18F-FDG SYNTHESIS AND QUALITY CONTROL AND COST EFFECTIVENESS IN NUCLEAR MEDICINE CENTER IN KHMC*

Pharm. Ala' A. Khwaj/ NM unit KHMC, Pharm. Faisal M. Al Obied/ NM unit KHMC
Pharm. Samer M. Ofeshat/ DRMS, Pharm. Amjad A. Yazjeen /Farah royal rehab. Center
Pharm. Qusai M. Migdadi / DRMS, Physicist. Noorhan O. Al Mahasees/ NM unit KHMC
* King Hussien Medical Center

ABSTRACT

The most widely used radiopharmaceutical in the expanding medical imaging technology of Positron Emission Tomography (PET) is 2-[18F]fluoro-2-deoxy-D-glucose (18FDG). The increasing demand for 18FDG requires reliable production in large amounts

In this article we will covers the fludeoxyglucose (18F-FDG) synthesis and quality control procedures and the cost effectiveness in KHMC with emphasis on practical synthesis currently; 18F-FDG is the most successful PET radiopharmaceutical so far.

It's started in Royal Medical Services in 2003 the advancement in synthesis and quality control of 18F-FDG, together with its approval by the US FDA and the availability of reimbursement, are probably the main reasons for the florin of clinical PET over the last 20 years. 18F-FDG can be synthesized by either electrophonic fluorination or nucleophilic fluorination reaction. Nucleophilic fluorination using mannose triflate as precursor and Kryptofix or tetrabutylammonium salts (TBA) is widely used because of higher yield and shorter reaction time.

The quality control requirements of 18F-FDG can be found in United States Pharmacopeia (USP), British Pharmacopeia (BP), European Pharmacopeia (EP) and the Chemistry, Manufacturing, and Controls (CMC) section from United States Food and Drug Administration (US FDA) PET draft guidance documents. Basic requirements include radionuclide identity, radiochemical purity, chemical purity, pH, residual solvent, sterility, and bacterial endotoxin level. Some of these tests (sterility, endotoxins and radionuclide purity) can be accomplished after the 18F-FDG has been released..

INTRODUCTION

The radioisotopes delivery system RDS eclipse in KHMC provides a low-cost automated efficient and easy to operate system for the production of PET/CT isotopes (F-18, C-11, N-13 and O-15) and radiochemical.

In cyclotron unit, the hydrogen gas is ionized under high voltage. These ions are changing direction in magnetic field and accelerated and strikes carbon foil. The ions which stroke the carbon foils loses their electrons and hence changes in to protons then those protons behaves differently in magnetic field and strikes the target . There are two beam lines, The O-18 loaded to the target before operation is changed in to F-18 through

(P, n) reaction after the end of bombardment.

O-18(p, n) F-18 means:

Stable nuclei can be bombarded with nucleolus (neutrons or protons) this process is referred to as a nuclear reaction.

The nomenclature for nuclear reaction is target nucleus (bombarding particle recoiling particles product nucleus. O-18 bombarded by protons results in F-18 and a neutron.

18F-FDG is a glucose analogue in which the hydroxyl group on the 2-carbon of a glucose molecule is replaced by a fluoride atom. Like glucose, 18F-FDG is taken up into living cells by facilitated transport and then phosphorylated by hexokinase. Unlike glucose, 18F-FDG cannot undergo further metabolism because the hydroxyl group at the 2-carbon is a requirement for the process. Nevertheless, 18F-FDG is a good indicator of

Glucose uptake and cell viability. The uptake of glucose analogues into living cells

Also depends on modifications of various carbons at different positions.

It has been shown that the specificity of 3-deoxyglucose (3-DG) and 4 deoxyglucose (4-DG) towards hexokinase reduced by 100-fold, hence3-DG and 4-DG were not retained inside the cells. Similarly, 3-fluoro-deoxyglucose and 4-fluorodeoxyglucose do not accumulate in living cells as much

As 18F-FDG. Although the nucleophilic substitution reaction is more widely used nowadays, the electrophilic fluorination reaction has an important place in the

Synthesis of 18F-FDG.

SYNTHESIS OF 18F-FDG BY NUCLEOPHILIC FLUORINATION

In our site we use Nucleophilic fluorination to produce FDG Many attempts have been made to develop a nucleophilic substitution for the synthesis of 18F-FDG. This included the use of 18F-CsF, 18F-Et4NF, and 18F-KHF.in 1986 they have used K222 as catalyst. The reaction had a consistent yield

of over 50% and the reaction time was shortened to 50 min. Nucleophilic substitution is a chemical reaction involving the addition of a nucleophilic molecule (highly negatively charged molecule) into a molecule with a leaving group (electron drawing group attached to the parent molecule through an unstable chemical bond).

Figure (1) is a general scheme for SN2 nucleophilic substitution reaction .The nucleophilic molecule has a high affinity towards the relatively electron deficient

Centre in the parent molecule created by the electron pulling leaving group. As a result, the nucleophilic molecule forms a covalent bond with the parent molecule

And displaces the leaving group. The stereo-configuration of the parent molecule is also changed.

In the synthesis of 18F-FDG, 18F ion is the nucleophile. The precursor is mannose triflate in which the 1,3,4,6 position carbons of a mannose molecule are protected with an acetyl group and triflate is the leaving group at the 2-carbon. In the presence of Kryptofix 222TM as catalyst and acetonitrile as solvent, 18F ion approaches the mannose triflate at the 2-carbon, while the triflate group leaves the protected mannose molecule to form 18F-FDG (figure 2). Although synthesis of 18F-FDG can be carried out in different computer controlled automatic synthesizers, the nucleophilic process proceeds in roughly same stages:

Removal of 18F from the 18O- water coming out from the Cyclotron target

Fluorine has a high hydration energy, so water is not a suitable solvent in this synthesis. Polar aprotic solvent such as acetonitile should be used in an SN2 nucleophilic substitution reaction. Since 18F- is produced by a O18 (p, n) 18F- reaction, it is necessary to isolate the 18F ion from its aqueous environment. The most convenient way to isolate is to use a light QMA (Quaternary ammonium anion exchange) Sep-Pak column (Accell plus QMA Sep-PakTM). The 18F- is retained by or via an ion-exchange reaction and allowed the 18O-water to flow through. The retained 18F- is then eluted with an acetonitrile solution of Kryptofix and potassium carbonate (figure 4).

In an aqueous environment, any negatively charged ions must be accompanied by positively charged counterparts. Usually, the 18F– washed out from the cyclotron target is accompanied by traces of metal ions from the surface of the target body. When passing through the light QMA anion exchange ion, the 18F – is retained and the metal ions will be lost in the 18O- water. Hence, it is necessary to introduce a positively charged counter ion to restore the 18F– reactivity before

Evaporation of residual 18O- enriched water. Several types of positively charged counter ions have been used, including large metal ions such as rubidium or cesium; potassium ion complexes by a large ring structure such as Kryptofix 222TM and tetrabutylammonium salts . Kryptofix 222TM is a cyclic crown ether (Figure 5), which binds the potassium ion, preventing the formation of 18F-KF. Thus, potassium acts as the counter ion of 18F- to enhance its reactivity but Does not interfere with the synthesis.

Since Kryptofix 222TM causes apnoea and convulsion, all automatic synthesis modules have multiple removal steps so that there is only negligible amount of Kryptofix in the final 18F-FDG products.

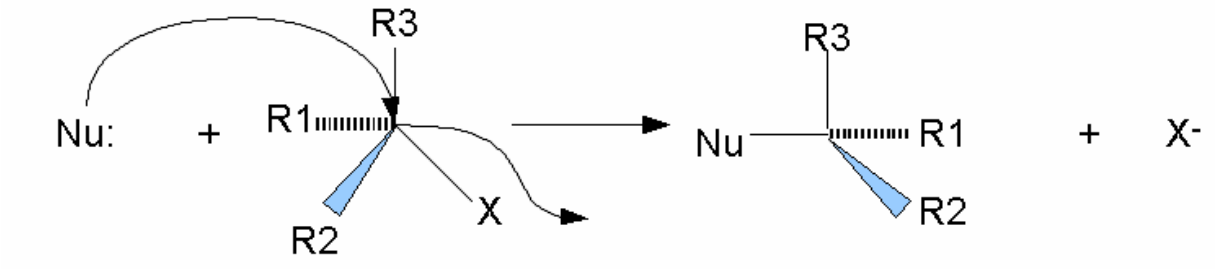


Figure (1) Nucleophilic substitution: Nu = nucleophilic molecule, X = leaving group.

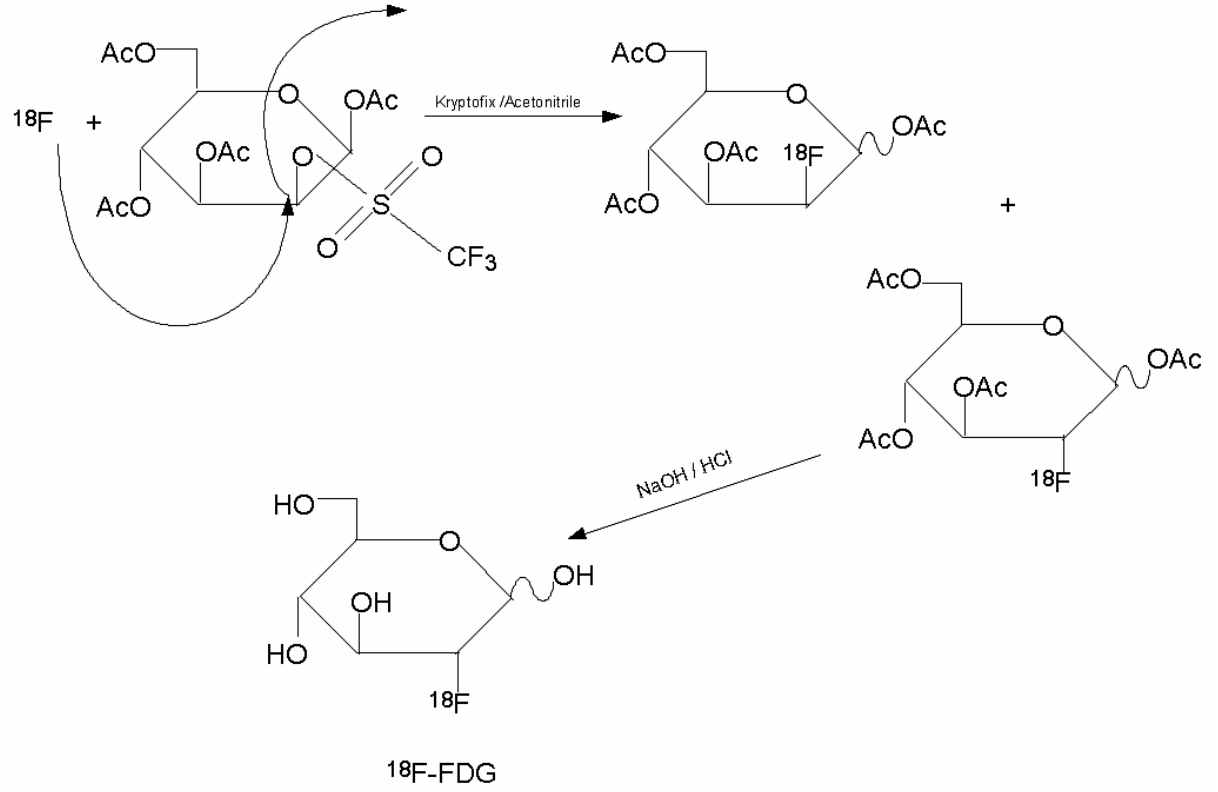


Figure (2) Synthesis of 18F-FDG by nucleophilic substitution.

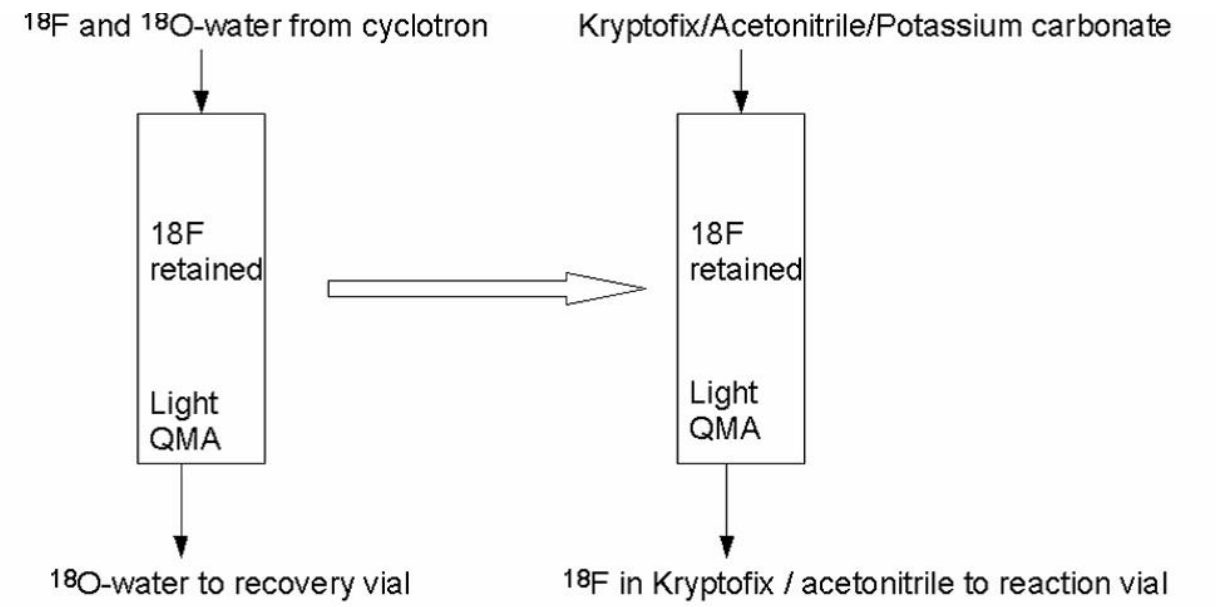


Figure (3) (a) Retention of 18F-FDG in light QMA ion exchange column; (b) elution of 18F from light QMA ion exchange column.

Evaporation of residual 18O- water from the 18F with Acetonitrile

After the 18F- is eluted into reaction vessel, it is necessary to evaporate any residual water from the solution. The advantage of using acetonitrile as the eluting solvent is that it forms an azeotropic mixture with water. Evaporation of the acetonitrile in a nitrogen

Atmosphere will at the same time remove any residual. The 18F. Most of the 18F-FDG automatic synthesizers perform the acetonitrile evaporation step several times to

Ensure all the residual 18O- water is removed. All components of the synthesis system are also rinsed with acetonitrile to remove moisture. Dry nitrogen (moisture content less than 3 pap) should be used in the synthesis

Addition of mannose triflate into the 18F- with Acetonitrile

The nucleophilic substitution takes place in this stage. After the evaporation of any residual water, the precursor is added to the 18F-. The choice of precursor depends on the ease of preparation, ease of producing the final product, consistency, yields, and so on. The most commonly used precursor molecule in synthesis of18F-FDG is 1, 3, 4, 6-O-Acetyl-2-O-trifluoromethanesulfonyl- beta-D-mannopyranose (mannose triflate). Its structure (Figure 5) is similar to that of FDG, except with a triflate group at the 2 carbon position and acetyl groups at 1,3,4,6 position carbons via ester bonds, which can be readily broken at a higher or lower phi The use of acetyl groups is to protect the hydroxy groups so that fluorination would not occur at these positions. The18F ion approaches the mannose triflate at the 2 position carbon, while the triflate group leaves the protected mannose molecule to form 18F-FDG (Figure 4). After the nucleophilic replacement of the triflate group by 18F-, the acetyl groups can be easily removed by hydrolysis to give rise to 18F-FDG the choice of leaving a group is an important consideration. A good leaving group should have the properties of leaving the parent molecule readily. Once it departs from the parent molecule, its negative charge is stabilized by delocalization and it will not re-enter the parent molecule.

Hydrolysis to remove the protective acetyl groups to Form 18F-FDG

The final step of the synthesis is to remove the protective acetyl groups on the 1,3,4,6 position carbons. This can be accomplished by either using hydrochloric acid (acid hydrolysis) or sodium hydroxide (base hydrolysis). Acid hydrolysis requires a longer time and higher temperature. Base hydrolysis, which is more commonly used currently, is faster and takes place at room temperature. One of the improved base hydrolysis

Is to absorb the 1,3,4,6 acetyl protected 18F labeled 2 deoxyglucose on to a C-18 reverse phase column. All other impurities can be removed by rinsing heavily with water. Sodium hydroxide is added to the column so that the base hydrolysis occurs on the column surface. The final 18F-FDG product can be eluted with water while the

unhydrolysed or partially hydrolyzed 1,3,4,6 acetyl protected 18F labeled 2 deoxyglucose remains on the column.

Purification of the final 18F-FDG product

Purification of the final 18F-FDG can be performed with a series of anion exchange column, C-18 reverse phase column and alumina column. Most automatic synthesizers can produce 18F-FDG of over 95% routinely.

FDG production in KHMC

In KHMC we use EXPLORA module to produce FDG by nucleophilic substitution, the reagent and consumbles what we supposed to used according to EXPLORA user manual as shown in table (1)

Table (1) :

			cost
K 222 *	3 ml		6.75 $
Mannose	4ml	100mg/4ml acetonitrile	
Ready column			$

*K222 preparation 600 mg cryptand + 110 mg potassium Carbonte which coast 45 $ for 20 ml solution .

In our site we change the concentration of kryptofix to be as what we use in CPCU module which cost as shown in table 2 , and we used self prepare column instead of ready column .

Kryptofix solution will be K222 250 mg 65 mg potassium in total solution 150ml coast 20 $. in column we use AG11 resin 3g + AG50 1.5 gm +Alumina N sep-pak + C18 sep pak , so in total value we save around 52 $ in each production .

With narrow yield to the user manual value and the result of QC was in the range of EuP standard as Table (2) :

shown follows .

		cost
K 222	3 ml	4 $
column	AG 50	1.05 $
	AG 11	3.6 $

QUALITY CONTROL OF 18F-FDG

The quality requirements of 18F-FDG are set out in various pharmacopoeia including the USP , BP , EP , etc. The US FDA has also published a draft Chemistry, Manufacturing and Controls (CMC) document concerning 18F-FDG . It should be noted that the quality control requirements of 18F-FDG differ among these references. An excellent comparison between them can be found elsewhere . In Asia,

Taiwan has established an official guideline for the compounding of PET drug products, as well as for the quality control of 18F-FDG Different countries my adopt a different set of standards. The BP is described in this article solely because this is the standard adopted by the author's country. Table 1 lists the quality control tests required by BP

.Due to short half-life of 18F-FDG, not all the listed tests can be completed before release of the18F-FDG product. The BP allows the 18F-FDG to be released before the radionuclide purity test, bacterial endotoxin test, and sterility test are finished. There are other tests not listed in the BP, but may be of significance. The BP Both USP and BP do not list the membrane filter integrity test. However, the test is does not list a test for ethanol, which is widely used in the synthesis of 18F-FDG. Essential as an indirect evidence of the 18F-FDG product sterility because the sterility test result will not be available until much later.

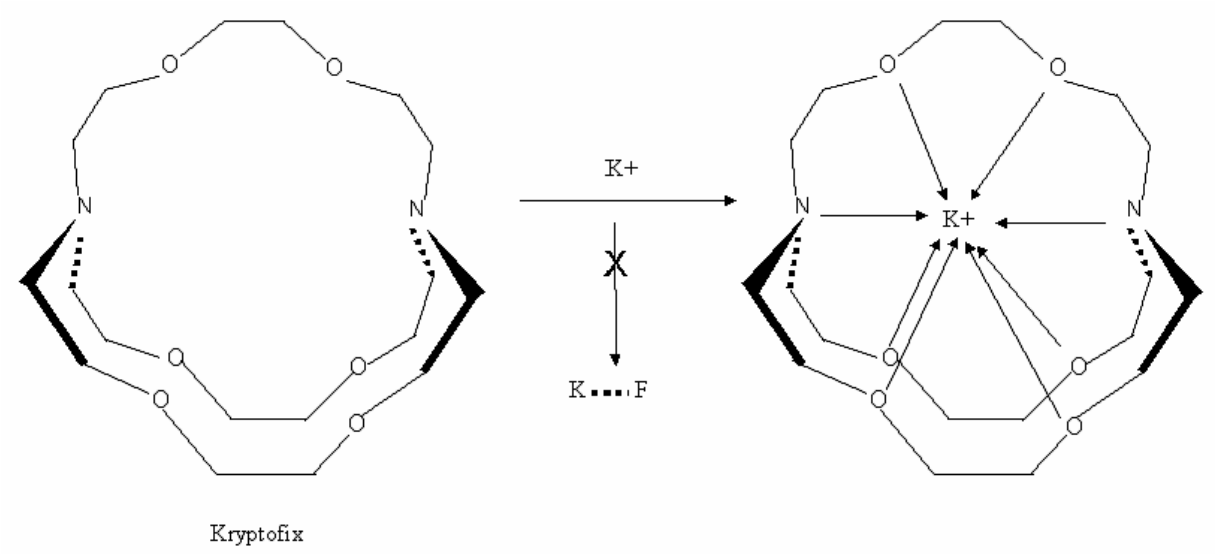


Figure (4) Kryptofix 222 ™ and K+.

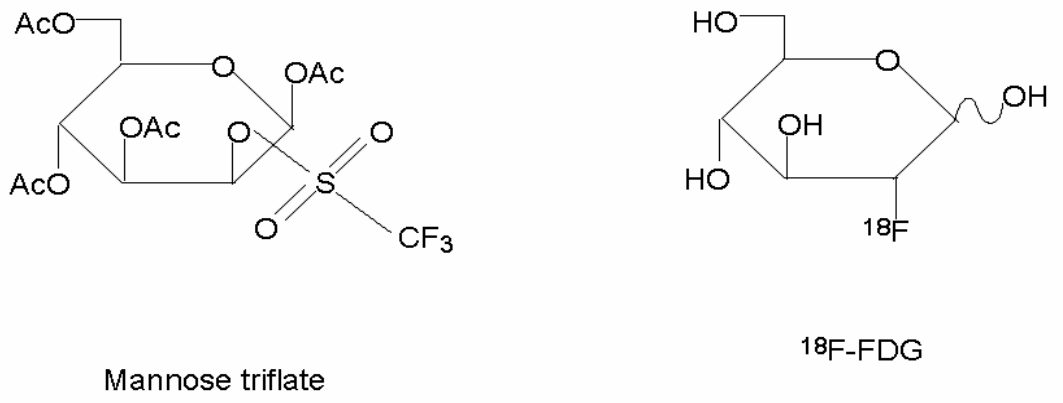


Figure (5) Structures of mannose triflate and 18F-FDG.

-attachment 1 Quality control form result of standard value
-attachment 2 Quality control form result of our modified version of reagent
-attachment 3 Quality control standard form according to EUP

Conclusion(s)

FDG production and hence the quality control procedures used to validate the drug Used to validate the drug are the most important issue of this useful drug release.

In addition this cost effective drug is widely applied in the detection and prognosis of cancer at an early stages which elaborate its value in the treatment of the cancerous tissue , hence improving the quality of life for many patients and reflects the sensitivity and the specify of the evaluation of disease

FDG Quality Form
Royal medical services
King Hussein Medical Center

Test	Method	Standard Result
Physically appearance	**vision**	**Colorless/ clear solution**
Radiochemical Identity (Purity)	**Thin layer chromatography**	**<95%** **RF >0.45**
Chemical Purity	**Gas chromatography GC**	**Acetonitil <0.04%** **Alcohol <0.5%**
pH	**Litmus Strips**	**7.5> pH >4.5**
Bacterial Endotoxin	**Limulus Amebocyte Lysate**	**<0.25 EU/ml usp endotoxin standards**
Sterility	**Typtic soybean broth/Fluid thioglycolate medium**	**Sterile**

***Attachment (1)**

FDG Quality Form
Royal medical services
King Hussein Medical Center

Test	Method	Standard Result	Results
Physically appearance	**vision**	**Colorless/ clear solution**	**Colorless/clear**
Radiochemical Identity (Purity)	**Thin layer chromatography**	**<95% RF >0.45**	
Chemical Purity	**Gas chromatography GC**	**Acetonitil <0.04% Alcohol <0.5%**	
pH	**Litmus Strips**	**7.5> pH >4.5**	
Bacterial Endotoxin	**Limulus Amebocyte Lysate**	**<0.25 EU/ml usp endotoxin standards**	
Sterility	**Typtic soybean broth/Fluid thioglycolate medium**	**Sterile**	

***Attachment (2)**

FDG Quality Form
Royal medical services
King Hussein Medical Center

Date: 9/9/2013 Monday

Production starting time: 8:05 Am

Test	Method	Standard Result	Results
Physically appearance	vision	Colorless/ clear solution	Colorless clear
Radiochemical Identity (Purity)	Thin layer chromatography	<95% RF >0.45	100 %
Chemical Purity	Gas chromatography GC	Acetonitil <0.04% Alcohol <0.5%	Pass
pH	Litmus Strips	7.5> pH >4.5	6.5
Bacterial Endotoxin	Limulus Amebocyte Lysate	<0.25 EU/ml usp endotoxin standards	Pass
Sterility	Typtic soybean broth/Fluid thioglycolate medium	Sterile	sterile

FDG Activity = (1024.0 mci.

Time of delivery= (8:50) am/pm. Production total time = (77)min

Decay corrected yield = (87.2) % .

*This is to certify that our FDG product has the above QC values that comply with international standards .

Analyzed by Verified by

Method: QuickStart File: 130909-0855.R001

Instrument Parameters

Method:	QuickStart	File:	130909-0855.R001
Evaluated:	09 Sep 2013 09:09:10	Created:	09 Sep 2013 09:09:10
Evaluation by:	I\\		
Collimator Type:	Hi Efficiency	Width:	10 mm
Elect. Resol:	Normal	Amp. Range:	50 - 2047
Resolution:	256 chan	Chan Size:	0.909 mm
Hi Voltage:	1502 Volts	Chan of Zero mm:	2.4
Run Time:	3.00 min		
Relative Pos:	0.0 mm		

Comments

ANALYZED BY AMER AL HOURANI

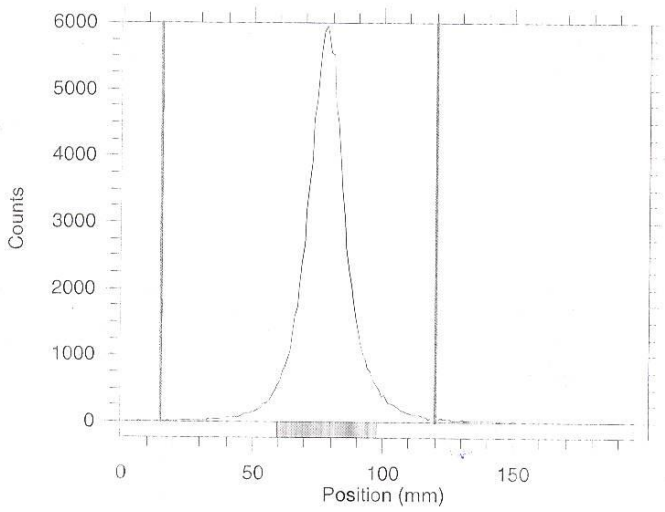
Analysis Parameters

Bkg Subtraction:	none	Origin:	15.0 mm
Normalization:	none	Front:	120.0 mm
Total Counts:	124361.0 (41453.7 CPM)	Region:	0.0 - 200.0 mm
Total File Counts:	124405		

Region Analysis

Definition:	Peak Search
Peak Slope:	1.0 counts/mm
Min Width:	11.7 mm
Min pct of Total:	0.0 %

Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	59.6	97.8	78.0	0.600	113605.0	37868.3	91.35	100.00
1 Peaks					113605.0	37868.3	91.35	100.00



WinScan Ver 3.12 - page 1 - 09-Sep-2013 09:12

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ACETONITRILE CONC =0.04%

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Sample Name      : Standard                          Location : Vial 1
Acq. Operator    : Amer Al Hourani                       Inj :   1
Acq. Instrument  : Instrument 1                   Inj Volume : Manually
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Last changed     : 9/25/2012 8:28:35 AM by Amer Al Hourani
new102
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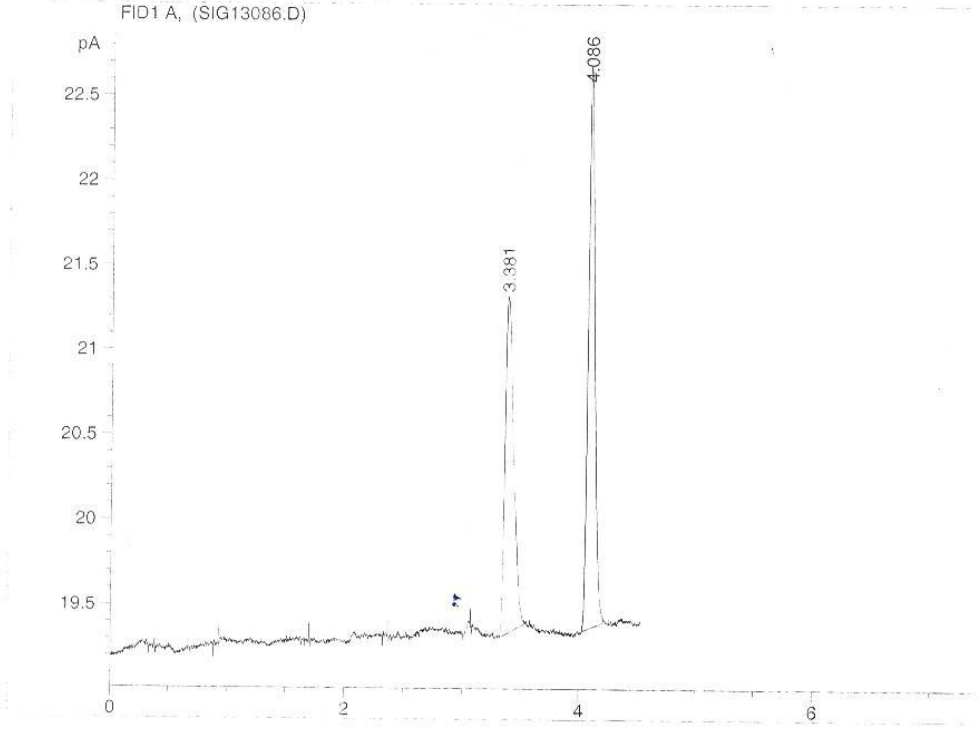

Area Percent Report

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Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type    Width     Area      Area     Name
  #   [min]           [min]   [pA*s]       %
----|-------|------|--------|----------|--------|--------------------
   1   3.381 PB      0.0764   10.09336 43.86474 Ethanol
   2   4.086 PB      0.0566   12.91683 56.13526 Acetonitrile

Totals :                      23.01020

 Results obtained with enhanced integrator!
```

*** End of Report ***

FDG Quality Form
Royal medical services
King Hussein Medical Center

Test	Method	Standard Result	Results
Physically appearance	**vision**	**Colorless/ clear solution**	
Radiochemical Identity (Purity)	**Thin layer chromatography**	**<95%** **RF >0.45**	
Chemical Purity	**Gas chromatography GC**	**Acetonitil <0.04%** **Alcohol <0.5%**	
pH	**Litmus Strips**	**7.5> pH >4.5**	
Bacterial Endotoxin	**Limulus Amebocyte Lysate**	**<0.25 EU/ml usp endotoxin standards**	
Sterility	**Typtic soybean broth/Fluid thioglycolate medium**	**Sterile**	

***Attachment (3)**

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