Automatic Production and Purification of [^{18}F]FMISO using the ABT BG75 system.

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SUMMARY:

Proof of concept for the synthesis of [^{18}F]FMISO using the disposable dose synthesis card (DSC) present configuration in the ABT BG75 system, and purification using solid phase synthesis and preparative HPLC is presented. Assessment of the radiochemical purity/identity, chemical purity and residual solvent determination in the final [^{18}F]FMISO product using ion exchange HPLC is also presented.

I. BACKGROUND:

The OUHSC-College of Pharmacy installed and commissioned the first Biomarker Generator (BG75) manufactured by ABT Molecular Imaging, Inc. in 2011. On-demand [^{18}F]FDG and [^{18}F]F$^-$ have been manufactured for pre-clinical and R&D purposes for more than two years and efforts towards aNDA application ([^{18}F]FDG) are currently on-going [1, 2]. Moreover pre-clinical [^{18}F]NaF was recently manufactured using the DSC configuration and bioequivalence with commercial product was established using mice imaging and biodistribution studies.[3]

Given the established workflow baseline for the BG75 system we wanted to study the potential to manufacture [^{18}F]FMISO, thus we proposed the following goals:

I.a. [^{18}F]FMISO synthesis (unpurified): Assess the feasibility to produce [^{18}F]fluoromisonidazole (FMISO) using a standard synthesis method (Fig. 1) and the same DSC configuration that is used for [^{18}F]FDG manufacture, with minimum changes to the hardware.

I.b. [^{18}F]FMISO purification: Compare reversed-phase high pressure liquid chromatography vs. solid-phase extraction as a suitable methods for[^{18}F]FMISO purification.

I.c. [^{18}F]FMISO quality control: Evaluate the capability of ion exchange HPLC to be incorporated in the following [^{18}F]FMISO quality control methods: Radiochemical Purity/Identity, Chemical Purity and Residual Solvent determination.

I.d. [^{18}F]FMISO imaging: Visualize different distribution of [^{18}F]FMISO and [^{18}F]FDG manufactured in the BG75 using a murine tumor xenograph.

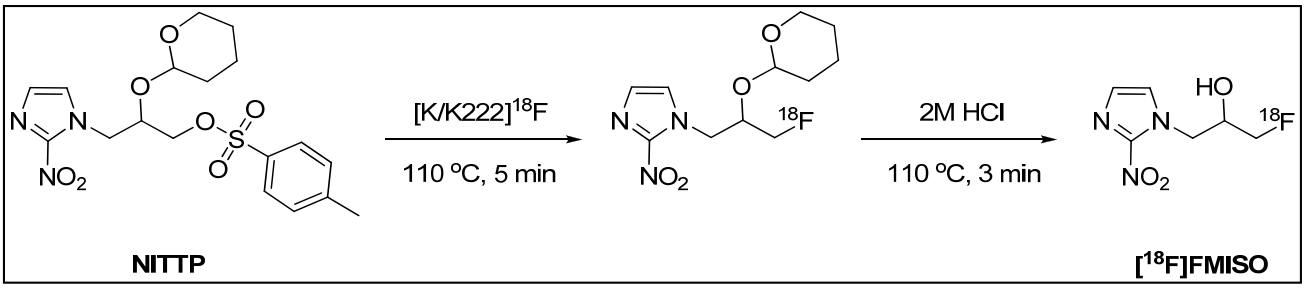

Figure 1: Scheme of [^{18}F]FMISO radiosynthesis.

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The Biomarker Generator comes with standardized [^{18}F]FDG synthesis, quality control and single-dose dispensing capabilities in a completely automated fashion. Reagents are loaded in a Reagent Metering System sub-assembly located at the top of the Card Chemistry System (CCS) and then measured/transferred to the sterile Dose Synthesis Card (DSC) where manufacture occurs in a close system, the dose is then purified using a solid phase column and transferred to a sterile syringe via a non-vented 0.2 µm filter (Fig. 2).

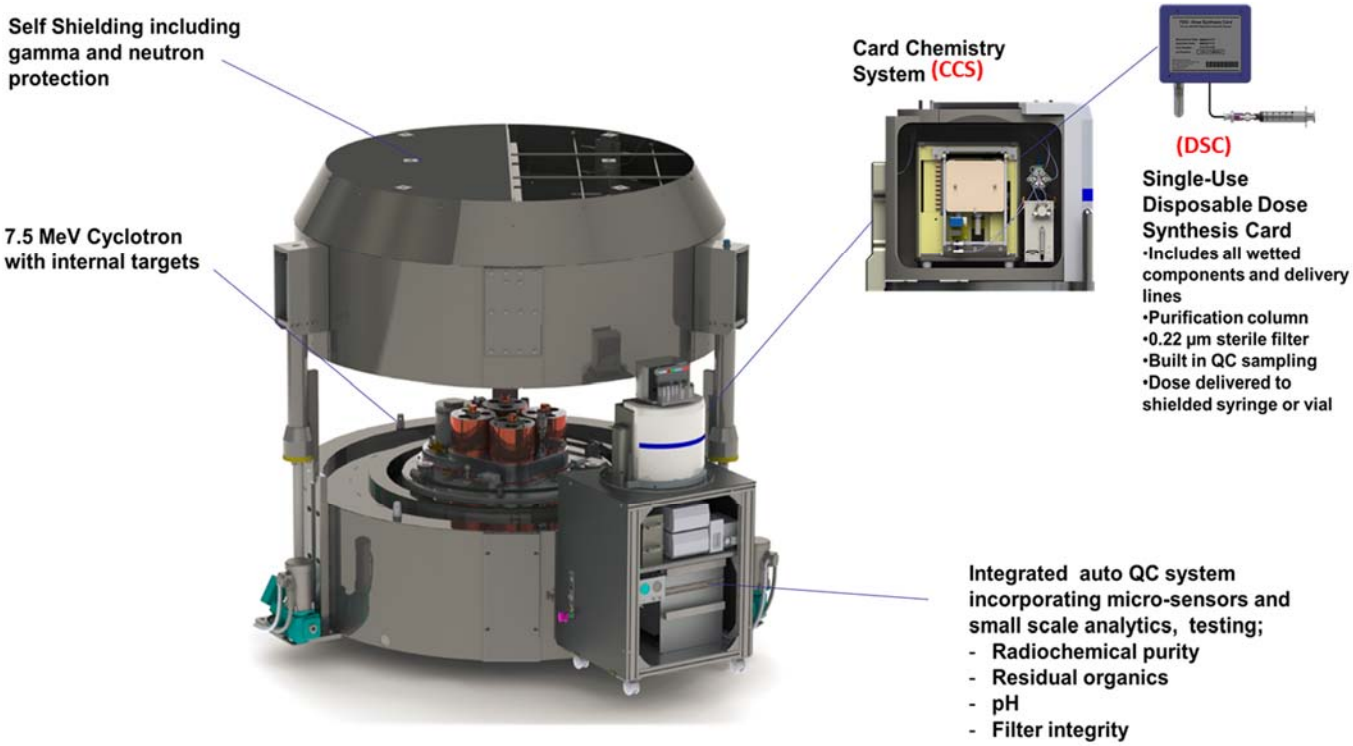

Figure 2: BG75 system showing its main components.

II. METHOD:

II.a. [^{18}F]FMISO synthesis (unpurified): The Biomarker Generator is a 7.5 MeV positive-ion cyclotron coupled with a customized CPM. Typically, no-carrier-added [^{18}F]fluoride was obtained through the ^{18}O(p,n)^{18}F nuclear reaction by irradiation (15 - 40 min, 3.5 µA) of a >95% enriched [^{18}O]water target (280 µl). [^{18}F]F^- (185-370 MBq) transferred to a reaction vial containing 200 µl of phase-transfer catalyst solution. The solvents were evaporated under a stream of nitrogen at 110°C. Azeotropic drying was repeated twice with 250 µl portions of acetonitrile to generate the anhydrous [K/K222]-[^{18}F]F^- complex. NITTP precursor [1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol] (10 mg, ABX, Germany) dissolved in anhydrous acetonitrile (600 µl) was transferred to the dried complex and the reaction was allowed to occur at 110°C for 5 min. The product was hydrolyzed by transferring 2 M HCl (1 ml) and heating the mixture at 110°C for 3 min. Finally, the reaction mixture was neutralized by transferring 120 µl of 10 M NaOH.

II.b. [18F]FMISO purification: The HPLC purifications were performed either on reverse phase chromatography (II.b.1.) or using solid-phase extraction (II.b.2.):

II.b.1. Beckman System Gold HPLC equipped with a Beckman Model 126 pump, 166 absorption detector (254 nm), and a Bioscan Model FC-3300 radioactivity detector. HPLC solvents consisted of water containing 0.1% trifluoroacetic acid (solvent A) and acetonitrile containing 0.1% trifluoroacetic acid (solvent B). A Sonoma C18 (ES Industries, 10 µm, 100 Å, 4.6 x 250 mm) column was used with a flow rate of 1.5 ml/min. The HPLC gradient system began with an initial solvent composition of 95% A and 5% B for 2 minutes followed by a linear gradient to 50% A and 50% B in 15 minutes.

II.b.2. Additional experiments were performed with built-in purification columns inside the DSC's containing strong cation exchange, C18 and alumina basic. The unpurified sample was run through the column and the final product was analyzed using a waters system with UV-vis (model 2489; Waters), refractive index (model 2489; Waters) and radiation detector (model FC-3300; Bioscan), with a reverse phase column, Synergi 4 µ Hydro-RP 80A, 250 X 4.6 mm (Phenomenex).

II.c. [18F]FMISO quality control: An ion exchange HPLC column (Rezex ROA-H^+, organic acid, Phenomenex) was used with HPLC grade water as mobile phase, flow was set to 0.55 ml/min and the column was kept at 85 °C. A waters system with UV-vis (model 2489; Waters), refractive index (model 2489; Waters) and radiation detector (model FC-3300; Bioscan) was used for this part of the study. Reference standards FMISO (PN 1410), desmethylmisonidazole (PN 1420)

II.d. [18F]FMISO imaging: About 100 microCi of the purified radiopharmaceuticals was injected intravenously and the distribution was allowed for 2 h. PET imaging (10 min) was performed in a PET/CT machine from Gamma Medica Ideas (California). The [18F]FMISO and [18F]FDG imaging studies were separated by 24 h.

III. RESULTS:

III.a. [18F]FMISO synthesis (unpurified): A total of seven automated runs were carried out to test the initial conditions. These runs were in addition to the innumerable step-by-step optimizations performed initially either manually or in an automated fashion. We obtained the unpurified product in all the automated runs (Table I). The confirmation of the product formation was based on the HPLC retention times of the cold FMISO standard and the [18F]FMISO (Figure 3).

The appearance, pH of the solution at the end of synthesis and decay-corrected radiochemical yield was recorded as shown in Table I. The entire synthesis was performed using a custom-written script for automated temperature control, solvent transfer, gas bubbling and reaction times.

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Table I: Summary of raw data obtained for unpurified [^{18}F]FMISO radiosynthesis.

Date (Batch #)	Script	Neutralization after hydrolysis	Appearance	pH	RCY(%)*	Total Synthesis Time (min)
5/4/12 (1)	FMISO V5	No	Clear, light brown	~1	46.4	22.0
5/4/12 (2)	FMISO V6	Yes	Clear, light brown	~1	54.4	22.3
5/7/12 (1)	FMISO V6a	Yes	Clear, light brown	2	69.9	24.0
5/7/12 (2)	FMISO V6a	Yes	Clear, light brown	2	63.2	22.5
5/8/12 (1)	FMISO V6a	Yes	Clear, light brown	2	53.2	250
7/13/12 (1)	FMISO V6a	Yes	Clear, light brown	2	31.3	24.0
7/13/12 (2)	FMISO V6a	Yes	Clear, light brown	2	48.7	24.0

*Decay Corrected

III.b. [^{18}F]FMISO purification: III.b.1. Unpurified samples from the previous section were purified either by reverse phase HPLC or solid phase synthesis (SPE), a summary of results are in table II. The main radiochemical impurity observed was free fluorine and only preparative reverse phase HPLC was able to produce a product complying with specifications (>95%) on this section of the project. The resins employed in all proof of concept experiments were based on C18 and strong cation exchange which did not have the capability of retaining the main radiochemical impurity. No assessment on chemical purity was performed on this section of the project.

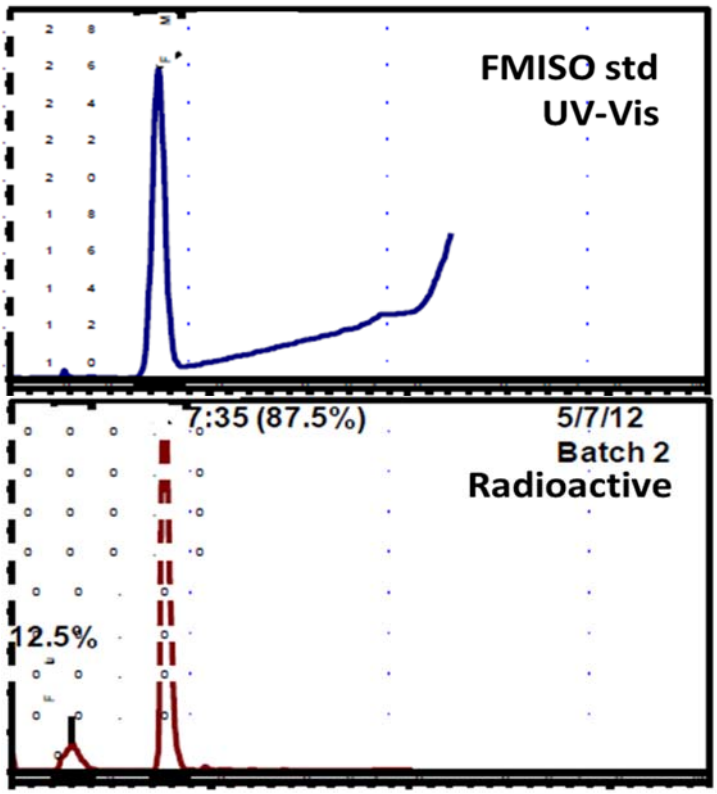

Figure 3: Comparison of UV-vis chromatogram of cold FMISO standard @ 254 nm (top) with the radio-chromatogram of a representative product from Table 1 (bottom). The only other radioactive impurity found was free [^{18}F]fluoride shown at the left of the main peak (bottom).

Table II: Summary of raw data obtained for unpurified [18F]FMISO radiosynthesis.

Date (Batch #)	Script	RCP (%)	SPE	Reverse Phase HPLC
5/4/12 (1)	FMISO V5	83.6	X	
5/4/12 (2)	FMISO V6	39.2	X	
5/7/12 (1)	FMISO V6a	86.5	X	
5/7/12 (2)	FMISO V6a	87.5	X	
5/8/12 (1)	FMISO V6a	100.0		X
7/13/12 (1)	FMISO V6a	100.0		X
7/13/12 (2)	FMISO V6a	90.8	X	

III.b.2. In a second attempt of purification via SPE alumina resin was included, this time the radiochemical purity was greatly improved as seen in figure 4. A total of five runs were performed using this cartridge prototype and a radiochemical purity of > 99% was obtained in all cases passing the specified criterion (>95%). Samples also exhibited a pH in the range of 6.5 – 7.0 and Kryptofix concentration below 50 ppm also passing the specified criteria.[4]

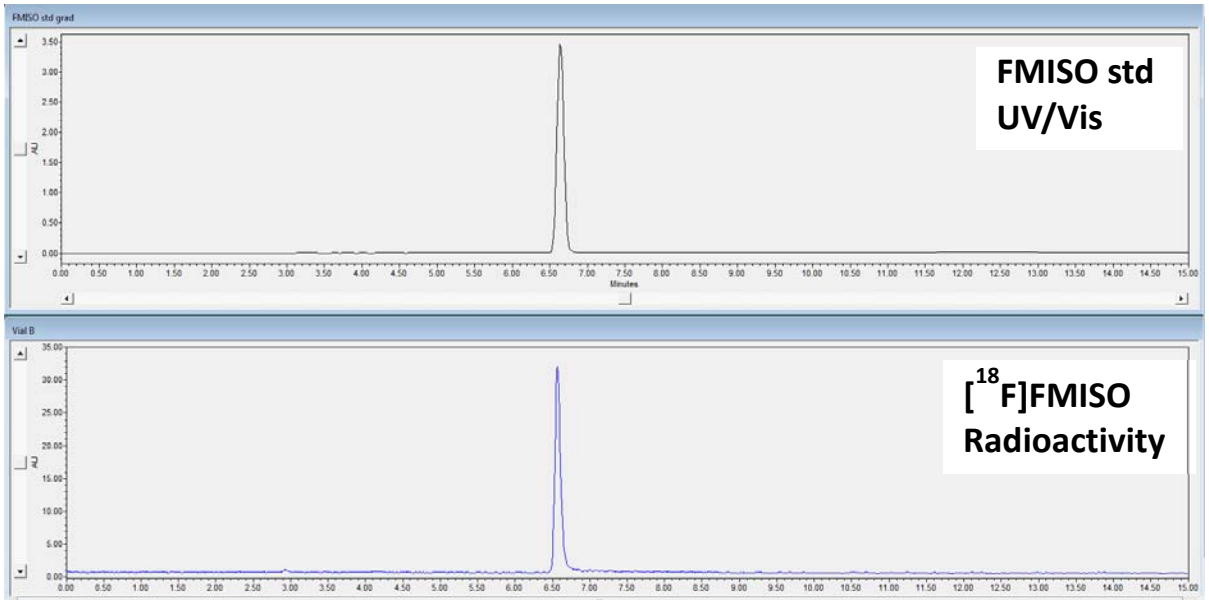

Figure 4: Radiochemical purity and identity for [18F]FMISO purified via SPE, radioactive trace (bottom) compared with UV-vis (254 nm) reference (top).

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III.c. Quality Control

The default HPLC column that is provided in the Quality Control Module (QCM) for the ABT BG75 system is based on ion exclusion, this type of column has proven useful in three of the automated quality control tests performed for [^{18}F]FDG i.e. determination of radiochemical purity/identity and residual solvent. On this work we aimed to characterize the approximate retention times for potential radiochemical and chemical impurities in [^{18}F]FMISO such that the potential for this HPLC column in automated analysis can be evaluated. BG75's HPLC system consists of three detection modes for UV/vis, refractive index and radiation.

Radiochemical Identity and Purity: The final product [^{18}F]FMISO and the main expected radiochemical impurity [^{18}F]F$^-$ elute with a half-height resolution of more than 14 (retention times of 15.5 min and 3.0 min respectively), which is very reasonable for method specificity, further experiments will assess the resolution to other potential radiochemical impurities.

Residual Solvents: acetonitrile and ethanol are the potential solvents that can be expected in the final product since DMSO is not used on the synthesis. There are specific windows for both solvents in the radioactive trace with a half-height resolution of more than 1 so the peaks don't overlap (the retention window for both solvents is shown in figure 5, E and F). This set-up is currently used for automated quality control of [^{18}F]FDG and has been validated according to ICH/FDA guidelines.[5,6] Moreover [^{19}F]FMISO and the main by-product of the reaction

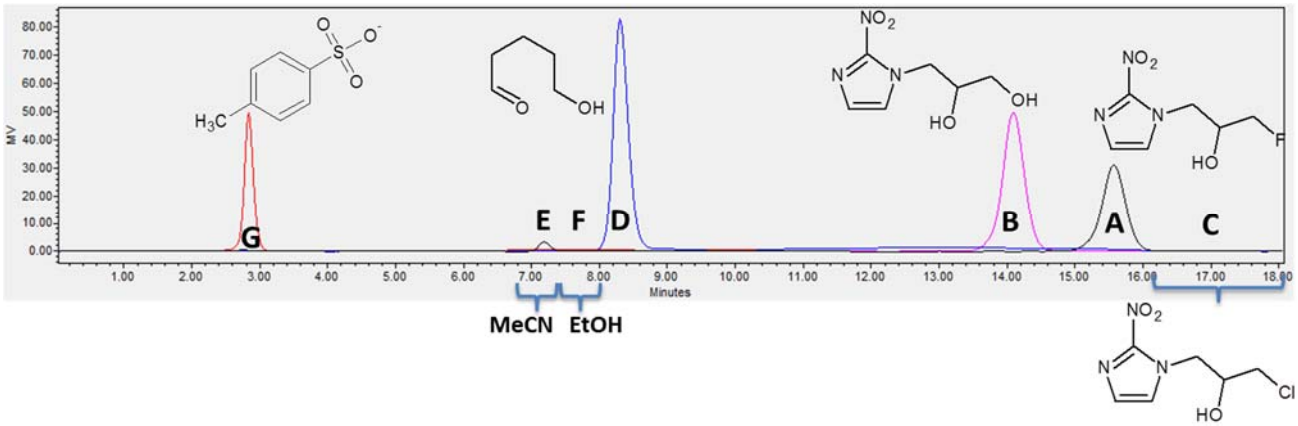

Figure 5: Chemical impurities potentially present in unpurified [^{18}F]FMISO, including the residual solvents acetonitrile (MeCN) and ethanol (EtOH) with corresponding elution windows. **A:** 1-Fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol; **B:** 1-(2,3-dihydroxy)propyl-2-nitroimidazole misonidazole; **C:** 1-Chloro-3-(2-nitro-imidazol-1-yl)-propan-2-ol; **D:** 5-hydroxypentanal; **E:** Acetonitrile; **F:** ethanol; **G:** tosylate.

Chemical Purity: NCI documentation for [^{18}F]FMISO specifies that concentration of all chemical contaminants shall not exceed 35 µg. Using the ion exclusion column in the QCM we showed that major chemical impurities are detected with good resolution using either the refractive index or UV/Vis detectors. As can be seen in figure 5 protecting groups and leaving groups (tosylate and tetrahydropyranyl G and D, respectively) in the precursor molecule are detected, as well as, potential by-products like the 1-(2,3-dihydroxy)propyl-2-nitroimidazole misonidazole (B), the "cold" [^{19}F]FMISO specie that can compromise specific activity in the final formulation and the chloro-derivative (C) recently identified in the literature[7].

Further we are testing new purification by SPE to minimize by-products coming from the synthesis in figure 6 assignation of the major species present in purified [18F]FMISO, these signals are monitored to adjust the design of resin type and quantity and reach the final design.

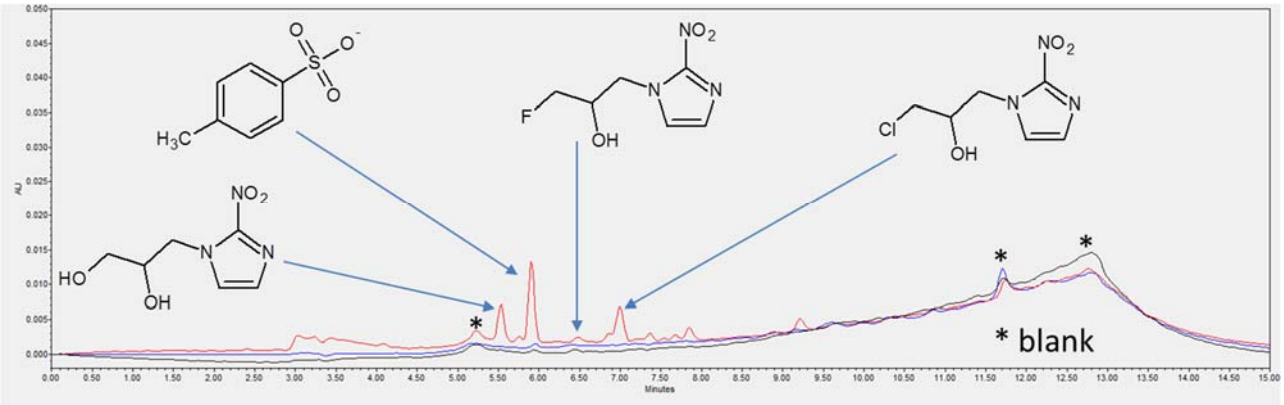

Figure 6: Assignation of chemical species present in SPE-purified [18F]FMISO, showing overlaying traces for a "blank" injection before and after (black, blue) the QC injection (red).

III.d. Imaging

The synthesized and HPLC-purified [18F]FMISO product was tested by PET imaging in a mouse model carrying HCT-116 colon cancer xenograft tumor. For comparison, the same mouse was also imaged with [18F]FDG. As can be seen in Figure 7, there is a difference in the distribution of the two radiopharmaceuticals in the tumor tissue. Whereas [18F]FMISO accumulated in relatively hypoxic core of the tumor, [18F]FDG was preferentially taken up by metabolically active circumferential tumor tissue.

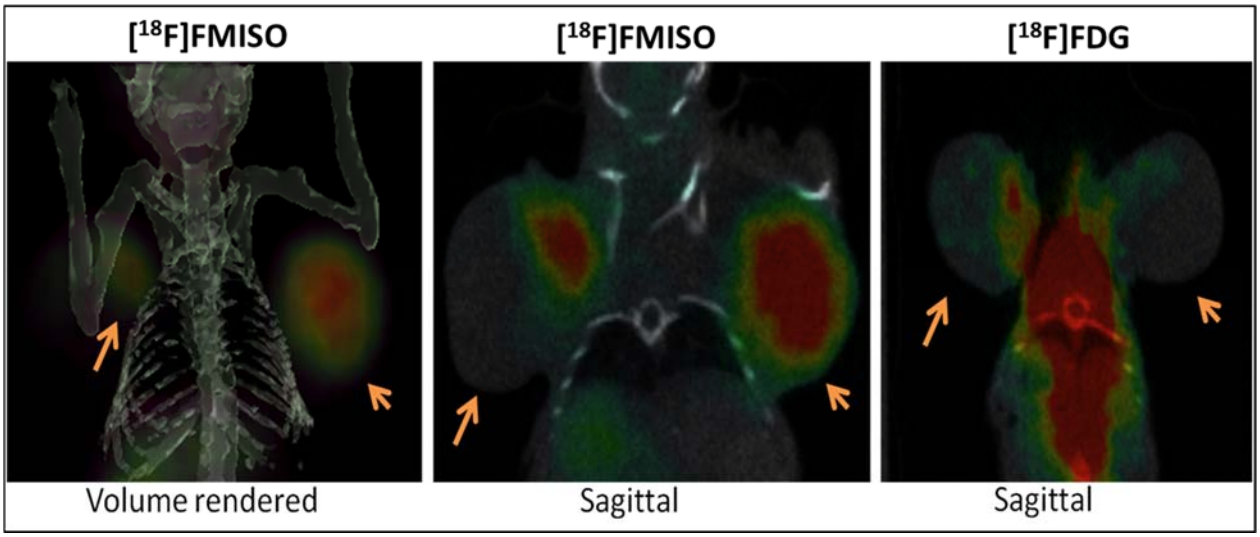

Figure 7. In vivo behavior of [18F]FMISO and [18F]FDG in a mouse carrying xenograft HCT-116 tumor.

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IV. CONCLUSIONS:

[^{18}F]FMISO was produced in the ABT BG75, using the DSC configuration and automatic script. A radiochemically pure product (RCP >95%) can be produced using preparative HPLC purification and in-card solid phase extraction. Biodistribution of the product in mice was verified as expected in a valid murine model.

A good potential for increasing [^{18}F]FMISO chemical purity, in agreement with current US and EU regulations, was observed based on a comprehensive understanding of the chemical species involved in the manufacture.

The proof of concept for the application of the current Quality Control Module (QCM) to [^{18}F]FMISO quality control without the need for hardware modification or re-configuration was also demonstrated. This fact supports the potential to execute pH and residual solvents determination, radiochemical identity/purity, chemical purity and filter integrity test on the final dose of [^{18}F]FMISO in an automated mode.

V. REFERENCES:

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