MULTIMILLICURIE PREPARATION OF 2-[^{18}F]-FLUORO-2-DEOXY-D-GLUCOSE VIA NUCLEOPHILIC DISPLACEMENT WITH FLUORINE-18 LABELLED FLUORIDE

1. Purification and Quality Control Procedures

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SUMMARY

High performance liquid chromatography (HPLC) has been utilized for the preparation of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-2FDG) in 98.3 ± 1.5% radiochemical purity. Employing an enriched water target for fluoride-18 labelled fluoride ion production, the time required for synthesis and purification of the finished product averaged 150 minutes from end of bombardment (EOB). The average radiochemical yield of the pharmaceutical product at EOB was 18%.

Key words: 2-[^{18}F]-fluoro-2-deoxy-D-glucose, high performance liquid chromatography, quality control.

INTRODUCTION

Since the introduction of 2-[^{18}F]-fluoro-2-deoxy-D-glucose(1,2) in 1976, numerous synthetic routes to this compound encompassing both electrophilic and nucleophilic displacements have been reported. Although these preparations indicate isomerically pure 2-fluoro-2-deoxy-D-glucose (2-FDG), several different groups have reported recently that varying concentrations of 2-fluoro-2-deoxy-D-mannose (2FDM) are incorporated with the final product (3-7) when the electrophilic fluorination is employed.

Suitable modifications to a nucleophilic displacement reaction of [^{18}F]fluoride anion with methyl-4,6-O-benzylidene-2,3-O-cyclic sulfato-β-D-mannopyranoside were made (9-10). We report the scaled-up preparative procedure, high performance liquid chromatography (HPLC) separation, and quality assurance that provide sterile, apyrogenic, and radiochemically pure fluorine-18 labelled 2-FDG at multimillicurie levels (40-60mCi) for clinical utility devoid of 2FDM (7) and other radiochemical impurities.

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EXPERIMENTAL

Materials. One molar solution of boron tris(trifluoroacetate) in trifluoroacetic acid was purchased from Aldrich Chemical Company. The reagent was transferred into single use vials sealed under argon. All solvents and methyl-4,6-O-benzylidene-2,3-O-cyclic sulfato-β-D-mannopyranoside were acquired from Burdick and Jackson Laboratories, U.S.A. and Mara Speciality Chemicals respectively. All were used without further purification.

Chromatography. High performance liquid chromatographic (HPLC) analyses were performed using two Altex Model 330 liquid chromotographs, one equipped with a LDC Refractomonitor III refractive index detector for mass analysis. The other solvent pumping system was connected to a Rheodyne Model 7000 switching valve to allow injection either onto an appropriate HPLC column or into a reference tube for comparison of radioactivity eluted from the column to the total activity injected. The eluate was monitored for radioactivity using a NaI(Tl) detector with a Nuclear Data Model 60A multichannel analyzer operated in the multichannel scaling mode. Preparative HPLC was performed using an Altex Model 110A solvent delivery pump equipped with a Rheodyne Model 7125 injector and a 1.0 ml loop. Detection of radioactivity was accomplished with an Eberline thin-window G-M tube and a Model RM-14 radiation monitor attached to a chart recorder. All conditions are shown in Table I.

Gas liquid chromatographic (GC) analyses were carried out with a Hewlett-Packard Model 5710A gas chromatograph equipped with a thermal conductivity detector. Columns of 10% SE-30 (6'x 1/8", isothermal at 200°C) and 3% OV-17 (5' x 1/8" isothermal at 160°C) both on Anakrom Q (70-80 mesh) were used. With a helium carrier gas at a flow rate of 20 ml/min, retention times for the silylated derivatives (8) of 2FDG were 7.6 min (α) and 9.6 min (β); and 8.5 min (α) and 11.5 min (β), respectively.

Table I

Conditions for High Performance Liquid Chromatographic Analysis of 2-Fluoro-2-deoxy-D-glucose*

	Column System				
Column Parameter	A	B	C	D	E
Void Volume, ml	3.68	3.60	3.84	6.88	20.0
Retention Time, min	5.12	6.44	7.54	13.76	15.52
Retention Volume, ml	10.24	12.88	15.08	27.52	77.60
Capacity Factor	1.78	2.58	2.93	3.00	2.88
Theoretical Plates	570	665	910	1970	1190
Plates/m	2280	2217	3640	3582	2380

*The solvent for column systems A-D was 90:10 acetonitrile:water at a flow rate of 2.0 ml/min. The columns used were: A) Alltech-NH_2 column (10μ, 25 cm x 4.6 mm i.d.) with a Brownlee Amino Guard column (10μ, 3 cm x 4.6 mm i.d.); B) Alltech 600 CH column (10μ, 30 cm x 4.1 mm) with a guard column of Analytichem-NH_2 (40μ, 3 cm x 4.6 mm i.d.); C) Alltech-NH_2 column (10μ, 25 cm x 4.6 mm i.d.) with a Brownlee Amino Guard Column (10μ, 3 cm x 4.6 mm i.d.); D) Column C and B in series with a Brownlee Amino Guard column; and E) Two Preparative Alltech-NH_2 columns (10μ, 25 cm x 10.0 mm i.d.) in series, with a guard column of Analytichem-NH_2 (40μ, 3 cm x 4.6 mm i.d.). The mobile phase was 84:16 acetonitrile:sterile water at a flow rate of 5.0 ml/min. The inlet frit of the guard column was 10μ.

Synthesis of ^{18}F-2FDG. ^{18}F-2FDG was synthesized, with subtle modifications, according to the literature procedures (9-10). Fluoride-^{18}F was produced from the ^{18}O(p,n)^{18}F reaction on oxygen-18 enriched water (11-13). Details of the small volume target are to be published at a later date. To the ^{18}F activity in 400 μl of water, 12 μl (20 μmols) of a 25% tetraethylammonium hydroxide solution and 3 mg (20 μmols) of tetraethylammonium fluoride were added followed by 2 ml of anhydrous acetonitrile. The mixture was vortex mixed for 15 seconds, cooled and evaporated under vacuum. Anhydrous acetonitrile (2 ml) was added to the activity, chilled in liquid nitrogen and evaporated under

vacuum. The last step was repeated twice -with caution- to avoid moisture. To the dried vessel containing the ^{18}F activity, a solution of 8 mg (23 μmols) of methyl-4,6-O-benzylidine-2,3-O-cyclic sulfato-β-D mannopyranoside in 3 ml anhydrous acetonitrile was added. The mixture was vortex mixed for 15 seconds then heated at 65°C for 20 minutes. Thin-layer chromatographic analysis was used to assess the yield (Table II) at this time. The mixture was evaporated under vacuum. To the dry residue, 2 ml of dry methylene chloride was added followed by 0.5 ml of 1M solution of boron tris(trifluoroacetate). The mixture was immediately stirred for 5 minutes then quickly frozen in liquid nitrogen. Following the evaporation of solvents under vacuum with intermittent application of very mild heat (40°C), 1 ml of water and 0.2 ml of 5N NaOH solution were added. The mixture was vortex mixed for 15 seconds and the pH was adjusted to ∿10. The mixture was transferred onto a chromatographic column (1 cm i.d.) containing a 6 cm bed of silica gel (60-200 mesh) topped with a 1.5 cm layer of neutral alumina (80-200 mesh). The reaction flask was washed with 1 ml of water, vortex mixed for 15 seconds, and transferred to the column. The column was next eluted with 2x4 ml aliquots of 90% aqueous acetonitrile. The slowly flowing eluent was collected, chilled, and concentrated under vacuum to 300 μl. This was injected onto the preparative HPLC column. The ^{18}F-2FDG fraction was collected and the solvent was evaporated to approximately 500 μl. The product was reconstituted to yield an isotonic solution and sterilized by micropore filtration.

Table II

Conditions of Thin-Layer Chromatographic Assessment of Fluorine-18 Incorporation*

	R_f	
Solvent System	Fluorinated Sulfate Products	Fluoride Anion
Ethylacetate/Ethanol (80/20)	0.51 and 0.58	0.0
Ethylacetate/Acetone (50/50)	0.50 and 0.55	0.0

*All chromatographic plates were Analtech Silica Gel GHLF (250 micron)

Purification of 2-FDG. Following partial purification on the silica-alumina column, a sample was removed to determine the amount of ^{18}F-2FDG by HPLC analysis, using system A or B in Table I. The amount of ^{18}F-2FDG ranged from 50-60%. Meanwhile, the solution was concentrated to 300 µl and loaded into the injector on the preparative system E. The flask was washed with 50 µl of water and 100 µl of 84:16 acetonitrile:water. The total amount of water should be below 400 µl. The material was injected with a flow rate of 5.0 ml/min. Several radioactive peaks were eluted between 6 and 10 minutes. When the ^{18}F-2FDG began to elute ($\sim$ 13 minutes), the flow rate was raised to 5.5 ml/min and the solvent collected for approximately 4 minutes. A sample (50 µl) was taken to determine the radiochemical purity of the product. After concentration of the eluate to 500 µl an aliquot (5-8 µl) was used for 2-FDG mass analysis.

During development of the procedure, samples were checked for radiochemical purity of ^{18}F-2FDG after elution from the preparative HPLC, after concentration, and final pharmaceutical preparation. No decomposition of ^{18}F-2FDG was detected during any of these steps.

RESULTS AND DISCUSSION

Several modifications were made to the published procedure (9-10) for the preparation of ^{18}F-2FDG by the reaction of [^{18}F]fluoride anion with methyl-4,6-O-benzylidene-2,3-O-cyclic sulfato- β -D-mannopyranoside. A fourfold reduction of 1M boron tris(trifluoroacetate) in trifluoroacetic acid proved sufficient for complete hydrolysis as well as lowering the amount of inorganic salts to be removed. Dilutions of the reagent were made with methylene chloride.

Purification of the product using ion retardation resins and flash chromatography on silica gel were of limited success in providing a radiochemically and chemically pure product. However the partial purification achieved on silica gel, resulted in the inclusion of a short column into the final purification process. Dual preparative HPLC columns (2x25cm) were utilized for final purification. The advantages over a single column include the ability of the system to handle larger injection volumes ($\sim$ 500 µl) without significantly

compromising the resolving capacity; and as chemically bonded primary amine columns undergo gradual irreversible degradation (14), only one column at a time has to be replaced.

It was observed that the final activity of ^{18}F-2FDG from the preparative HPLC system was consistently less than that calculated from the analytical sample (Table I). Since there appeared to be negligible decomposition of ^{18}F-2FDG between the various steps during partial purification, an examination of the solution was performed with two analytical HPLC column in series (Table I, System D). This analysis (Figure 1A) indicated the presence of two radioactive peaks in the (2-FDG) region, one at 12.35 minutes corresponding to 2-FDG and a second partially resolved peak at 13.43 minutes. The ratio of the activity in the two peaks was ∿4:1.

After elution of the ^{18}F-2FDG from the preparative columns, the remainder of the injected activity retained on the tandem columns was analyzed. Most of the retained activity could be eluted with water and was examined by HPLC (System D). As shown in Figure 1B there were two peaks, one with a retention time identical with 2-FDG and another at 13.45 minutes. Analysis using column C of a mixture of pure 2-^{18}FDG and the activity washed from the first preparative column is shown in Figure 1C. When the unidentified peak was less than 20%, no

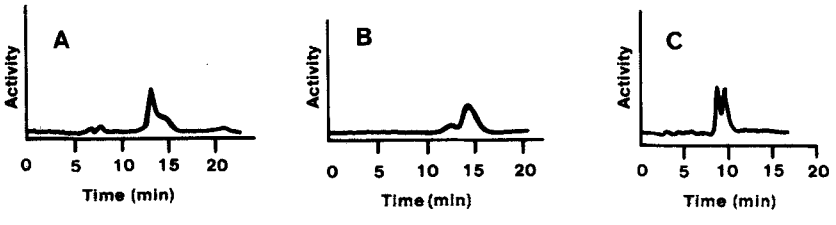

Figure 1. (A) Analysis of partially purified ^{18}F-2FDG on column system D. (B) Analysis of radioactivity removed from preparative system with water wash using column system D. (C) A 1:1 mixture of pure ^{18}F-2FDG and wash from the preparative column using column system C; flow rate 1.8 ml/min.

resolution was observed. Systems A and B, which had lower selectivity, were incapable of resolving the peaks in any ratio.

The radiochemical purity of the 2-^{18}FDG was routinely examined using either system B or C. The product purity was 98.3 ± 1.5% (n = 31). Examination using system D also showed no impurity. A mass determination was performed using system C and compared to the calculated amount of 2-FDG that should be formed based on added F^-. The calculated mass (655 μg) and the amount determined by HPLC agreed within ± 11%.

The finished radiopharmaceutical was also analyzed using gas liquid chromatography. The activity associated with 2-FDG was the same as determined by HPLC. Analysis by GC of the impurity retained on the second HPLC preparative column indicated no 2-FDG. This investigation clearly showed that by following routine analysis using HPLC columns specifically developed for carbohydrate analysis, one and possibly two impurity peaks remain unresolved from 2-FDG. We surmize that one of these impurities might be a fluorinated altrose (15). Possibly, the reaction of fluoride with the cyclic sulfate may not be completely regio specific (9-10). Using two preparative columns in series (16) for purification results in the complete separation of all impurities from 2-FDG. The effects of temperature, time, and solvent for optimum yield at various steps are presently being studied as well as attempts to identify the impurity peaks.

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