Fast Method for the Determination of Residual Solvents in Radiopharmaceutical Products

MIRELA MIHON[1,2]*, CATALIN STELIAN TUTA[2], ALINA CATRINEL ION[1], DANA NICULAE[2], VASILE LAVRIC[1]

[1]University Politehnica of Bucharest, 313 Splaiul Independentei,060042, Bucharest, Romania

[2]National Institute for Physics and Nuclear Engineering Horia Hulubei, 30 Reactorului Str., 077125, Magurele, Ilfov, Romania

The aim of this work was the development and validation of a fast analytical method to determine the residual solvents content in radiopharmaceuticals such as: ^{18}F-Fluorodeoxyglucose (^{18}F-FDG), ^{18}F-Fluoroestradiol (^{18}F-FES), ^{18}F-Fluorothymidine (^{18}F-FLT),^{18}F-Fluoromisonidazole (^{18}F-FMISO). Radiopharmaceuticals are radioactive preparations for medical purposes used in nuclear medicine as tracers in diagnostic imaging and treatment of certain diseases. Positron Emission Tomography (PET) is a medical imaging technique that consists in introducing into the body of a small amount of a biologically active chemical compound labelled with a short lived positron-emitting radioisotope (^{18}F, ^{11}C, ^{68}Ga). Residual solvents are critical impurities in radiopharmaceuticals that can affect labelling, stability and physicochemical properties of drugs. Therefore, the determination of these solvents is essential for quality control of radiopharmaceuticals. Validation of the control method for residual solvents by gas chromatography is referred by the European Pharmacopoeia using a special injection technique (head space). The parameters of the method, which comply with International Conference on Harmonization guidelines, are: accuracy, precision, linearity, limit of detection, limit of quantification and robustness. The proposed method (direct gas chromatography injection) proved to be linear, precise, accurate and robust. Good linearity was achieved for all the solvents and correlation coefficients (R^2) for each residual solvent were found more than 0.99.

Keywords: residual solvent, ethanol, gas chromatography, radiopharmaceuticals, validation

Radiopharmaceuticals are radioactive compounds used for diagnosis and therapy of human diseases. Radiopharmaceuticals formulated as liquid solutions are sterile, isotonic and pyrogen free. Even some solvents are required in the production process (acetonitrile, methanol), final product purification (ethanol), cleaning the synthesis module (acetone, 2-propanol) and cannot be excluded from the fabrication process [1], the content of solvents in the final product must not exceed the limits required by the pharmaceutical regulation.

The quality parameters of any radiopharmaceuticals are very important, as in all pharmaceutical industry, so the qualitative and quantitative determination of residual solvent impurities in the final products is essential. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the European Pharmacopoeia (Eur.Ph.) provide limits of residual solvents [2]. Because the Positron Emission Tomography (PET) requires the injection of radiopharmaceutical solution, the Eur.Ph requirements recommend appropriate limits for any toxic potential ingredients. The content of residual solvent in radiopharmaceuticals is analysed by using headspace gas chromatography (HS-GC), in accordance with the recommendations of Eur.Ph. [3].

Residual solvents are critical impurities in radiopharmaceuticals because of the side biological effects that can affect the efficacy of the labelling and physicochemical properties of drugs. The amount of the solvent in the radiopharmaceutical must be safe from a toxicological point of view. Class 1 solvents are known for human carcinogens or environmental hazards. Class 2 solvents (acetonitrile and methanol) are suspected of other significant but reversible toxicities, below certain limits. ICH guideline Q3C lists acetone and ethanol as a Class 3 solvents and their presence in radiopharmaceuticals may be regarded as being of low risk to human health. Ethanol is used as a scavenger in radiopharmaceuticals preparation, being demonstrated its presence is improving the stability, the FDG stability increase with the use of ethanol up to 10% [4]. The ethanol concentration is considered within the tolerance for injectable solution [5]. Currently, the allowed dose of ethanol is 0.5%, if considered as residual solvent.

Analytical methods for the determination of residual solvent in pharmaceuticals are based on gas chromatography: static headspace [6], purge and trap [7], headspace- programmed temperature vaporization and gas chromatography/mass spectrometry [8]. Also, headspace – solid phase microextraction (HS-SPME) and spray dried dispersion [9, 10] has been proposed for determination of solvents in the pharmaceutical preparation.

Only few reports concerning methods of determination of residual solvents in radiopharmaceuticals have been published both direct injection and headspace technique [11, 12].

One of the most important aspects of working with radiopharmaceuticals emitting positrons is the short time, about 30 minutes, which can be spent on quality control testing. The analytical test should be fast and effective, since the radioisotope has a short half-life (109.8 min for ^{18}F). Channing et al. described the analysis of ethanol, acetone and acetonitrile with a lower resolution of ethanol and acetonitrile for concentrations of ethanol up to 10 % [13].

This work is devoted to the development and validation of an improved analytical method to quantify the residual levels of methanol, acetonitrile, acetone, ethanol and 2-propanol in radiopharmaceutical preparations such as ^{18}F FDG, ^{18}F FMISO, ^{18}F FLT and ^{18}F FES. Gas chromatography

* email: mirela.mihon@nipne.ro; Tel: +40723871076

GC Parameter	
Inlet temperature	180 °C
Carrier (He) flow rate	48 ml/min
Pressure	15.68 kPa
Inlet split ratio	15:1
Oven temperature gradient / Ramping to	40 °C (2 min) – 90 °C (0 min) 15 °C / min
FID temperature	250 °C
Detector gas flows Air & Hydrogen	30 L/min.

Table 1
CHROMATOGRAPHIC PARAMETERS

method was developed based on the chromatographic separation of residual solvents considering the shortest retention time (faster analysis) and better separation (resolution higher than 1.5). The method was characterized by: precision, linearity, accuracy, limit of detection, limit of quantification and robustness [14].

Experimental part

Materials

All solvents were purchased from Merck (Germany) and were of gas chromatography grade. Ultrapure water was prepared using the Millipore MilliQ Direct 8/16 water purification system.

Chromatographic studies

Chromatographic separation was performed on Agilent 6850 equipped with FID detector and ALS G4513A auto sampler. The GC column is a J&W DB – 624 (6% cyanopropylphenyl / 94% dimethylpolysiloxane) fused silica capillary column, 30 m lenght, 0.53 mm internal diameter, 3 μm film thickness. As mobile phase we used helium gas. The optimised chromatographic conditions are listed in table 1.

Preparation of standard stock solution

About 1.5 mg methanol, 2.5 mg ethanol, 2.5 mg acetone and 0.25 mg acetonitrile were accurately weighed into a 25 mL volumetric flask. Working standard solutions were prepared by diluting the standard stock solution with ultrapure water.

The sample preparation

Sample from different batches (^{18}F-FDG, ^{18}F-FLT, ^{18}F-FES) were diluted 5 times with purified water and analysed.

Method validation

The analytical method validation was carried out based on ICH method validation guideline. The validation parameters addressed were specificity, precision and linearity, limit of detection and limit of quantification, accuracy and system suitability.

Specificity of the method was tested by injecting standard solutions of each residual solvents and a mixture of standard solutions for these compounds and comparing the retention time of each standard. The individual retention times of residual solvents were noted. No peak was observed from the chromatogram obtained by injecting the blank solution (ultrapure water).

The precision of the method was tested on six replications of following mixture 5000 ppm acetone, ethanol and 2-propanol, respectively 500 ppm acetonitrile and 3000 ppm methanol, from standard solution, was injected into the chromatograph. For each solvent peak areas, standard deviation (SD) and relative standard deviation (RSD) were calculated. For the precision of the method and of the system the %RSD, five solvents comply with the acceptance criteria of less than 5%.

The method's accuracy was validated through recovery experiments by spiking with known amount of each solvent at 80, 100 and 120%. Each concentration was prepared in triplicate and percent recovery was calculated. The recovery of each residual solvent should be in between 80-120%.

The system suitability was evaluated by injecting the blank solution (duplicate) and standard test solution (six injections) using the optimized method. The chromatograms were recorded, evaluated and the relative standard deviations were calculated. The resolution between two successive peaks was measured.

Results and discussions

A faster method was developed to determine the residual solvents, methanol, ethanol, acetone, 2-propanol and acetonitrile which can be found in ^{18}F radiopharmaceutical products.

The proposed method has significant changes in comparison with the current methods such us: GC oven temperature gradient, flow rate of the carrier gas and sample split ratio [11-13]. The ramp temperature was optimized in the 40 – 90°C intervals. All the solutions (standards and samples) were diluted with water. The GC cycle was optimized at 7 min.

The parameters of the improved GC direct injection method are listed in table 1. In this method the maximal used temperature was 90°C because all the residual solvents present vaporization temperatures below this value.

Specificity is the ability of the method to confirm only the analyte from other interferences. Specificity of the method was confirmed by the resolution between the peaks, both in sample and in standard solution. No interference from the blank at each retention time of analytic peaks was observed. A chromatogram obtained from standard solution is shown in figure 1.

The data obtained for standard solutions are presented in Table 2. The resolution between two successive eluting peaks was higher than 2.0, which meets the acceptance criteria for methanol, ethanol, acetone and acetonitrile.

The method has been shown to be linear by plotting minimum 6 points in the range 100 – 5000 ppm for acetone and 2-propanol, 60-3000 ppm for methanol and 10 – 500 ppm for acetonitrile. The linearity range was determined

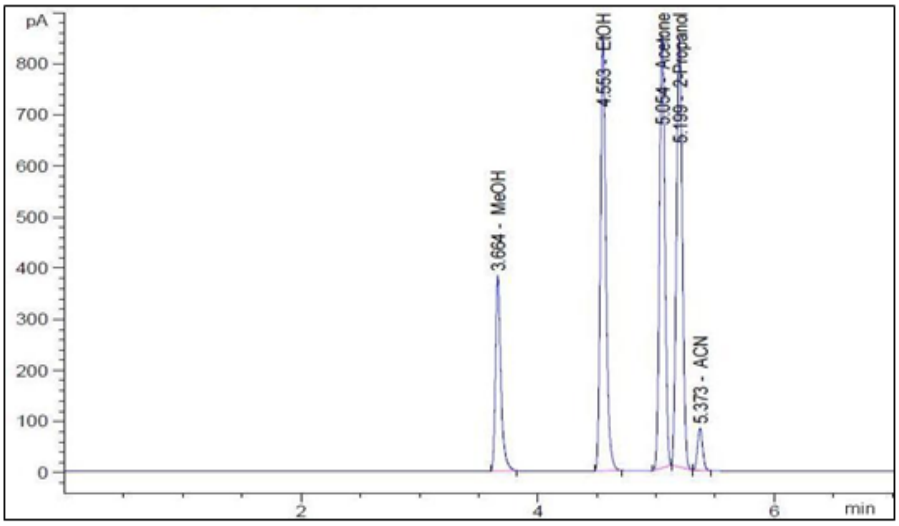

Fig. 1. Chromatogram of standard residual solvents for the GC method

Solvent	Retention Time (min)	Relative Retention Time	Resolution
Methanol	3.66	1.0	-
Ethanol	4.55	1.24	10.97
Acetone	5.05	1.38	5.90
2-Propanol	5.20	1.42	1.65
Acetonitrile	5.37	1.47	2.00

Table 2
SPECIFICITY OF THE METHOD

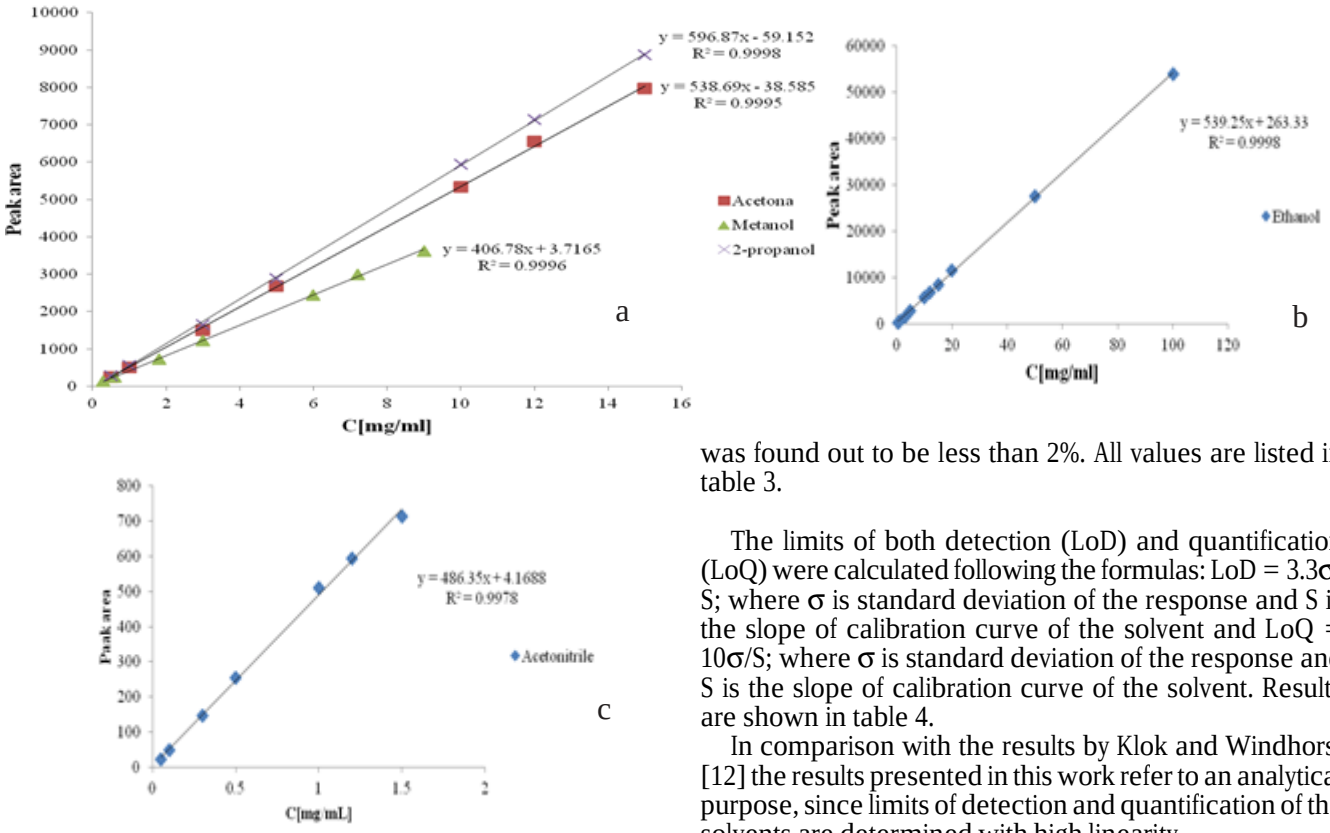

Fig. 2. Calibration lines for residual solvents: a. Acetone, Methanol, 2-Propanol; b. Ethanol; c. Acetonitrile

in between 100 – 10000 ppm for ethanol. Good linearity was achieved for all solvents (fig. 2). The method was found to be linear with correlation coefficients (R^2) greater than 0.99 for all investigated analytes. The calibration curves lines are presented in figure 2.

The precision of this method was expressed in term of %RSD data and was performed by injecting 6 standard test solutions. Standard deviation (SD) and relative standard deviation (RSD) were calculated for each solvent. The RSD was found out to be less than 2%. All values are listed in table 3.

The limits of both detection (LoD) and quantification (LoQ) were calculated following the formulas: LoD = 3.3σ/S; where σ is standard deviation of the response and S is the slope of calibration curve of the solvent and LoQ = 10σ/S; where σ is standard deviation of the response and S is the slope of calibration curve of the solvent. Results are shown in table 4.

In comparison with the results by Klok and Windhorst [12] the results presented in this work refer to an analytical purpose, since limits of detection and quantification of the solvents are determined with high linearity.

System suitability has been demonstrated by analyzing the standard solution during the validation study. The system performance was checked by the resolution, % RSD and the asymmetry. The results obtained for system suitability with this method are presented in table 5.

In comparison with the above cited results, the results presented in this work (table 6) show that the order of elution of ethanol and acetonitrile is reversed, the difference between the retention times being higher than in Klok et al.[12]. The difference between the retention times of these solvents increases, without reversing the elution order as in Channing et al.[13].

Number	Area				
	Methanol	Ethanol	Acetone	2-Propanol	Acetonitrile
1 injection	1226.1	2844.1	2693.5	2875.3	253.2
2 injection	1231.3	2855.5	2738.5	2897.3	257.5
3 injection	1219.7	2821.0	2688.5	2857.7	260.1
4 injection	1244.4	2880.5	2771.6	2930.4	255.0
5 injection	1224.2	2828.1	2690.4	2870.2	253.2
6 injection	1214.3	2876.2	2696.1	2831.6	252.3
Average	1226.6	2850.9	2713.1	2877.08	255.2
Standard deviation	8.81	29.87	28.94	28.65	2.56
% RSD	**0.72**	**0.73**	**1.07**	**1.00**	**1.00**

Table 3
PRECISION OF THE METHOD

Solvent	Limit of detection [mg/mL]	Limit of quantification [mg/mL]
Methanol	0.008	0.025
Ethanol	0.007	0.023
Acetone	0.005	0.015
2-Propanol	0.004	0.024
Acetonitrile	0.002	0.007

Table 4
VALUES OF THE LoD AND LoQ FOR RESIDUAL SOLVENTS

Solvent	System Suitability Parameters		
	Resolution >1.5	% RSD <5	Asymmetry 0.8 – 1.2
Methanol	-	0.97	0.80 – 0.85
Ethanol	10.97	1.23	0.84 – 1.20
Acetone	5.20	1.01	0.87 – 0.97
2-Propanol	1.65	0.94	0.85 – 0.94
Acetonitrile	2.00	0.98	0.85 – 0.95

Table 5
SYSTEM SUITABILITY PARAMETERS FOR THE GAS CHROMATOGRAPHY METHOD

Table 6
COMPARISON OF THE RESULT WITH LITERATURE DATA

Solvent	Maximum allowed concentration(ppm)	Optimized method		R.P.Klok, A.D.Windhorst[12]		M.A.Channing, B.X.Huang [13]	
		t_R	R^2	t_R	R^2	t_R	R^2
Methanol	3000	3.65	0.9996	2.1	0.9996	-	-
Ethanol	5000	4.55	0.9998	4.5	0.9946	2.26	0.9994
Acetone	5000	5.05	0.9995	5.8	0.9959	2.67	0.9997
2-Propanol	5000	5.20	0.9998	6.4	Int. standard	-	-
Acetonitrile	410	5.37	0.9978	4.1	0.9988	1.76	0.9998
Analysis Time		7 minutes		12 minutes		4 minutes	

Robustness was set by analyzing the sample in six replicates with the optimized method and by changing the carrier gas flow rate and the inlet temperature.

The parameters which characterize the robustness of the method are presented in table 7.

The RSD calculated for methanol, ethanol, acetone, 2-propanol and acetonitrile were found to be less than 2%.

Solvent	Parameters	Variation of parameters			
		Inlet temperature 170°C	Inlet temperature 190°C	Column flow 2.8 mL/min	Column flow 3 mL/min
Methanol	t_R	3.65	3.66	3.76	3.58
	area	1203.6	1200.9	1195.9	1198.02
	RSD%	0.39	0.51	0.48	1.03
Ethanol	t_R	4.54	4.55	4.65	4.46
	area	2794.7	2783.7	2771.6	2777.4
	RSD%	0.5	0.62	0.50	1.33
Acetone	t_R	5.04	5.05	5.16	4.95
	area	2449.4	2451.6	2414.9	2437.8
	RSD%	0.45	0.44	0.47	1.88
2-Propanol	t_R	5.19	5.19	5.3	5.1
	area	2824.4	2833.5	2806.4	2828.3
	RSD%	0.22	0.27	0.47	1.15
Acetonitrile	t_R	5.36	5.37	5.48	5.27
	area	237.1	238.5	234.6	226.9
	RSD%	0.82	0.41	0.51	1.36

Table 7
ROBUSTNESS OF THE METHOD

Conclusions

The proposed chromatographic method has been successfully applied for different radiopharmaceuticals and has been validated according to ICH international standards. This method is suitable for chemical purity evaluation of ^{18}F-radiopharmaceuticals in a routine laboratory. The advantage of this approach over others previously described is the shorter analyse time of 7 min per sample in comparison to 12 min.

The direct injection-gas chromatography is specific method suitable for our purpose, especially for a percentage of ethanol up to 10 %. Because of the short time of analysis, this method is suitable for radiopharmaceuticals labeled with ^{11}C (half life 20 min).

Acknowledgments: This work is supported by the Sectorial Operational Program Human Resources Development (SOP HRD), financed by the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390/.

References

1. RICHARDS, M.L., SCOTT, P.J.H., Radiochemical Syntheses, Radiopharmaceuticals for Positron Emission Tomography, First Ed., **vol 1**, Wiley Inc., Scott, P.J.H., Hockley, B.G., New Jersey, 2012, p.3–13.

2. International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Impurities: Guideline for Residual Solvents, Q3C(R5), 2011.

3.*** Residual Solvents (5.4), European Pharmacopoeia, European Directorate for the Quality of Medicines of the Council of Europe, Strasbourg, 8.0 Ed., 2014, p. 638–646.

4. JACOBSON, M.S., DANKWART, H.R., MAHONEY, D.W., Appl. Radiat. Isot., **67**, 2009, p. 990–995.

5. STRICKLEY, R. G., Pharmaceutical Research, **21**, 2004, p. 201–230.

6. FLISZAR, K., WIGGINS, M.J., PIGNOLI, C.M., MARTIN, G.P., LI, Z., J. Chromatogr. A, **1027**, 2004, p. 83–91.

7. LAKATOS, M., J. Pharm. Biomed. Anal., **47**, 2008, p. 954–957.

8. PEREZ PAVON, J.L., SANCHEZ, M.N., FERNANDEZ LAESPADA, M.E., PINTO, C.G., CORDERO, B.M., J. Chromatogr. A, **1141**, 2007, p. 123–130.

9. RAGHANI, A.R., J. Pharm. Biomed. Anal., **29,** 2002, p. 507–518.

10. QUIRK, E., DOGGETT, A., BRETNALL, A., J. Pharm. Biomed. Anal., **96**, 2014, p. 37–44.

11. KLICK, S., SKOLD, A., J. Pharm. Biomed. Anal., **36**, 2004, p. 401–409.

12. KLOK, R. P., WINDHORST, A.D., Nucl. Med. Biol., **33**, 2006, p. 935–938.

13. CHANNING, M. A., HUANG, B.X., ECKELMAN, W.C., Nucl. Med. Biol., **28,** 2001, p. 469–471.

14.*** International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology, Q2(R1), 2005

Manuscript received: 15.03.2016