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# Radiochemical Characterization of a $^{68}$Ga-Labelled PSMA Inhibitor for Prostate Cancer Imaging

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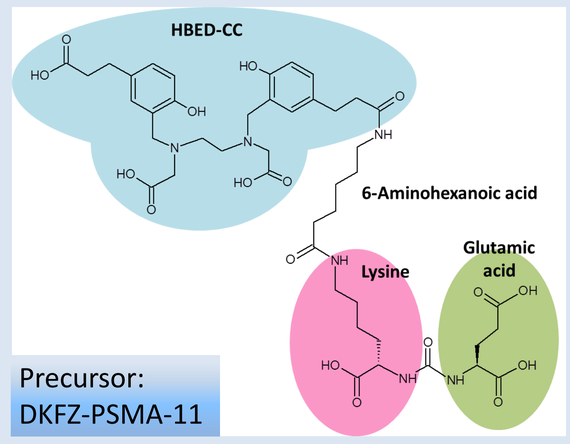
## BACKGROUND

Prostate-specific membrane antigen (PSMA) is a membrane associated zinc protease, expressed in nearly all prostate cancers with increased expression in poorly differentiated, metastatic and hormone-refractory carcinomas. The urea-based inhibitors of PSMA exhibiting Glu-NH-CO-NH-Lys are excellent pharmacophores to bind PSMA. Its labelling with $^{68}$Ga is an attractive generator-based alternative to cyclotron PET radiopharmaceuticals. HBED-CC is an acyclic complexing agent that allows efficient radiolabeling with $^{68}$Ga, even at room temperature (RT). This chelator attached to Glu-NH-CO-NH-Lys has an improved binding to PSMA due to the interaction of their aromatic groups with a lipophilic part of the binding site, which is different from that interacting with urea-based inhibitors.




**Our aim was the radiolabelling optimization of Glu-NH-CO-NH-Lys(Ahx)-HBED-CC (DKFZ-PSMA-11) with $^{68}$Ga and the stability evaluation at RT of different incubation conditions. Also the radiochemical purity (RCP) determination was optimized in order to separate different diastereomers as well as potential impurities.**

## METHODOLOGY

**Labelling optimization (precursor)**
- $^{68}$Ga: 361 ± 11 MBq in 1000 µL HCl 0.05M, available from a $^{68}$Ge/$^{68}$Ga generator system (ITG)
- DKFZ-PSMA-11 (ABX): 0.04-1.25 µg (0.04-1.32 nmol)
- Reaction pH: 4.0, adjusted with 250 µL NaOAc 0.25M
- Incubation at 100ºC for 5 min.


**$^{68}$Ga incorporation (kinetics)**
- $^{68}$Ga: 240 MBq in 1000 µL HCl 0.05M
- DKFZ-PSMA-11 (ABX): 0.3 µg (0.32 nmol)
- Reaction pH: 4.0, adjusted with 250 µL NaOAc 0.25M
- Incubation at 100ºC: 0-5 min

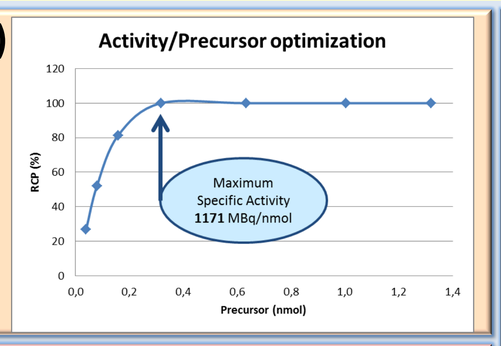
The reaction was stopped at different incubation times (0 to 5 min) by mixing 20 µL of reaction solution with 100 µL of $^{nat}Ga(NO_3)_3$ 50 mM

**Stability studies** (at different incubation times)
- $^{68}$Ga: 240 ± 10 MBq in 1000 µL HCl 0.05M
- DKFZ-PSMA-11 (ABX): 0.3 µg (0.32 nmol)
- Reaction pH: 4.0, adjusted with 250 µL NaOAc 0.25M
- Incubation at 100ºC: 0, 1, 2, 3, 4, 5 min [room temperature (RT) to complete a total incubation time of 5 min]

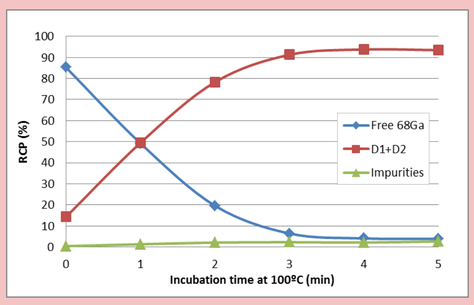
Stability post-labelling was studied at room temperature up to 60 min

## RESULTS

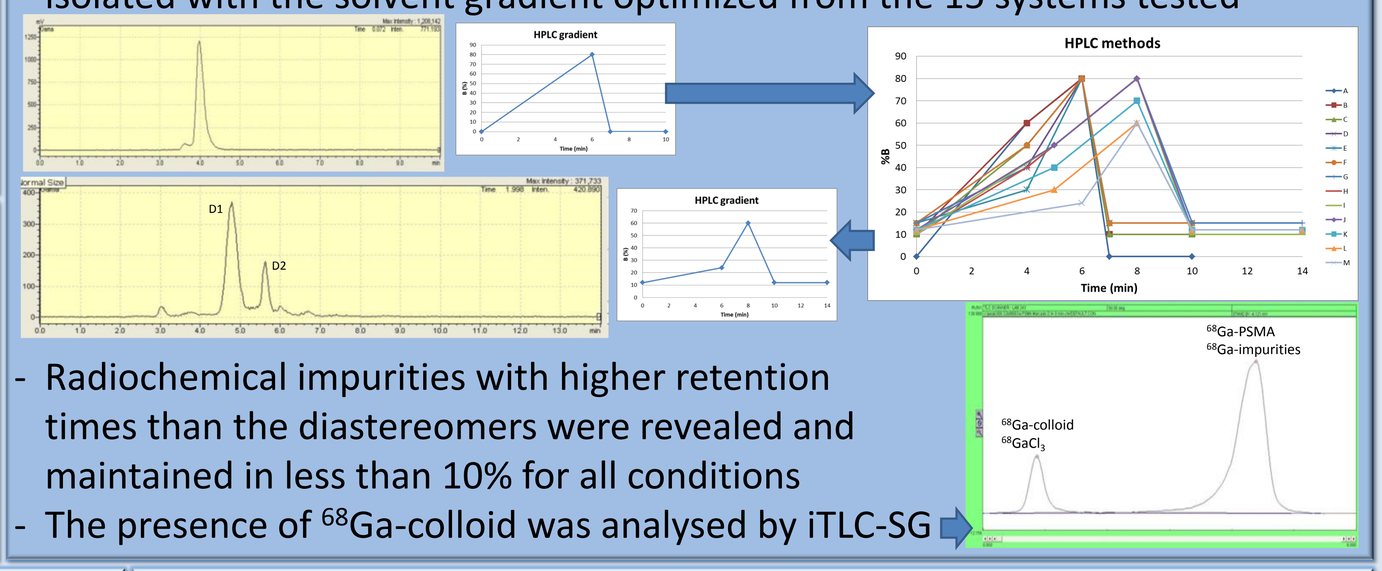
**Labelling optimization (precursor)**
- RCP reached 100% (sum of 2 diastereomers) for precursor amounts > **0.32 nmol**
- Specific Activity max: **1171 MBq/nmol**




**$^{68}$Ga incorporation (kinetics)**
- RCP (D1+D2) overcame 90% from 3 min incubation at 100ºC
- Conversion of diastereomers: D2 into D1 with time elapsed even after the reaction had been stopped with $^{nat}$Ga




**Radiochemical purity** (RCP)
- $^{68}$Ga incorporation to precursor yielded 2 diastereomers (D1-D2), which were isolated with the solvent gradient optimized from the 15 systems tested



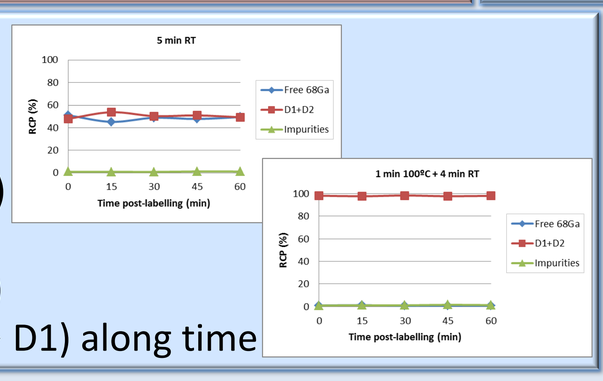

- Radiochemical impurities with higher retention times than the diastereomers were revealed and maintained in less than 10% for all conditions
- The presence of $^{68}$Ga-colloid was analysed by iTLC-SG




**Stability studies (D1+D2)**

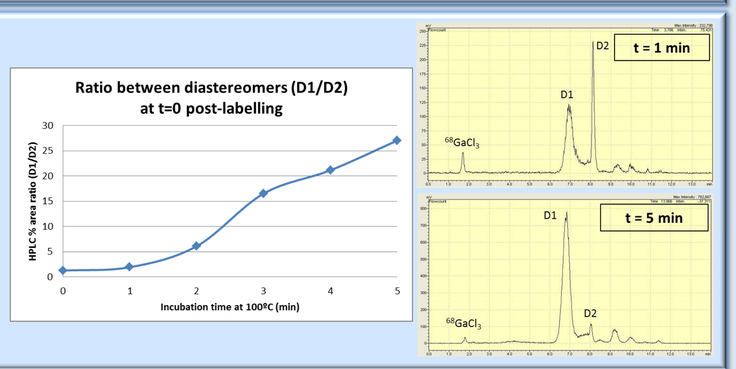
Incubation for:
- 5 min at RT: RCP ≈ 50% (unchanged over time)
- (1-5) min 100ºC + (4-0) min RT: RCP ≈ 98% (unchanged over time)

Conversion of diastereomers (D2 → D1) along time




**Diastereomers conversion**

Rate of conversion (D2 → D1) was positively correlated with:
- temperature incubation
- time of incubation
- time elapsed after labelling




## CONCLUSION

Two diastereomers were successfully resolved from the radiochemical impurities and from free $^{68}$Ga with the optimized HPLC system. More research is required to determine the molecular recognition of each diastereomer. The high specific activities required for the clinical application of this promising agent for diagnosis of prostate cancer, require careful control of the ratio activity/ precursor as well as incubation temperature and time. These achievements in optimization allowed to start clinical studies at CUDIM with auspicious results.

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