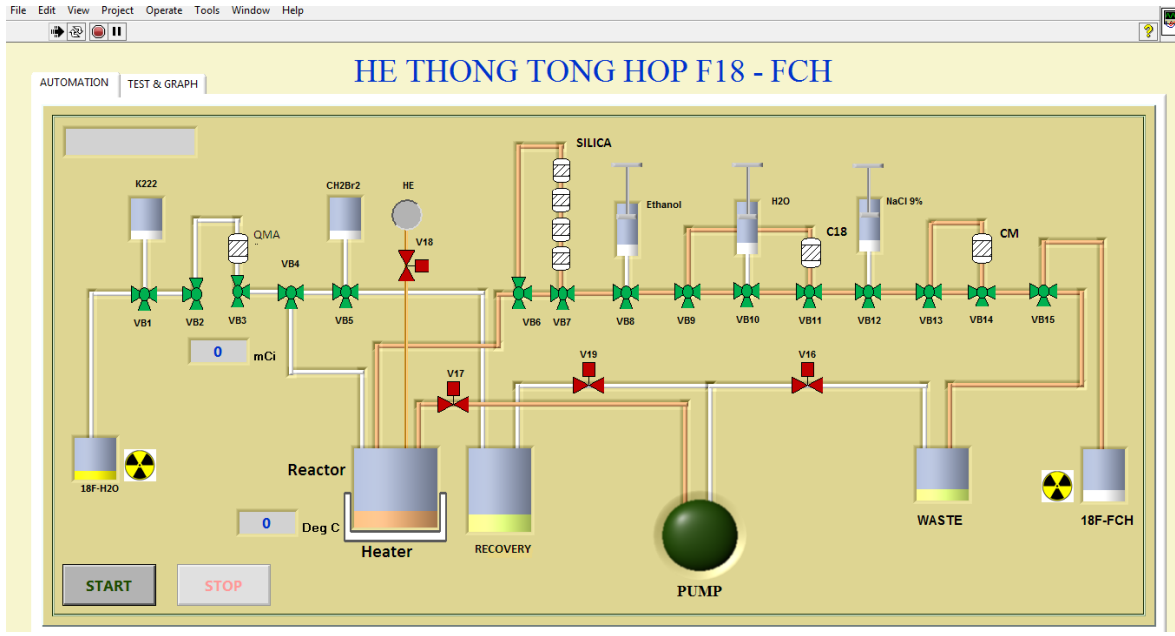

Figure 2. Control software interface of the [^{18}F]FCH automatic synthesis

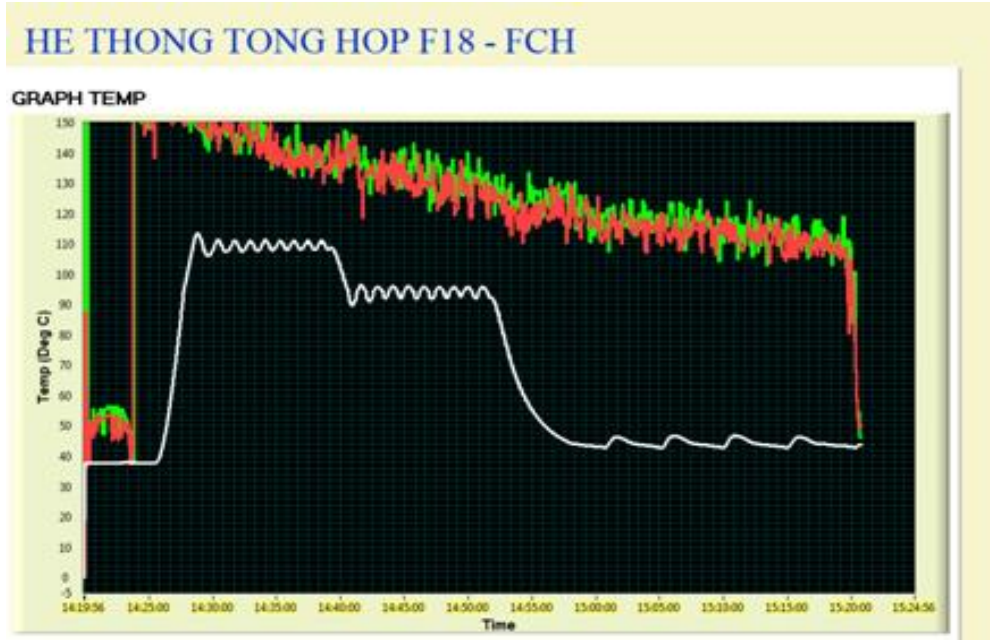

Figure 3. Temperature and Radioactivity diagram of [^{18}F]FCH synthesis process

Steps 1 and 2 take place at room temperature. The radioactivity at QMA cartridge in step 1 has a pulse pattern that increases rapidly from 0 to 231.75 mCi (figure 4 a) and decreases rapidly to 14.95 mCi at the end step 2 (figure 4 b). Duration time of the each steps were fixed for 2min. Base on the equation 1, the uptake yield of Fluorine-18 on QMA were determined (n=3) to be more than 90% and tabulated in table 1.

Y_uptake, % = 100 x (A_absorp - A_elut)/ A_absorp (1)

In which: Y_uptake,% is the uptake yield of Fluorine-18 on QMA cartridge; A_absorp is the radioactivity on QMA cartridge at the end of step 1, mCi, A_elut is the radioactivity on QMA cartridge at the end of step 2, mCi.

Temperature of the reaction vessel was controlled at 110±2^{0}C for 3 minutes then 95±2^{0}C for 5 minutes and then 40±2^{0}C for 11 minutes in steps 3, 4 and 5 respectively. In these steps, radioactivity decreases by natural decay. The radioactivity rapidly decreases in step 8 due to [^{18}F]FCH solution is transferred to a product vial placed in a single dose dispense hotcell. According to equation 2 and 3, synthesis yield with non decay correction is determined to be 11.7% and synthesis yield with decay correction is 15% average(n=3) presented in table 2.

Y_nondecay,% = 100 x (A_FCH/37)/(A_absorp x Y_uptake/100) (2)

Y_correction,% = 100 x(A_FCH/37)/[(A_absorp x Y_uptake/100)x(1/2)$^{t/109,77}$](3)

In which: Y_nondecay(%) is the synthesis yield with non decay correction; Y_ correction(%) is the synthesis yield with decay correction; A_FCH(MBq) is radioactivity of the final product at the end of step 8 and t(min) is total time of the synthesis of a [^{18}F]FCH batch.

Figure 4. Control interface at the end of step 1(a) and step 2(b)

Table 1. Uptake yield of [^{18}F] on QMA cartridge.

Batches	A_absorp,mCi	A_elut,mCi	Y_uptake,%
1	230,74	29,05	87,41

2	231,75	14,95	93,5
3	305,44	29,23	90,43
Average of the Y_uptake			90±3%

Table 2. Synthesis yield of [^{18}F]FCH

batch	A_absorp, mCi	Y_uptake, %	A_FCH MBq	t, synthesis time, mine	Y_nondecay, %	Y_correction, %
1	230,74	87,41	250,8	40	3,36	4,33
2	231,75	93,5	1550,68	38	19,34	24,59
3	305,44	90,43	1265,98	42	12,39	16,15
Average synthesis yield of ^{18}F-FCH					11,70	15,02

1.4. Quality control of [^{18}F]FCH

The QC test results of [^{18}F]FCH were presented in Table 3. The quality of [^{18}F]FCH synthesized by using this method met the requirements of European Pharmacopoeia 2017: The appearance of the [^{18}F]FCH solution was clear; The HPLC retention time of standard sample contained Saline, DMAE and Choline Chloride were 7.05±0.7, 11.18±1.2 and 12.50±1.3 min. respectively (Fig. 5); Radio-HPLC chromatogram of [^{18}F]FCH sample have only one peak with retention time of 13.67min (Fig. 6). Radio-TLC chromatogram of[^{18}F]FCH have an unique peak with retention time of 79.7 mm.(Fig 7). The radiochemical purity of [^{18}F]FCH was greater than 99.99% (Figs. 6 and 7) and the GC chromatogram of [^{18}F]FCH solution shown that there are four peaks of solvents with retention time of 3.28, 4.06, 16.05 and 24.13 min. corresponded to Ethanol, Acetonitrile, DMAE and DMSO (Fig. 8).

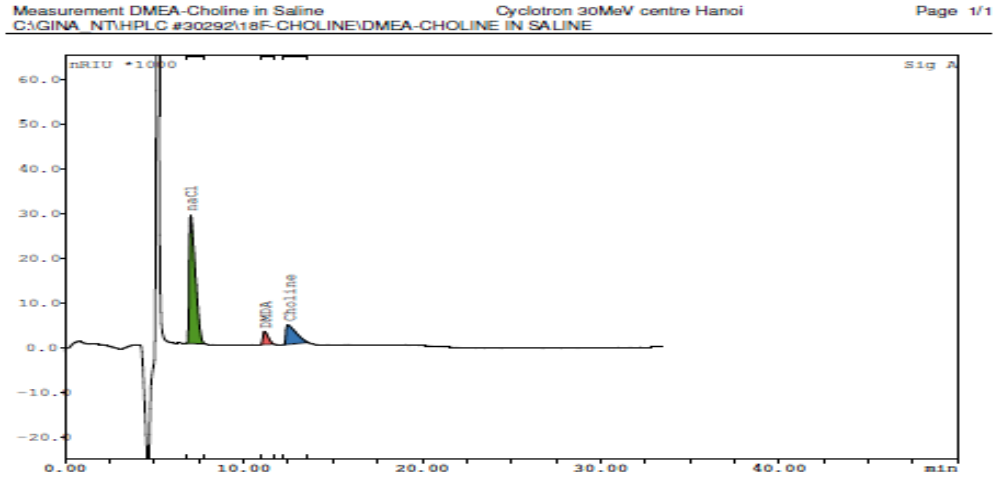

Figure 5. HPLC chromatogram of Sanline, DMAE and Choline standard sample

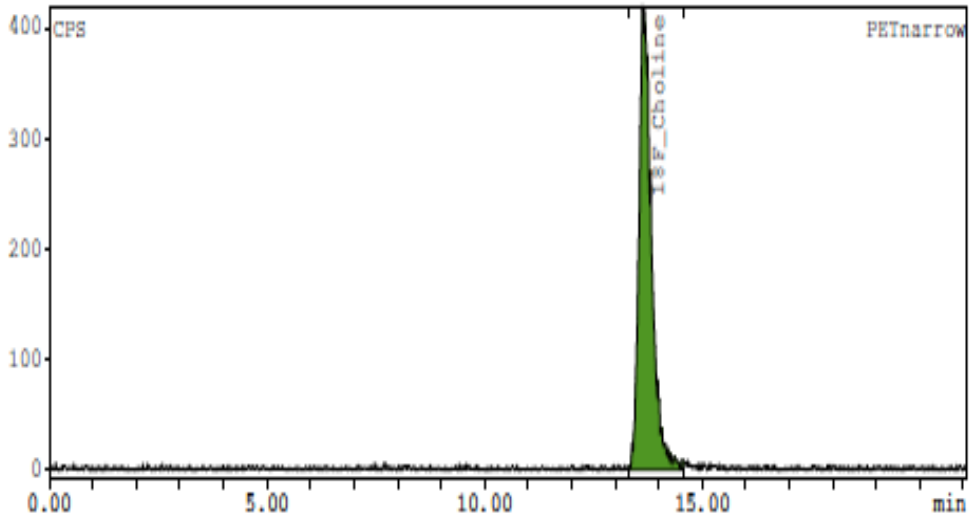

Figure 6. Radio-HPLC chromatogram of [^{18}F]FCH sample

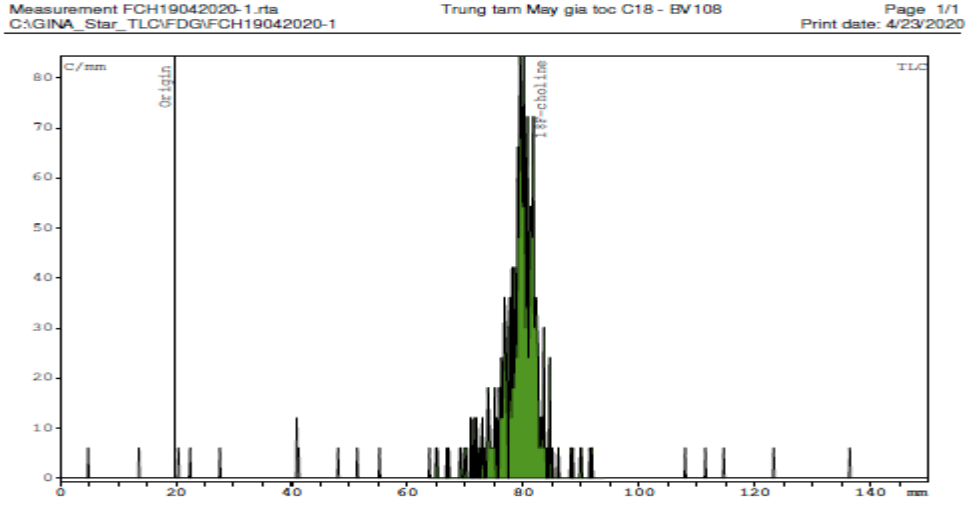

Figure 7. Radio-TLC chromatogram of [^{18}F]FCH sample

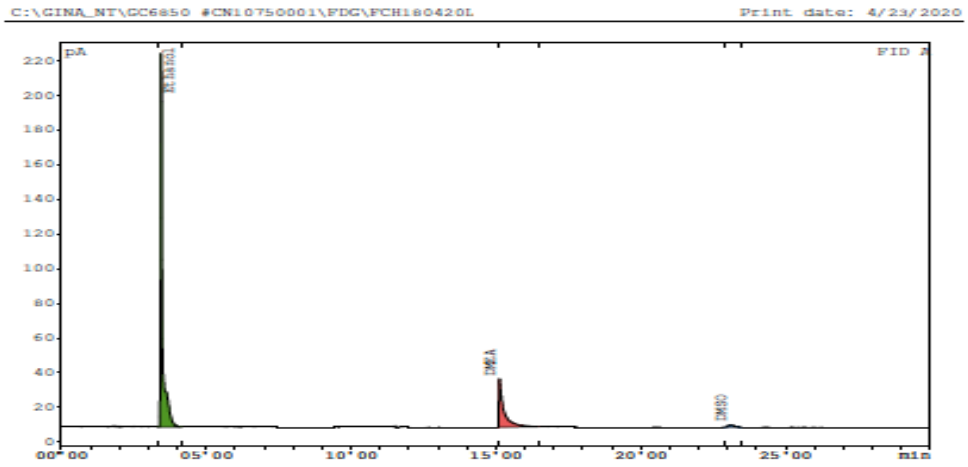

Figure 8. GC chromatogram of [^{18}F]FCH sample

1.5. Stability tests of [^{18}F]FCH

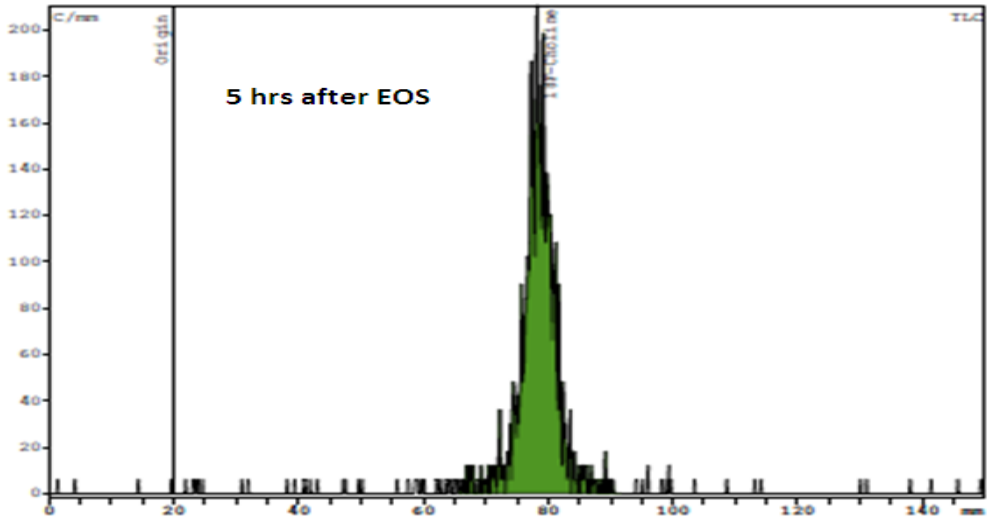

Figure 9. Radio-TLC chromatogram of [^{18}F]FCH at 5hrs after EOS

The Radio-TLC chromatogram of [^{18}F]FCH is monitored at the moment of 5 hrs after EOS to have an unique peak with retention time of 79.7 mm.(Fig 9). The stability test showed that [^{18}F]FCH to be stable at room temperature for up to 5 h after EOS.

Table 3. Quality control for ^{18}F-FCH

Test	EuPh2017 Criteria	Rerults (n=3)
Appearance	Clear, colorless solution	Clear, colorless solution
Identification	Gamma counter determines peak of 511KeV;	$T_{1/2}$=111±3 min.

	$T_{1/2}$=105-115 min.	
pH	4,5-8,5 pH paper indicator	6,5±0,1
DMEA	<100 µg/ml	60±10 µg/ml
DMSO	<10 µg/ml	< 6±2 µg/ml
Kriptofix	<220 µg/ml	<220 µg/ml
Acetonitrile	<400 µg/ml	<100±10 µg/ml
Ethanol	<5.000 µg/ml	< 4.000±10 µg/ml
^{18}F	<5%	< 0.1%,
Radiochemical purity	>95%	>99,9%

2. Conclusion

Automatic synthesizers are designed and manufactured to produce [^{18}F]FCH via 8 steps. It includes Labview programming software to control and monitor hardware through RS232-COM communication standard. The Hardware consists of a box containing a communication circuit board connected to computer and the circuit boards controlling motors, vacuum pump, temperature of reaction vessel, helium flow and radiation detectors and the synthesis disposal kit is designed with 1 row of 15 three way valves using chemicals and cassettes for [^{18}F]FCH from ABX. The automatic synthesizer is easy to use and stable in operation. All commands control 8 steps of the synthesis process are performed accuracy 100%. Test for [^{18}F]FCH synthesis using this synthesizer confirmed that total time of the synthesis of a [^{18}F]FCH batch is 50 ± 5 min; synthesis yield is 11.7± 0.2% with no decay correction and radiochemical purity is more than 99,9% that meet the requirements of European Pharmacopoeia 2017 and could supply a sufficient amount of [^{18}F]FCH for both of pre-clinic studies and clinical practice, especially for early detection of prostate cancer and hepatocellular carcinoma in Vietnam.

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