Automated two-steps manufacturing of [^{11}C]glyburide for PET imaging in Humans[1]

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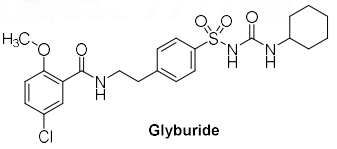
[1]Caillé F. et al., *Nucl. Med. Biol.* **2020**, 20

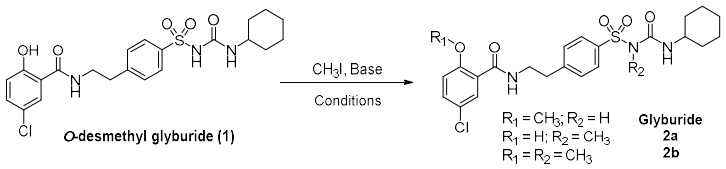
Context

Glyburide is an approved drug to treat diabetes which binds to the sulfonylurea receptors (SUR-1, ABCC8) and is a substrate of OATP and ABC transporters. Positron emission tomography (PET) using radioisotopically labelled [^{11}C]glyburide would be a powerful tool to quantitatively image those proteins and assess pharmacokinetic parameters of functional or pathological tissues. Radiolabelling of glyburide with carbon-11 ($t_{1/2}$ = 20.4 min.) has already been described in one step by methylation using *O*-desmethyl-glyburide and [^{11}C]methyl triflate in the presence of sodium hydroxide but lack of reproducibility. Aiming at investigating the use of [^{11}C]glyburide in a clinical trial, we have developed a reproducible one pot two-steps radiomethylation followed by urea formation and a complete quality control compliant with to the European Pharmacopeia 9.7 guidelines for the manufacturing of [^{11}C]glyburide as a radiopharmaceutical preparation for human injection.



Chemistry

One-step approach



O-desmethyl glyburide (1)

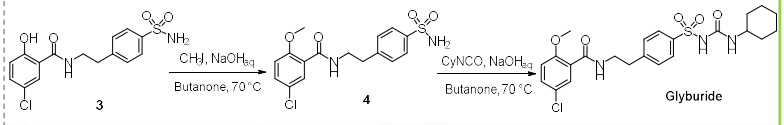
Entry	Base	Conditions[a]	Results[b]
1	NaH	DMF, 110 °C	Degradation of **1**
2	NaH	DMF, 70 °C	Formation of **2a**
3	DBU	Butanone, 70 °C	No reaction
4	KF + K_{222}	DMA, 70 °C	Formation of **2b**
5	$NaOH_{aq}$ (3 M)	Butanone, 70 °C	Mixture of glyburide and **2a**

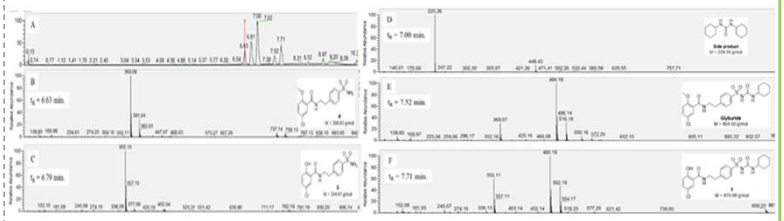
[a]All reactions were carried out for 30 minutes with 1.0 equiv. of methyl iodide compared to **1**

[b]The crude reaction was analysed qualitatively by UPLC-MS

Selectively alkylating the phenol moiety from the sulfonylurea was difficult due to the high capacity of sulfonylurea to deprotonate at pH above 7.

Two-steps approach

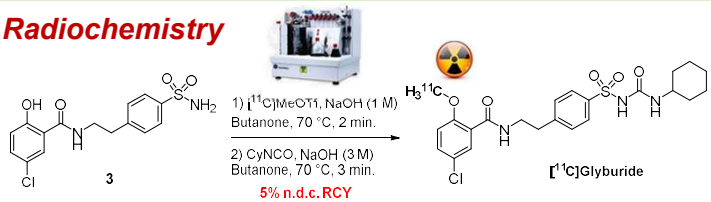


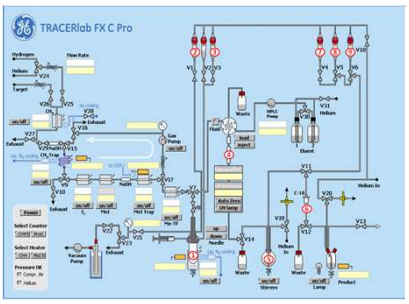


UPLC-MS analysis of the two-steps one pot non-radioactive synthesis of glyburide from **3** after 1 hour. A) Full chromatogram; B) Mass spectrum of the 6.63 min. peak; C) Mass spectrum of the 6.79 min. peak; D) Mass spectrum of the 7.00 min. peak; E) Mass spectrum of the 7.52 min. peak; F) Mass spectrum of the 7.71 min. peak.

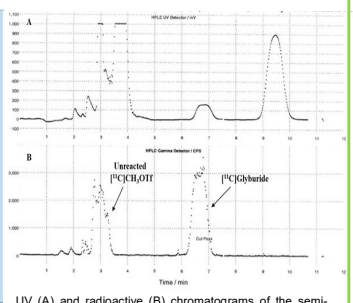
This one pot two-steps approach demonstrated orthogonality as no other methylated products were observed. The side products observed will not be detrimental moving to radiochemistry and will be discarded during purification.

Radiochemistry





TRACERlab® FX C Pro synopsis for the automated production of [^{11}C]glyburide. 1) **3** (1 mg) and $NaOH_{aq}$ (1 M, 3.5 µL) in butanone (200 µL); 2) CyNCO (4 µL) and $NaOH_{aq}$ (3 M, 7 µL) in butanone (200 µL); 3) H_2O/CH_3CN/TFA (45/55/0.1 v/v/v, 1 mL); 4) Waters Symmetry® C18 7.8 x 300 mm, 7 µm; 5) H_2O (20 mL); 6) Sep-Pak® C18 cartridge; 7) H_2O (10 mL); 8) EtOH (2 mL); 9) 0.9% $NaCl_{aq}$ (18 mL)

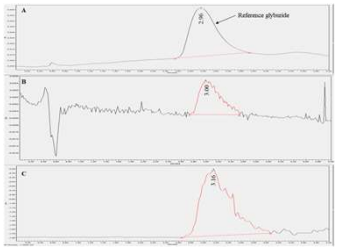


UV (A) and radioactive (B) chromatograms of the semi-preparative HPLC purification for the preparation of [^{11}C]glyburide

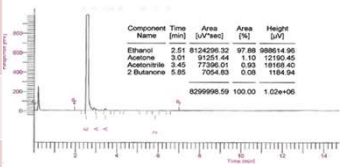
No side product was observed during the methylation step. A large excess of cyclohexyl isocyanate and sodium hydroxide were used to reach full conversion of [^{11}C]glyburide. Purification by HPLC followed by reformulation and sterilization afforded 1.5 GBq of [^{11}C]glyburide with high reproducibility to produce PET images in Humans.

Quality control

Test	Specifications	Results
Organoleptic test	Limpid colourless liquid	Compliant
pH	5.0 - 8.0	6.2
Chemical identification	t_R = 2.7-3.3 min	Compliant
Chemical purity	≥ 95%	≥ 99%
Radiochemical purity	≥ 95%	≥ 99%
Molar activity	≥ 4 GBq/µmol	14 ± 2 GBq/µmol
Residual solvents	Ethanol ≤ 0.79 g/inj. Acetone ≤ 50.00 mg/inj. Acetonitrile ≤ 4.10 mg/inj. Butanone ≤ 50.00 mg/inj.	Compliant
Radionuclide purity	Photons energy : 511 keV Half-life : 19.9 – 20.9 min.	≥ 99% 20.3 min.
Filter integrity	≥ 50 psi	60 psi
Sterility	Sterile	Sterile
Bacterial endotoxins	≤ 50 EU/inj.	3.5 EU/inj.



Analytical radio-HPLC for the quality control of [^{11}C]glyburide for A) glyburide reference; B) [^{11}C]glyburide (UV detection); C) [^{11}C]glyburide (gamma detection).



Residual solvents analysis by gas chromatography of the [^{11}C]glyburide preparation with areas under the curve measured for ethanol, acetone, acetonitrile and butanone

The quality control of the [^{11}C]glyburide preparation was compliant with the European Pharmacopeia 9.7 guidelines. The pre-release quality control operations were realized within 15 minutes, leaving 900 MBq of [^{11}C]glyburide for injection.

Conclusion

We have described a reproducible automated two-steps radiosynthesis of [^{11}C]glyburide with a complete quality control to qualify as a radiopharmaceutical for human injection. This manufacturing process is currently used to conduct a clinical trial to elucidate the hepatic transport of drugs.

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