# Quality Control Procedures In The Preparation Of 2-Deoxy-D-[1-$^{11}$C]Glucose Radiopharmaceutical

Manhar M. Vora*, Thomas E. Boothe, Ronald D. Finn, Patricia M. Smith and Albert J. Gilson

Baumritter Institute of Nuclear Medicine, Mount Sinai Medical Center, Miami Beach, Florida 33140 U.S.A.

## SUMMARY

Preparation and quality control of 2-deoxy-D-[1-$^{11}$C]glucose ($^{11}$C-2DG, 1) have been accomplished by a combination of cation-exchange and high performance liquid chromatography (HPLC) to give the radiopharmaceutical in greater than 99% radiochemical purity and an overall 25-35% radiochemical yield in 45-50 min. Quality control procedures utilizing GC and HPLC have been developed to determine not only chemical and radiochemical purity of the final product, but also to examine the reactants used and the products formed during the preparation of 1.

Key words: 2-deoxy-D-[1-$^{11}$C]glucose, quality control, positron emission tomography(PET), 18-crown-6, preparative HPLC.

## INTRODUCTION

Based upon several investigations with 2-[$^{18}$F]-fluoro-2-deoxy-D-glucose (1-10) and 2-deoxy-D-[1-$^{14}$C]glucose(11-15), carbon-11 labeled 2-deoxy-D-glucose(16-20) has been proposed as a viable analog for regional cerebral glucose metabolism studies using positron emission tomography (PET). Detailed procedures for rapid synthesis of the 2-deoxy-D-[1-$^{11}$C]glucose radiopharmaceutical were first reported by MacGregor et al(19) and subsequently by Padgett et al(20).

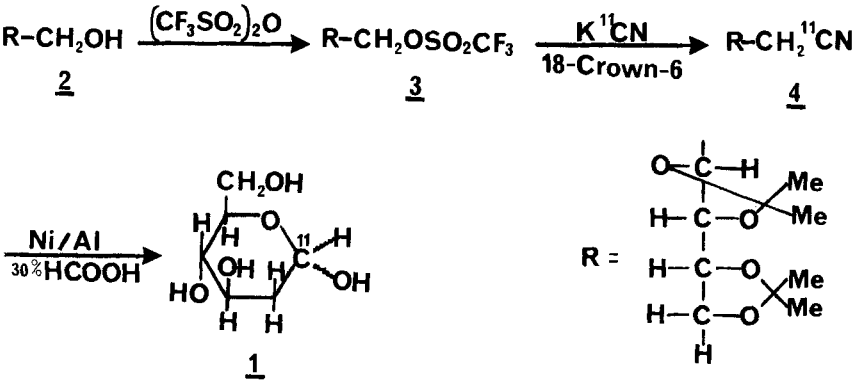
In this paper we report quality control procedures during preparation of the $^{11}$C-2DG radiopharmaceutical and also a modified synthesis with improved yield in conversion of the triflate 3 to the $^{11}$C-nitrile 4. Although our

* Author to whom correspondence should be addressed.

0362-4803/83/030417-11$01.10

Received May 12, 1982
Revised October 29, 1982

synthetic scheme is based upon the original approach(19), we have developed a




procedure in which a more efficient conversion of 3 to 4 is achieved by using 18-crown-6* (1,4,7,10,13,16-hexaoxacyclooctadecane) as a catalyst.

In addition, we have modified the purification procedure by employing preparative and analytical HPLC to obtain consistent and accurate quality control of the $^{11}$C-2DG radiopharmaceutical.

## METHODS AND MATERIALS

All solvents were high purity "distilled in glass" grade from Burdick and Jackson, USA and were degassed ultrasonically before use. Formic acid (96%), 2-deoxy-D-glucose, 18-crown-6 and trifluoromethanesulfonic anhydride were purchased from Aldrich Chemical Company, U.S.A. Nickel/ Aluminum catalyst and D-arabinitol were obtained from Alfa Products, Thiokol/Ventron U.S.A. The ion-exchange resins, AG50W-X8 ($H^+$ form, 200-400 mesh) and AG1-X8 ($Cl^-$ form, 200-400 mesh), were acquired from Bio-Rad, U.S.A. 2,3:4,5-di-O-isopropylidene-D-arabinitol (2) was prepared according to literature procedures(18,19,21). H$^{11}$CN was prepared by a proton reaction on a $N_2/H_2$ gas mixture(22). Gas chromatographic(GC) analyses were performed on a Hewlett-Packard model 5710A gas chromatograph. HPLC was carried out using an Altex model 330 liquid chromatograph equipped with an Altex model 155-40 variable

* 18-crown-6 is toxic and should be handled with care (ref. 26).

wavelength UV detector operated at 195 nm. Eluate from the HPLC was continuously monitored for radioactivity using an on-line analysis system consisting of a Nuclear Data model 60A multichannel analyzer equipped with a NaI detector and operated in the multichannel scaling(MCS) mode. Also, the eluate was collected at 0.5 min intervals for radioassay using a NaI well-counter.

Synthesis of 2,3:4,5-Di-O-isopropylidene-1-O-trifluoromethanesulfonyl-D-arabinitol (3)

Compound 3 was synthesized as described in the literature(18,19). Progress on the formation of 3 was assessed by GC analysis at 0.5 hr intervals using a 6 ft. x 0.083 in. i.d. column of 10% SE-30 on 70-80 mesh Anakrom at 170°C with a helium flow rate of 30 ml/min. The retention times of 2 and 3 were 4.2 and 5.4 min respectively. Within the first half an hour the yield of 3 was in the range of 30-40%; however, within two hours,3 could be obtained in greater than 90% yield. Approximately 30 min prior to the delivery of K$^{11}$CN, the reaction mixture was filtered through a 2 x 0.5 cm column of anhydrous sodium sulfate and the solvent removed under reduced pressure. The flask containing 3 (approximately 35-40 μmoles) was left under vacuum until just prior to the delivery of K$^{11}$CN at which time it was dissolved in 500 μl of acetonitrile containing 5 mg (19 μmoles) of 18-crown-6 and 0.325 mg (5.0 μmoles) of carrier KCN ("triflate/KCN/18-crown-6" solution).

Synthesis of 2-Deoxy-D-[1-$^{11}$C]glucose ($^{11}$C-2DG)

"No carrier added "H$^{11}$CN (100-150 mCi) was collected in a vessel ( ∿2 ml) containing 0.325 mg (5 μmoles) of KOH in 100 μl of 95% ethanol, and then the solvent was removed under vacuum. To the residue was added the "triflate/KCN/18-crown-6" solution in acetonitrile and the mixture stirred at room temperature for 5 min. After evaporation to dryness under reduced pressure, 1 ml of 30% aqueous formic acid and 150 mg of Raney alloy (Ni/Al catalyst) were added, and the stoppered vessel was heated at 100°C for 10 min. After cooling, the mixture was passed through a column containing 0.5 x 0.7 cm section of celite above a 1.5 x 0.7 cm section of AG50W-X8 ($H^+$ form) resin.

The column was washed with ~2.5 ml of 80% aqueous acetonitrile and the combined filtrate was collected in a flask containing 4-5g of AG1-X8 ($HCO^-_3$ form) resin to neutralize the formic acid. After a brief stirring, the resin was removed by filtration; the filtrate concentrated to 0.5ml under reduced pressure (bath temperature ~40-45°C), and injected on the preparative HPLC column(Table 1). The fraction containing $^{11}$C-2DG as indicated by the radiochromatogram (Figure 1) was collected and concentrated to approximately 1 ml under reduced pressure. Using sterile conditions, the final solution was rendered isotonic in a final volume of 3 ml and sterilized by passage through a 0.22 μm millipore filter.

Analysis and Quality Control

Analytical HPLC (columns 2 and 3 in Table 1) analyses were performed at various stages as indicated in Table 2, in order to evaluate the effectiveness of the synthesis and the purification procedures. The corresponding radiochromatograms are shown in Figures 2 and 3.

The $^{11}$C-2DG radiopharmaceutical was analyzed for its chemical and radiochemical purity as described below. Possible chemical entities present include, arabinitol, carrier 2-DG, 18-crown-6 and acetonitrile. Arabinitol, 2DG and 18-crown-6 were analyzed by HPLC under the conditions listed in Table 1. In addition, arabinitol and 2DG were further analyzed as trimethylsilyl derivatives(23) on a 3 ft x 0.083 in i.d. column of 3% OV-17 on 70-80 mesh Anakrom at 180°C and flow rate of 30 ml/min (retention time for arabinitol was 2.5 min and that of the anomers of 2DG was 4.3 and 5.5 min). Acetonitrile was analyzed by GC analysis on a 6 ft. x 0.083 in i.d. column of 50-80 mesh Porapak Q at 140°C with helium flow rate of 30 ml/min. Finally, the radiochemical purity of the radiopharmaceutical was determined by HPLC (Figure 4), and the apyrogenicity was confirmed by Limulus Lysate Test(24,25).

## RESULTS AND DISCUSSION

The catalytic utility of crown ethers in nucleophilic displacement

TABLE 1. CONDITIONS FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATIONS

| | Column | Flow Rate(ml/min)[a] | Retention Time (min) | | |
|---|---|---|---|---|---|
| | | | 2-DG | Arabinitol | 18-Crown-6 |
| 1. | Whatman Partisil 10 PAC M9/50, 50 cm x 9.4 mm i.d. | 5.4 | 9 | 12 | 13.5 |
| 2. | Alltech Partisil 10 PAC 25 cm x 4.4 mm i.d. | 1.6 | 4.8 | 5.2 | 7.2 |
| 3. | Alltech 600 CH Carbohydrate 30 cm x 4.1 mm i.d. | 1.8 | 5.5 | 6.9 | - |

a) The solvent in each case is $CH_3CN/H_2O$ (80/20)

TABLE 2. $^{11}$C-2DG DISTRIBUTION AT VARIOUS STEPS

| Step | Total Activity(mCi)[a] | % Activity as $^{11}$C-2DG | % of Initial Activity as $^{11}$C-2DG |
|---|---|---|---|
| 1. $K^{11}CN$ | 100 | - | - |
| 2. Preparation of $^{11}$C-Nitrile (4) | 93 ± 5 | 75 ± 5[b] | - |
| 3. After Reduction | 89 ± 5 | 35 ± 5 | 31 ± 6 |
| 4. After Partial Purification With AG50W-X8 ($H^+$) | 52 ± 9 | 55 ± 5 | 29 ± 8 |
| 5. After neutralization with AG1-X8($HCO_3^-$) and concentration | 50 ± 9 | 55 ± 5 | 28 ± 7 |

a) Corrected to Initial Activity in $K^{11}CN$

b) $^{11}$C-Nitrile (4)

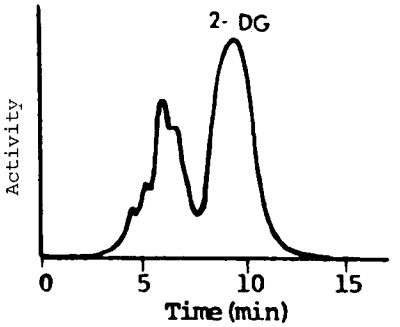



Figure 1. Preparative HPLC profile of product mixture after partial purification with AG50W-X8($H^+$) resin

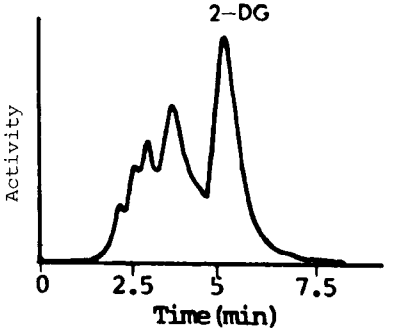



Figure 2. Analytical HPLC profile of product mixture before purification

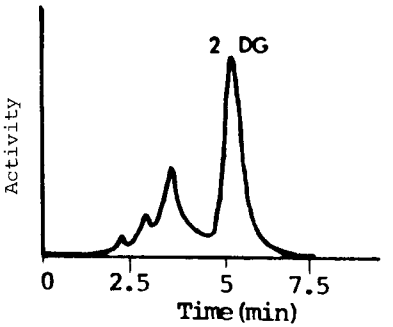



Figure 3. Analytical HPLC profile after partial purification with AG50W-X8($H^+$) resin

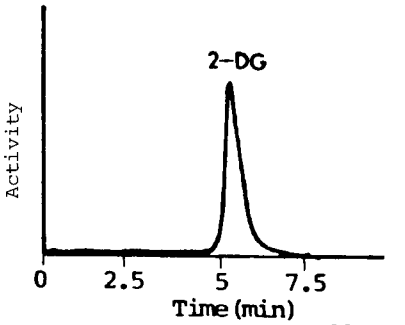



Figure 4. Quality control of $^{11}$C-2DG radiopharmaceutical

reactions is well documented in the literature(26-32). 18-Crown-6 effectively solubilizes potassium salts in relatively nonpolar solvents to generate in solution 'naked' counter anions possessing increased nucleophilicity and basicity(28-30). Use of this catalyst during conversion of the triflate 3 to the $^{11}$C-nitrile 4 in acetonitrile provided an almost quantitative as well as rapid incorporation of the initial $^{11}CN^-$ activity in this step. TLC analysis indicated 70-85% of this activity in the labelled nitrile 4, compared to 50-60% from reaction in DMF(19) in absence of crown ether. However, due to poorer yield of 35-45% compared to 60-70% in the reduction step(19), the over-all yield of $^{11}$C-2DG ranged from 25-35%. Nevertheless, with this modification, starting with 100-150 mCi of $H^{11}CN$, we have obtained within 45-50 min 5-10 mCi of greater than 99% pure $^{11}$C-2DG radiopharmaceutical having specific activity of 20-50 mCi/mg.

In order to obtain precise quality assurance of the $^{11}$C-2DG radiopharmaceutical, purification was accomplished using HPLC equipped with variable wavelength UV detector and an on-line sodium iodide detector in connection with a multichannel analyzer operated in MCS mode. With this arrangement, elution of $^{11}$C-2DG and its radioactive contaminants could be monitored continuously and accurately. While all the quality control and other analyses were performed on the analytical columns, purification of $^{11}$C-2DG was achieved through the use of a preparative column (Table 1).

Since a large number of experimental variables could influence yield as well as purity of the $^{11}$C-2DG radiopharmaceutical, it was deemed beneficial to examine the radiolabeled side-products formed during various stages of synthesis and purification procedures (Table 2). Figures 2 and 3 show the activity profiles as a function of time from which the percent distribution of $^{11}$C-2DG and the radioactive impurities at various stages of preparation can be determined. First step in the purification involving cation exchange column removed ~35% of the activity which is assumed to be the $^{11}$C-amine formed as a major side product during reduction (19). This is well reflected when one compares Figures 1 and 2. Composition of the product mixture remained

unchanged after neutralization with the anion-exchange (bicarbonate form) resin as well as the subsequent volume reduction before HPLC separation.

Prep HPLC allows an adequate separation of the $^{11}$C-2DG from other radiochemical impurities as is evident in Figure 1. In addition, we found that no radioactivity was retained on the column. Radiochemical purity of $^{11}$C-2DG radiopharmaceutical collected from the prep column averaged 99-100%; in the majority of the cases no detectable radiochemical impurity was observed (Figure 4). Chemical analyses using HPLC and GC as described in the materials and methods section revealed the following amounts of chemical entities present in the final solution: 0.0-0.2 mg of arabinitol; 0.2-0.25 mg of carrier 2-DG; 0.0-0.06 mg of 18-crown-6 and no detectable amount of acetonitrile.

During our experience with this procedure, we found that it was necessary to neutralize formic acid before the HPLC separation. Failure to do so could result in decomposition of the $^{11}$C-2DG during concentration(19) prior to the HPLC separation and also inadequate separation of $^{11}$C-2DG from arabinitol and 18-crown-6. In addition, the HPLC column life-time is shortened considerably with successive use. The use of anion-exchange resin before the HPLC separation effectively neutralizes the formic acid, providing adequate separation of $^{11}$C-2DG from impurities and prolonging the column-life.

In summary, the use of crown ether considerably increased the yield of one of the key intermediates in the synthesis of $^{11}$C-2DG. Use of preparative and analytical HPLC equipped with an on-line radioactivity detector and the multichannel analyzer to continuously monitor the column effluent provides accurate and predictable quality control.

## ACKNOWLEDGEMENTS

This research was supported by PHS Grant NINCDS 1 NS 15639. The authors express their appreciation to Ms. Ana Abreu and Ms. Jan Holle for aiding in manuscript preparation.

REFERENCES

1. Reivich M. - Neurosci. Res. Program Bull. 14: 502 (1976).

2. Reivich M., Kuhl D., Wolf A., Greenberg J., Phelps M., Ido T., Casella V., Fowler J., Gallagher B., Hoffman E., Alavi A. and Sokoloff L. - Acta Neurol. Scand. 56(Suppl. 64): 190 (1977).

3. Reivich M., Kuhl D., Wolf A., Greenberg J., Phelps M., Ido T., Casella V., Fowler J., Hoffman F., Alavi A., Som P. and Sokoloff L. - Circ. Res. 44: 127 (1979).

4. Phelps M.E., Huang S.-C., Hoffman E.J., Selin C., Sokoloff L. and Kuhl D.E. - Ann. Neurol. 6: 371 (1979).

5. Huang S.-C., Phelps M.E., Hoffman E.J., Sideris K., Selin C.J. and Kuhl D.E. - Am. J. Physiol. 238(Endocrinol. Metabolism 1): E69 (1980).

6. Brownell G.L., Ackerman R.H., Strauss H.W., Elmaleh D.R., Cochavi S., Alpert N., Correia J.A., Kearfott K. and Taveras J. - J. Comput. Assist. Tomogr. 4: 473 (1980).

7. Alavi A., Reivich M., Greenberg J., Hand P., Rosenquist A., Rintelmann W., Christman D., Fowler J., Goldman A., MacGregor R. and Wolf A. - Seminars in Nuclear Medicine 11: 24 (1981).

8. Greenberg J.H., Reivich M., Alavi A., Hand P., Rosenquist A., Rintelmann W., Stein A., Tusa R., Dann R., Christman D., Fowler J., MacGregor B. and Wolf A. - Science 212: 678 (1981).

9. Reivich M., Alari A., Greenberg J., Farkas F. and Wolf A.- Progress in Nuclear Medicine 7, Juge, O. and Donith, A., eds., S. Karger, Basil, Switzerland 1981, p. 138.

10. Bushsbaum M.S., Ingvar D.H., Kessler R., Waters R..N., Cappelletti J., van Kammen D.P., King A.C., Johnson J.L., Manning R.G., Flynn R.W., Mann L.S., Bunney Jr. W.E. and Sokoloff L.- Arch Gen Psychiatry 39: 251 (1982).

11. Bachelard H.S. - J. Neurochem. 18: 213 (1971).

12. Kennedy C., DesRosiers M., Reivich M., Sharpe F., Jehle J. and Sokoloff L. - Science 187: 850 (1975).

13. Sokoloff L., Reivich M., Kennedy C., Des Rosiers M., Patlak C. Pettigrew

K., Sakurada O. and Shinohara M. - J. Neurochem. 28: 897 (1977).

14. Sokoloff L. - Brain 102: 653 (1979).
15. Ginsberg M., Busto R., Boothe T. and Campbell J. - Brain Research 230: 165 (1981).
16. Fowler J.S., Lade R.E., MacGregor R.R., Shiue C.-Y., Wan C.-N. and Wolf A.P. - J. Labelled Compd. Radiopharm. 16: 7 (1979).
17. Mestelan G., Aubert F., Beaucourt J.-P., Comar D. and Pichat L. - J. Labelled Compd. Radiopharm. 16: 661 (1979).
18. Shiue C.-Y., MacGregor R.R., Lade R.E., Wan C.-N. and Wolf A.P. - Carbohydr. Res. 74: 323 (1979).
19. MacGregor R.R., Fowler J.S., Wolf A.P., Shiue C.-Y., Lade R.E. and Wan C.-N. - J. Nucl. Med. 22: 800 (1981).
20. Padgett H.C., Barrio J.R., MacDonald N.S. and Phelps M.E. - J. Nucl. Med. 23: 739 (1982).
21. Zinner H. and Kristen H., Chem. Ber. 97: 1654 (1964).
22. Christman D.R., Finn R.D., Karlstrom K.I. and Wolf A.P. - Int. J. Appl. Radiat. Isotopes 26: 435 (1975).
23. Sweeley C.C., Bentley R., Makita M. and Wells W.W. - J. Amer. Chem. Soc. 85: 2497 (1963).
24. The United States Pharmacopeia, 20th Revision, The National Formulary, 15th ed, United States Pharmacopeial Convention, Inc. Rockville, Md.,1980, p.888.
25. Pearson F.C. - Amer. J. Med. Tech. 45: 704 (1979).
26. Pederson C.J.- J. Amer. Chem. Soc. 89: 7017 (1967).
27. Liotta C.L. and Harris H.P.- J. Amer. Chem. Soc. 96: 2250 (1974).
28. Sam D.J. and Simmons H.E.- J. Amer. Chem. Soc. 96: 2252 (1974).
29. Cook F.L., Bowers C.W. and Liotta C.L. - J. Org. Chem. 39: 3416 (1974).
30. Ido T., Irie T. and Kashida Y. - J. Label. Comp. Radiopharm. 16: 153 (1979).
31. Mizruno A., Hamada Y. and Shioiri T.- Synthesis: 1007 (1980).
32. Gnade B.E., Schwaiger G.P., Liotta C.L. and Fink R.W. - Int. J. Appl. Radiat. Isotopes 32: 91 (1981).