Stability Evaluation of ^{18}F-FDG at High Radioactive Concentrations

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The objective of our study was to determine the concentration of ethanol, a known radiolytic stabilizer, needed to maintain stability for 12 h at an ^{18}F-FDG concentration of 19.7–22.6 GBq/mL (533–610 mCi/mL) at the end of synthesis (EOS). **Methods:** ^{18}F$^-$ was formed by the ^{18}O(p, n)^{18}F reaction using 16.5-MeV protons on a cyclotron. ^{18}F-FDG was synthesized using a synthesis platform. The final product was formulated in 15 mL of phosphate buffer. The synthesis took 22 min, delivering up to 336.7 GBq (9.1 Ci) of ^{18}F-FDG at the EOS. A series of 9 runs, 19.7–22.6 GBq/mL (533–610 mCi/mL), was completed. Three runs were doped with 0.1% ethanol, 3 with 0.2% ethanol, and 3 with no ethanol added. The radiochemical purity (RCP) was tested at about 1-h increments over a 12-h period. RCP was found by radio–thin-layer chromatography using aluminum-backed silica gel plates, acetonitrile, and water 90:10. An ^{18}F-FDG standard of 1 mg/mL was used to confirm radiochemical identity. The chromatography plates were analyzed on a radio–thin-layer chromatograph using a β-detector. Residual solvents were also tested using gas chromatography with flame ionization detection and a capillary column. Other quality control measurements performed were pH and appearance. **Results:** The 3 runs doped with 0.1% ethanol failed RCP after 5 h. The 3 runs using an ethanol concentration of 0.2% maintained stability through 12 h beyond the EOS. For these 3 runs, the radiolytic impurities were relatively constant at 6.1% $\pm$ 0.7% after 3 h. The runs using no ethanol failed RCP at 1 h. The pH varied between 5.3 and 6.1. Visual inspection was always clear and particulate-free. For the runs with 0.2% and 0.1% ethanol, the residual solvents were 0.21% $\pm$ 0.02% and 0.10% $\pm$ 0.02%, respectively. Regardless of ethanol concentration, chemical purity and identity passed quality control measurements. **Conclusion:** With the addition of 0.2% ethanol, ^{18}F-FDG (19.7–22.6 GBq/mL [533–610 mCi/mL]) kept stability through 12 h beyond the EOS. Each run passed stability parameters related to radiolysis—that is, radiochemical identity and RCP, chemical purity and identity, appearance, pH, and residual solvents.

Key Words: ^{18}F-FDG; high radioactive concentration; radiochemical purity; stability evaluation; stabilizer

The most commonly used PET radiopharmaceutical is ^{18}F-FDG. ^{18}F-FDG is a glucose analog labeled in the 2 position of the glucose ring by the PET radionuclide fluoride. Once injected into a subject, the radioactivity can be detected using a PET camera. Locations throughout the body that have an increased uptake of ^{18}F-FDG can be observed.

In our institution, a recent influx of studies using ^{18}F-FDG has resulted in the necessity of producing the drug in high radioactive quantities. This increase has resulted in an intandem increase in radioactive concentration. Multiple runs of ^{18}F-FDG result in an increased radiation exposure to the radiochemists and additional time, money, and equipment maintenance. Furthermore, the higher radioactive concentration has created a serious final product quality issue that affects the time ^{18}F-FDG maintains its radiochemical purity (RCP) within the limits allowed by the United States Pharmacopeia (USP) monograph for ^{18}F-FDG injection (*1,2*).

Ethanol is a stabilizer commonly used to aid in lowering the occurrence of radiolytic decomposition by absorbing the free radicals before they have time to break the bond between fluoride and the deoxyglucose molecule (*3,4*), thus increasing the useable life of ^{18}F-FDG by reducing the rate of radiolysis. The current USP monograph and the Food and Drug Administration (FDA) specify that no more than 0.5% w/v of the final drug formulation can be made up of ethanol; however, current good manufacturing practice encourages that the least amount of chemical impurities should be added to the formulation (*1,5*).

The objective of our research was 3-fold: collect radiolytic decomposition profiles (RDPs) in the range of 19.7–22.6 GBq/mL (533–610 mCi/mL) for ^{18}F-FDG produced with the FASTlab synthesis unit (GE Healthcare), determine the concentration of ethanol required to stabilize ^{18}F-FDG of radioactive concentration in the range of 19.7–22.6 GBq/mL (533–610 mCi/mL), and extend the shelf-life of ^{18}F-FDG to 12 h from the current 10 h.

MATERIALS AND METHODS

^{18}F-FDG Synthesis

^{18}F-fluoride was produced by the ^{18}O(p,n)^{18}F reaction using 16.5-MeV protons on a PETtrace cyclotron (GE Healthcare). ^{18}F-FDG was synthesized using the FASTlab platform.

The final product was formulated in 15 mL of phosphate buffer. The synthesis took approximately 22 min, delivering up to 336.7 GBq (9.1 Ci) of ^{18}F-FDG at the end of synthesis (EOS).

^{18}F-FDG Run Details

A series of 9 high-radioactive-concentration runs, 19.7–22.6 GBq/mL (533–610 mCi/mL), was completed. All radioactivities were measured in a dose calibrator (CRC15-PET; Capintec USA) and decay-corrected to the EOS. The EOS is defined as the time at which the final PET drug product formulation is assayed (6). Runs 1, 2, and 7 were doped with 0.1% ethanol. The FASTlab ^{18}F-FDG cassette contained a water bottle. For each synthesis, a 1-mL tuberculin syringe was used to add specific amounts of ethanol into the water bottle to obtain the required concentration of ethanol in the final product formulation. The concentration of ethanol was increased to 0.2% for runs 3, 4, and 5. For runs 6, 8, and 9, no ethanol was added to the synthesis.

Radiochemical Stability Evaluation

Radiochemical stability was evaluated using guidance from both the USP and the FDA (1,5). Stability concerns were primarily due to radiolysis. Parameters, as outlined by the FDA, to evaluate the stability of ^{18}F-FDG were tested including RCP and identity, chemical purity, pH, and appearance (5). These quality control (QC) measurements were performed for 3 production runs at each ethanol concentration.

RCP

RCP was tested at approximately 1-h increments over an extended period for runs containing ethanol to ensure a 12-h shelf life. Testing for runs without ethanol added was continued approximately hourly until the sample consistently failed the RCP acceptance criteria.

The detector used was a flow-count FC-3600 (Bioscan), a plastic scintillator–photomultiplier tube ideal for the detection of high-energy β-emitters such as positrons.

RCP was determined by radio–thin-layer chromatography (TLC) in accordance with the USP monograph 34-National Formulary 29 (1). Samples were analyzed using aluminum-backed silica gel plates (Whatman) in acetonitrile and water 90:10. The TLC plates were analyzed on a radio–thin-layer chromatograph (Mini-Scan; Bioscan) using a β-detector (4.3-cm [1.7 in] × 0.25 mm plastic scintillator tube [FC-3600]). Free ^{18}F^{-}-fluoride remains at the origin while ^{18}F-FDG rises up the TLC plate (7). The ratio of free ^{18}F^{-}-fluoride to bound ^{18}F-FDG was determined and assigned a percentage value by comparing the amount of counts in each location. The most current USP monograph and the FDA stipulate that no more than 10% of the radioactivity can be composed of radiochemical impurities or free ^{18}F^{-}-fluoride (1,5).

Radiochemical Identity

A 1-μL ^{18}F-FDG reference standard (>95%; Sigma Chemicals) of 1 mg/mL was spotted onto the TLC plate and used to confirm radiochemical identity. The addition of p-anisidine and heat turned the reference standard brown. The ^{18}F-FDG sample spot did not change color because there was insufficient mass of ^{18}F-FDG to allow for a visual change in color. An R_f value was assigned to both the standard and ^{18}F-FDG samples. R_f was determined for the standard by dividing the distance traveled from the origin by the distance to the solvent front. The distance traveled by ^{18}F-FDG was determined by measuring the velocity of the plate and the time it took for the ^{18}F-FDG radiodetector peak to reach a maximum peak relative to the origin. The minimum acceptance level of radiochemical identity as stipulated by the USP monograph and FDA is that the R_f value of ^{18}F-FDG should correspond to that of the standard solution, which is about 0.4 (1,5).

Chemical Purity: Residual Solvent Content

Chemical purity was tested at approximately 1-h increments using gas chromatography with flame ionization detection. Samples were analyzed using gas chromatography (8610C model; SRI Instruments) and a capillary column (30 m in length × 0.53 mm in inside diameter; MXT Crossbond Carbowax [Restek]) in accordance with the USP monograph 34-National Formulary 29 (1). The samples were run at 10 mL/min using helium carrier gas. The initial oven temperature was 40°C for 2 min and increased at a rate of 20°C per minute up to 130°C. Gas chromatography allowed for the determination of the amount of residual solvent (i.e., ethanol and acetonitrile) present in the ^{18}F-FDG product formulation. The minimum acceptance levels for ethanol and acetonitrile as per USP and FDA are not more than 0.5% and 0.04%, respectively (1,5).

pH

For the samples, pH was measured at approximately hourly increments using indicator strips. The pH indicator strips had a range of 4.0 to 7.0 ± 0.2. The QC measurement was performed in accordance with the USP monograph 34-National Formulary 29 (1). The acceptance level of pH as stipulated by the USP was between 4.5 and 7.5 (1).
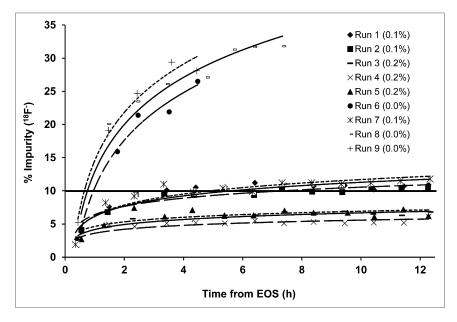
Visual Inspection

In accordance with the FDA guidance, visual inspection was also performed as a QC measurement (5). Visual inspection ensured that the solutions were clear, colorless, and particulate-free.

RESULTS

RCP

A radiolytic decomposition profile (RDP) was established for the nine ^{18}F-FDG runs. The RDPs, showing percentage of impurity of free ^{18}F^{-}-fluoride over time for the samples, are illustrated in Figure 1. Data for the 9 radioactive concentration runs conducted are presented and summarized in Table 1. [**Fig**. 1]

[Table 1]

Three runs (runs 6, 8, and 9) were performed without the addition of ethanol. For these runs, RCP failed before 1 h after the EOS (Fig. 1). A plateau occurred at approximately

FIGURE 1. Radiolytic decomposition profile for ^{18}F-FDG with radioactive concentrations of 19.7–22.6 GBq/mL (533–610 mCi/mL) doped with ethanol concentrations of 0%, 0.1%, and 0.2%. Horizontal solid line represents 10.0% threshold for amount of ^{18}F$^-$ impurities in solution.

4 h, with the mean percentage impurity being approximately 28.8% at the completion of the test period (Table 1).

QC measurements for the 3 runs doped with 0.1% ethanol (runs 1, 2, and 7) failed RCP approximately 5 h after the EOS, when it was determined that they exceeded the 10% impurity for free ^{18}F$^-$-fluoride (Fig. 1). The RDP plateau occurred approximately 7 h after the EOS, with a mean percentage impurity of approximately 11.1% after 12 h (Table 1).

The 3 runs conducted using an ethanol concentration of 0.2% (runs 3–5) maintained stability through 12 h after the EOS. The radiochemical impurities averaged 6.4% through 12 h (Table 1).

RCP and Radiochemical Identity

For all runs, the R_f value for ^{18}F-FDG was approximately 0.6.

Chemical Purity: Residual Solvent Content

Regardless of the amount of ethanol contained in each sample, chemical purity showed solvent impurities below the 0.5% limit for ethanol. Results for runs 6, 8, and 9 confirmed that there was no ethanol in the final solutions. Runs 1, 2, and 7 had results indicating that 0.1% of ethanol was in the formulated products. Results for runs 3, 4, and 5 revealed a concentration of 0.2% ethanol in the formulated products. No acetonitrile was detected in any of the samples.

pH

For the runs containing no ethanol, pH ranged from 5.5 to 6.1. Runs containing 0.1% maintained pH values ranging from 5.8 to 6.1. Runs containing 0.2% ethanol had pH values ranging from 5.3 to 6.1.

Visual Inspection

For all runs, visual inspection showed that the solutions remained clear and particulate-free.

DISCUSSION

The Mayo Clinic in Rochester, Minnesota, has recently received an approval for a request to change the strength (i.e., radioactive concentration) of ^{18}F-FDG from that of the reference listed drug product (i.e., from 11.1 GBq/mL [300 mCi/mL] at the EOS to 18.5 GBq/mL [500 mCi/mL] at the EOS). As such, our study examined the radiolytic stability of ^{18}F-FDG at high radioactive concentrations in the FASTlab unit. It is known that ethanol is an effective stabilizer of ^{18}F-FDG (*3,4*). A concentration of 0.1% ethanol successively stabilized up to 14.2 GBq/mL (385 mCi/mL) of ^{18}F-FDG out to 10 h (*3*).

TABLE 1
Relationship of ^{18}F$^-$-Fluoride Contents at EOS and Final Hour Versus Various Ethanol and Radioactive Concentrations

Ethanol concentration (% w/v)	Radioactive concentration at EOS*		Percentage of ^{18}F$^-$-fluoride content . . .	
	mCi/mL	GBq/mL	EOS	Final hour[†]
0.00	563.33	20.84	3.7	31.8 (7)
0.00	559.33	20.70	5.2	28.1 (4)
0.00	599.76	22.19	3.9	26.5 (4)
0.10	539.35	19.96	2.7	10.9 (12)
0.09	596.88	22.08	4.1	10.5 (12)
0.12	540.00	19.98	1.9	11.8 (12)
0.21	555.17	20.54	3.0	6.8 (12)
0.21	533.60	19.74	1.9	6.1 (12)
0.21	609.10	22.54	2.7	6.2 (12)

*Solutions in 15-mL final volume.
[†]Final hour is in parentheses.

The data in Figure 1 apply a logarithmic regression analysis, suggesting that radiolysis is the cause of the decline in RCP. This decrease in RCP is a result of a known phenomenon referred to as radiolysis or radiolytic decomposition. Radiolysis is caused by the high-density γ-photon flux produced from the decay of the ^{18}F nuclei. Increased high radioactive concentration creates high-energy species or free radicals, which in turn have the ability to break chemical bonds, including the bond connecting ^{18}F to the deoxyglucose molecule [2].

Radiolysis results in free, unbound, $^{18}F^-$-fluoride, which, once injected, would not go to areas of increased sugar uptake but rather be absorbed by bone. This uptake would be detrimental to the scan quality, standardized uptake values, and ultimately the diagnosis of disease [8]. It is essential that the levels of free $^{18}F^-$-fluoride are kept to a minimum. If the amount of impurity rises above 10%, ^{18}F-FDG can no longer be administered to patients because of USP stipulations [1].

Our results show that runs performed without the addition of ethanol failed to maintain the RCP necessary for patient administration. At merely 1 h after the EOS, the percentage impurity exceeded 10% for runs 6, 8, and 9 (Fig. 1). These results are considered a failure for RCP because the goal was to remain below 10% impurities for 12 h.

The addition of 0.1% ethanol also resulted in the failure to maintain RCP within the acceptance criteria. The percentage impurity in runs 1, 2, and 7 was found to be significantly lower than in the runs containing no ethanol; however, after approximately 5 h these percentages also exceeded the 10% impurity level (Fig. 1).

Stability was successfully maintained for 12 h after the EOS for runs 3–5, which contained an ethanol concentration of 0.2% (Fig. 1). The radiolytic impurities for these runs remained well below the 10% limit throughout 12 h, ensuring that RCP acceptance criteria were met so the solution could be used for patient administration.

For all runs, the R_f values were approximately 0.6. The standard solution also had a matching R_f value of 0.6. According to the USP, the R_f value should be about 0.4 [1]. In our experience, 0.6 is typical and acceptable. Therefore, for all runs, radiochemical identity was considered to be successfully affirmed.

We suspected that solutions of ^{18}F-FDG at high radioactive concentrations (i.e., more than 18.5 GBq/mL, or 500 mCi/mL) would need greater than 0.1% of ethanol to meet USP guidelines for RCP. As the data in Figure 1 demonstrate, 0.1% ethanol nearly maintained RCP for 12 h but ultimately did not consistently keep RCP below the 10% radiochemical impurity threshold. One might theorize that 0.15% ethanol would be sufficient to maintain RCP, but further evaluation is needed. The USP guidelines stipulate that up to 0.5% of ethanol can be present in the final formulation of ^{18}F-FDG [1]. However, because of the spirit of current good manufacturing practice, our research focused on finding the least amount of ethanol that was necessary to achieve the desired result [5].

All of the data met the acceptance levels for ethanol and acetonitrile. The USP monograph and the FDA stipulate that the solution cannot contain more than 0.5% and 0.04% ethanol and acetonitrile, respectively [1,5].

Ethanol content at 0.2% maintained an RCP above the 90% threshold, suggesting that it could stabilize a radioactive concentration beyond that which was studied. Once again further evaluation is necessary for ^{18}F-FDG in concentrations greater than 22.6 GBq/mL (610 mCi/mL).

For all runs, pH values were within the acceptance limits of 4.5–7.5 as stipulated by the USP [1].

The current USP monograph does not specifically state what is required for appearance testing; however, the FDA requires completing a visual inspection before patient administration [5]. Our results show that all solutions were clear, colorless, and particulate-free, which would be suitable for injection.

CONCLUSION

Levels of $^{18}F^-$ were measured at approximately hourly intervals to characterize radiolytic decomposition of high-radioactive-concentration ^{18}F-FDG produced with FASTLab. ^{18}F-FDG produced with FASTlab exhibits radiolytic decomposition, correlating with findings using high-radioactive-concentration ^{18}F-FDG produced with the TRACERlab FX-FDG synthesis unit. ^{18}F-FDG doped with less than or equal to 0.1% ethanol could not maintain RCP throughout the course of 12 h from the EOS for a concentration of 19.7–22.6 GBq/mL (533–610 mCi/mL). It was found that the addition of 0.2% ethanol to ^{18}F-FDG (radioactive concentration of 19.7–22.6 GBq/mL [533–610 mCi/mL]) at the EOS stabilized the formulation through 12 h after the EOS. Therefore, we can feel confident in administering the solution to patients for out to 12 h from the EOS. Each run containing 0.2% ethanol passed all QC stability testing as required by the USP and FDA (i.e., radiochemical identity and RCP, appearance, pH, and residual solvents). Because all QC tests were passed, ^{18}F-FDG in a volume of 15 mL with an activity of up to 336.7 GBq (9.1 Ci), meeting all USP and FDA acceptance criteria for stability related testing, can be manufactured.

ACKNOWLEDGMENTS

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