VALIDATION OF A HEADSPACE GAS CHROMATOGRAPHY METHOD FOR VOLATILE RESIDUAL SOLVENTS IDENTIFICATION IN RADIOPHARMACEUTICALS

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The following study presents the development and validation of a headspace gas chromatography (HS-GC) analytical method for determining five residual solvents, frequently encountered in radioactive drugs. Measurement of residual solvents is a critical step in the testing and release of pharmaceutical products. The method demonstrated high accuracy and precision, being specific and fast, under the regulations of the European Pharmacopoeia.

Keywords: residual solvent, chemical purity, HS-GC, validation, [^{64}Cu]Cu-DOTA-NT

1. Introduction

Neurotensin (NT) is one of the highly specific peptides used in nuclear medicine to image the neurotensin receptors (NTR1) overexpressed in the colon [1] and prostate [2] cancers through positron emission tomography (PET) [3]. The use of [^{64}Cu]Cu-DOTA-NT radiopharmaceutical in oncological applications requires meeting certain criteria provided by the European Pharmacopoeia (Ph.Eur.), among which: high radiochemical purity (RCP ≥ 95%), high radionuclide purity (RNP ≥ 99.9%), stable *in vivo* and *in vitro*, low toxicity and endotoxin content (≤ 175 EU/mL), and predominant accumulation in the target tissue [4], [5], [6]. Headspace gas chromatography (HS-GC) is the election choice method, used for the separation and analysis of compounds that can be volatilized without decomposition, by using a flame ionization detector (FID) and headspace injection [7]. The qualitative and quantitative determination of the content of residual solvents (Table 1) in radiopharmaceutical products is essential in the pharmaceutical industry, in preclinical or clinical studies, being an indispensable quality parameter that can affect the quality assessment of the final drug [8], [9].

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From a chemical point of view, residual solvents are volatile organic substances, frequently used as processing agents in the synthesis of compounds with pharmacological action [9]. The amount of residual solvents in the radiopharmaceutical product must be within the limits of safety for intravenous (IV) injectable solutions, meaning low toxicity, as accepted by the International Council for the Harmonization of Technical Requirements for Pharmaceutical Products for Human Use (ICH) [9], [10]. An increased level of residual solvents represents a high risk for human health [9]. According to the ICH, residual organic solvents are divided into 3 classes, depending on the level of toxicity, namely: solvents in toxicity class 1 present an increased risk level for human health, being avoided in pharmaceutical products (concentration limits is between 2-8 ppm); class 2 solvents have a medium level of toxicity, has to be tested and used in limited concentrations (50-3880 ppm); and class 3 solvents have a low degree of toxicity, the accepted limit is larger, in concentrations up to 5000 ppm [9], [10].

Table 1

Volatile organic solvents reported in radiopharmaceuticals

Solvent	**ICH Class**	**Concentration limit [ppm]**	**Application**	**Radiopharmaceuticals** [11], [12], [13]
Methanol	2	3000	stabilizing agent	[^{211}At]At α-particles
Ethanol	3	5000	stabilizing agent	[^{11}C]C-flumazenil, [^{18}F]F-FLT, [^{18}F]F-FDG, [^{18}F]F-FET, [^{11}C]C-methionine, [^{18}F]F-DOPA, [^{18}F]F-MISO, [^{11}C]C-choline, [^{68}Ga]Ga-DOTA-TATE
Acetone	3	5000	cleaning vials and tubes	[^{11}C]C-methionine, [^{11}C]C-raclopride, [^{18}F]F-FDG, [^{11}C]C-flumazenil
Isopropanol	3	5000	equipment cleaning	[^{18}F]F-FDG
Acetonitrile	2	410	reaction medium	[^{11}C]C-choline, [^{18}F]F-FET, [^{18}F]F-FDG, [^{18}F]F-FMISO, [^{18}F]F-FLT

This study aims to validate an improved analytical gas chromatography method to quantify the level of residual solvents, frequently used in the synthesis of radiopharmaceuticals, namely: methanol, ethanol, isopropanol, acetonitrile, and acetone.

2. Experimental

2.1 Chemicals and reagents

The solvents used in this study were purchased from commercial sources: absolute ethanol and isopropanol (Supelco, Merck KGaA, Germany); acetonitrile and methanol (for GC residue analysis, Scharlau, Scharlab S.L, Barcelona); acetone (100% purity, VWR BDH CHEMICALS, USA). The ultrapure water used to dilute the solvent samples was obtained from the water purification system, Millipore 8/16 Systems (Millipore SAS, France). The enriched isotope Nickel-64 (99.5%) was purchased from Isoflex Company (San Francisco, USA), the DOTA-NT peptide (>95%) was obtained from Eurogentec (Belgium) and the saline solution (0.9% NaCl, 308 mOsm/L theoretical osmolarity) was purchased from Hemofarm (Serbia).

2.2. HS-GC method

The residual solvents evaluation was performed using the gas chromatograph Agilent 7890A (Agilent Technologies Inc., USA) equipped with a flame ionization detector, a 7697A Headspace, and ALS G4513A Autosampler. The chromatographic separation was performed on fused silica capillary column (6% poly[(cyanopropyl)-(phenyl)] siloxane and 94% poly(dimethyl) siloxane) with dimensions 30 m × 0.53 mm, 3 μm (Agilent J&W DB-624) [14]. The headspace vials used for analytical samples were crimped with an aluminium cap provided with a Teflon-lined septum. OpenLab CDS software (Agilent Technologies Inc., USA) was used to perform system control, data acquisition, and processing.

For method validation, the stock standard solution was prepared by diluting appropriate amounts of investigated solvents (99.99% analytical purity) with ultrapure water to reach a mixture of solvents with the following concentrations: 60 mg/mL of methanol, 100 mg/mL of ethanol, 100 mg/mL of acetone, 100 mg/mL of isopropanol, and 10 mg/mL of acetonitrile.

The work standard solutions were prepared by diluting the standard stock solution with ultrapure water, according to ICH Q2(R2) and ICH Q3(R8) acceptable limits[4], [15], [16]. The peptide solution was obtained by diluting 100 μL of the [^{64}Cu]Cu-DOTA-NT solution with 400 μL of ultrapure water.

The method used for determining residual solvent content has been validated employing the limits and standards specified in the ICH guideline and the Ph. Eur. The method's robustness, linearity, precision, accuracy, specificity, limit of quantification (LOQ), limit of detection (LOD), and system testing were all evaluated as part of the validation process. All these method quality parameters were evaluated using the recommendations of the ICH Q2(R2) standard [4].

2.3 Radiosynthesis of [^{64}Cu]Cu-DOTA-NT

Copper-64 is a radioisotope with huge potential in nuclear PET imaging due to its β^+ emission (17.86%). Besides this emission, the radioisotope decays by β^-

emission (39.03%) and undergoes electron capture decay (43.10%), resulting in Auger electrons of very low energy, making it a potential therapeutic agent in targeted radiotherapy. ^{64}Cu is obtained via ^{64}Ni(p, n)^{64}Cu nuclear reaction, by irradiating enriched ^{64}Ni target at TR-19 cyclotron (ACSI, Canada) with 14.2 MeV protons, for 6 h. The [^{64}Cu]$CuCl_2$ solution was obtained by processing and purifying the irradiated solid target [17] using the ALCEO module (COMECER, Italy). The chemical form of copper used in peptide labelling is chloride (Cu^{2+}), which forms complex combinations with the DOTA (2, 2′, 2″, 2‴- (1, 4, 7, 10-Tetraazacyclododecane-1, 4, 7, 10-tetrayl) tetraacetic acid) chelator. For the radiolabelling, 1 mL of [^{64}Cu]$CuCl_2$ solution (radioactive concentration ~ 750 MBq/mL) was added over 20 nmol of DOTA-NT dissolved in 50 µL ultrapure water. The process involves certain reaction conditions, including high temperature (> 95°C), at pH 4.0, and 20-30 minutes reaction time. After radiosynthesis, the final solution is purified using a specific cartridge (StrataTM-X 33 µm RP cartridge, Phenomenex); the volume was reduced via evaporation and recovered in a saline solution [18].

3. Results and discussion

The analysis of the residual solvents and implicitly the validation of the method described in this study were carried out following the adaptation and the optimization of certain parameters of the method in accordance with the current needs, starting from our previously reported work [8], [14] and the conditions provided by the Ph.Eur. [19]. The application of the method described in the Ph.Eur. presents a disadvantage in terms of quality control of radiopharmaceutical products, due to the long time for equilibration of the head-space system (45-60 min). As a result, we attempted to develop a faster and more precise method for determining the residual solvent concentration in the radiopharmaceuticals synthesised by our team. After optimizing the method parameters, the pressure was increased from 2.27 psi to 7.95 psi, the flow rate was reduced to 33 mL/min leading to a better separation of the components in the analysed sample, the inlet split ratio was modified from 15:1 to 20:1 and the FID detector temperature value was set at 300 °C. The parameters of the optimized method are shown in Table 2.

Table 2

GC-HS parameters for residual solvent analysis

	Parameters	**Eur.Ph.** [19]	**Initiated** [8], [14]	**Optimized**
GC	Carrier gas	He		
	Inlet split ratio	5:1	15:1	20:1
	Carrier flow rate (mL/min)	-	48	33
	Pressure (psi)	-	2.27	7.95

	Oven temperature gradient (°C)	40-240	40-100	35-85
	FID temperature(°C)	250	250	300
	Analysis time (min)	35	7	7
Headspace	Equilibration temperature(°C)	80 -105	105	100
	Equilibration time (min)	45-60	2	3
	Transfer-line temperature (°C)	80-110	110	100
	Injection duration (min)	-	0.2	0.5

3.1 Linearity

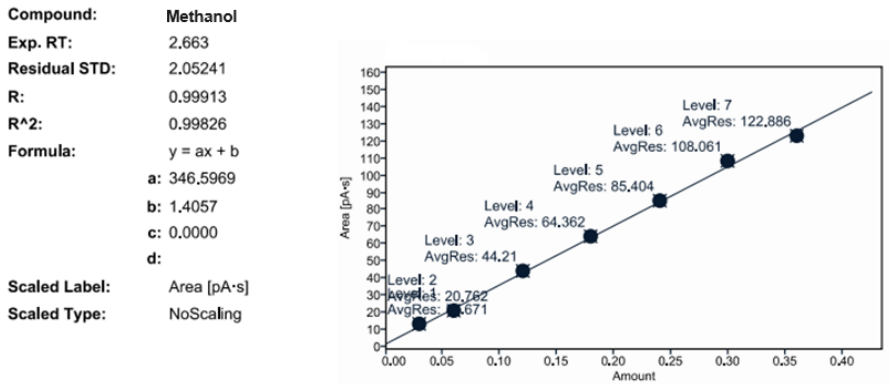
The linearity of the method was studied on seven different concentrations within the 10%-120% range of the maximum concentration allowed for each solvent. The following reference solutions: 10%, 20%, 40%, 60% 80%, 100%, and 120% concentration were prepared from the stock solution. Figure 1 shows the calibration curve (absorbance as a function of the analyte concentration) and the linear regression determined using the least squares method. The regression slope (m), residual standard deviation values, and correlation coefficient (R^2) were evaluated according to the criteria presented in ICH Q2 (R2) [4]. R^2 higher than 0.995 was considered the acceptance criteria for each solvent. As can be observed in Figure 1, linearity was demonstrated for all residual solvents analyzed. The results of the linearity test are presented in Table 3.

Table 3

The linearity 10-120% of the method

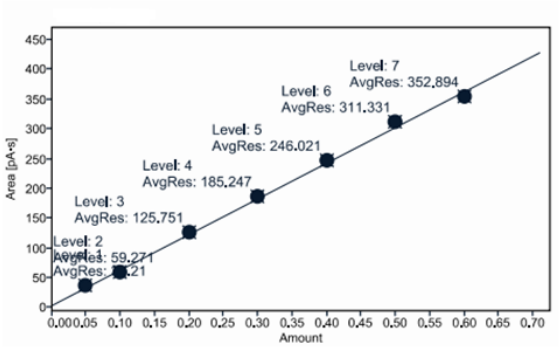
Solvent	**t_R [min]**	**m -** regression slope	**RSD***	**R^2**
Methanol	2.67	346.60	2.05	0.998
Ethanol	3.31	598.78	6.12	0.998
Acetone	3.79	943.82	6.45	0.999
Isopropanol	3.89	643.99	6.45	0.998
Acetonitrile	4.12	695.47	0.63	0.999

*RSD= residual standard deviation or residual variation



(a)

Compound:	**Ethanol**
Exp. RT:	3.302
Residual STD:	6.12431
R:	0.99906
R^2:	0.99813
Formula:	y = ax + b
a:	598.7751
b:	3.6697
c:	0.0000
d:	
Scaled Label:	Area [pA·s]
Scaled Type:	NoScaling



(b)

Compound:	**Acetone**
Exp. RT:	3.780
Residual STD:	6.44615
R:	0.99958
R^2:	0.99916
Formula:	y = ax + b
a:	943.8173
b:	4.4273
c:	0.0000
d:	
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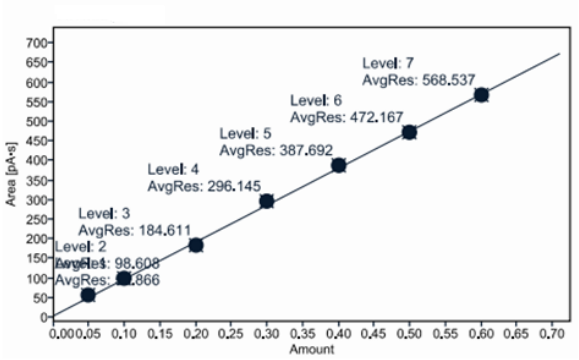

(c)

Compound:	**Isopropanol**
Exp. RT:	3.877
Residual STD:	6.45124
R:	0.99910
R^2:	0.99820
Formula:	y = ax + b
a:	643.9847
b:	4.8361
c:	0.0000
d:	
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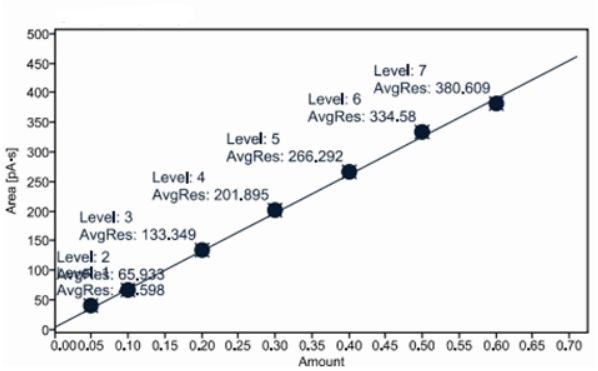
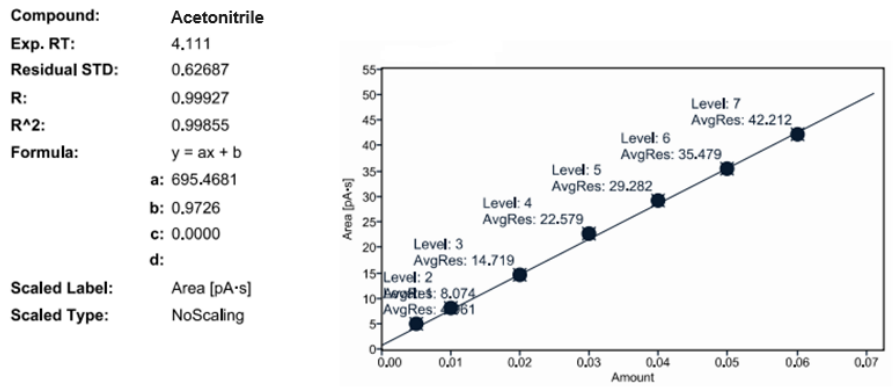


(d)



(e)

Fig 1. Residual solvent calibration curves: (a) Methanol, (b) Ethanol, (c) Acetone, (d) Isopropanol, and (e) Acetonitrile

3.2 Selectivity/Specificity

A method is considered specific when it has high selectivity [4]. Selectivity is investigated to demonstrate the ability of the method to differentiate the examined analyte from other substances (potential residual solvents) that may be present in the sample. A standard mixture of residual solvents at 100% concentration of the accepted limit was analysed using HS-GC to assess the method selectivity. Following the chromatographic analysis, the resolution between each two successive peaks was determined. The resolution (R_s) was calculated according to the equation [5]:

$$R_s = \frac{1.18 \times (t_{R2} - t_{R1})}{w_{h1} + w_{h2}} \quad (1)$$

Where:

- t_{R1} and t_{R2} are the retention times of the first and second peaks, respectively.
- wh_1 and w_2 are the widths of the first and second peaks at their base, respectively.

A resolution >1.0 is considered suitable for an adequate separation of two compounds. As can be observed in Figure 2, all the solvents of interest were identified and showed good separation (both as resolution values between two peaks and symmetry factor, being consistent with the acceptance criteria, as presented in Table 4). The developed method proved to be specific for the investigated solvents.

Table 4

The specificity of the method

Solvent	t_R (min)	Resolution		Symmetry	
		Acceptance criteria	Determined	Acceptance criteria	Determined

Methanol	2.66	-	-		0.87
Ethanol	3.30	≥ 1.0	9.81	0.80 – 1.80	0.88
Acetone	3.78		6.88		0.94
Isopropanol	3.88		1.31		0.98
Acetonitrile	4.10		3.00		0.87

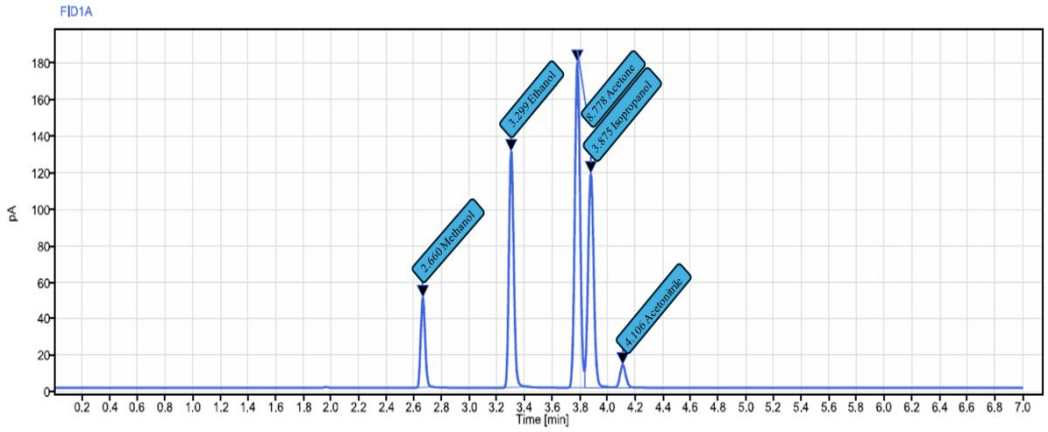


Fig 2. The GC chromatogram of the standard residual solvents

3.3 Accuracy

The accuracy of the method was assessed by calculating the mean percentage of recovery for each residual solvent [4], [5]. This is calculated using the following formula:

$$Accuracy\ [\%] = \frac{\overline{c_d}}{\overline{c_r}} \times 100 \qquad (2)$$

Where:

- $\overline{c_d}$ is the determined/recovery concentration
- $\overline{c_r}$ is the real concentration.

The most common solvents found in radiopharmaceutical preparations are methanol, ethanol, acetone, isopropanol, and acetonitrile. The accuracy was investigated using three different concentrations of the standard solution of solvents mixture (80%, 100%, and 120% of limit concentration), each being injected in triplicate. The accuracy of the method is within 90-110% recovery of the accepted real concentration. The results obtained are presented in Table 5.

Table 5

The accuracy of the method

Solvent	$\overline{c_d}\ [mg/mL]$*	$\overline{c_r}\ [mg/mL]$**	Accuracy [%]
80%			
Methanol	0.24	0.24	101
Ethanol	0.42	0.40	105
Acetone	0.44	0.40	110
Isopropanol	0.44	0.40	110
Acetonitrile	0.04	0.04	105

100%			
Methanol	0.30	0.24	102
Ethanol	0.53	0.40	105
Acetone	0.55	0.40	109
Isopropanol	0.55	0.40	110
Acetonitrile	0.05	0.04	104
120%			
Methanol	0.37	0.24	103
Ethanol	0.62	0.40	104
Acetone	0.62	0.40	104
Isopropanol	0.63	0.40	104
Acetonitrile	0.06	0.04	100

* $\bar{c}_d$ = determined/recovery concentration
** $\bar{c}_r$ = real concentration

3.4 Precision

According to the ICH Q2(R2) guideline [4], the precision test is performed by evaluating six consecutive measurements for a solution containing 100% of the maximum allowed value of residual solvent in the sample to be analyzed [4], [15]. The final concentration of the injected solutions for each residual solvent was: 0.3 mg/mL methanol, 0.5 mg/mL ethanol, 0.5 mg/mL acetone, 0.5 mg/mL isopropanol, and 0.05 mg/mL acetonitrile. For each peak of residual solvent obtained after the analyses, the average of the obtained areas, the standard deviation (SD), and the relative standard deviation (%RSD) were calculated. The RSD for each residual solvent was obtained after injecting six samples with the same solution was less than 15%. The results obtained are presented in Table 6.

Table 6

The precision of the method

Injection		**Methanol**	**Ethanol**	**Acetone**	**Isopropanol**	**Acetonitrile**
1		105.00	309.93	487.74	342.26	35.60
2		109.33	327.09	502.28	364.99	36.66
3	**Peaks areas**	103.96	309.12	493.32	345.79	35.65
4		114.93	335.16	484.57	362.07	36.31
5		108.43	316.63	343.35	343.35	35.30
6		119.31	342.63	363.62	363.62	36.81
Average areas		110.16	323.42	488.18	353.68	36.06
SD		5.92	13.81	9.17	10.92	0.63
% RSD		5.37	4.27	1.88	3.09	1.73

3.5 Detection and quantification limits

The detection (LOD) and quantification (LOQ) limits were determined in accordance with the requirements for identification and quantification presented in the ICH Q2(R2) guide [4]. The LOD is defined as the lowest amount of analyte that can be detected against the blank, while the LOQ is known as the lowest amount of

analyte in a sample that can be determined with an acceptable level of repeatability and accuracy. The residual standard deviation (RSD) and regression slope (m) values were used to calculate the LOD and LOQ, applying the following formulas [4]:

$$LOD = \frac{3.3 \times RSD}{m} \quad (3)$$

$$LOQ = \frac{10 \times RSD}{m} \quad (4)$$

The results are presented in Table 7.

Table 7

LOD and LOQ values for residual solvents

Solvent	***RSD***	***m** - regression slope*	***LOD [mg/mL]***	***LOQ [mg/mL]***
Methanol	2.05	346.60	0.020	0.060
Ethanol	6.12	598.78	0.034	0.102
Acetone	6.45	943.82	0.023	0.068
Isopropanol	6.45	643.98	0.033	0.100
Acetonitrile	0.63	695.47	0.003	0.009

3.6 Robustness

The robustness of the method was evaluated by measuring the test solution of the system under controlled changes of the following parameters: injection temperature and separation column temperature [4]. The values of retention times, resolution and symmetry of the peaks were evaluated. The results obtained following minor changes in the operational parameters mentioned above demonstrated that the method is robust and that they do not show significant variations [4].

3.7 Method evaluation on radiopharmaceuticals

System testing was carried out to show that the technique can identify, and separate residual solvents present in radiopharmaceuticals. The retention time, symmetry, and resolution between two consecutive peaks (if more than 1 is present) were determined for the residual solvents identified in the chromatogram. These parameters must be consistent with the acceptance criteria for resolution (≥ 1.0) and symmetry (0.80-1.80) provided by the Eur. Ph. The [^{64}Cu]Cu-DOTA-NT was analysed for method evaluation (Fig. 3).

A specific peak, corresponding to the ethanol, appeared at a retention time of 3.364 min (Fig 3.). The determined peak's symmetry was 1.005. The amount of ethanol in the sample under analysis was 0.08 mg/mL, a value within the limitations specified by the European Pharmacopoeia.

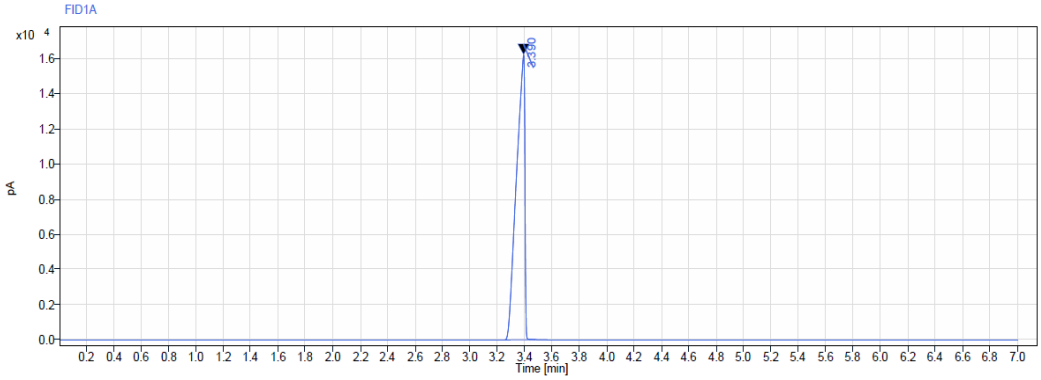


Fig 3. GC chromatogram for [^{64}Cu]Cu-DOTA-NT solution

4. Conclusions

We developed, validated, and implemented a straightforward, reliable, and efficient HS-GC method for the simultaneous determination of five residual solvents (methanol, ethanol, acetone, isopropanol, and acetonitrile) in our laboratory's routine analysis. The suggested method is a fast way to determine the residual solvent content (analysis time 7 min) and compared with the previously reported method [14], the peaks of the solvents of interest are observed earlier; therefore, it can save time when analyzing various radiopharmaceuticals. The system was calibrated using seven distinct concentration levels in the reference range of 10-120%, and the linear regression correlation coefficient (R^2) is more than 0.995 for all the linearity solutions examined. The method's repeatability was tested by injecting a mixture of standard solvents into the system six times. After averaging the areas for each residual solvent, the coefficient of variation %RSD is ≤10%. Acetonitrile presented the smallest residual variation. The method to determine the residual solvent content was tested using the optimal operating settings to analyse the [^{64}Cu]Cu-DOTA-NT radiopharmaceutical, with ethanol as the residual solvent of interest. All radiopharmaceutical series tested carried out the acceptance standards, and the residual solvent content assayed was less than the limit concentration values.

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