Efficacy and Safety of [211At]At-Substance P as Adjuvant Therapy in Recurrent Glioblastoma Multiforme: A Pilot Study

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2. Context of the project (800 characters max. including spaces)

In this project, we aimed to develop radiolabelling processes for [211At]At-Substance P for future application in preclinical and clinical studies. The project was carried out at ICNAS, University of Coimbra, Portugal.

The aim was to identify and characterize the behaviour and to establish the reference calibration factors for future At-211 experiments. This report describes our preliminary results with the first delivery batch of At-211 from Arronax (Nantes, France), the calibration of the dose calibrators, and the preliminary TLC and HPLC studies.

3. Results and discussion (1000 characters max. including spaces)

The results of the preliminary tests are summarized below.

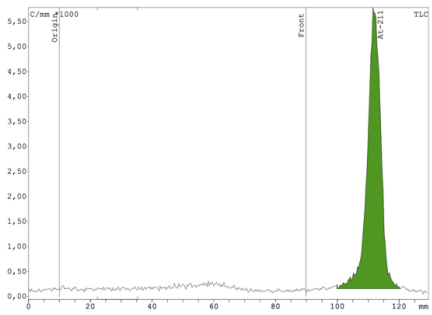
1. Dose calibrator calibration: calibration factor #052 (measurements performed in 3 distinct dose calibrators at about 20 hours after time of reference.

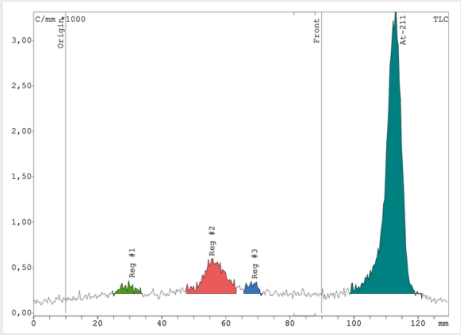
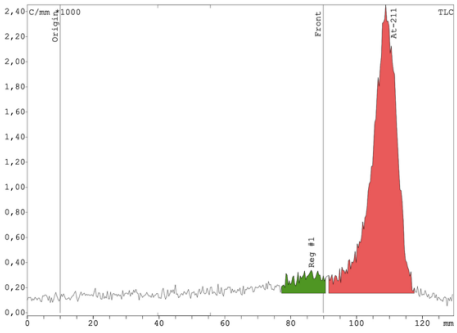
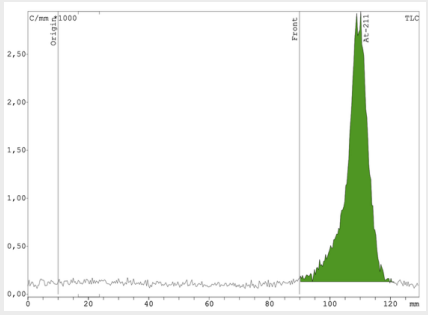
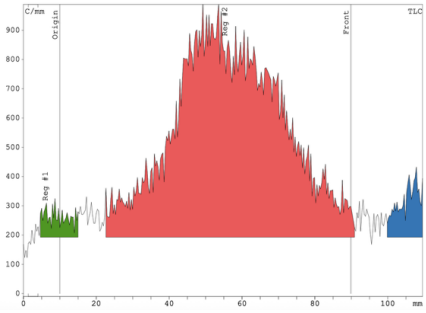
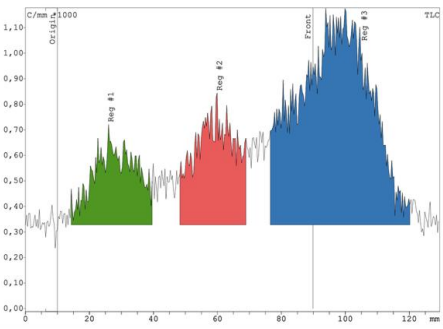
2. Chromatographic studies

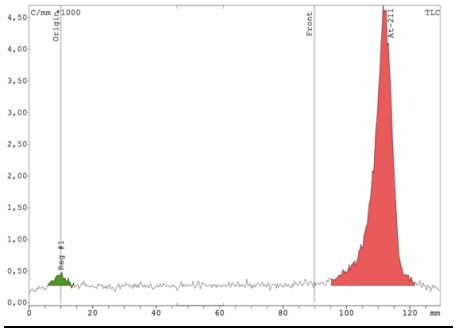
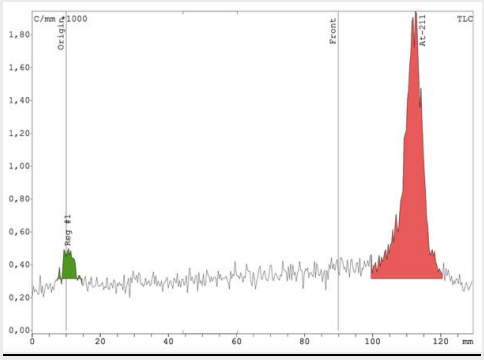
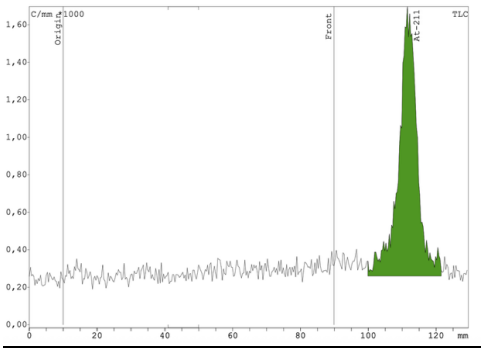
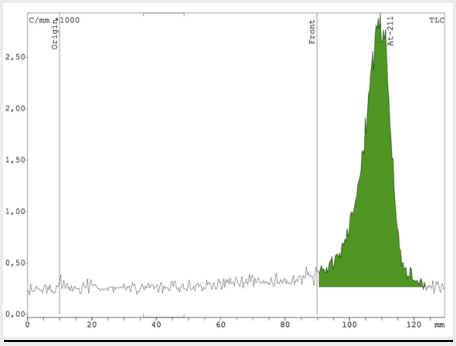
The radiochemical purity and test different mobile phase was assessed in two solutions.

Solution 1: Dry At-211 was dissolved in 50 µL of MeOH and 450 µL of PBS. A sample of 5 µL of the solution 1 was used for the tests.

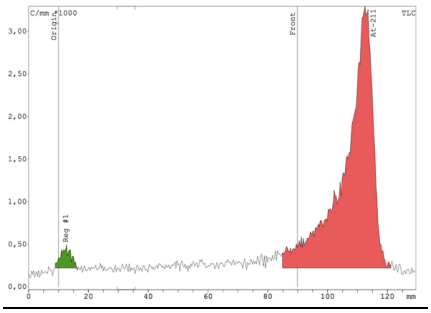
Solution 2: The pH of the solution 1 was adjusted to pH 8 with NaOH 0.1M. A sample of 5 µL of the solution 2 was used for the tests.

Solution 1		
Mobile phase	**iTLC chromatogram**	
MeOH		**RCP 211At = 100%**

MeOH:H2O		**RCP 211At = 81.68%**
NaOH 0.1M		**RCP 211At = 92.72%**
Acetone/HCl 0.02M (9:1)		**RCP 211At = 92.72%**
NaCl 0.9%		**RCP 211At = 92.94%**
		**RCP 211At = 19.80% ?(red zone)** **0r 64.82%? (blue zone)**

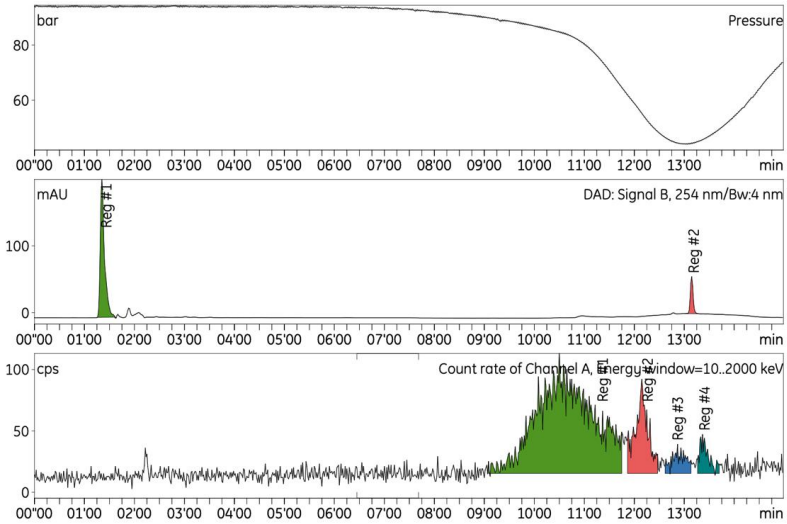
Solution 2		
Mobile phase	**iTLC chromatogram**	
MeOH		*RCP ^{211}At = 97.67%*
MeOH:H_2O		*RCP ^{211}At = 94.72%*
NaOH 0.1M		*RCP ^{211}At = 100%*
Acetone/HCl 0.02M (9:1)		*RCP ^{211}At = 100%*

NaCl 0.9%



RCP ^{211}At = 97.01%

Radio-HPLC



Integration Count rate of Channel A, Energy window=10..2000 keV

Substance	R/T s	Type	Area Counts	%Area %
Reg #1	10'29	DD(M)	6533.108	76.96
Reg #2	12'09	DD(M)	1242.324	14.63
Reg #3	12'50	DD(M)	354.946	4.18
Reg #4	13'22	DD(M)	358.568	4.22
Sum in ROI	-	-	8488.946	100.00
Area (total)	-	-	8557.432	-
BKG1 (CPS)	-	-	15.3243	-
Remainder	-	-	68.49	0.80

Integration DAD: Signal B, 254 nm/Bw:4 nm

Substance	R/T s	Type	Area mAU*s	%Area %
Reg #1	01'21	BB(M)	1195.447	85.46
Reg #2	13'09	BB(M)	203.467	14.54
Sum in ROI	-	-	1398.915	100.00
Area (total)	-	-	-4489.526	-

Figure 1. Radio-HPLC chromatograms of Solution 1.

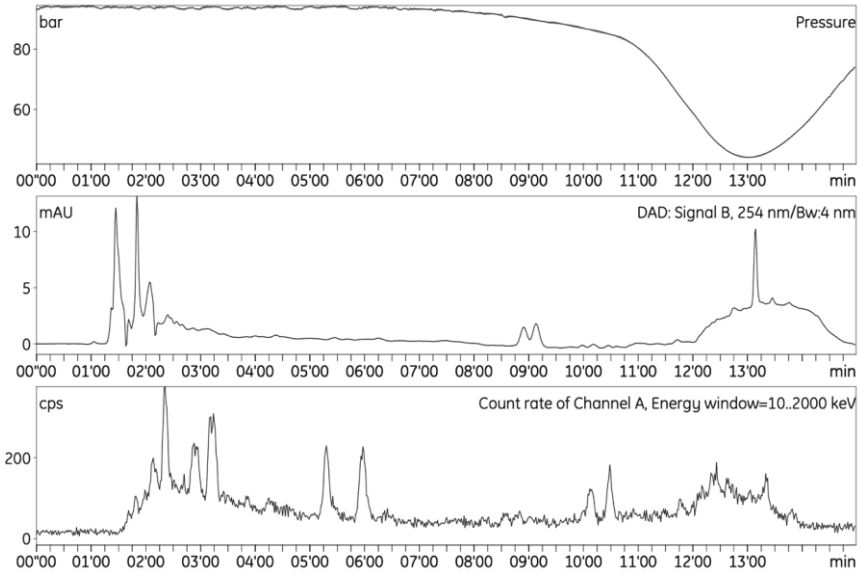


Figure 2. Radio-HPLC chromatograms of Solution 2.

4. Conclusions (800 characters max. including spaces)

- Around 60 MBq of At-211 was received from Arronax. This activity received at ICNAS represents a decay of three half-lives.
- The radioactivity and half-life of At-211 were measured using three dose calibrators (Capintec, Florham Park, NJ) that had been pre-calibrated with At-211.
- After dissolution of the At-211 in MeOH/PBS (1:9), the target solution appeared clear and colorless.
- The radiochemical purity and identity were analyzed using TLC and HPLC for two different solutions (solution 1 and 2 with pH values of 2 and 8, respectively).
- The HPLC column (Luna C18) was selected based on the properties of the modified Substance P molecule to be used, but further HPLC studies are required to confirm the appropriate method and column for the final radiopharmaceutical product.

In our preliminary results, the quality control test of At-211 was performed approximately 20 hours after production, which may have affected the radiochemical purity of the radionuclide. Further quality control tests are required to clarify the chemical form of At-211.

5. Involvement of the PRISMAP services (600 characters max. including spaces)

The preliminary results obtained during the project, which are summarized in this report, were achieved with the batch received on the 24th September 2025.

At-211 Batch Information

- Activity sent (MBq/µCi): 402.4 MBq @ 24/09/2025 @ 04:00 p.m.
- Activity Received (MBq/µCi): 63.8 MBq @ 25/09/2025 @ 10:11 a.m (average of 401.7 ± 0.7 MBq @ 24/09/2025 @ 04:00 p.m. from measurements performed with 3 distinct dose calibrators.

- $^{210}At/^{211}At$ ratio: 3.6 x 10^{-4} Arronax vs 2.9 x 10^{-4}ICNAS (HPGe Ortec GEM30P4-76).

6. Feedback to PRISMAP (600 characters max. including spaces)

We demonstrated the feasibility to deliver pre-clinical level doses from Arronax to Coimbra, approximately 2 half-lives, 14 hours. We need to plan for future a scale-up to supply clinical trials or to explore future shipments via air travel.

7. Publications and other dissemination activities (conferences etc.)

This project included a training mission to provide knowledge and insights into the technical requirements of radiochemistry laboratories for implementation of 211At radiolabelling processes.

Appendix 1. Dissemination guidelines for user projects as agreed in the signed User Agreement

Dissemination rules

Only user groups that are allowed to disseminate the results which they have generated under the project may benefit from the access, unless they are working for SMEs.

For each user group project, a publishable project summary and a publishable summary of the results will be published on the European Union Horizon 2020 PRISMAP project website www.prismap.eu. The publication of results in journals or at conferences is strongly encouraged.

To ensure the long-term sustainability of the PRISMAP initiative, proper recognition of the contributing facilities, their services and the involved persons is necessary. All participating PRISMAP facilities shall be acknowledged in the publication. Acknowledgement and co-authorship of PRISMAP staff members who participated in the experiment shall be considered according to the research field best practices and verified with the PRISMAP Technical Manager before any publication.

The user group shall contact the PRISMAP Technical Manager 30 days prior to submission of publications or other communications of results that were obtained by making use of services provided by PRISMAP (radionuclides delivered or medical services provided). The Technical Manager will communicate to the user group the list of PRISMAP facilities and persons that have contributed to each specific project and the way this contribution must be acknowledged in the publication/communication or where co-authorship is required to reflect specific scientific contributions.

Users must comply with Horizon 2020 dissemination rules (i.e. acknowledge that their work was financially supported by the European Union's Horizon 2020 Research and Innovation Programme by including the following acknowledgement: "This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008571 (PRISMAP)"), and grant open access to resulting publications and related data.

Dissemination shall take place only once legitimate interests regarding intellectual property have been safeguarded. A maximum publication delay of 90 days may be granted for this purpose.

Acknowledgements

The list of name(s) to be mentioned in the acknowledgment section is sent to the technical manager by the main contact of the involved facilities.

A general sentence will be added by the corresponding author of the article (user side):

"The authors would like to thank the members of the PRISMAP consortium and of the PRISMAP user selection panel, coordination and management team for their advice and support."

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