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A generic gas chromatography method for determination of residual solvents in PET radiopharmaceuticals

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ABSTRACT

A novel gas chromatography (GC) method for quantitation of volatile organic compounds (VOCs) in ^{18}F- and ^{11}C-radiopharmaceuticals listed in the European Pharmacopoeia (Ph. Eur.) was proposed. Optimized chromatographic parameters were used for separation of ethanol, acetone, acetonitrile, tetrahydrofuran (THF), dibromomethane (DBM), 2-dimethylaminoethanol (deanol), *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) which could be detected in radioactive drug samples. The calculated peak resolutions (R_S) were higher than 2.0 at ethanol concentration of up to 11 m/m%. Reproducible results could be obtained using base deactivated fused silica wool as packing material of inlet liner. Validation parameters showed excellent linearity ($r^2 \geq 0.9998$) in the range from 10 to at least 120% of concentration limit of solvents. The accuracy was determined as recovery of concentrations which ranged from 99.3% to 103.8%. Additionally, the relative standard deviation (RSD) of each solvent for inter-day and intra-day precision were in the range of 0.5–4.2% and 0.4–4.4%, respectively. The limit of quantitation (LOQ) for ethanol, acetone, acetonitrile, THF, DBM, deanol, DMF and DMSO was 0.48, 0.42, 0.43, 0.46, 4.35, 0.73, 0.68 and 0.50 mg/L, respectively. The developed procedure was successively applied for quantitation of ethanol, acetone, acetonitrile and deanol in radioactive drug samples of [^{11}C]methionine, [^{11}C]choline, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET). The proposed GC method applying flame ionization detection (FID) could be adapted in routine quality control of most frequently used positron emission tomography (PET) radiopharmaceuticals to perform the determination of residual solvents with analysis time of 12 min

1. Introduction

Positron emission tomography is a minimally invasive functional imaging method in nuclear medicine. Computed tomography (CT) and magnetic resonance imaging (MRI) were successfully combined with PET and the emerged multimodality systems revolutionized the clinical imaging and disease characterization in the past two decades. Recently, PET/CT and PET/MRI hybrid techniques have been widely used in oncology, cardiology, neurology and could be applied in drug development as well [1]. Pathological and physiological processes can be examined in vivo by PET using biologically active molecules labeled with positron emitting isotopes. Radiotracers are usually formulated as sterile injections for intravenous administration. The European Pharmacopoeia comprises quality standards of several radiopharmaceuticals predominantly with oncological indications [2]. For instance, [^{18}F]FDG could be applied for visualization of tumors having significantly enhanced glucose uptake compared with normal cells [3]. [^{11}C]methionine and 6-[^{18}F]fluoro-3,4-dihydroxyphenylalanine ([^{18}F]DOPA) could be characterized with low accumulation in normal brain cells which allows the detection of low-grade gliomas and facilitates more precise tumor delineation [4]. These radiolabeled amino acids together with [^{18}F]FET show high sensitivity and specificity for detecting brain tumors and differentiating recurrent tumors from post-therapeutic changes [5]. [^{18}F]fluoromisonidazole ([^{18}F]MISO) could be applied in assessment of tumor hypoxia for radiation therapy planning [6]. [^{11}C]choline and [^{18}F]fluorocholine are frequently used tracers in PET imaging of prostate cancer [7]. 3′-Deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) is eligible for studying tumor cell proliferation and applicable in assessment of glioma grading and differentiating tumor recurrence from necrosis [8].

Radiopharmaceuticals could be released for human use only after assessment of specific quality parameters. Among others, determination of residual solvent content is a required analysis applied in quality control of radioactive injections. Volatile organic compounds in the final product may arise from the reaction system of

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radiolabeling procedure and rinsing solution used for purification of synthesis modules and dispensers [9]. Additionally, ethanol is frequently used in the formulation step as free radical scavenger for stabilization of radiotracer against autoradiolysis [10,11]. The quantitation of residual solvents in the pharmaceutical industry is generally performed by gas chromatograph equipped with flame ionization detector using either direct sample injection mode or headspace analysis combined with various extraction techniques [12]. Accurate and reliable analytical methods could be developed for analysis of organic solvents with different polarity by choosing the appropriate dilution medium [13,14]. Several generic GC methods are accessible for determination of up to 44 solvents of Class 2 and Class 3 of International Conference of Harmonization (ICH) guideline Q3C [15,16]. However, radiopharmaceuticals may contain residue of volatile compounds not classified into any categories, namely 2-dimethylaminoethanol and dibromomethane, that are used as precursors in [^{11}C]choline and [^{18}F]fluorocholine synthesis [2]. Furthermore, the maximum permitted concentration level in some cases may exceed the typical value determined for solvents of lowest toxicity by the ICH guideline [17]. For instance, ethanol can be used as excipient in [^{18}F]FDG formulation with concentration of up to 12 m/m% [18]. Consequently, improved analytical procedures should be applied for determination of residual solvent content in PET radiopharmaceuticals. Channing et al. [19] proposed an accurate GC-FID method for determination of concentration of ethanol, acetone and acetonitrile in [^{18}F]FDG. The validation results were in accordance with the suggested requirements of the United States Food and Drug Administration (FDA). Klok et al. [18] developed two methods for fast and quantitative analysis of residual solvents in radiopharmaceutical formulations with ethanol content in the range of 5–12 m/m%. No special sample treatments or injection techniques were used. It was observed, that *N,N*-dimethylformamide and dimethyl sulfoxide had high affinity for column packing and elevated temperatures were necessary to use throughout the whole analysis. On the other hand, solvents such as acetone and acetonitrile could only be separated from ethanol at lower initial temperatures. This method met the requirements of the ICH and United States Pharmacopeia (USP) for the detection and quantification of residual solvents. Santos Costa et al. [20] proposed a GC-FID method for direct determination of acetonitrile and ethanol content in [^{18}F]MISO, [^{18}F]FLT and [^{18}F]fluoroestradiol ([^{18}F]FES). This procedure was applicable for analysis of samples with ethanol content of up to 11 v/v %. Organic solvents could be determined in a 1.5 min run. The fast chromatographic separation was achieved on a DB-WAX GC column, 30 m × 0.25 mm, 0.5 µm with isothermal oven temperature. Kilian et al. [21] developed a method for determination of acetonitrile and ethanol in the most frequently used radiopharmaceuticals, namely [^{18}F]FDG and [^{11}C]methionine. Short sample preparation and chromatographic separation time as well as minimum manual operation facilitate the application of the procedure in routine quality control.

Several gas chromatography methods could be found in the literature for determination of residual solvents in pharmaceuticals. Nevertheless, a generic procedure for analysis of volatile organic compounds that may occur in radioactive preparations for PET is still lacking. The goal of this work is to develop a single GC-FID method for quantitation of residual solvents which could be detected in ^{18}F- and ^{11}C-radiopharmaceuticals listed in the European Pharmacopoeia. The study is aimed to overcome the challenges of separation of VOCs with different polarity and maximum allowable concentration.

2. Materials and methods

2.1. Chemicals

Ethanol was purchased from Merck (Darmstadt, Germany). Tetrahydrofuran, dimethyl sulfoxide, dibromomethane and deanol were obtained from Sigma (St. Louis, USA). Methanol, acetonitrile, acetone and *N,N*-dimethylformamide were products of VWR (Rue Camot, France). All solvents used for the experiment were GC grade and applied without additional purification. Water used for dilution was provided by a Simplicity water purification system (Merck, Darmstadt, Germany) and controlled for the content of organic impurities.

2.2. Instrumentation

Experiments were carried out on Shimadzu (Japan) GC-2010 Plus and Perkin Elmer (USA) Clarus 500 gas chromatograph equipped with flame ionization detector and split/splitless injector. SKY liner, Split 3.5 mm× 5.0 × 95 (Restek, USA) were used for Shimadzu GC. Inlet sleeves, 4 mm, Split for PE Split/Splitless injector (Supleco, USA) and wool packed Straight Focus liner (Supleco, USA) were used for Clarus 500 GC. Liners were filled with silane treated glass wool (Supelco, USA), base deactivated fused silica wool (Restek, USA) or deactivated glass wool (Restek, USA). Gas chromatographs were controlled as well as data acquisition and processing were accomplished using GCsolution (Version 2.43.00, Shimadzu) and TotalChrom workstation (Version 6.3.2, Perkin Elmer) chromatography data system. Compounds were separated on ZB-624 (30 m x 0.32 mm × 1.80 µm) capillary column (Phenomenex, USA) formed by 6% Cyanopropylphenyl and 94% Dimethylpolysiloxane. GC systems were supplied with hydrogen (99.9999%, Linde, Germany), helium (99.9999%, Linde, Germany) and air (99.9990%, Linde, Germany).

2.3. Sample preparation

Standard solutions for method development and validation were prepared by dilution of organic solvents in water to achieve the appropriate concentrations. Samples were either freshly prepared or stored at 2–8 °C and brought to room temperature before use. Quantitation was performed using methanol as internal standard (ISTD). Sample preparation was accomplished by adding 95 µL of analyzed sample to 5 µL of internal standard solution with methanol concentration of 40 g/L. 1 µL of the obtained mixture was injected on gas chromatograph with Hamilton syringe. Every analysis was carried out in triplicate.

Standard solutions for method development were taken with the following concentration of solvents: 113,794 mg/L (ethanol), 6122 mg/L (acetone), 512 mg/L (acetonitrile), 886 mg/L (THF), 20 mg/L (DBM), 226 mg/L (deanol), 1073 mg/L (DMF) and 6052 mg/L (DMSO). Samples used for linearity investigation were prepared by sequential dilution of stock mixture solution to 10 concentration level in ranges of 11,379–113,794 mg/L (ethanol), 612–6122 mg/L (acetone), 51–512 mg/L (acetonitrile), 89–886 mg/L (THF), 2–20 mg/L (DBM), 23–226 mg/L (deanol), 107–1073 mg/L (DMF) and 605–6052 mg/L (DMSO).

Samples used for determination of limit of detection (LOD) and limit of quantitation (LOQ) were taken with concentrations of 18.8–14.1 - 9.4–4.7 - 2.4–1.2–0.2 mg/L (ethanol), 16.1–12.1 - 8.0–4.0 - 2.0–1.0–0.2 mg/L (acetone), 17.9–13.4 - 9.0–4.5 - 2.2–1.1–0.2 mg/L (acetonitrile), 20.7–15.5 - 10.4–5.2 - 2.6–1.3–0.3 mg/L (THF), 15.4–11.6 - 7.7–3.9 - 1.9–1.0–0.2 mg/L (DBM), 20.4–15.3 - 10.2–5.1 - 2.6–1.3–0.3 mg/L (deanol), 23.0–17.2 - 11.5–5.7 - 2.9–1.4–0.3 mg/L (DMF) and 17.1–12.8 - 8.6–4.3 - 2.1–1.1–0.2 mg/L (DMSO).

3. Results

3.1. Potential volatile organic compounds in radiopharmaceuticals

According to the European Pharmacopoeia monographs and scientific papers most frequently used ^{18}F- and ^{11}C-labeled radiopharmaceuticals might contain residual solvents which are listed in Table 1 [2,22–29]. Volatile organic compounds play important role in

Table 1
Detectable volatile organic compounds in ^{18}F- and ^{11}C-radiopharmaceuticals.

Solvent	ICH Class	Boiling point, (°C)	PDE*, (mg/day)	Concentration limit, (mg/L)	Radiopharmaceutical
Acetone	3	56	50	5000	[^{11}C]methionine, [^{11}C]raclopride, [^{18}F]FDG, [^{11}C]flumazenil
Tetrahydrofuran	2	66	7.2	720	[^{11}C]flumazenil, [^{11}C]sodium acetate
Ethanol**	3	78	950	95000	[^{11}C]flumazenil, [^{18}F]FLT, [^{18}F]FDG, [^{18}F]FET, [^{11}C]methionine [^{18}F]DOPA, [^{18}F]MISO, [^{11}C]choline
Acetonitrile	2	82	4.1	410	[^{11}C]choline, [^{18}F]FET, [^{18}F]FDG
Dibromomethane	n/a	97	0.1	10	[^{18}F]fluorocholine
2-Dimethylaminoethanol	n/a	133	1.0	100	[^{11}C]choline, [^{18}F]fluorocholine
N,N-Dimethylformamide	2	153	8.8	880	[^{11}C]raclopride, [^{18}F]DOPA, [^{11}C]flumazenil
Dimethyl sulfoxide	3	189	50	5000	[^{11}C]raclopride

* Maximum injectable volume of radiopharmaceutical is 10 mL.
** Ethanol is taken with increased concentration due to its function as stabilizing agent.

production of radioactive drugs. For instance, acetonitrile is usually applied in production of [^{18}F]FET and [^{18}F]FDG as a reaction medium for accomplishing the radiofluorination step [22,23]. DMSO, DMF and THF could be also served as solvent for labeling procedures, namely in synthesis of [^{11}C]raclopride, [^{11}C]flumazenil and [^{18}F]DOPA [27–29]. Deanol and DBM are non-radioactive precursors for synthesis of [^{11}C]choline and [^{18}F]fluorocholine [2]. Acetone could be used as solvent in cleaning procedure of vials and tubings of synthesizer modules. Ethanol is applied as stabilizing agent against autoradiolysis in case of several radiopharmaceuticals [10]. The production of ^{18}F- and ^{11}C-radiopharmaceuticals is usually based on the application of the above listed volatile organic compounds. While deanol and dibromomethane could not be avoided in the synthesis of choline tracers, among aprotic solvents acetonitrile is used predominantly, but for harsh radiolabeling conditions DMF and DMSO is preferred. Additionally, ethanol is frequently used as excipient for effective stabilization of radioactive drugs. Production sites may use few of these VOCs for the optimized procedures. However, quality control of ^{18}F- and ^{11}C-radiopharmaceuticals should address the determination of residual solvents listed in Table 1 in almost all cases.

In Table 1 permitted daily exposure (PDE) as well as concentration limit (CL) of solvents could be found which are in correlation with toxicity of organic compounds. According to the ICH guideline [17] acceptable level of Class 3 solvents is 5000 mg/L at 10 mL of maximum injectable volume, while this limit decreases by an order of magnitude in case of Class 2 solvents. Additionally, European Pharmacopoeia monographs determine special concentration limit for deanol and DBM based on 1.0 and 0.1 mg/day PDE, respectively [2].

3.2. Optimization of GC separation

To separate VOCs identified in ^{18}F- and ^{11}C radiopharmaceuticals (Table 1) a medium polarity capillary column of Phenomenex ZB-624 (30 m x 0.32 mm × 1.80 µm) was applied. The method development was started based on the following chromatographic parameters. The column temperature was maintained at 40 °C for 6 min, and then increased to 200 °C at a rate of 30 °C/min and hold at 200 °C for 10 min. The inlet split ratio was 1:5 and the carrier gas were helium at velocity of 1.46 mL/min. The temperature of inlet and detector was adjusted to 250 and 275 °C, respectively. The flow rate of hydrogen and air was 40 mL/min and 400 mL/min, respectively. The injection volume of sample with limit concentration of residual solvents was 1 µL. The examination time was ca. 15 min. Using the above listed chromatographic conditions, the obtained results showed that the identified solvent peaks were separated with resolution in the range of 2.1–24.2. However, resolutions between peaks of ethanol and acetone (R_1) as well as acetone and acetonitrile (R_2) proved to be the lowest values and consequently were selected as critical parameters for optimization purposes. To achieve adequate peak resolution and sensitivity, several GC parameters were evaluated during the method development (Supplementary material, Table S1). For instance, increasing the initial column temperature from 40 °C to 80 °C led to slight decrease of R_1 and increase of R_2 from 7.7 to 4.5 and 2.8–3.8, respectively (Table 2). The optimal value of 7.1 and 4.0 for R_1 and R_2 was obtained at initial column temperature of 60 °C. On the other hand, the time of analysis was dropped from 13 to 9 min. Variation of ramp rate caused only slight effect on peak resolution (Supplementary material, Table S2). Increasing the carrier gas velocity from 1.04 to 1.92 mL/min increased the resolution values from 3.7 to 6.3 (R_1) and 2.8–3.4 (R_2). The optimal value could be found at 1.46 mL/min with 7.7 (R_1) and 2.8 (R_2). Fortunately, during the method development resolutions between identified peaks did not drop below 2 despite the high ethanol concentration. On the other hand, in case of DBM and deanol the sensitivity of the analysis is crucial for successful application of the chromatographic method due to low PDE values. Expectably, the signal-to-noise ratio (s/n) in case of DBM and deanol was dropped by increasing the inlet split ratio. The maximum s/n for dibromomethane was achieved at 1:1 split ratio. However, the carrier gas velocity and oven temperature had lower effect on the sensitivity

Table 2
Effect of critical GC conditions on separation parameters.

Parameter	Oven temperature, (°C)					Split ratio			Carrier gas velocity, (cm/min)		
	40	50	60	70	80	1:1	1:3	1:5	1.04	1.46	1.92
R_1	7.7	6.4	7.1	5.2	4.5	4.2	5.6	7.7	3.7	7.7	6.3
R_2	2.8	2.8	4.0	3.8	3.8	2.1	3.4	2.8	2.8	2.8	3.4
T_F (ethanol)	0.6	0.6	0.8	1.7	2.2	2.1	1.3	0.6	0.7	0.6	0.8
s/n (DBM)	21	20	18	14	9	40	24	21	11	21	14
Analysis time, (min)	13	12	11	10	9	11	11	13	12	13	10

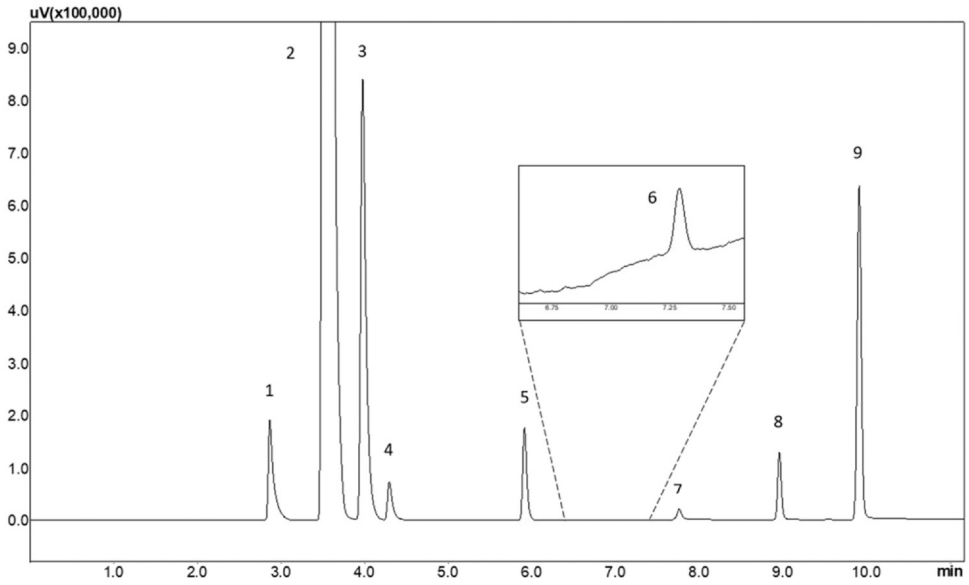

Fig. 1. Typical chromatogram of sample containing residual solvents with concentration limit analyzed by optimized gas chromatography method. (1 - methanol, 2 - ethanol, 3 - acetone, 4 - acetonitrile, 5 - THF, 6 - DBM, 7 - deanol, 8 - DMF and 9 - DMSO).

and the previously determined optimal values of 1.46 mL/min and 60 °C resulted acceptable signal-to-noise ratio for DBM at 1:5 split ratio. On the other hand, low split ratio degrades tailing factor (T_F) of ethanol peak as well as decreases R_1 and R_2 resolution. For instance, at 1:1 split ratio the tailing factor of ethanol increased to 2.1 and R_2 decreased to 2.1 while the signal-to-noise ratio for DBM was 40. Consequently, optimal sensitivity could be observed for solvents with lowest sample concentration at split ratio of 1:3. In this case the acceptable values of 3.4 (R_2), 1.3 (T_F) and 24 (s/n) could be achieved. After the optimization step, the following GC method could be recommended for determination of volatile organic compounds in PET radiopharmaceuticals. The inlet split ratio is 1:3 and the carrier gas velocity is 1.46 mL/min. The temperature of inlet and detector should be adjusted to 250 and 275 °C, respectively. The column temperature should be maintained at 60 °C for 4 min, and then increased to 200 °C at a rate of 30 °C/min and hold at 200 °C for 2 min. The examination time is ca. 11 min. The obtained chromatogram using the optimized chromatographic conditions is shown in Fig. 1.

3.3. Selection of packing material of inlet liner

The early results of optimization process of chromatographic parameters have shown that the type of packing material of inlet liner significantly determines the repeatability of the measurements. It was observed, that using various treated wools had a considerable effect on the relative standard deviation of peak area ratio of components to internal standard. For instance, silane treated glass wool (Supelco) gave RSD lower than 5% only for ethanol and acetonitrile (Table 3). Surprisingly, the deviation reached 41.50% in case of DMSO. While silanized glass wool used in Straight Focus liner (Supelco) and Quartz wool (Restek) also ensured unacceptably high RSD for deanol, DMF and DMSO, the maximum value of relative standard deviation exceeded 10% in case of other solvents. Using deactivated glass wool (Restek) RSD ranged from 0.63% to 19.38%. Presumably, the significant variation in response ratios could be in connection with interaction of solvents and packing materials of inlet liner. Among the examined packing materials only the base deactivated fused silica wool (Restek) gave RSD value below 5% for all solvents. Even in case of the reactive deanol and DMF the deviation was dropped to 2.21% and 2.66%, respectively. Dibromomethane and DMSO gave RSD higher than 4%. The relative deviation in case of ethanol, acetone, acetonitrile and tetrahydrofuran ranged from 1.39% to 3.44%. Consequently, the base deactivated fused silica packing material was applied in GC measurements and used for the validation procedure.

3.4. Method validation

The proposed gas chromatography method for separation of detectable solvents in radiopharmaceuticals was validated according to Q2(R2)/Q14 ICH guideline [30]. To demonstrate that the developed GC procedure is suitable for the analysis of volatile compounds the following parameters were evaluated: linearity, precision, recovery, specificity, range, limit of detection (LOD) and limit of quantitation (LOQ). The obtained validation results are shown in Table 4.

3.4.1. Linearity

Standard solutions of organic solvents at 10 concentration level were used for investigation of method linearity. The maximum concentration of compounds was adjusted to at least 120% of the permitted level and in case of deanol and dibromomethane 200% of the concentration limit was taken. For linearity test, stock solution with highest concentration of ethanol, acetone, acetonitrile, THF, dibromomethane, deanol, DMF and DMSO was diluted to obtain working solutions with concentrations ranging from 11,379 to 113,794 mg/L, 612–6122 mg/L, 51–512 mg/L, 89–886 mg/L, 2–20 mg/L, 23–226 mg/L, 107–1073 mg/L and 605–6052 mg/L, respectively. The linearity of solvents between the peak area ratio of component to ISTD and the concentration was determined by least squares linear regression analysis. (Supplementary material, Fig. S1-S2). Linear regression was used to assess linearity for each solvent. The obtained regression coefficients (r^2) for solvents were within the range of 0.9998–0.9999, which exhibited excellent linearity (Table 4).

3.4.2. Precision

Precision was determined using standard solutions of solvents at 3 level covering the examined concentration range. Repeatability results obtained at highest concentrations of 113,794 mg/L (ethanol), 6122 mg/L (acetone), 512 mg/L (acetonitrile), 886 mg/L (THF), 20 mg/

Table 3
Effect of the type of inlet liner filling material on the reproducibility.

Inlet liner filling material	RSD, (%)							
	Ethanol	Aceton	Acetonitrile	THF	DBM	Deanol	DMF	DMSO
Silane treated glass wool (Supelco)	2.89	8.61	4.62	11.53	12.35	23.42	31.22	41.50
Silanized glass wool (Supelco)*	2.50	3.86	3.71	3.01	5.69	24.04	22.22	31.24
Deactivated glass wool (Restek)	0.63	2.01	7.17	3.95	4.81	19.38	8.28	9.93
Quartz wool (Restek)	1.67	5.73	2.79	8.46	7.26	16.96	21.57	57.54
Base deactivated fused silica wool (Restek)	1.39	2.73	1.52	3.44	4.42	2.21	2.66	4.07

* Straight Focus liner.

L (dibromomethane), 226 mg/L (deanol), 1073 mg/L (DMF) and 6052 mg/L (DMSO) are shown in Table 4. Intra-day precision was obtained by injecting the same sample six times on the same day. Inter-day precision was determined by injecting the same sample six times in six consecutive days. Both precisions were checked by calculating the RSD values of the obtained concentrations using calibration curves of the linearity test. The RSD for intra- and inter-day precision were in the range of 0.5–4.2% and 0.4–4.4%, respectively. Moreover, at 10% and 50% of concentration limit the obtained inter-day precision values ranged from 0.5% to 2.6% and 0.1–3.5%, respectively (Supplementary material, Table S3). These results indicated that this GC method had reasonable precision within the analytical range of determinations.

3.4.3. Accuracy

Recovery was used for determination of accuracy of the developed method. Standard solutions of solvents at three concentration levels of the examined range were analyzed six times. Concentration of organic compounds were calculated using calibration curves of the linearity test. Recovery was obtained from the experimental concentrations of the tested solvents divided by the theoretical concentrations. Table 4 presents recoveries obtained at highest concentrations of solvents which were ranged from 99.3% to 103.8%. Additionally, at 10% and 50% of concentration limit the calculated recovery values ranged from 97.3% to 103.6% and 96.6–103.9%, respectively (Supplementary material, Table. S3). The obtained results showed that the method had sufficient accuracy for analyzing the examined solvents.

3.4.4. Specificity

Retention times of organic compounds were determined by analyzing samples of each solvent at concentration of 100 mg/L using GC method with optimized parameters. As indicated in the retentions of solvents in Table 4, every component could be separated from each other. Examination of standard solution of solvents with concentration limits showed no peak overlapping despite of the high ethanol content and the resolution of peaks ranged from 2.8 to 15.9. The specificity results showed that the developed method was suitable for identification of the examined VOCs in sample. However, it should be noted that radiopharmaceuticals usually contain only few of the examined solvents.

3.4.5. Range

The experimental results showed that the developed gas chromatography method was suitable for separation of organic solvents of radiopharmaceutical samples. Evaluated validation parameters indicated that the analytical procedure had excellent linearity ($r^2 > 0.9998$), good precision (RSD% < 5%) and accuracy (±5% recovery) in the concentration range of 11,379–113,794 mg/L, 612–6122 mg/L, 51–512 mg/L, 89–886 mg/L, 2–20 mg/L, 23–226 mg/L, 107–1073 mg/L and 605–6052 mg/L for ethanol, acetone, acetonitrile, THF, DBM, deanol, DMF and DMSO, respectively (Table 4). The concentration ranges involved at least 120% of the concentration limit of organic solvents.

3.4.6. LOD and LOQ

The quantitation limit with a signal-to-noise ratio of 10 and limit of detection with s/n of 3.3 was determined using a concentration range of 0.2–23.0 mg/L. Signal noise and a calibration curve of peak height ratio of component to ISTD was used to calculate LOD and LOQ values (Supplementary material, Fig. S3-S4). The LOQ for ethanol, acetone, acetonitrile, THF, deanol, DMF and DMSO was 0.48, 0.42, 0.43, 0.46, 0.73, 0.68 and 0.50 mg/L, respectively, while dibromomethane had relatively higher LOQ at 4.35 mg/L, as it showed in Table 4. The LOD for the solvents were ranged from 0.13 to 1.32 mg/L. The experiment showed that the method sensitivity is appropriate for determination of solvents at trace level.

3.5. Determination of residual solvents in ^{18}F- and ^{11}C-radiopharmaceuticals

The proposed gas chromatography method was applied to determine concentration of residual solvents in [^{11}C]methionine, [^{11}C]choline, [^{18}F]FDG and [^{18}F]FET samples (Supplementary material, Table S4). Three batches of radioactive drugs were analyzed. Ethanol, acetone and acetonitrile were detected in [^{18}F]FDG samples. Ethanol

Table 4
Validation parameters of the proposed gas chromatography method.

Solvent	Retention time, (min)	Linearity, (r^2)	Precision (RSD%, n = 6)		Recovery, (%)	Specificity, (R_S)	LOQ, (mg/L)	LOD, (mg/L)	Range, (mg/L)
			Inter-day	Intra-day					
Ethanol	3.57	0.9998	0.5	0.4	100.7	–	0.48	0.15	11,379–113,794
Acetone	3.97	0.9998	2.1	0.5	102.8	2.8	0.42	0.13	612–6122
Acetonitrile	4.29	0.9999	0.8	0.7	100.7	7.7	0.43	0.13	52–512
THF	5.91	0.9998	2.1	1.8	103.8	15.9	0.46	0.14	89–886
DBM	7.29	0.9998	1.7	4.4	99.9	15.5	4.35	1.32	2–20
Deanol	7.76	0.9998	1.0	3.3	102.8	4.9	0.73	0.22	23–226
DMF	8.95	0.9998	2.9	2.1	101.4	11.3	0.68	0.21	107–1073
DMSO	9.90	0.9998	4.2	3.0	99.3	8.7	0.50	0.15	605–6052

was used as excipient in manufacturing of injection. The obtained concentrations ranged from 770 to 1621 mg/L and did not exceed the 5000 mg/L limit. The acetonitrile content reached only 10% of the concentration limit. It means that the evaporation step of the synthesis was performed with high efficiency. The elimination of acetone from the synthesis module during the cleaning procedure was performed also successfully, since the obtained concentrations did not exceed 10 mg/L. Acetonitrile level in [^{18}F]FET was of the same order of magnitude than that of observed in [^{18}F]FDG samples. Since ethanol was used as excipient in manufacturing of [^{18}F]FET injection the measured concentration was higher and ranged from 12,033 to 19,667 mg/L. At the same time, in [^{11}C]methionine the highest ethanol content reached 47,667 mg/L. Deanol could be identified in [^{11}C]choline samples with concentrations from 16.3 to 41.8 mg/L which were lower than the 100 mg/L limit. The results of the analysis of ^{18}F- and ^{11}C-radiopharmaceuticals revealed that the developed gas chromatography method was suitable for determination of residual solvent concentrations in quality control samples (Supplementary material, Fig. S5-S8).

4. Discussion

The developed gas chromatography method is suitable for determination of typical residual solvents that could be identified in most frequently used PET radiopharmaceuticals. This novel analytical procedure could be applied as a generic method for quality control a radioactive drug and consequently, the expensive maintenance of different GC methods in Good Manufacturing Procedure (GMP) environment could be avoided. Although in the literature several GC procedures could be found for quantitation of volatile organic compounds in pharmaceuticals, none of them applicable for simultaneous determination of residual solvents studied in this work. For instance, Cheng et al. [16] proposed a generic head space procedure for determination of residual solvents in drugs, however, it could not be used for quantitation of dibromomethane and deanol that could be detected in [^{11}C]choline and [^{18}F]fluorocholine. The European Pharmacopoeia monograph gives recommendations toward analysis of these solvents, but it is not extended to other compounds [2]. Kilian et al. [21] developed a method only for analysis of solvents in [^{18}F]FDG.

Additionally, the most important advantage of the method developed in this study is the possibility to separate volatile compounds at ethanol concentration of up to 11 m/m%. At the same time, dibromomethane could be quantified in the range of 2–20 mg/L. Consequently, solvents could be successfully analyzed with concentration limits differing by three orders of magnitude. The developed GC method fulfills validation requirements and therefore could be applied generally for determination of residual solvents in ^{18}F- and ^{11}C-radiopharmaceuticals. Since the synthesis routes of these radiotracers are universally used, the impurity profile will be similar, and the proposed analytical procedure could be successfully integrated into quality control system for most PET centers.

5. Conclusion

In this work, a generic GC-FID method was developed for determination of eight volatile organic compounds which could be detected in ^{18}F- and ^{11}C-radiopharmaceuticals listed in the European Pharmacopoeia. Optimized chromatographic conditions as well as appropriate inlet liner packing material should be applied to perform the analysis with good peak resolution and repeatability. The proposed method is suitable for quantitation of ethanol, acetone, acetonitrile, tetrahydrofuran, dibromomethane, 2-dimethylaminoethanol, *N,N*-dimethylformamide and dimethyl sulfoxide in samples with concentration of ethanol up to 11 m/m%. Additionally, the concentration of DBM could be determined in the range from 2 m to 20 mg/L. The developed method was validated which showed good linearity, accuracy and precision as well as offered sufficiently low detection limit and short analysis time. The proposed method was also successfully applied to detect residual solvents in most frequently used ^{18}F- and ^{11}C-labeled tracers. Consequently, this investigation offers a single gas chromatography method which could be used in routine quality control of PET radiopharmaceuticals.

CRediT authorship contribution statement

István Jószai: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, **Nándor Vékei:** Methodology, Validation, Resources, **Dávid Bajnai:** Validation, Resources, **István Kertész:** Writing - review & editing, **György Trencsényi:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2021.114425.

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