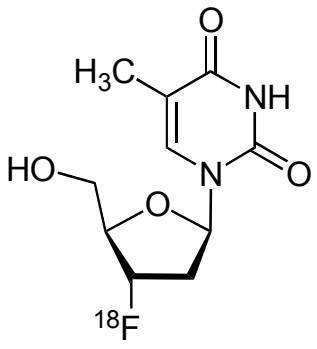
7 Chemistry, Manufacturing, and Control (Version 3/01/05)

7.1. Product Summary 3'-deoxy-3'-[^{18}F]fluorothymidine, ([^{18}F]FLT)

7.1.1 Chemical Structure



[^{18}F]FLT

7.1.2 Specifications for the [^{18}F]FLT final product

Table 1

Radiochemical Purity(TLC):	**R_f = 0.4-0.7** **Purity ≥ 95%**
Residual Solvent Levels:	**Acetone < 5000 ppm** **Acetonitrile < 400 ppm** **DMSO < 5000 ppm**
Radionuclidic Purity:	**Measured half-life 100-120 minutes**
Bacterial Endotoxin Levels:	**< 175 EU per dose**
pH:	**6-8**
Sterility:	**no growth observed in 14 days**
Residual Kryptofix® [2.2.2]:	**< 50 µg/ mL Kryptofix®**
Radiochemical Purity (HPLC)	**> 95%**
Chemical Purity (HPLC):	**FLT < 6.1 µg per injected dose**
Chemical Purity (particulates):	**Clear and Colorless**

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7.1.3 [^{18}F]FLT Drug Product Components and Composition

The final product has been characterized and is as follows:

TABLE 2

COMPONENTS		
[^{18}F]FLT, 3'-deoxy-3'-[^{18}F]fluorothymidine	**Same as for [^{19}F]FLT**	**≤ 5.0 mCi**
[^{19}F]FLT, 3'-deoxy-3'-[^{19}F]fluorothymidine	**NSC #140025 for [^{19}F]FLT**	**≤ 6.1 µg**
Sodium phosphates	**USP**	**0.01 M (92% by volume)**
Ethanol, absolute	**USP**	**8% by volume**
Saline for injection	**USP**	**0.15 M**

TABLE 3

IMPURITIES	Acceptance Criteria See Section 7.9.3	Highest Values in Qualification Runs
Kryptofix® [2.2.2]	< 50 µg/mL	
Acetonitrile	< 400 ppm	
DMSO	< 5000 ppm	
Acetone	< 5000 ppm	

The name of the drug is 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT). FLT is the only active ingredient and it is in a solution of ≤ 10 mL of 92% 0.01 M phosphate in 0.15 M saline (PBS): 8% ethanol (v:v). The drug product solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial with an expiration time of 8 hours. The injectable dose of [^{18}F]FLT for this study will be ≤ 0.07 mCi/kg not to exceed 5.0 mCi with a specific activity greater than 200 Ci/mmol at the time of injection. The amount of injected drug is ≤ 6.1 µg (≤ 25 nmol) of FLT. [^{18}F]FLT is administered to subjects by intravenous injection of ≤ 10 mL. In the dose of [^{18}F]FLT only a small fraction of the FLT molecules are radioactive. There is no evidence that nonradioactive and radioactive FLT molecules display different biochemical behavior.

The synthesis of [^{18}F]FLT is shown schematically in Figure 1. The synthesis procedure follows that reported by Machulla *et al*.(2001). There is a difference in the methods we propose for manufacture of [^{18}F]FLT from those used for some of the patient data supplied in the toxicity section. The most significant difference is the use of a different protecting group, benzoic acid, on the nonradioactive precursor. The purpose of the protecting group is to prevent ^{18}F from labeling an undesired site on the molecule. Benzoic acid was used as the protecting group in the syntheses for the human data reported by Smyczek-Gargya (2004). Some of the other human use data involved radioactive syntheses that used a dimethoxytrityl protecting group in the nonradioactive FLT precursor. Benzoic acid was chosen because it gives reliable yields of [^{18}F]FLT, it is commercially available from a GMP facility and it is known to be nontoxic in the amount used.

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Another difference from what was reported for the [^{18}F]FLT for human use (toxicity section) is the use of a different preparative HPLC column and mobile phase for purification of the final [^{18}F]FLT product. There is no anticipated toxicity associated with the use of preparative chromatography because only USP injectables are used for the mobile phase. Data are included to show that the product is clean and has the correct identity using the preparative separation method employed (section 7.5.5).

An automated procedure will be used instead of manual radiosynthesis. The use of automation should minimize the variance in the chemical reaction when compared with previously published syntheses of [^{18}F]FLT. This is particularly important in the case of a multi-center trial where the manufacturing is performed at the individual sites and not at a central location. Thus, the use of a semi-closed and automated system should reduce the risk to the subjects.

7.2. Controls for Components and Raw Materials

A complete list of forms and standard operating procedures, have been developed to follow the manufacturing materials and process from ordering to final approval for injection. As outlined in **GEN-M001**, "Writing and Revising Reagent & Supply Form," SS refers to the Specification Sheet for a reagent or supply (Appendix 1 in a separate binder labeled CMC Appendix 1-Standard Operating Procedures (SOPs) for [F-18] FLT). Each reagent or supply has a unique internal specification code of three characters. **GEN-Q003**, "Receiving, Handling, Quarantine, & Release of Reagents & Supplies," outlines how the individual sites track each lot of reagents and supplies needed for manufacturing and quality control using both a location code generally denoted as "L" and a unique sequential number, for each lot, starting from the number series 0001 and counting up, (0002, 0003, etc.) appended to the internal specification code. When an item is ordered it is given a unique internal tracking number for the reagent or supply. The specification sheets list all of the reagent and supply manufacturers' addresses and contact information, including any pertinent certificate of analysis or certificate of quality criteria that these reagents/supplies must meet prior to their release for further use. Any additional testing beyond what may be listed on the C of A or C of Q is described in this IND submission.

7.2.1 Organic Substrate Used for the Radiosynthesis

The [^{18}F]FLT precursor must be tested before release by a GMP/GLP facility. This is intended to provide rigorous confirmatory analytical testing. Each time a new lot of precursor is received for use, the precursor will be qualified by first conducting one to three qualification runs to ensure that the precursor provides the expected product within the target specification ranges.

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TABLE 4

REAGENT	INTERNAL SPECIFICATION CODE	CURRENT SUPPLIER	ADDITIONAL TESTING REQUIRED
[^{18}F]FLT Precursor; 5'-*O*-Benzoyl-2,3'-anhydrothymidine	**SS-BAT**	**ABX**	Confirmatory GMP/GLP testing

7.2.2 Target Material for Preparing [^{18}F]-Fluoride Reagent, a Key Intermediate

The radioactive fluoride precursor will be produced on site at each manufacturing site listed in the IND. The following target material will be used for the production of radioactive fluoride precursor:

TABLE 5

PRECURSOR	INTERNAL SPECIFICATION CODE	CURRENT SUPPLIER	ADDITIONAL TESTING REQUIRED
$H_2{}^{18}O$ target water, > 80% enriched in ^{18}O	**SS-O18**	**Rotem, Isonics, Isotec, Cambridge Isotope Laboratories, or Eastern Isotope**	**None**

7.2.3 Other Ingredients

The following ingredient(s) are used in the formulation of 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT) injection.

TABLE 6

REAGENT	PURPOSE	CURRENT SUPPLIER	INTERNAL SPECIFICATION CODE
23.4 % Sodium Chloride Solution, for Dilution, USP (234 mg/mL)	**To make the formulation isotonic prior to injection.**	**Any USP supplier**	**SS-NCC**

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7.2.4 Reagents, Solvents, Gases, Purification Columns, Solutions, and Other Auxiliary Materials

TABLE 7: Reagents/Supplies Used for Radiosynthesis of [^{18}F]FLT

REAGENT/SUPPLY	INTERNAL SPECIFICATION CODE	CURRENT SUPPLIER
Ethyl Alcohol, Absolute USP	SS-ETH	Any USP supplier
Sterile Water for Injection, SWFI, USP	SS-WFI	Any USP supplier
Kryptofix® [2.2.2], (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane)	SS-KRY	Sigma-Aldrich, EMD (VWR International) or Rotem Industries
Potassium carbonate, K_2CO_3, high purity	SS-KCA	Sigma-Aldrich or Mallinckrodt Baker
Sodium hydroxide, pellets, NaOH, NF	SS- NAH	Sigma-Aldrich or Mallinckrodt Baker
Dimethyl Sulfoxide (DMSO), anhydrous >99.9%	SS-DMS	Sigma-Aldrich or Mallinckrodt Baker
Sodium Dihydrogen Phosphate, USP	SS-DPM	Any USP supplier
Sodium Phosphates for Injection, USP	SS-NAP	Any USP supplier
Acetonitrile, anhydrous 99.8%	SS-ACN	Sigma-Aldrich or Mallinckrodt Baker
Acetone 99.5 +% ACS	SS-ACT	Sigma-Aldrich or Mallinckrodt Baker
Sterile Vent Filter 0.2 µm Millex SLGV V25 5F	SS-SVF	Millipore Corporation
Alumina N Light Sep-Pak Cartridge	SS-SPC	Waters Corporation
[^{18}F] Separation Cartridge, an anion exchange solid phase extraction cartridge	SS-FSC	Macherey Nagel
HPLC Preparative Column (Luna C_{18} 10 x 250 mm with or without guard column)	SS-LUN	Phenomenex
Sterile Vented Filter Needle	SS-VFN	International Medical Industries
Sterilizing Product Filter 0.2 µm	SS-SPF	Costar Corp. (Cambridge,MA), Millipore Corporation, or VWR International
Empty Sterile Vials, 10 mL, 20 mL or 30 mL borosilicate glass with gray butyl septa, sterile, nonpyrogenic	SS-SBV	Hospira (Lake Forest, IL), SLK Abello, or Miller Analytical
Liquid Nitrogen or liquid argon .	check label	Any local supplier
Argon Gas, > 99.9% pre-purified	Certificate of Analysis	Byrne gas (Seattle, WA), AirGas (Radner, PA) Air Liquide (Houston, TX) Matheson Gas, Airco (Murray Hill, NJ), General Welding Supply (Seattle, WA)
Helium Gas, (>99%)	Certificate of Analysis	
Nitrogen Gas, HP from a cylinder	Certificate of Analysis	

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TABLE 8: Solutions Used in the Radiosynthesis of [^{18}F]FLT

SOLUTIONS	SPECIFICATION	COMPOUNDING RECORD NUMBER (if applicable)	QUANTITY
70% Volume Ethanol	Compounded from USP reagents	ETH70-L	≥ 200 mL
Sodium Phosphates 10 mM : Ethanol (92:8 v:v)	Compounded from USP reagents	HBS-L	≥ 750 mL
0.04 M K_2CO_3 Solution	High purity potassium carbonate in USP Water for Injection	KCO-L	0.5 mL
Kryptofix® [2.2.2] Solution (15 ± 2 mg in 1 mL acetonitrile)	Anhydrous CH_3CN, reagent grade [2.2.2]	N/A	1.0 mL
1% NaOH	Compounded from USP/NF reagents	NAH-SOLN-L	0.35 mL
0.2N Sodium dihydrogen phosphate	Compounded from USP/NF reagents	DHP-L	0.75 mL

TABLE 9: Disposable Supplies Used in the Radiosynthesis of [^{18}F]FLT

SUPPLY	DESCRIPTION	SUPPLIER
Sterile Syringe, 1 mL	No silicone or latex (for vial 1-6 additions and ^{18}F separation cartridge wash)	Sigma Aldrich or GE Medical Systems
Disposable Needles 25G x 3½"	To fill Vials 1-6.	Becton Dickinson & Co.
Sterile Disposable Syringe, 10 mL	No silicone or latex (for cleaning with acetone)	Sigma Aldrich or GE Medical Systems
Syringes, various sizes, disposable plastic	Sterile, nonpyrogenic	Beckton Dickinson & Co. (Franklin Lakes, NJ)
Disposable Needles 22G or smaller	Sterile, nonpyrogenic	Beckton Dickinson & Co (Franklin Lakes, NJ)
Alcohol Swabs	Sterile Individually Wrapped	Various including Cardinal Health
Tubing	Teflon® and PEEK and polyethylene	Alltech Associates (Deerfield, IL), Upchurch Scientific (Oak Harbor, WA) or equivalent
Tube fittings and connectors:	Miscellaneous made of Delrin, Kel-F, PEEK, polypropylene, nylon or stainless steel. Brass for gas lines only	Alltech Associates (Deerfield, IL), Upchurch Scientific (Oak Harbor, WA) or equivalent Swagelok or equivalent

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7.3 Reference Standards

The following reference standards are used in the quality control methods of 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT) injection:

TABLE 10

REAGENT/SUPPLY	INTERNAL SPECIFICATION CODE	CURRENT SUPPLIER
3'-deoxy-3'-[^{19}F]fluorothymidine ([^{19}F]FLT)	**SS-FLT**	**Sigma-Aldrich or ABX**
Kryptofix® [2.2.2], (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane)	**SS-KRY**	**Sigma-Aldrich, VWR International or Rotem Industries**
Acetone, 99.5+%, ACS, spectrophotometric grade	**SS-ACT**	**Sigma-Aldrich or Mallinckrodt Baker, Inc.**
Acetonitrile (CH_3CN), anhydrous 99.8%	**SS-ACN**	**Sigma-Aldrich or Mallinckrodt Baker, Inc.**
Dimethyl Sulfoxide (DMSO), anhydrous > 99.9%	**SS-DMS**	**Sigma-Aldrich or Mallinckrodt Baker, Inc.**

7.4 Manufacturing & Testing Facilities:

The Sponsor will maintain responsibility for oversight of quality assurance and will work closely with the principal chemistry investigators at each manufacturing site to ensure correct implementation of the written SOPs, procedures, Master Production Record, Master Batch Record, Master Compounding Records, Specification Sheets, Forms, and all other written documentation that is not specifically referenced here. **GEN-Q012**, "Quality Systems Administration," describes the overall plan for implementing and maintaining process, in-process, and product quality control. **GEN-R503**, "Software Administration and Change Control for the nucleophilic synthesis module," describes the change control procedures for the nucleophilic synthesis module software to be followed at the site prior to implementation. (GEN-R503 to be provided by the Sponsor)

The [^{18}F]FLT will be synthesized and tested at the following site. Each site must complete six qualification runs to be submitted to FDA prior to initiation of human studies. The site is listed below.

1) **Name:** Site Name
   Principal Chemistry Investigator
   Address:
   Phone:
   Fax:

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7.5 Manufacture of the Drug Substance

7.5.1 General Description of the Method for Preparation of the [^{18}F]FLT

The general chemical scheme for the radiochemical synthesis of [^{18}F]FLT is illustrated in Figure 1. The general procedure for the synthesis of [^{18}F]FLT is shown as a flow chart in Figure 2. Briefly, ^{18}F is made by irradiating enriched [^{18}O]-water with protons. The ^{18}F is separated from the [^{18}O]-water by trapping the ^{18}F on an anion exchange cartridge. Then aqueous potassium carbonate is used to elute the [^{18}F]-fluoride into the reaction vessel. Acetonitrile and the phase transfer reagent, Kryptofix® [2.2.2], are added to the reaction and the reaction is heated through three temperature steps, 55°C, 95°C and 120°C, to azeotropically remove the water and then to remove the acetonitrile. Next the organic precursor for the reaction, 5'-O-benzoyl-2,3'-anhydrothymidine, in dimethylsulfoxide is added to the reaction vessel. The reaction is heated at 160°C for 10 minutes to promote the substitution of the ^{18}F into the sugar ring in the 3' position. Next aqueous sodium hydroxide is added and the reaction is heated at 50°C for 10 minutes to remove the benzoate protecting group. Aqueous 2N sodium dihydrogen phosphate is added to neutralize the reaction mixture prior to loading it on the preparative HPLC column for purification.

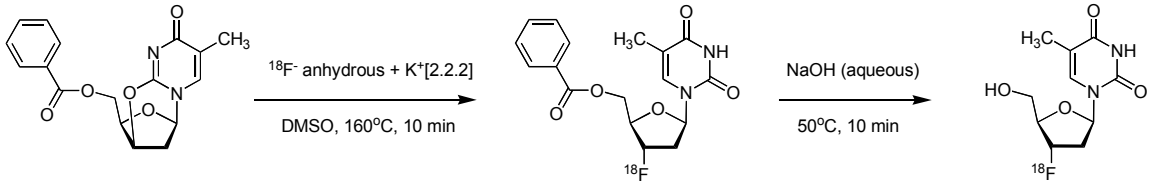

Figure 1: Synthetic scheme for preparation of [^{18}F]FLT

The resulting [^{18}F]FLT is separated from the other compounds in the reaction mixture using preparative high performance liquid chromatography (HPLC). A sterile mobile phase consisting of 92% 0.01M phosphate buffered saline and 8% ethanol is the preparative mobile phase that is used to elute the purified [^{18}F]FLT.

The typical decay corrected chemical yield is 15%. The drug substance is not isolated. Instead the drug substance is collected directly through a 0.22 μm sterilizing filter into a vented sterile vial containing 0.50 to 0.60 mL of concentrated NaCl (23.4% USP) to make the final drug product isotonic. Samples are removed for analysis of product quality (see section 7.6).

The radiosynthesis of [^{18}F]FLT will be conducted at the manufacturing site using an automated synthesis procedure that is described in much greater detail in Appendix 1 in a separate binder labeled CMC Appendix 1- Standard Operating Procedures (SOPs) for [F-18] FLT under the Master Production Record "MPR [^{18}F]FLT". The control information for each batch is captured in a copy of the Master Batch Record that is assigned a unique batch number for every production run as described in **GEN-R004**, "Assigning Batch & Compounding Record Numbers to Production Runs." The

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automation unit that will be used for the manufacturing of [^{18}F]FLT is a nucleophilic synthesis module (Figure 3). (Appendix 2, Nucleophilic Synthesis Operating Manual, is to be supplied by the sponsor.)

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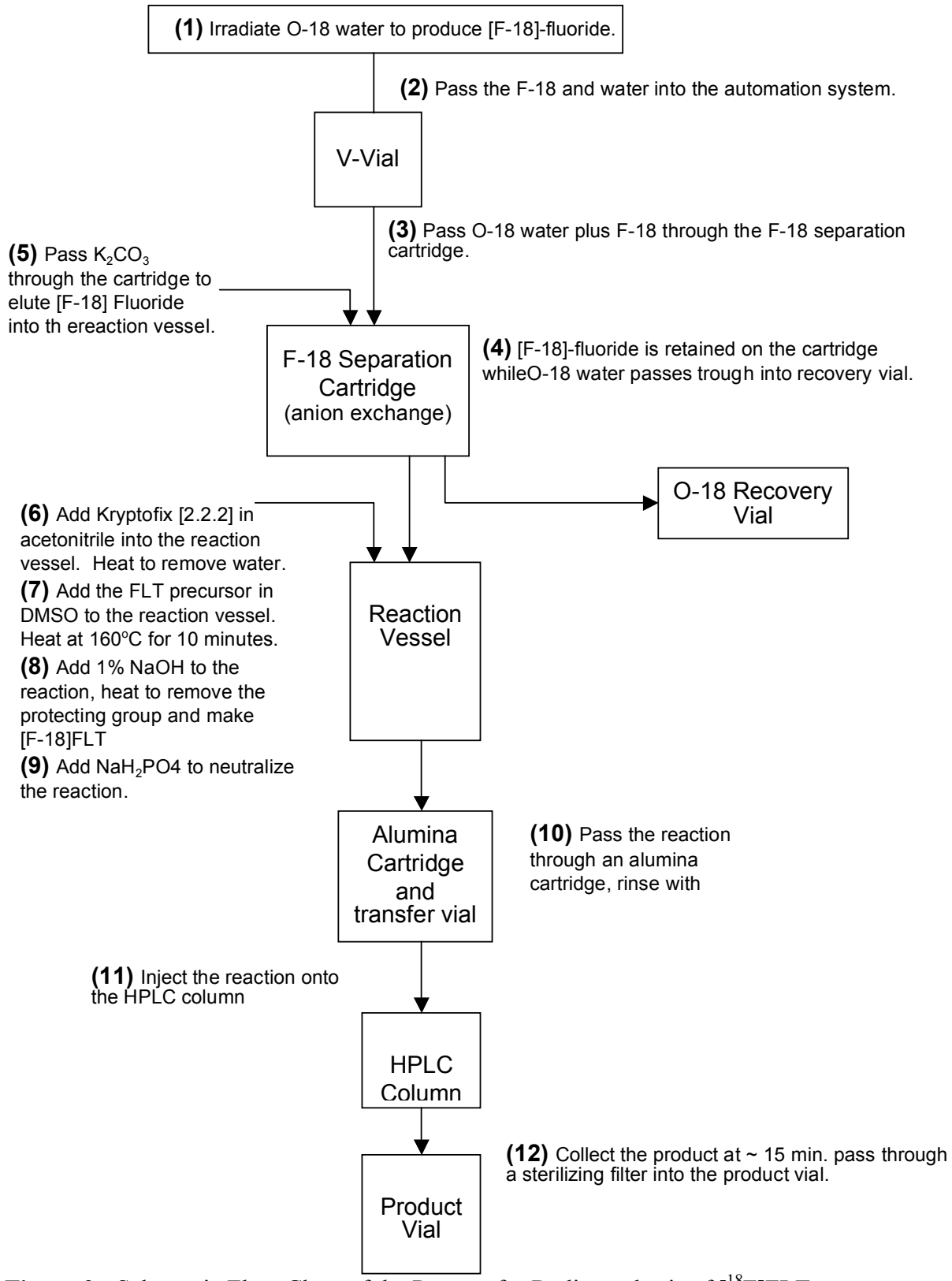

Figure 2: Schematic Flow Chart of the Process for Radiosynthesis of [^{18}F]FLT

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Figure 3: Insert picture of nucleophilic synthesis module (provided by sponsor)

7.5.2 Cyclotron Production of [^{18}F] Fluoride

[^{18}F]fluoride is produced by irradiating ^{18}O enriched water with protons in a silver, titanium, niobium or tantalum target. The target entrance window is made of Havar, Arnavar, titanium, or tantalum. One to two targets are irradiated for 10-120 min with 10-80 µA of proton beam for one batch synthesis of [^{18}F]FLT. Yield is approximately linear with the beam current but is different at each institution due to the different cyclotron energies.

The information below describes the typical target and irradiation conditions for production of ^{18}F. However, none of these parameters are critical variables for the quality of the final [^{18}F]FLT drug product.

Particle Accelerator (Cyclotron) Used:
Make:
Model:

Operating Parameters

- During irradiation a beam current of 10 µA to 80 µA is used on one to 2 targets.
- Irradiation times of 10 minutes to 120 minutes are used. High-pressure targets are used, irradiations are performed under approximately 400-500psi of pressure.

Specifications for Target Body

- Volume of the target(s): between 1 and 5 mL.
- The target is made of silver, titanium, niobium or tantalum.
- The target windows are made of Havar, Arnavar, titanium, or tantalum.

7.5.3 [^{18}F] Fluoride.

The [^{18}F] fluoride is transferred from the target to the nucleophilic synthesis module using inert gas pressure. Once in the box, the fluoride is passed through an anion exchange cartridge to retain the [^{18}F] fluoride and to separate it from the [^{18}O] water. The [^{18}F] fluoride is eluted from the anion exchange cartridge using 0.5 mL of 0.04 M potassium carbonate (K_2CO_3) and transferred to a glassy carbon reaction vessel that is supplied with the synthesis box. A solution of anhydrous acetonitrile (CH_3CN) 1.0 ± 0.1 mL containing 15 ± 2 mg of Kryptofix® [2.2.2], a phase transfer reagent, is then added to the reaction. This fluoride / Kryptofix solution is dried at 95-120°C for ~10 minutes to remove the water.

7.5.4 Radiosynthesis of [^{18}F]FLT

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Anhydrous dimethylsulfoxide (DMSO) containing 10 ± 2 mg of 5'-*O*-benzoyl-2,3'-anhydrothymidine (1.0 ± 0.1mL) is added to the reaction vessel. The [^{18}F]fluoride is transferred into the organic phase, presumably carried as an anionic counterion to the potassium ions in the Kryptofix® [2.2.2]. The fluoride reacts with the organic precursor to break the ether bond and substitutes onto the sugar ring in the 3' position. The fluoride ring addition is reacted for 10 minutes at 160°C.

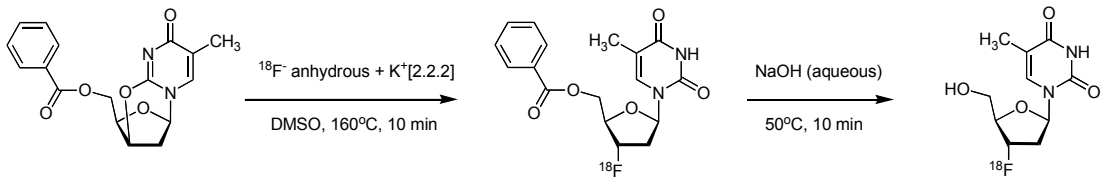

Figure 1: Method of Preparation for [^{18}F]FLT

Once the fluorination reaction is finished, the benzoate protecting group is removed by base hydrolysis. Specifically, 0.35 ± 0.1 mL of 1% sodium hydroxide is added to the reaction and it is heated at 50°C for 10 minutes. The reaction is neutralized by adding 0.75 mL of 2N NaH_2PO_4 and then the reaction mixture is passed through an aluminum oxide cartridge to remove unreacted fluoride. The cartridge is rinsed with 0.5 mL of the HPLC mobile phase.

7.5.5 Purification of [^{18}F]FLT

The resulting [^{18}F]FLT is separated from the other compounds in the reaction mixture using preparative high performance liquid chromatography (HPLC). A sterile mobile phase consisting of 92% 0.01M phosphate buffered saline and 8% ethanol is used to elute the purified [^{18}F]FLT from a C_{18} HPLC column (Luna C_{18}, 10 µm, 250 x 10 mm from Phenomenex, Inc.). The mobile phase is pumped at a flow rate of 6 mL/min at room temperature. The product, [^{18}F]FLT, is typically eluted at 16 min (96 mL, k′= 7) with a FWHM of 6 mL. The [^{18}F]FLT is baseline separated from the other UV absorbing (254 nm) compounds and from the other radioactive products.

The typical decay corrected chemical yield is 15%. The [^{18}F]FLT solution is collected through a 0.22 µm sterilizing filter into a vented sterile vial containing 0.50 to 0.60 mL of concentrated NaCl (23.4% USP) to make the final drug product isotonic. Samples are removed for analysis of product quality (see section 7.6).

At the end of synthesis an HPLC cleaning program is run so that the preparative HPLC column and injector are stored in 70% ethanol, USP. The product output luer fitting is sealed with a 0.2 µm sterilizing filter between uses. The column is prepared for use by passing >300 mL of the preparative mobile phase through the column and injector less than 3 hours prior to injection of the ^{18}F product reaction mixture onto the column. The vessels and tubing for the nucleophilic synthesis box are rinsed with acetone using a clean-up program after the synthesis. All of the clean-up procedures are also detailed

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in the Master Production Record (Appendix 1 in a separate binder labeled CMC Appendix 1- Standard Operating Procedures (SOPs) for [F-18] FLT).

7.6 Post Synthesis Processing and Quality Assurance of the [^{18}F]FLT

7.6.1 Sampling for Quality Assurance and Storage

The drug product is assayed for total radioactivity and is examined for particulates. Two samples totaling nominally 2.4 mL are removed for measurement of pH, apyrogenicity, sterility, analytical HPLC measurements of specific activity, chemical and radiochemical purity, GC measures of solvents and for conducting assays to determine levels of Kryptofix® [2.2.2] in the product. Approximately 1.0 ml of sample is retained for further testing as necessary. The integrity of the sterilizing filter is tested. The product dose is drawn, labeled, and once all but the sterility tests have been passed, the product dose is released for injection. A 20 minute endotoxin test may be used to release the dose but a 60 minute endotoxin test must be completed. The sample for sterility testing is inoculated within 48 hours but after the sample has decayed to a background radiation level.

7.6.2 Master Production and Master Batch Records

Six to eight qualification runs will be conducted at each manufacturing site as the sites start the synthesis processes. Each site is required to complete at least six qualification runs with full QC testing and analysis, prior to initiating manufacturing operations for human use.

7.6.3 Reprocessing of PET Drug Product

The final [^{18}F]FLT drug product will be reprocessed for only one reason. The final drug product may be resterilized in the event of an integrity test failure as per **GEN-Q110** and **GEN-Q108.**

7.7 Container/Closure

The drug substance is manufactured in a semi-closed automated system, a nucleophilic synthesis box using in-process controls such as high purity solvents to synthesize the radiopharmaceutical, automated software that controls the temperature & timing parameters and as well as recording the information from the production run. Sterile water for injection is used to compound the solutions for the preparative HPLC purification. The drug substance is then sterile filtered directly into the final drug product vial, the sterile USP Type I, Glass, Gray Butyl Rubber Stopper, Vial, SS-SBV. An integrity test is performed on the sterilizing filter, in addition to performing a LAL and a sterility test on the final drug product.

7.8 Controls For Finished Drug Product

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The quality control oversight will remain at the respective site. The quality control SOPs were written to be general enough for each site to use their own equipment while maintaining a high level of assurance that the final product is safe for administration to human subjects. Several quality control tests are run on the [^{18}F]FLT product prior to release for human administration, to assure the quality of the final product as per **GEN-R009**, "QC Testing, Review, and Final Review." The quality control tests and the numbers for their SOPs are presented in Appendix 1 in a separate binder labeled CMC Appendix 1- Standard Operating Procedures (SOPs) for [F-18] FLT.

TABLE 11: Quality Control Tests for the [^{18}F]FLT Product

QUALITY CONTROL TEST	DESCRIPTION	REQUIREMENTS FOR PASS	REQUIRES TEST PASS PRIOR TO PRODUCT RELEASE	GEN-SOP NUMBER (APPX I)
Filter Integrity	Test bubble point	**Meet pressure specified by manufacturer**	yes	**GEN-Q110**
Radiochemical Purity	TLC	**Rf = 0.4-0.7 and Purity ≥ 95%**	yes	**GEN-Q111**
Residual Solvent Levels	Gas Chromatography	**Acetone < 5,000 ppm Acetonitrile < 400 ppm DMSO < 5,000 ppm**	yes	**GEN-Q112**
Radionuclidic Purity	Half-life Determination	**100 – 120 minutes**	yes	**GEN-Q113**
Bacterial Endotoxin Levels	Limulus Amebocyte Lysate (LAL)	**< 175 EU per dose**	yes (20 min test acceptable)	**GEN-Q114**
pH	pH	**pH must be between 6 and 8**	yes	**GEN -Q115**
Sterility	USP sterility test	**No growth observed in 14 days**	no	**GEN-Q117**
Residual Kryptofix® [2.2.2]	Color spot test	**< 50 µg/mL Kryptofix[2.2.2] by comparison with standard**	yes	**GEN-Q118 or GEN-Q118A**
Chemical and Radiochemical Purity	HPLC	**Radiochemical Purity > 95% Chemical purity: FLT < 6.1 µg**	yes	**GEN-Q119**
Chemical Purity	Visual inspection for color and particulates	**Clear and Colorless**	yes	**GEN-Q121**

As outlined in GEN-R009, all of the quality control tests will be completed prior to product release except for the sterility test. In the event of a positive sterility test result, action will be taken within 24 hours. The treating physician will be notified with a summary from the principal physician investigator at each site and a report will be sent to IND sponsor.

If all tests, with the exception of the pending sterility test, are within acceptable limits, the product will be released for administration to the subject. If one or more of the quality assurance tests do not meet the required specifications listed above, then one of two actions will be taken. For all tests with the exception of GEN-Q110 and GEN-Q113, if the test does not meet the specifications listed above, the product will be "failed" and will not be released. As a result of this "failure" an Out of Specification Investigation will be conducted as outlined in GEN-Q015, "Investigating Out of

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Specification QC Test Results," to determine the cause of the aberrant test result. If the 0.22 µm filter used to filter the final product fails the integrity test both initially and upon rewetting of the filter, as per GEN-Q110, "Filter Integrity Test by Bubble Point," then the final product will be resterilized using a new sterile filter as per GEN-Q108, "Resterilization of Final Product in the Event of a Failed Filter Integrity Test." If the half-life determination does not meet specifications, it may be due to insufficient time points. In this case, the dose is not to be released and the half-life determination (GEN-Q113) may be repeated or more time points may be collected for the determination to improve the counting statistics. If the half-life determination passes after the second set of counts, then the dose may be released and an Out of Specification Investigation will be conducted as well. If the half-life determination does not pass after the second set of counts, the product will be "failed" and will not be released.

7.9 Analytical Test Procedures

7.9.1 Chemicals for Quality Control Analyses (QC)

TABLE 12: Reagents Used for Quality Control Analyses (QC)

REAGENT	INTERNAL SPECIFICATION CODE	CURRENT SUPPLIER
Potassium Iodide, Granular, USP	SS-KIG	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Iodine, USP	SS-IOD	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Methanol, HPLC Grade	SS-MTH	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Acetonitrile, snhydrous 99.8%	SS-ACN	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Acetone, 99.5% spectrophotometric grade	SS-ACT	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Dimethylsulfoxide, anhydrous	SS-DMS	Sigma-Aldrich or Mallinckrodt Baker, Inc.
Water, HPLC Grade	SS-HGW	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Chloroplatinic Acid, ACS Reagent grade	SS-CPA	Sigma-Aldrich
Benzoic Acid, ACS Reagent	SS-BZA	Sigma Aldrich
Limulus Amoebocyte Lysate (LAL) single test vials	SS-LAL	Associates of Cape Cod, Charles River or Cambrex
Control Standard Endotoxin (CSE)	SS-CSE	Associates of Cape Cod, Charles River or Cambrex
LAL Reagent Water (LRW)	SS-LRW	Associates of Cape Cod, Charles River or Cambrex
Ammonium Hydroxide Solution, 28% in water ≥ 99.99%	SS-NHO	Sigma-Aldrich and Mallinckrodt Baker
Hydrochloric Acid, 36.5-38.0%, NF	SS-HCL	Sigma-Aldrich or Mallinckrodt Baker, Inc.

TABLE 13: Solutions Used for Quality Control Analyses (QC)

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Solutions	Compounding Record Number (if applicable)
HPLC Aquasil Column Mobile Phase, 14% Methanol, 86% HPLC Water (v:v)	HES-L
Preparation of GC Standard Solution	GCS-L
Preparation of FLT Standard Solution for HPLC	STDFLT-L

7.9.2 Materials for Quality Control Analyses (QC)

TABLE 14: Supplies Used for Quality Control Analyses (QC)

SUPPLY	INTERNAL SPECIFICATION CODE/ MANUFACTURER'S LOT NUMBER	CURRENT SUPPLIER
pH strips, Panpeha® pH Indicator Strips (0-14)	SS-PHP	Sigma-Aldrich
TLC plates, Silica Gel 60 F_{254} Aluminum Backed	SS-TLA	Alltech Associates and VWR Scientific
TLC plates, Silica Gel 60 F_{254} Plastic Backed	SS-TLP	Alltech Associates and VWR Scientific
Column analytical Aquasil C18 2.1 X 150 mm (5µm)	SS-AQH	Thermo-hypersil Keystone
Helium(UHP) (UHP)	NA	Byrne gas (Seattle, WA), AirGas (Radner, PA) Air Liquide (Houston, TX) Matheson Gas, Airco (Murray Hill, NJ), General Welding Supply (Seattle, WA)
Hydrogen (UHP if use FID)	NA	
ATWAx column 20m long 0.25 mm ID 0.25µm	NA	Alltech Associates
Culture tube, capped, sterile, pyrogen free,disposable Falcon brand 12x75 mm, polypropylene, product no. 2005 17X100 mm, polypropylene, product no. 2006 12x75 mm, polystyrene, product no. 2003 17X100 mm, polystyrene, product no. 352001	NA	Beckton Dickinson (Rutherford, NJ) VWR
Pipet tips, various sizes, polypropylene	NA	VWR (San Francisco, CA)
Vacutainer tubes 16 X 100 mm, glass, product no. 3208	NA	Beckton Dickinson (Rutherford, NJ)
Vacutainer tube, 12 X 75 mm, glass, product no. 6530	NA	Becton Dickinson (Rutherford, NJ)
Deionized water	NA	made in house

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7.9.3 Validated Standard Test Procedures

All of the analytical test procedures are performed using high-quality solvents and reagents, which have been carefully logged in, controlled, and verified in the same manner as the reagents for the manufacturing process. Testing forms have been developed to ensure consistency for some of the more involved documentation of test information that is being collected.

The radionuclidic purity using half-life determination (GEN-Q113) is a variation of the test listed in the USP. This test is used to determine the identity of the radioactive nuclide should not vary with the compound being tested. The half-life test result for ^{18}F must be between 100 and 120 minutes. The chemical purity by visual inspection is straightforward; the final drug product in the vial should be clear and colorless without any visible particulates. The pH should be between the values 6-8.

The use of Radio-Thin Layer Chromatography to determine the radiochemical purity and identity were validated using acetonitrile as the mobile-phase, with varying concentrations of 0 to 5% water to mimic normal laboratory conditions over time. The average R_f values obtained from these studies were 0.4-0.7 for the final [^{18}F]FLT. The specification for the purity is greater than or equal to 95% using this methodology. This is primarily a test for free fluoride. If present, unlabeled ^{18}F will remain at the origin, R_f value equal to 0 and so is an adjunct test to the analytical HPLC. The identity is confirmed by running the nonradioactive FLT standard with the drug product to confirm the R_f values are consistent.

HPLC chromatography analysis, as written in GEN-Q119, will be used to determine chemical and radiochemical purity for this drug product. The final radiochemical purity is proposed to be greater than 95%, and the nonradioactive FLT must be less than 6.1 µg in the final product dose. The amount of nonradioactive FLT is measured by UV absorbance at 254 or 266 nm of the FLT eluted from a C18 Aquasil column with a mobile phase of 14% methanol: 86% water. The amount of FLT is quantitated by measuring the peak area response of a minimum of five FLT standards to bracket the sample or bracket the minimum acceptable mass limit. A linear regression is determined for UV absorbance peak areas of the standards. This constitutes the "calibration curve." Then the peak area of the FLT drug product is fit on the calibration curve to determine the FLT concentration in the drug product sample. Typically the final concentration of the [^{19}F]FLT in the [^{18}F]FLT product to be tested will be between 0.01 and 1 µg/mL.

The [^{18}F]FLT organic precursor does not elute from the HPLC column in the 15 minutes of the analytical separation unless it is eluted with 35% methanol as opposed to the 14% methanol for the [^{18}F]FLT. We expect it to behave the same on the preparative column. After synthesis the preparative HPLC column is washed with 70% ethanol to remove any retained organic material. The FLT precursor should not survive the synthesis. We have done tests of the stability and as shown in the figure 4 chromatograms, the FLT precursor when heated at 103°C for 10 minutes in slightly more diluted base than is used for the radiosynthesis, is destroyed and <2% is left. The figure 4 chromatogram used the analytical HPLC conditions as per GEN-Q119 except the mobile phase was 35% methanol rather than 14% methanol to elute the precursor and mass detection was used to specifically identify the peak. The degradation of

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the precursor was confirmed by observing the benzoate mass peak (loss of protecting group) in the base hydrolyzed experiment (data not shown).

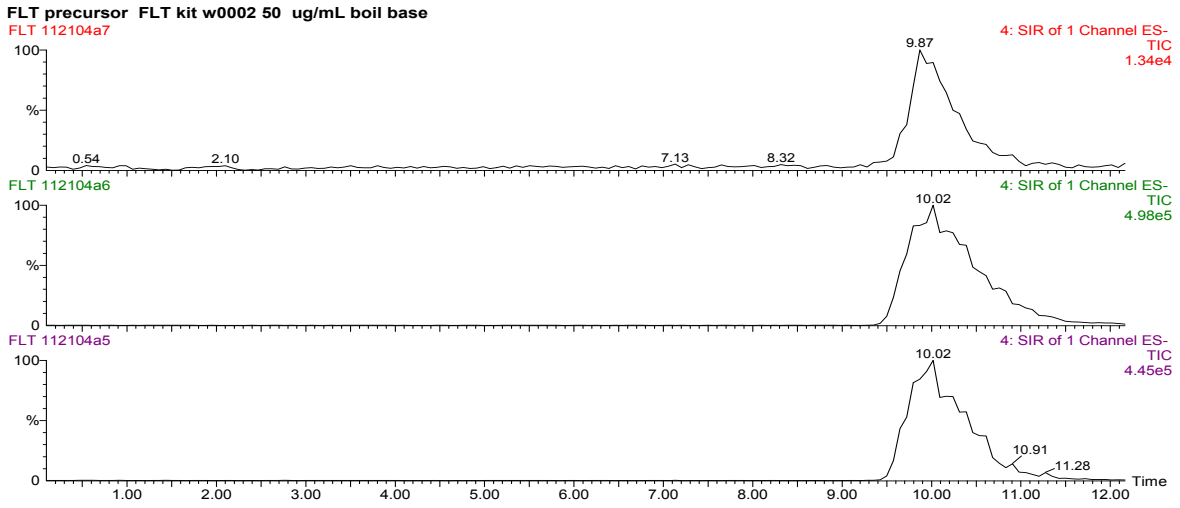

Figure 4: Specific mass spectra (351 M/Z ES-) of the FLT precursor in water. The bottom trace shows the precursor in water, the middle trace shows the precursor after 12 minutes at 103°C in water and the top trace shows the precursor after 10 minutes at 103°C in base (100 µL of precursor solution + 350 µL of 1% NaOH). The signal height is given below the word (TIC) on the right side of each trace for comparison.

Other impurities that can be expected to absorb UV are Kryptofix [2.2.2] which elutes at ~ 2 minutes, but is assayed using a chemical spot test (GEN-Q118), benzoate, impurities in the USP solutions and phosphates. Other chemical impurities must be less than 12 µg/mL total for any retention time between 3 and 15 minutes assuming the same UV molar absorption coefficient as FLT. The maximum amount of benzoic acid (the leaving group) possible in the final reaction is less than 3.5 mg, a nontoxic amount, so benzoic acid is not assayed. All standards must be baseline resolved (resolution > 1.5) for a valid analysis.

Residual Solvent Levels are determined using the methods described in GEN-Q112, GC analysis of solvents, once the final product has been manufactured. DMSO is the solvent for the FLT precursor. Acetone is used to clean the system. Acetonitrile is used to dissolve the Kryptofix® [2.2.2]. The permissible level of acetonitrile in the final product is less than or equal to 400 ppm. This value is the same as that for the USP permissible level of acetonitrile in 2-[^{18}F]FDG which may not exceed 400 ppm. The allowable levels for both acetone and DMSO are less than 5000 ppm. Both of these are Class three solvents; this class of solvents includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. Therefore this limit is based upon the FDA's Guidance for Industry ICH Q3C-Tables and List (November 2003 Revision 1), page 7, where it considers 5,000 ppm in 10 mL i.e., 50 mg or less per day, of these Class 3 residual solvents as an acceptable limit, without additional justification.

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The qualified Kryptofix® [2.2.2] test, GEN-Q118, is based on the method by Mock *et al.* (1997) using a "color spot test" for the detection of residual Kryptofix® [2.2.2] in the final drug product for this IND. The FDA has proposed a maximum permissible level of 50 µg/mL of Kryptofix® [2.2.2] in 2-[^{18}F]FDG, therefore this maximum permissible level will also apply to the [^{18}F]FLT final product. For this IND, there are two SOPs to test for residual Kryptofix® [2.2.2] that can be used for testing the final drug product. The USP test is actually the alternate test, GEN-Q118A. The preferred method, GEN-Q118, is based on the method by Mock using a "color spot test" for the detection of Kryptofix® [2.2.2], which is more sensitive but slightly less selective than the standard method listed in the USP.

Sterility will be tested using the direct inoculation method as per GEN-Q117. A report of the validation tests for the [^{18}F]FLT product and the USP sterility method is presented in Appendix 3 (provided by sponsor).

Bacterial Endotoxin Levels are tested and qualified using GEN-Q114, which was written based on the USP methodology, but using control standard endotoxin referenced to the USP's RSE. All of our bacterial endotoxin levels were <175 EU per batch for the initial qualification syntheses.

7.10 Information to Support the Stability of the Drug Substance

[^{18}F]FLT was made according to the procedures described in this IND application. The final drug product was left at room temperature for up to 24 hours. At periodic times over the 24 hours, the product was measured for radiochemical purity using analytical HPLC and TLC as described in SOPs GEN-Q119 and GEN-Q111. In addition, the FLT product has been examined for changes in UV absorbance of the product peak with time. Results are summarized below: (provided by sponsor)

Table 15
Site Qualification Run Data

Batch Number	Time Post End of Synthesis of Assay*	% Radiochemical Purity by HPLC	% Radiochemical Purity by TLC	Mass of FLT in product (by UV Absorbance 254 nm)

Expiration dating: We propose to use an expiration time of 8 hours. This is a conservative measure compared with the 12 hours now allowed for [^{18}F]FDG. Stability data is shown in the table below.

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Table 16

[^{18}F]FLT Stability Test Results

Time post synthesis(hr)	% pure by HPLC	% pure by TLC

7.11 Drug Product Vial Labeling

Two labels are attached to the patient dose and examples are shown for each site below:

Dose Labels for Site

UX PET RADIOCHEMISTRY LABORATORY
^{18}F-3'-Fluoro-3'-deoxythymidine in 92: 8 PBS: Ethanol
MBR-[^{18}F]FLT-X_____

Patient:_____
Physician: _____
Activity: _____ mCi in _____ mL
Calibration Date: _____ Time: _____

Caution

Radioactive Material

This radiopharmaceutical is for intravenous injection. Do not use if cloudy or if it contains particulate matter. Expires 8 hours after calibration. Calculate injection dose from date & time of calibration. $T_{½}$ is 109.8 minutes.

CAUTION: New Drug – Limited by Federal (or United States) law to investigational use.

7.12 Environmental Assessment

We request a categorical exclusion for the Environmental Impact Statement requirement in section 21 CFR part 25 because this radiopharmaceutical meets the conditions stipulated in Section 25.24(c)(4). Namely [^{18}F]FLT involves a short-lived radionuclide ($T_{1/2}$ = 109.8 min) prepared in unit dose and resulting in a total mass of active drug that does not exceed 20 µg, and the ^{18}F decays to a stable nuclide. This procedure will be done no more then 100 times per year. Thus the amount of the waste that is expected to enter the environment may be reasonably expected to be nontoxic.

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Machulla H.J. et al. Procedure for Routine Synthesis of [^{18}F] FLT in High Activities. J. Nucl. Med; 42 *(5):* 257P, 2001

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