Research Article

Assessing the influence of neglected GC-FID variables on the multiple responses using multivariate optimization for the determination of ethanol and acetonitrile in radiopharmaceuticals

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Abstract

Analytical gas chromatography in line with a flame ionization detector (GC-FID) method was developed and validated for direct determination of organic solvents in [^{18}F]fluoro-ethyl-tyrosine ([^{18}F]FET), [^{18}F]fluoromisonidazole ([^{18}F]FMISO) and [^{18}F]fluorothymidine ([^{18}F]FLT). Variables of the splitless time (min) and injection temperature (°C) on the response of analysis time and resolution were optimized with the assistance of a two-level full factorial design and desirability function of Derringer. The proposed procedure was validated following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R1) guideline. Excellent linearity, $R^2 > 0.990$, indicated that approximately 99% of the response variance could be predicted from ethanol and acetonitrile concentrations ranging from 0.5 to 6.0 mg mL^{-1} and 0.1 to 0.8 mg mL^{-1}, respectively. The proposed procedure has proved to be selective, sensitive, and accurate (90–110%), with excellent repeatability and precision (RSD < 2%). In the robustness analysis, the findings from the calculated Standardized Effects Values (SE) were insignificant ($p > 0.05$) and demonstrated that the proposed method was robust for a splitless time of 1.0 ± 0.5 min and an injection temperature of 210 ± 10 °C. The proposed method was also successfully used for the quantitative determination of ethanol and acetonitrile in [^{18}F]FET, [^{18}F]FMISO, and [^{18}F]FLT. Both solvents were well separated (R, 4.1–4.3) within 4.5 min. Therefore, the proposed method is relevant for routine quality control analysis of all ^{18}F-radiopharmaceutical derivatives for the direct determination of ethanol and acetonitrile.

Keywords Organic solvent analysis · Quality control · Gas chromatography-flame ionization detection (GC-FID) · Splitless time · Injection temperature · Radiopharmaceutical

Abbreviations

[^{18}F]	Fluorine-18
[^{18}F]FET	[^{18}F]fluoro-ethyl-tyrosine
[^{18}F]FLT	[^{18}F]fluorothymidine
[^{18}F]FMISO	[^{18}F]fluoromisonidazole
%RE	Percentage of relative error
CDNI	Centre for Diagnostic Nuclear Imaging
d_i	Individual desirability
d_{iAt}	Individual desirability for analysis time
d_{iR}	Individual desirability for resolution
D	Global desirability
FID	Flame ionization detection

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GC	Gas chromatography
GC-FID	Gas chromatography flame ionization detection
ICH	The International Conference on Harmonisation of Technical Specifications for the Registration of Pharmaceuticals for Human Use
IJ	Injection temperature
LOD	Limit of detection
LOQ	Limit of quantification
min	Minute
mg mL^{-1}	Milligram per milliliter
OLSM	Ordinary least squares method
r	Correlation coefficient
R	Resolution
R^2	Regression analysis
RSD	Relative standard deviation
RSDIP	Relative standard deviation from intermediate precision
RSDr	Relative standard deviation from repeatability
S	Weight
SE	Standardized effects
ST	Splitless time
UPM	Universiti Putra Malaysia
w/v	Weight by volume
y_i	Response
y_{mim}	Lowest experimental response
y_{max}	Highest experimental response

1 Introduction

Organic solvents play an indispensable role in most radiopharmaceutical production phases to facilitate reactions, separation, purification and drying. Therefore, it is almost impossible to remove them entirely in the final product formulation [1–5]. The aforementioned has sparked interest in the community due to organic solvents' potential ability to pose a health risk due to their toxicity [6]. The International Conference on Harmonization of Technical Specifications for the Registration of Pharmaceuticals for Human Use (ICH) Guideline Q3C, updated in 2018, listed acetonitrile and methanol as Class 2 solvents due to their inherent toxicity and potentially harmful to human health. In the meantime, acetone, ethanol, and dimethyl sulfoxide as Class 3 are moderately harmful in large quantities [7].

In pursuance of overcoming the potential effects of organic solvents on the patient, the international and local regulatory authorities have decided that organic solvents' determination is compulsory in radiopharmaceutical quality control practices. The previously mentioned is achieved by limiting the amount of Class 2 solvents in the final formulation of radiopharmaceuticals to a maximum of 0.41 mg mL^{-1} and 5 mg mL^{-1} for Class 3 solvents [2, 3, 7].

The current consensus is that the analytical gas chromatography method has been identified as the most efficient method for analyzing organic solvents [8, 9]. With the development of gas chromatography technology, now it is possible to determine the concentration of organic solvents which remained in the final formulation in a short time with high specificity and sensitivity [10–12].

Over the last ten years, much research has examined, and different pharmacopeias have discussed several gas chromatography analyses [13]. As the gas chromatography equipment and its sub-component may differ one to another, depending on the specification, the optimal procedure also may be different. The influence of variation in the column temperature, injection split ratio, carrier gas flow (mL min^{-1}), and carrier gas on gas chromatography responses have been extensively investigated in previous research [2, 3, 10, 14–16].

Work in this area is extensive but is primarily concerned with the influence of variation in the column temperature, injection split ratio, carrier gas flow (mL min^{-1}), and carrier gas on gas chromatography responses. Limited number of studies have been conducted to investigate the effect of the splitless time and injection temperature on gas chromatography multiple responses to the analysis time and resolution in the radiopharmaceutical field [13]. To our knowledge, these two variables are always overlooked, and the method's potential has not yet been identified. The splitless time parameter is repeatedly used for analyzes involving adulteration in food products, pesticides, and environmental monitoring, to name a few [17–22].

Therefore, this study is set to explore the influence of splitless time and injection temperature on multiple responses to analysis time and resolution using multivariate analysis for method optimization. The present study's goal was to test the hypothesis that variation in the splitless time and injection temperature could significantly result in rapid analysis time and excellent resolution of adjacent peaks. At the latter stage, this recent work focuses on optimizing and validating an improved gas chromatography analytical method for the determination of ethanol and acetonitrile in [^{18}F]fluoro-ethyl-tyrosine ([^{18}F]FET), [^{18}F]fluoromisonidazole ([^{18}F]FMISO), and [^{18}F]fluorothymidine ([^{18}F]FLT).

2 Materials and methods

2.1 General

[^{18}F]fluoride produced via the $^{18}O(p,n)^{18}F$ nuclear reaction on a 16.5 meV cyclotron PETtrace® (GE Healthcare

Technologies, USA) was supplied by the National Cancer Institute (NCI) and delivered in the aqueous form to the Centre of Diagnostic Nuclear Imaging (CDNI), Universiti Putra Malaysia (UPM). The no-carrier-added [^{18}F]fluoride solution was then transferred to the Scintomics GRP 4 V module (Scintomics GmbH, Germany) to produce [^{18}F]FET, [^{18}F]FMISO, and [^{18}F]FLT.

2.2 Reagent and chemicals

Ethanol and acetonitrile with the purity of 99.9% and 99.5%, respectively, were purchased from Merck (Darmstadt, Germany) and used to prepare analytical solutions. Reagent kits and sterile cassettes used in the preparation of [^{18}F]FET, [^{18}F]FMISO, and [^{18}F]FLT were purchased from ABX Advanced Biochemical Compounds (Radeberg, Germany). All precursors and eluents were stored following ABX Advanced Biochemical Compounds instruction.

2.3 Instrumentation

All experiments were performed on a Shimadzu (Japan) GC-2010Plus AF gas chromatography equipped with an FID-2010Plus flame ionization detector (FID) and an integrated AOC-20i autoinjector. The system was controlled by LabSolutions (Version 5.82). Chromatographic separation was performed on the Agilent J&W DB-200 column ((35% trifluoropropyl)-methylpolysiloxane column, mid-polarity), 30 m in length, 0.53 mm inner diameter, and 1 µm film thickness. For each injection, a sample volume of 1 µL was used.

The ultrapure nitrogen gas, 99.9995% purity (Air Products, Malaysia), was used as the carrier gas and set at 30 mL min^{-1}. For combustion of the FID, as a rule of thumb, the ratio of air to hydrogen gas was 10:1; the airflow (Air Products, Malaysia) was set at 400 mL min^{-1}, while for hydrogen gas, 99.9992% purity (Air Products, Malaysia) it was set at 40 mL min^{-1}. The temperature of the FID detector was set at 250 °C.

The carrier gas flow was set at 2.4 mL min^{-1}. The column temperature was set at 50 °C and hold for 1 min before linearly increased at 10 °C min^{-1} to 90 °C. In an attempt to test the current study hypothesis, the splitless time and injection temperature were evaluated using multivariate optimization of the two-level full factorial design.

2.4 Optimization of the experimental factors

Multiple responses to the analysis time and resolution of adjacent peaks directly affected by the splitless time and injection temperature were assessed using a two-level full factorial design. To allow these responses to be optimized simultaneously, the desirability function was used [2, 23]. Each response (y_i) was converted to an individual desirability function (d_i), which varies between 0 and 1 ($0 \le d_i \le 1$). The individual desirability (d_i) was then determined using the Eqs. (1) and (2) for the analysis time and resolution of adjacent peaks.

$$di = \left(\frac{ymax - yi}{ymax - ymim}\right)^{S=1}, ymim \le yi \le ymax \quad (1)$$

$$di = \left(\frac{yi - ymim}{ymax - ymim}\right)^{S=1}, ymim \le yi \le ymax \quad (2)$$

y_i is any experimental response, while y_{mim} and y_{max} are the lowest and highest experimental response, and S is the weight (when equal to 1 = linear desirability function). The two individual desirability scores, d_i; analysis time and resolution, were then combined into a single global desirability D.

Analysis of Variance (ANOVA) is a statistical method used to analyze variations among or between groups. It is also commonly used for the treatment of gas chromatographic data [24]. In this study, a statistical analysis of a Factorial ANOVA was carried out on data to ascertain which factors significantly impact the response. All statistical analyses were performed using the SPSS Statistics 26 (IBM, USA) software.

2.5 Validation of the proposed procedure

The procedure was validated following the ICH analytical procedure validation guideline [25]. The matrix effect, linearity, precision (repeatability and intermediate precision), the limit of detection (LOD) and quantification (LOQ), sensitivity, selectivity, accuracy (recovery), and robustness of the proposed procedure were evaluated.

2.6 Application of the procedure

Production of [^{18}F]FET, [^{18}F]FMISO, and [^{18}F]FLT were performed in Scintomics GRP 4 V automated synthesis module (Scintomics, Germany) at CDNI, UPM. Two consecutive productions were carried out for each of the ^{18}F-derivatives. Before the sample collection in a sterile vial, samples were passed through and filtered using a 0.22 µm membrane filter attached to the end of the dispensing line (Millipore, USA). The automated synthesis module usually controlled the step. Samples were then analyzed using the proposed procedure. The peaks obtained from the samples were determined by comparing the retention times with the ethanol and acetonitrile standards under the same chromatographic conditions and with the test samples.

3 Results and discussion

3.1 Optimization of the experimental factors

Experiments were executed using a 0.9% (w/v) NaCl solution spiked with ethanol and acetonitrile concentrations equivalent to 1 mg mL^{-1} for the simulation of a radiopharmaceutical matrix. The two-level full factorial design was performed on two-levels: high (+) and low (–). Table 1 shows the coded and actual values of the selected factors at this level, the central point triplicates, and the d_i values for the resolution and analysis time. The factors were optimized using a two-level full factorial design, which includes the central point triplicates for estimating the experimental error.

Table 1 Experimental factors with individual desirability values for the analysis time and resolution response

Experiment	Splitless time (min)	Injection temperature (°C)	Individual desirability (d_i)	
			Analysis time (d_{iAt})	Resolution (d_{iR})
1	0.5 (–1)	200 (–1)	0.35	0.49
2	0.5 (–1)	220 (1)	0.61	0.53
3	1.5 (1)	200 (–1)	0.34	0.42
4	1.5 (1)	220 (1)	0.49	0.34
5	1 (0)	210 (0)	0.30	0.67
6	1 (0)	210 (0)	0.44	0.50
7	1 (0)	210 (0)	0.37	0.55

d_{iAt} = Individual desirability for analysis time realised through application of Eq. (1)

d_{iR} = Individual desirability for resolution realised through application of Eq. (2)

However, in contrast to previous studies, splitless time and injection temperature were selected as factors [2, 3]. There are no previous literature reports about whether the splitless time and injection temperature directly affected both the analysis time and resolution, particularly in radiopharmaceutical analysis. Analysis time and resolution of adjacent peaks were chosen as responses to assess the proposed method's suitability in terms of rapid analysis time and separation efficiency.

The influence of column temperature was not evaluated here as the preliminary results have shown that at the isothermal temperature of below 100 °C, the separation between ethanol and acetonitrile was poor and not convincing (Fig. 1). It was surprising because both solvents have a relatively low boiling point, below 100 °C, which can usually be quite satisfactorily separated under isothermal conditions [26]. When the column was set isothermally between 100 and 200 °C, ethanol and acetonitrile formed a single peak (Fig. 1). The finding is expected since the temperature was set at too high, the lighter components will co-eluate, resulting in poor resolution [27]. It was interesting to note that throughout these experimental designs,

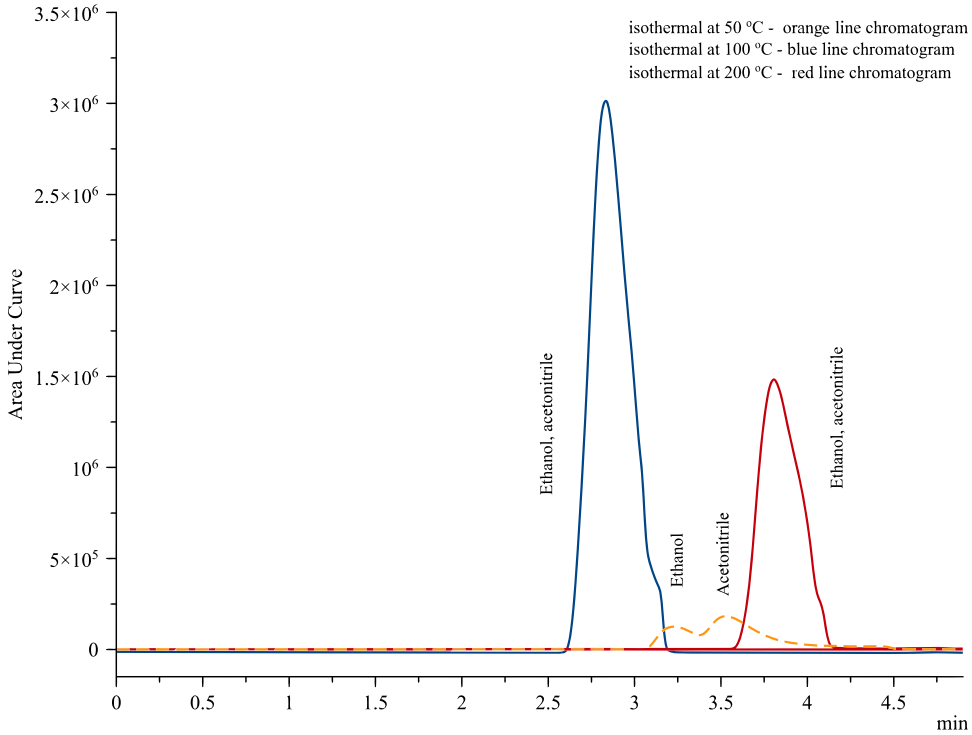

Fig. 1 GC-FID chromatogram of 0.9% (w/v) NaCl solution spiked with ethanol and acetonitrile (1 mg mL^{-1}) at isothermal column temperature of 50, 100, and 200 °C

the temperature control program was set at 50 °C and held for 1 min before the column temperature was linearly increased at 10 °C min^{-1} to 90 °C.

The statistical analysis of Factorial ANOVA revealed a relatively low observed power, 0.61. However, there was a significant interaction between the splitless time and injection temperature on the analysis time ($p < 0.05$). The Partial Eta squared revealed about 56% of the variance in the analysis time can be predicted from the splitless time. In comparison, only about 33% was predicted for the injection temperature (Table 2).

As the interaction between these factors on the analysis time was significant, further Games-Howell posthoc analysis was performed to determine the combination of experimental matrices that yield statistically significant results on the analysis time. It is unsurprising to find that the increase in the splitless time increased the analysis time. Our most intriguing finding is the combination of 0.5 min splitless time and 220 °C of injection temperature produced a resolution of more than 4 (R > 4), analysis time within 4.5 min with a precision of relative standard deviation of less than 3%. On the contrary, no significant difference was found on the interaction between splitless time and injection temperature on the resolution ($p > 0.05$). The finding of this statistical analysis reinforces the general belief that only the injection split ratio affects resolution [2].

Table 2 Statistical analysis of factorial ANOVA

Parameters	Partial Eta squared	Observed power	Sig
Splitless time (ST)	0.562	0.998	0.000
Injection temperature (IJ)	0.332	0.746	0.018
ST*IJ (interaction)	0.268	0.607	0.044

After the validation and optimization of the two-individual desirability (d_i) for analysis time and resolution, both functions could be combined into the overall desirability (D), which was computed using Eq. (3)

$$D = \sqrt{diAtdiR} \tag{3}$$

d_{iAt} and d_{iR} are denoted as the individual desirability for the analysis time and resolution, respectively.

From the calculation, the optimum conditions for the ethanol and acetonitrile in the sample to be well resolved with a rapid analysis time, within 5 min and highest D (0.57) were: 0.5 min of the splitless time at 220 °C of injection temperature before the solvents entering the column (Fig. 2). Under these conditions, the resolution values were between 4.1 and 4.3, with a relative standard deviation (RSD) of less than 3%. The statistical analysis of Two-Way ANOVA performed earlier significantly strengthened the optimum conditions obtained using the overall desirability (D). In summary, the optimal experimental conditions used for validation in the later part were as follow (Table 3).

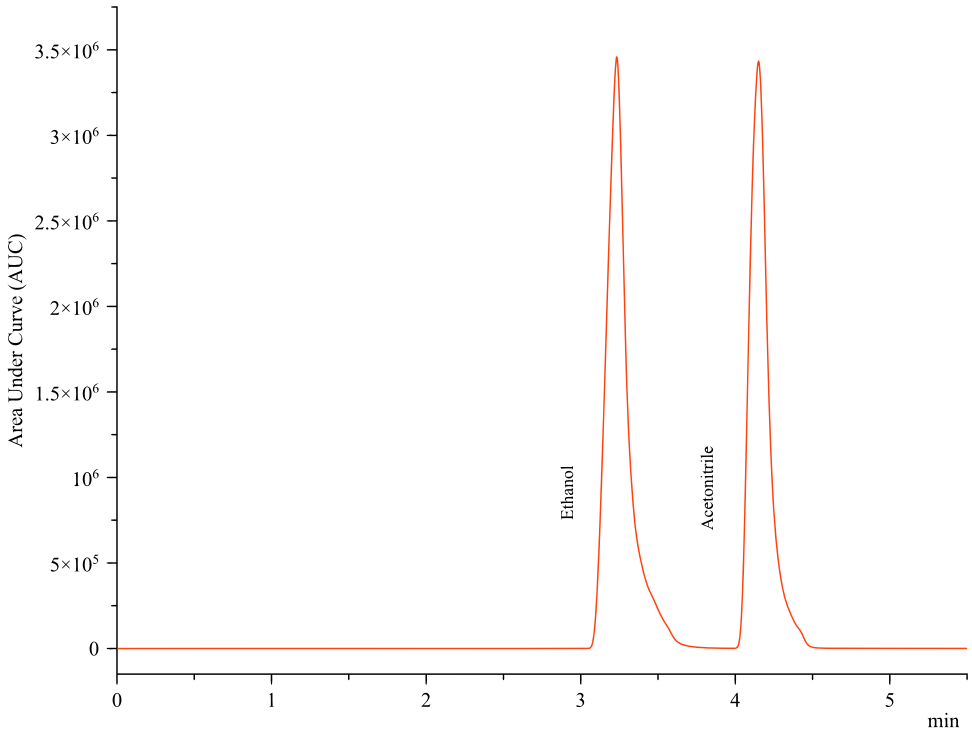

Fig. 2 GC-FID chromatogram of 0.9% (w/v) NaCl solution spiked with ethanol and acetonitrile (1 mg mL^{-1}) under optimized chromatographic conditions

Table 3 Optimal experimental conditions

Parameters	Optimal conditions
Injection volume	1 µL
Splitless time	0.5 min
Injection temperature	220 °C
Carrier gas flow	2.4 mL min^{-1}
Column temperature	50 °C for 1 min, 10 °C min^{-1} to 90 °C
Detector temperature	250 °C

3.2 Validation of the proposed procedure

Following a statistical analysis of Student's *t*-test, the matrix slopes and standard calibration curves for both solvents showed a significant result of the matrix effect ($p < 0.05$). It could be due to the interaction between the matrix and both solvents, which produces a significant difference in response. For this reason, fortified samples were used to construct the calibration curve.

In the linearity study, Levene's test was performed to support homoscedasticity ($p > 0.05$) before the use of the ordinary least squares method (OLSM) for slope, intercept, residual, and correlation coefficient (r) estimation. Notably, a close correlation existed between the standard solution at the five concentrations levels and the area's response under the curve for both solvents, $r > 0.990$. Logistic regression analyses were performed to assess the association between concentration levels and response. Through the regression analysis (R^2), about 99% of the response's variance can be predicted from the concentration.

All calibration points were found within the prediction interval (Fig. 3a), and the present value of R^2 was 0.992. The studentized residual plot (Fig. 3b) also shows all the computed residues within the ± 2 limits and appears normally distributed and centered at zero. The response factor for ethanol tends to become unstable at a medium level, with values were exceeded ± 5% (Fig. 3c). Nevertheless, superior

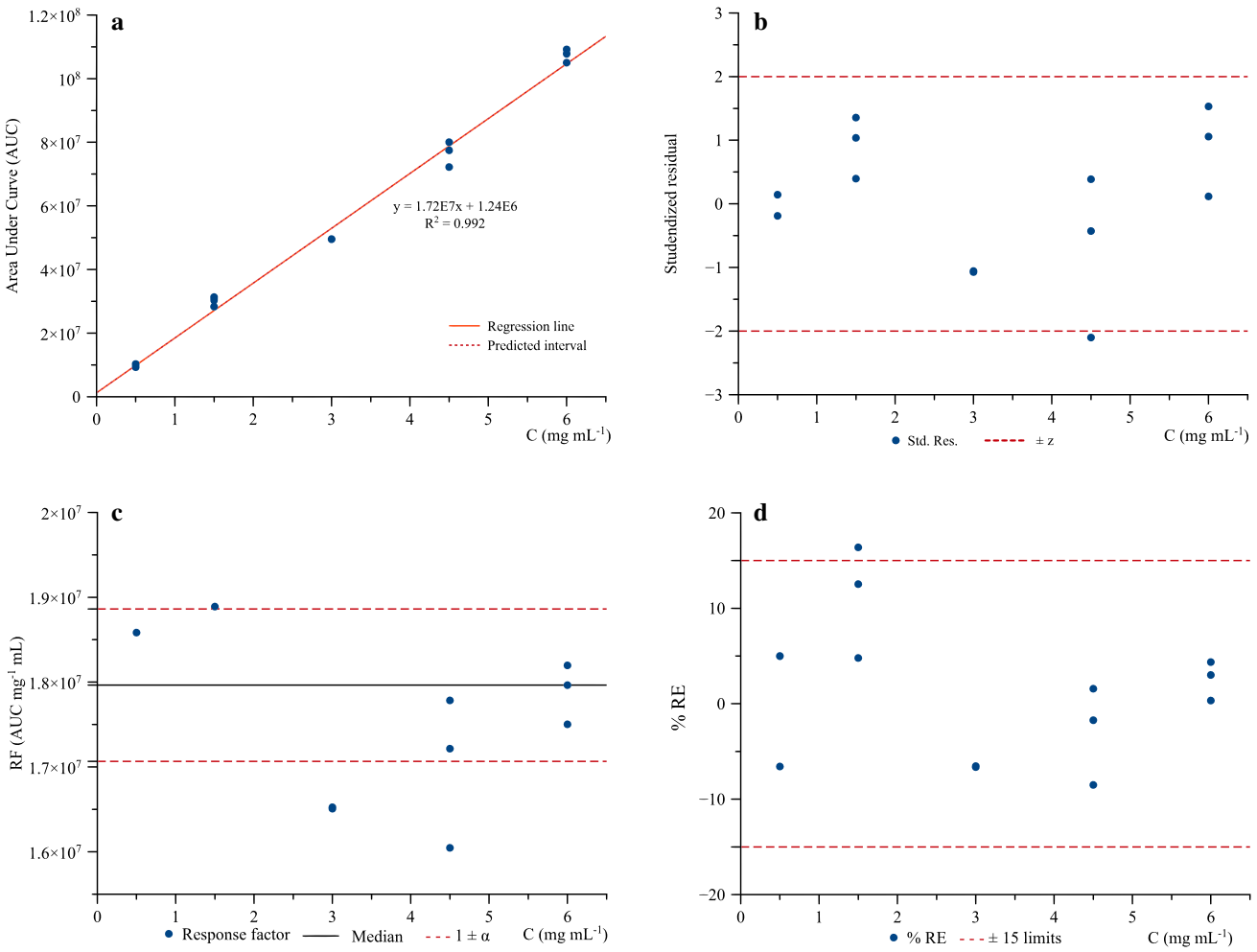

Fig. 3 Linearity study for the calibration curve of ethanol by GC-FID in the calibration range, 0.5–6.0 mg mL^{-1}

results were seen in the back-calculated relative errors (%RE) against standard solutions concentration (Fig. 3d). From the author's point of view, this proposed method exhibits excellent performance in almost all cases.

In Fig. 4, all calibration points are found within the prediction interval (Fig. 4a), and the present value of R^2 0.998 for concentration ranges from 0.1 to 0.8 mg mL^{-1}. The studentized residual plot (Fig. 4b) also shows all the computed residuals within the ± 2 limits with the back-calculated %RE were lower than 5% (Fig. 4d). In the case of the acetonitrile response factor, excellent results were achieved compared to ethanol's response factor (Fig. 4c).

The LOD and LOQ were calculated adopting the equation: $LOD = 3.3 \times s_a/b$, and $LOQ = 10 \times s_a/b$, where s_a is the standard deviation of the calibration curve intercept, while b is the calibration curve slope. The estimated LOD and LOQ were subsequently verified by the independent analysis of solutions prepared solutions at these ranges ($n = 6$). The LOD and LOQ were higher for ethanol than acetonitrile as the calibration curve's concentration range was not similar [3]. The LOD and LOQ, as presented in Table 4, demonstrated excellent precision (RSD below 3%) and accuracy (recovery from 93 to 103%) for both solvents.

Selectivity (α) is defined for peak pairs and is determined by the ratio of retention factors of the more retained component (k_2), acetonitrile, and the less retained component (k_1), ethanol in this experiment [28]. It was calculated following Eq. (4). The selectivity (α) should be greater than one. If the selectivity (α) is equal to one, it indicates that the sample's two components cannot be separated and that their peaks overlap.

$$\text{Selectivity}\ (\alpha) = k_2 / k_1 \tag{4}$$

In this experiment, the selectivity (α) was 4.88, indicating higher selectivity and therefore means better separation. The method has also proved to be sensitive. The method's sensitivity was greater for acetonitrile than ethanol, as the method could detect the smallest difference for acetonitrile. The finding was also supported by the LOD previously

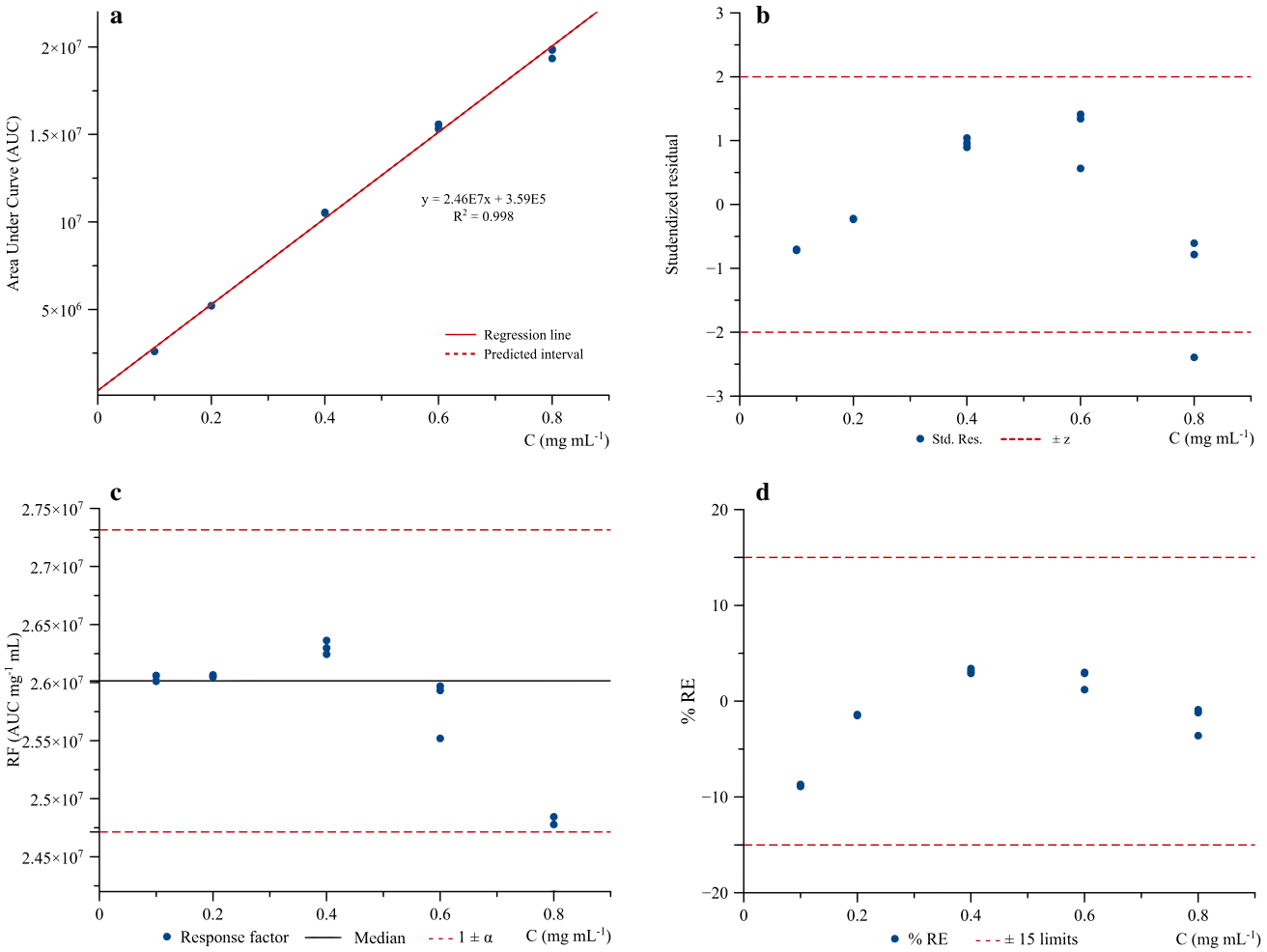

Fig. 4 Linearity study for the calibration curve of acetonitrile by GC-FID in the calibration range, 0.1–0.8 mg mL^{-1}

Table 4 Analytical figures obtained from the proposed procedure of GC-FID analytical

Analyte	Correlation coefficient (R^2)	LOD	LOQ	RSD_{IP} (%)	Recovery (%) ± RSD_r %		
					Low level	Middle level	High level
Ethanol	0.992	0.136	0.412	1.6	93 ± 1.5	94 ± 0.8	96 ± 1.7
Acetonitrile	0.998	0.004	0.012	0.16	97 ± 0.2	103 ± 0.4	100 ± 0.6

RSD_{IP} = Relative standard deviation from intermediate precision (repeated on the next day)

RSD_r = Relative standard deviation from repeatability

presented, and in agreement with Lozano & Cantero, as the higher the analytical sensitivity, the lower the detection limit [29].

The assessment of precision for repeatability and intermediate precision was carried out by the RSD % acquired from the recovery analysis. A statistical analysis of the Student's *T*-test was then carried out, and the analysis did not demonstrate any significant differences in the result ($p > 0.05$). In the accuracy assessment, the recovery values for ethanol and acetonitrile ranged from 93 to 96% and 97 to 103%, respectively. Good agreement was found when comparing results from this work against published data [2, 3, 10]. Table 4 above summarized the regression analysis, LOD, and LOQ, assessment of precision and recovery.

Another integral element for validating the proposed method is the robustness of the method. Robustness is best described as the analytical method's ability to remain unaffected, even in the presence of minor changes that have been intentionally introduced under experimental conditions [30, 31]. In pursuance to assess robustness, the two-level full factorial design approach was adopted in this study [30]. To date, the two-level full factorial design has been the approach of choice due to its efficiency for assessing robustness when the number of factors is not high [30]. The standardized effects values (SE) were calculated as in Eq. 5 and compared to the critical value of the Student's *t*-test (α = 0.05). SE is the ratio between the effect value and the dispersion of the value (S_{effect}) obtained during the optimization process. In order to obtain the calculated SE, one may have to refer to the previous work by Ferreira et al., which discussed the robustness evaluation in depth using the two-level full factorial experimental design [30].

$$SE = \text{Effect value} / S_{effect} \quad (5)$$

In assessing the robustness of the proposed method, the experimental matrix was developed based on adjusting factors associated with the optimized conditions, considering the retention time of ethanol and acetonitrile as a response. The two factors and their interactions are not significant for the established experimental matrix (Table 5). One of the key findings of this study showed that the proposed method is robust for the splitless time of

Table 5 Standardised effects from the robustness analysis

Parameters	Standardised effects	
	Ethanol	Acetonitrile
Splitless time (min)	2.06	1.57
Injection temperature	− 0.75	− 0.59
Splitless time x Injection temperature	− 0.44	− 0.37

1.0 ± 0.5 min and an injection temperature of 210 ± 10 °C. All the standardized effect values attained were below 4.30, the critical value of Student's *t*-test for the degree of freedom of 2 at a confidence level of 95%. The proposed method is, therefore, robust for the experimental matrix set out in the robustness analysis.

3.3 Application of the procedure

Following the production of [^{18}F]FET, [^{18}F]FMISO, and [^{18}F]FLT, ethanol, and acetonitrile in the samples were evaluated using the method developed. Ethanol and acetonitrile were well resolved and determined within 4.5 min, with an overall analysis time of fewer than 5 min, including sample injection and separation. The separation of the two peaks was excellent. The total analysis time of fewer than 5 min was acceptable, as the procedures described in the literature required a total separation time of 7 min [3].

From our perspective, although the splitless time and injection temperature have always been overlooked in the previous studies, the present results showed that the combination of these two factors also contributes to rapid analysis time and excellent resolution. Although the splitless time should be long enough to allow most of the vaporized sample to be transferred to the column, an excessive splitless time of more than 1.5 min may produce tailing peaks and broad peaks, which are always mistakenly thought due to the column aging [32]. On the other hand, opening the split vent too early, as a result of shorter splitless time, may risk losing the analyte [33]. Therefore, in this study, 0.5 min is the optimum splitless time to achieve good reproducibility and sensitivity in the splitless injection mode. In the meantime, the injection temperature must be relatively high enough to allow the sample to

Table 6 Comparison of the results with the other literature

Solvent R_t	Optimized method	Klok and Windhorst [13]
Ethanol	2.61	4.50
Acetonitrile	3.45	4.10
Splitless time	0.5 min	*
Injection temperature	220 °C	200–230 °C
Resolution	4.2	*

R_t: retention time

*Not specifically mentioned the exact value

Table 7 Ethanol and acetonitrile concentration in [^{18}F]FET, [^{18}F]FMISO and [^{18}F]FLT samples

Sample	Concentration (mg mL^{-1})	
	Ethanol	Acetonitrile
[^{18}F]FET-1	Not detected	0.33
[^{18}F]FET-2	2.84	0.20
[^{18}F]FMISO-1	13.5	0.03
[^{18}F]FMISO-2	1.93	0.01 (< LOQ)
[^{18}F]FLT-1	0.42	0.01 (< LOQ)
[^{18}F]FLT-2	Not quantified	0.16

Maximum acceptance limit: 5 mg mL^{-1} for ethanol and 0.41 mg mL^{-1} for acetonitrile

vaporize completely, but not too high to cause the sample's degradation, especially for the organic solvent, which has a low boiling point.

The tabulated data in Table 6 below showed that the proposed method could provide rapid analysis time. Ethanol and acetonitrile were well resolved and determined within 4.5 min. The separation between the two peaks was excellent compared to Klok and Windhorst [13] method. The difference in retention times between ethanol and acetonitrile, 0.4 min observed in the respective method, was too small for a good baseline separation. Table 7 shows the concentration values for radiopharmaceuticals, and all the samples tested were within the range of acceptance, except for one [^{18}F]FMISO sample with a relatively high concentration of ethanol.

4 Conclusion

The experimental factors of splitless time and injection temperature were successfully optimized through a multivariate approach. Although these two factors are doubtful of contributing to a substantial impact, the proposed method nevertheless contributes to rapid analysis time and excellent resolution (R > 1.5). The proposed method has been validated to meet all acceptance criteria and provides excellent linearity and resolution of an adjacent peak, adequate analysis time, and robustness. The proposed method also has been successfully used for the quantitative determination of ethanol and acetonitrile in [^{18}F]FET, [^{18}F]FMISO, and [^{18}F]FLT. The proposed method is relevant for the direct determination of ethanol and acetonitrile for any ^{18}F-radiopharmaceutical derivatives.

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Author contributions All authors contributed to the study conception and design. Hishar Hassan performed material preparation, data collection, and analysis. Hishar Hassan wrote the first draft, and all authors made comments. All authors read and approved the final manuscript.

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Availability of data and materials The datasets used and analyzed during the current study are available on a reasonable request from the corresponding author.

Code availability LabSolutions (Version 5.82) (Shimadzu, Japan) and SPSS Statistics 26 (IBM, USA).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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