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Evaluation of factors with potential influence on [^{18}F]FDG radiochemical synthesis yield

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ABSTRACT

[^{18}F]FDG radiopharmaceutical production is performed with automatic synthesis modules, which enables to carry out the production process safely, reliably and reproducibly.

This study aimed to investigate the factors which may influence and give inconsistent radiochemical synthesis yield, as well as common pitfalls in the synthesis process which can occur. We evaluated more than 500 batches in the overall study.

The results confirmed that irradiation parameter affect the production yield of the final product, also transport capillary capability and some synthesis factors have an impact on [^{18}F]FDG radiochemical synthesis yield.

1. Introduction

Fluorine-18 ($T_{1/2} = 109.8\,min$, $\beta^+ : 97\,\%$)- [^{18}F]F, is a commonly used radionuclide over the last twenty years for the production of different radiopharmaceuticals due to its excellent nuclear properties for PET imaging, 97% of the decay is by positron emission with fairly low positron emission energy of 0.635 MeV, and a short positron range (maximum 2.4 mm in the living tissue) which consequentially influences the spatial resolution of a PET scanner and it is better for [^{18}F]F than the other PET isotopes, such as ^{11}C, ^{13}N, and ^{15}O (Jacobson and Chen, 2010; Cole et al., 2014; Goud et al., 2019; Nerella et al., 2022). [^{18}F]F as most important radioactive halogen radionuclide is primary reagent for the production of glucose analog 2-[^{18}F]fluoro-2-deoxy-D-glucose (also referred to as FDG, [^{18}F]FDG, or fluorodeoxyglucose) radiopharmaceutical.

Radioactive ^{18}F can be produced with a cyclotron in either electrophilic ^{18}F-Fluorine ([^{18}F]F_2) or nucleophilic ^{18}F-Fluoride ([^{18}F]F^-) followed by two different synthesis processes of electrophilic and nucleophilic substitution, respectively (Ido et al., 1977; Hamacher et al., 1986; Yu, 2006; ZamanUz et al., 2014).

An analog of glucose in which the hydroxyl group is substituted with fluorine-18 at the C-2 position in the glucose molecule is [^{18}F]FDG with a molecular formula of $C_6H_{11}FO_5$ and low molecular weight drug molecule (181.26 Da) (IAEA/International Atomic Energy Agency, 2012). [^{18}F]FDG is the most widely used radiopharmaceutical for molecular imaging by positron emission tomography (PET). As a solution for intravenous administration, it is subjected to quality control tests to assure its safety and efficacy before injecting to patients.

The modules for [^{18}F]FDG radiopharmaceutical production use standardized and validated synthesis scripts (sequences) for routine production. In general, the radiochemical synthesis yield (RCY %, the radiochemical efficiency) is the most important indicator for the efficiency of the synthesis process. A high and reproducible RCY is desirable, although it may not be always attainable under the same physicochemical conditions and the same synthesis sequence. By definition, the "radiochemical yield" is the amount of activity in the end product expressed as a percentage (%) of a related starting activity utilized in the considered process (e.g. synthesis, separation, etc.). The quantity of both must relate to the same radionuclide and be decay corrected to the same time point before the calculation is made (Coenen et al., 2018).

Various factors that are known to have a major impact on RCY are

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generally basic factors that directly affect synthesis efficacy (reaction time, temperature, pressure of fluorination, hydrolysis and drying, precursor and reagents concentration). This study aimed to evaluate the factors and variances other than ones with major impact, which can notably affect the production process efficiency in everyday practice. The parameters, such as produced activity of [^{18}F]F, transfer capability of capillaries, and synthesis process parameters as elution solutions, residual activity on trapping cartridges of [^{18}F]fluoride and purification cartridges were subject of analysis. This is the first reported study evaluating the mentioned factors.

2. Material and methods

We analyzed several factors with potential influences that could result in inconsistent synthesis yield of [^{18}F]FDG. The factor of production of [^{18}F]F$^-$ is described as factor 1 (F1), transport capillaries' capability is described as factor 2 (F2), and the factors from the synthesis process are described as factor 3 (F3). In total, we observed more than 500 batches for all factors. In F1, all batches were considered, 201 batches were observed in F2, and for F3 a total of 160 batches were investigated. About 30 batches were excluded from the study for which we provided a list of possible issues that could result in low yields, that are yields of less than 50%.

2.1. Chemicals and reagents

Enriched [^{18}O]-water (NUKEM isotopes, Germany); nucleophilic integrated fluidic processor (IFP cassettes), reagents, and precursor for the synthesis of [^{18}F]FDG (ABX, Radeberg, Germany); Sep-Pak QMA Accell Plus Light cartridge, Sep-Pak Accell Plus QMA Carbonate Plus Light cartridges with carbonate counter-ion, Sep-Pak Alumina B Plus Long cartridge with a highly active grade of alumina with a basic surface chemistry, Sep-Pak C18 Plus Short Cartridge with silica-based bonded phase with strong hydrophobicity and Oasis HLB plus short cartridge with polymeric 225 mg reversed-phase sorbent (Waters, Massachusetts, USA); SCX Cartridge or strong cation exchange SPE cartridge, silica-based benzenesulfonic acid-based with negatively charged sulfonic acid and the benzene ring (S Pure, Nordcom One, Singapore).

2.2. Instrumentation

PETtrace 16.5 MeV GE Cyclotron (General Electric Medical System, Uppsala, Sweden); BBS2–O hot cell (Comecer, Italy); Synthera V2 synthesis module (IBA RadioPharma Solutions, Belgium); VIK 202 activity calibrator (Veenstra, Comecer Netherlands) and Atomlab 500 activity calibrator (Biodex, New York, USA), Gamma spectrometer - radiometer MKGB-01 RADEK (Radek, Russia).

2.3. Production of [^{18}F]F$^-$

In this study, we are discussing the production of nucleophilic ^{18}F-fluoride: [^{18}F]F$^-$, as the most common chemical ionic form in aqueous solution which is used as nucleophilic fluorinating agent in the synthesis of [^{18}F]FDG. PETtrace cyclotron was used for the production of radionuclide [^{18}F]F$^-$ with proton irradiation of enriched water ($H_2^{18}O$) in the niobium target by the reaction of ^{18}O(p,n)^{18}F. The irradiation was performed with a proton beam energy of 16.5 MeV, directed into a target with a chamber volume of 3 mL, filled with enriched water with 98% purity, at different beam currents and different irradiation times depending on the required activity. After the production, the activity of [^{18}F]F$^-$ was sent through transport capillaries to an [^{18}F]F$^-$ collecting vial in a shielded hot cell installed in a radiopharmaceutical production laboratory.

2.4. Synthesis process

[^{18}F]F$^-$ ion in an aqueous solution as a starting reagent (3 mL) was collected before the start of synthesis (SOS). IBA Synthera V2 synthesis module (IBA, Louvain la Neuve, Belgium) was used for the synthesis of glucose analog (2-[^{18}F]fluoro-2-deoxy-D-glucose) in sequential steps. The first step after SOS is [^{18}F]F$^-$ trapping on an anion-exchange cartridge and recovery of oxygen-18 enriched water. Trapped [^{18}F]F$^-$ anions were eluted from the anion exchanger into the reaction vial with a cryptand solution (elution solution). After that, the next step was drying and preparation of reactive [^{18}F]F$^-$. Water was removed by azeotropic distillation under inert He gas at 120 °C. Chemically, this is a critical reaction for the reactivity of fluoride because hydrated fluoride is generally thought to be poorly nucleophilic. Solubility and nucleophilicity of fluoride ions in an organic solvent are essential to carry out the next reactions. Prepared K$^+$[^{18}F]F$^-$ complex is the main reactant in the next step of radiofluorination for the production of [^{18}F]F$^-$ fluorinated intermediate via nucleophilic bimolecular (SN_2) substitution reaction mechanism. A 20 mg precursor 1,3,4,6-Tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose (containing a suitable leaving group – trifluoromethanesulfonyl group) dissolved in 1.5 mL dry acetonitrile was added and the fluorination was performed on heating the reaction mixture at 100 °C for 3 min. Then, the reaction mixture was cooled and evaporated to dryness, followed by base-catalyzed hydrolysis with sodium hydroxide solution (1 mL, 1 M NaOH). The hydrolysis to remove protecting acetyl groups from the [^{18}F] fluorinated intermediate was performed at 110 °C for 80 s, producing [^{18}F]FDG and converting unreacted precursor into L-glucose. The resulting mixture after hydrolysis was passed through cartridges for purification. Due to the short half-life of [^{18}F]F$^-$ ion, the method of purification is one of the key aspects of radiopharmaceutical production. Commercially available solid-phase extraction cartridges are ideal for this purification, usually set of three cartridges is commonly used - cation exchange cartridge for extraction of positively charged basic compounds, aluminum oxide cartridge for extraction of unreacted fluoride, and reverse-phase cartridge for extraction of compounds with weak hydrophobicity from aqueous solutions.

Two different anion exchange cartridges with two different elution solutions were investigated during the synthesis. Synthesis A was performed with Sep-Pak QMA Accell Plus Light cartridge (QMA-A) (preconditioned with 5.0 mL of 8.4% w/v solution of $NaHCO_3$ and 5 mL water) eluted with 0,6 mL cryptand solution containing Cryptand 222, 22.6 mg, K_2CO_3, 4.2 mg in the 1:1 acetonitrile-water. Synthesis B was performed with Sep-Pak Accell Plus QMA Carbonate Plus Light cartridges (QMA-B) with carbonate counter-ion (preconditioned with 5 mL water) eluted with 0,6 mL cryptand solution containing Cryptand 222, 22.6 mg, K_2CO_3, 4.2 mg in 4:1 acetonitrile-water. Synthesis A process was performed using were use strong cation exchange (SCX-A) and Sep-Pak Alumina B (Alu B-A) connected in series and Sep-Pak C18 Plus Short Cartridge (C18-A). Purification in Synthesis B process was performed using strong cation exchange (SCX-B), Sep-Pak Alumina B (Alu B–B), and Oasis HLB (HLB-B). Fig. 1 shows the main difference between Synthesis A and Synthesis B.

2.5. Analysis of factors with potential influence on RCY

The activity of produced [^{18}F]FDG was measured with VIK 202 activity calibrator (Veenstra, Comecer Netherlands). Radiochemical yield was calculated based on the activity of produced [^{18}F]FDG in GBq expressed as a percentage (%) of related EOB activity, decay-corrected (d.c.) at EOB time.

The factors of production of [^{18}F]F$^-$ described as F1 include data from produced activity of [^{18}F]F$^-$ (EOB activity, well known as incoming activity).

With F2 we describe an observational study where we observe the effect of TEFZEL - ethylene tetrafluorethylene (IDEX, USA) transport

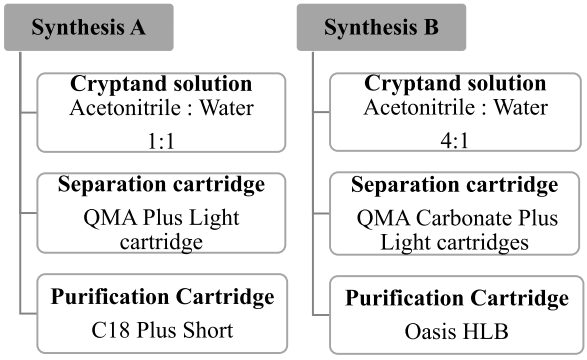

Fig. 1. Schematic representation of the difference between Synthesis A and Synthesis B.

capillaries' capability on RCY, a seven-month period that begins from new capillary installation for the capillaries from cyclotron - target switch - synthesis hot-cell and the ones from synthesis hot-cell - dispensing hot-cell. The RCY was compared on a monthly level, expressed as a mean value.

The factors of F3 described the residual activity on cartridges in Synthesis A and Synthesis B. F3a refers to Synthesis A aiming to present residual activity on QMA-A, SCX-A, Alu B-A, C18-A and F3b refers to Synthesis B aiming to present residual activity on QMA-B, SCX-B, Alu B–B, and HLB-B. The residual activity was measured using Atomlab 500 activity calibrator (Biodex, New York, USA), 10–18 h after the end of synthesis (EOS) and decay-corrected at EOB time. The results are expressed as a percentage (%) of a related EOB activity.

In this paper, we also listed the issues that could result in low yield or yields less than 50%. We evaluated 30 batches with a low yield by analysis of possible causes for a loss of yield.

In addition, during the measurement of activity, we applied all the necessary radiation protection principles.

Pearson's correlation coefficient was used to test whether the relationship is significant, r is reported alongside its degrees of freedom and p value. For two groups comparison *t*-test was used. For examination of the dependent variables, analysis of variance (ANOVA) was used by the significance level of all statistical analyses set at $\alpha = 0.05$. All the statistical analyses were performed using the SPSS v.26.0 software (IBM Corporation, Chicago, IL, USA).

3. Results and discussion

As key factors for running successful production and good RCY (more than 50% d.c.) we can highlight the following: types of cartridges (for efficient trapping of $[^{18}F]F^-$ and purification of the product), precursor (concentration, mass, volume); radionuclidic purity of the $[^{18}F]F^-$ solution, recovery of $[^{18}F]F^-$ after elution, effective drying of $[^{18}F]F^-$ (time, temperature and pressure), fluorination reaction (effective labeling of the mannose triflate with the $[^{18}F]F^-$ - time, temperature and pressure), hydrolysis reagent and reaction parameters (effective removing of the protective acetyl groups - time, temperature and pressure) and effective purification. Except for these key factors, there are many others process factors affecting the RCY. This study shows the correlation between other factors with the radiochemical yield. The goal was to check if these factors influence the RCY.

For the analyzed batches, all important checks and tests were performed (synthesis modules, cassettes, and reagents) to prevent any malfunction or failure during the production process. Also, quality control was performed for all investigated batches and the results of the tests were within the acceptance criteria defined in the $[^{18}F]$FDG specification as it is set in Ph. Eur. monograph for Fludeoxyglucose (^{18}F) injection (European, 2020).

Radiochemical synthesis yield depends on $[^{18}F]F^-$ production yield, which besides its dependence on proton beam energy (in our case it is fixed and set to 16,5 MeV by the cyclotron type) is directly correlated with the beam current and the irradiation time (IAEA/International Atomic Energy Agency, 2012). Within the analysis of the influence of F1 factors on RCY, we evaluated the dependence between EOB activity and RCY (Table 1). The results of $[^{18}F]$fluoride (GBq) EOB activity from 40 to 160 GBq was divided into 12 groups and the mean of RCY d.c. was calculated for each group.

A weak negative linear relationship exists between EOB activity and RCY (Table 1). A Pearson correlation coefficient was −0.221. The $p = <$ 0.0001 less than the alpha significance level means that the differences between the groups were statistically significant. Taking into consideration the unequal sample size in the groups, the statistical analysis should be further adjusted with a similar number of measurements in all groups, especially for the first two groups (40–50 GBq and 50–60 GBq). The measured RCY at EOB activity from 60 GBq to 130 GBq was consistent, the p-value of 0.117 (α values of less than 0.05) confirmed no statistically significant differences between RCY. For EOB activity higher than 130 GBq there was marked decrease in RCY. The p-value for these 3 groups (130–140 GBq; 140–150 GBq; 150–160 GBq) of 0.014 indicated differences between the RCY means. It is worth emphasizing that the EOB activity as incoming activity from a cyclotron in our study was not measured in each case before synthesis, it was assessed from the irradiation parameters. Owing to the fact that the targets for production of $[^{18}F]F^-$ are regularly calibrated and the saturation yield is obtained from this procedure and the calibration procedure lasts long enough (approximately 2h) for the short-lived positron emitter impurities to completely decay. Completing the analysis, the decay curve is plotted and fitted with a bi-exponential model, allowing us to resolve only the $[^{18}F]F^-$ component. With increasing the proton charge (irradiation current and time) will increase the number of nuclei produced, which in turn may lead to an increase in produced activity of $[^{18}F]F^-$ but also with production of many impurities in $[^{18}F]F^-$ solution, formed through (p,α) nuclear reaction. The trend of decreasing RCY after 130 GBq point to it and the possible conclusion for the negative trend is the fact that production of $[^{18}F]F^-$ through proton irradiation of $[^{18}O]H_2O$ from a niobium target chamber with a HAVAR entrance window, results in the production of a variety of radionuclide and chemical impurities (Avila-Rodriguez et al., 2008; Bowden et al., 2009; Köhler et al., 2013; Kambali et al., 2017). Therefore, the specific and molar activity may be influenced by the distribution of parts of these radionuclides and non-radioactive atoms present in the solution before synthesis and not in the final product. Taking into account the important role of those unknown chemical species present in the solution, which have the chemical potential to compete with the $[^{18}F]F^-$, we can say that this is a potential reason for this trend. Although it is clear that by passing this solution through the anion exchange cartridge, the $[^{18}F]F^-$ ions are trapped while the most of other ions from the solution go into the recovery water vial, also the final product is purified through a combination of purification cartridges we can consider that the final product is free from these impurities. As the origin of these impurities is the

Table 1
RCY d.c. (mean % ± SD %) as a function of $[^{18}F]$fluoride (GBq) EOB activity.

EOB activity (GBq)	RCY d.c (mean % ± SD %)	Number of analyzed batches
40–50	65.29 ± 3.56	14
50–60	56.93 ± 5.10	14
60–70	58.07 ± 5.09	29
70–80	59.64 ± 5.69	22
80–90	61.56 ± 5.03	31
90–100	61.55 ± 5.34	32
100–110	59.09 ± 5.98	54
110–120	59.97 ± 5.45	63
120–130	60.06 ± 5.29	95
130–140	56.99 ± 4.53	70
140–150	57.63 ± 4.72	48
150–160	55.04 ± 3.52	41

HAVAR foil, it also depends on the targets' workload which is not taken into consideration in this paper. Anyhow, if we compare the EOB activity with the activity from the impurities in the final product it is even less than a permille. Higher amount from already examined incoming activity, should not be a major issue or strong downtrend of RCY, as most commercial synthesis modules and kits are capable of operation with a large quantity of $[^{18}F]F^-$ activity (Krasikova, 2007; Dalle et al., 2017; IAEA., 2021).

We were observing the influence of transport capillaries' capability, presented as factor F2, for a period of 7 months starting from new capillary installation. The method of observing was through RCY d.c. comparation on a monthly level, expressed as a mean value. We evaluated one case about capillaries' capability for Synthesis A (case 1-A) and one case about capillaries' capability for Synthesis B (case 2-B). Both cases refer to new capillaries' installation from cyclotron - target switch - synthesis hot-cell and from synthesis hot-cell - dispensing hot-cell performed at the same time in two different periods for each case.

As shown in Table 2, the yields slightly decreased over the period considered, about Synthesis A (Case 1 – A) from a level of 58,14% ± 1.48% in the first month to the level of 53,39% ± 2.21% in the seventh month and about Synthesis B (Case 2 – B), from a level of 68,69% ± 3.65% in the first month to the level of 64,06% ± 1.80% in the seventh month. Pearson correlation coefficient −0.523 and −0.425 respectively, indicating a strong negative relationship. The $p = < 0.0001$ less than the alpha significance level for both cases, confirmed statistically significant differences between RCY on a monthly level for 7 months. The results confirm that the $[^{18}F]F^-$ and $[^{18}F]$FDG tend to be adsorbed on the inner walls of the transfer capillary to slightly decrease the optimal transfer capability of capillary tubing and contribute to lower RCY. This is a common challenge for capillaries from cyclotron - target switch - synthesis hot-cell and for capillaries from synthesis hot-cell - dispensing hot-cell. Transfer capability may deteriorate over time, and replacement with new ones is consequently needed for consistent yield (Füchtner et al., 2008; Savisto et al., 2018).

In the context of different RCY values in Synthesis A and Synthesis B, illustrated in Table 2, additionally, we further explain this phenomenon within the framework of the following Factor 3 (F3). F3 presents two groups of results for residual activity on the cartridges from Synthesis A (group F3a: QMA-A, SCX-A, C18-A and Alu B-A) and Synthesis B (group F3b: QMA-B, SCX-B, HLB-B and Alu B–B), together with comparative effect of elution solutions on RCY Synthesis A (1:1 acetonitrile-water in cryptand elution solution) and Synthesis B (4:1 acetonitrile-water in cryptand elution solution). To interpret the results, we divided the batches from each group (F3a and F3b) into 4 subgroups depending on the RCY: 50–55%, 55–60%, 60–65%, and more than 65%.

Table 2
A variation in RCY from the length of use of capillaries after their installation in the next seven months.

Length of use of capillaries (months)	RCY d.c (mean % ± SD %) (N = number of batches)	
	Synthesis A (Case 1 – A)	Synthesis B (Case 2 – B)
1st	58.14% ± 1.48% (n = 12)	68.69% ± 3.65% (n = 18)
2nd	58.34% ± 3.90% (n = 12)	67.42% ± 3.81% (n = 14)
3rd	57.62% ± 2.23% (n = 15)	65.31% ± 3.07% (n = 14)
4th	57.16% ± 2.87% (n = 10)	66.22% ± 3.73% (n = 16)
5th	56.62% ± 2.29% (n = 10)	65.22% ± 2.63% (n = 16)
6th	55.39% ± 2.66% (n = 15)	64.98% ± 2.98% (n = 18)
7th	53.41% ± 2.21% (n = 15)	64.06% ± 1.80% (n = 16)

F3a results for QMA-A, SCX-A and C18-A presented in Table 3 showed that there were no significant differences between residual activity in the 4 RCY subgroups from group F3a. The results confirm that residual activity on those cartridges has no impact on RCY in Synthesis A ($p > 0.05$, for all correlations). Also, F3b results (Table 3) show a similar trend between the residual activity on the QMA-B, SCX-B and HLB-B in the 4 RCY subgroups.

As well known, residual activity for QMA-A and QMA-B arises from non-eluted $[^{18}F]F^-$. The instability of yield can be affected by activity residual on the QMA. In our study, high recovery of fluoride ions is achieved in both (98,7% and 98,5%, respectively) and we hereby confirm that residual activity on QMA-A (p-value 0.94) and QMA-B (p-value 0.15) has no impact on RCY. The type of anion exchange cartridges affects the elution efficiency of $[^{18}F]F^-$ as well as the subsequent radiolabeling, but this is not the case in our study because the counter ion is the same. In the first case was OH^- ions, and in the other case CO^{3-} ions, but after preconditioning both types were carbonate ions.

For the other examined cartridges, a strong cation exchange cartridge used for neutralizing the solution with retaining of the cations and reversed-phase cartridge C18-A/HLB-B for retaining incompletely hydrolyzed intermediate was expected very low residual activity on them due to the nature of retained particles. According to the result in SCX-A (p-value 0.10) and C18-A (p-value 0.69) we confirm that $[^{18}F]$FDG is not retained on that sorbent, which means that residual activity on those cartridges has no impact on RCY. Although p-value for residual activity on SCX-B (p-value 0.08) and HLB-B (p-value 0.06) showed certain trend toward significance or close to being statistically significant, we can confirm that only physical adsorption occurs on the cartridges and that its extent is negligible.

The results for residual activity on Alu B-A (F3A) and on Alu B–B (F3B), are presented in Table 4 revealed a negative correlation between the 4 RCY subgroups in both cartridges. Pearson correlation coefficient - 0.838 of Alu B-A results indicated very strong negative relationship, with p value less than 0.001 which confirms statistically significant differences between the mean values in the groups.

Pearson correlation coefficient - 0.65, in the case of the Alu B–B, showed also a strong negative correlation, with no statistically significant difference between the mean (p-value 0.12). A negative correlation confirmed that as the residual activity increases, the RCY tends to decrease, which means that at lower RCY there was a higher content of unreacted fluorine. Free $[^{18}F]$F- (unreacted fluoride) after fluorination is a potential chemical impurity that should be retained on Alu B-A or Alu B–B. If there is low residual activity on this cartridge that means high efficiently synthesis reactions that contribute to a greater RCY, considering a minimum of 95% radiochemical purity of total activity in the end

Table 3
Residual activity d.c. (mean % ± SD %) on the cartridges Synthesis A (group F3a) and Synthesis B (group F3b).

Types of cartridges	50–55% RCY	55–60% RCY	60–65% RCY	>65% RCY
	Residual activity d.c. (mean % ± SD %)[a]			
QMA-A	1.24 ± 0.19	1.27 ± 0.32	1.23 ± 0.28	1.28 ± 0.24
QMA-B	1.63 ± 0.03	1.50 ± 0.12	1.38 ± 0.14	1.38 ± 0.19
SCX-A	0.91 ± 0.23	0.73 ± 0.17	0.75 ± 0.38	0.79 ± 0.31
SCX-B	0.59 ± 0.01	0.56 ± 0.17	0.53 ± 0.06	0.40 ± 0.09
C18-A	0.96 ± 0.30	0.95 ± 0.36	0.97 ± 0.59	1.13 ± 0.53
HLB-B	1.55 ± 0.36	1.66 ± 0.28	1.38 ± 0.29	1.09 ± 0.31

[a] Number of batches processed: 23, 26, 18 and 15, respectively of groups from Synthesis A (total 82) and 12, 18, 26, and 24, respectively from Synthesis B (total 80).

Table 4
Residual activity d.c. (mean % ± SD %) on the Alu B-A and Alu B–B cartridges.

RCY (% d.c)	Residual activity d.c. (mean % ± SD %)	
	Alu B-A	**Alu B–B**
50–55	21.50 ± 2.02 (n = 28)	6.25 ± 0.26 (n = 12)
55–60	19.15 ± 2.30 (n = 21)	6.11 ± 0.41 (n = 18)
60–65	17.63 ± 2.87 (n = 18)	6.07 ± 0.38 (n = 26)
> 65	9.55 ± 1.58 (n = 15)	5.40 ± 0.37 (n = 24)

product. The results of the radiochemical purity testing show that the [^{18}F]FDG content is more than 99% of the total radioactivity, in all of the batches. As revealed from results about Alu B-A (max 21.50%, min 9.55%) and Alu B–B (max 6.25%, min 5.40%) it was noted that the residual activity on the Alu B-A notably affects the yield. Taking into consideration the challenges of fluoride chemistry, the success of nucleophilic fluorination is highly dependent on the reactivities of [^{18}F]F$^-$ ions as well as the leaving group. This environment may be enhanced using a polar aprotic solvent such as acetonitrile. Furthermore, to confirm the important impact of elution solutions on the reactivity of [^{18}F]F$^-$ ions and subsequently leaving of the group to reach efficient radiolabeling and higher RCY, we compared the RCY results from Synthesis A (50 batches) and Synthesis B (50 batches).

A comparative study of these two groups of RCY results is showed in Fig. 2. The results from RCY in Synthesis B (mean 65.01% ± 4.52%) with 4:1 acetonitrile-water in cryptand elution solution indicate higher RCY compared with RCY in Synthesis A (57.83% ± 3.61%) with 1:1 acetonitrile-water in cryptand elution solution. Because the standard deviations for the two groups are similar (4.52 and 3.61), we used the *t*-Test of equal variances assumed of these two groups of results, which confirmed statistically significant differences between RCY in Synthesis A and Synthesis B ($p < 0.001$).

As well known, elution solution substantially influence the efficacy of [^{18}F]F$^-$ elution as well as the subsequent radiolabeling (Cai et al., 2008; Krasikova., 2022).

To measure the loss of activity in the entire synthesis process, it is necessary to measure all critical components during the production. Adsorption activity on the [^{18}F]F$^-$ transport line wall to the hot cell, recovery vial, reactor vessel, IFP lines, [^{18}F]FDG transport line, vented filter in the final vial were not reported in the study. Also, azeotropic drying of [^{18}F]F$^-$ is associated with radioactivity losses, as well as drying steps after fluorination.

Batches with low RCY and less than 50% were excluded from the complete observational study, in which the behavior of several factors with potential influence on RCY was investigated more deeply and systematically. In the list below are given the following issues for low synthesis yields:

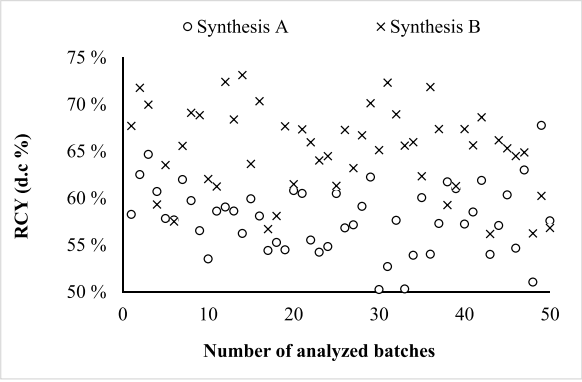

Fig. 2. The comparative effect of elution solutions on RCY Synthesis A (1:1 acetonitrile-water) and Synthesis B (4:1 acetonitrile-water).

a. Incomplete volume of delivered enriched water from the target before starting the synthesis;
b. No proper transfer of irradiated water to the modules (into the trapping cartridge for [^{18}F]F);
c. Low recovery of [^{18}F]F$^-$ due to leakage of elution solution or not properly preconditioning of trapping cartridge;
d. Failure synthesizer communication (communication dropping);
e. Defects of the cassettes despite the completed cassette self-test, such as cassette leakage, blocking of a valve;
f. Low efficacy of fluorination due to not complete transfer of precursor, drying after elution or pressure/vacuum failure;
g. Delaying the preventative maintenance of the synthesizer;
h. Delaying the replacement of transfer capillary;
i. Transfer issue of [^{18}F]FDG, such as slow transferring or minor valve leakage;
j. Issue not detected.

From all the listed issues, we can appoint only a few that can occur more frequently than others. Common issues such as the low recovery of [^{18}F]F$^-$ due to leakage of elution solution, low efficacy of fluorination due to not complete transfer of precursor or leakage, transfer issue of [^{18}F]FDG, such as slow transferring or minor valve leakage and defects of the cassettes despite the completed cassette self-test, such as cassette leakage, or blocking of a valve should be first checked in case of low synthesis yields. Synthesizer performance is usually affected by various factors, so the module must always be maintained and controlled to maximize the yield of the desired product. Overall, the described issues above were casual and infrequent, not causes of persistent low yields as described by Dalle et al. (2017).

4. Conclusion

This study aimed to evaluate how different potential factors, other than the key factors, impact radiosynthesis efficiency and give inconsistent radiochemical synthesis yield. The results from the analyzed F1 showed a decrease in RCY at high EOB activity, because of the production of impurities in [^{18}F]F- solution, formed through (p,α) nuclear reaction. Transport capillary capability (F2) also has an impact on RCY. It may deteriorate over time, and replacement with new ones is consequently needed for a consistent yield. Based on the study of residual activity (F3), there is a statistically significant correlation between the RCY and the amount of residual activity on the alumina cartridge, which adsorbs the unreacted [^{18}F]F$^-$. No statistically significant difference was found for other cartridges. It can be concluded that the success of nucleophilic fluorination is highly dependent on the reactivities of the [^{18}F]F$^-$ ion reaction in which elution solution has an important role, confirmed with statistically significant differences between RCY in Synthesis A and Synthesis B. By providing comprehensive analysis of pitfalls in [^{18}F]FDG production, we can contribute to the fast detection and recognition of potential challenges which negatively influence the RCY.

Although this type of synthesis is not highly sensitive as in the case of many other [^{18}F]F$^-$ radiopharmaceuticals, many aspects and essential requirements have to be satisfied for a consistent and efficient radiochemical synthesis process.

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CRediT authorship contribution statement

Maja Chochevska: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maja Velichkovska:** Writing – review & editing, Formal analysis,

Conceptualization. **Marija Atanasova Lazareva:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Katerina Kolevska:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Filip Jolevski:** Investigation. **Jasmina Razmoska:** Investigation. **Zlatko Filipovski:** Investigation. **Sasho Nikolovski:** Writing – review & editing, Investigation. **Marina Zdraveska Kocovska:** Writing – review & editing. **Ana Ugrinska:** Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

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