Production of high specific activity ^{195m}Pt-cisplatinum at Necsa for Phase 0 clinical trials in healthy individual subjects

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RUNNING HEAD

^{195m}Pt-cisplatinum's ability to optimise patient dose demonstrated

SHORT ABSTRACT

Platinum agents continue to be the main chemotherapeutic agents used in first and second line treatment of cancer patients. It is important to fully understand the biological profile of these compounds in order to optimize the dose given to each patient. ^{195m}Pt-cisplatinum (commonly referred to as cisplatin) was produced from $^{194}PtCl_2$ that was irradiated in *SAFARI-1* for up to 200 h. Final product yield was 51.7 ± 5.2% while the chemical and radionuclidic purity for each production run passed quality control providing a GMP compliant product that was administered to ten healthy volunteers as part of an ethical approval phase 0 clinical trial. Volunteers received 108-126 MBq of radioactivity and 6.8-10 mg of carrier cisplatinum which was well-tolerated. The majority of the injected dose 27.53 ± 5.81% was excreted in the urine within 5 h post injection (p.i). Only 8.45 ± 3.07% of cisplatinum remained in blood pools at 5 h which gradually cleared over the six day monitoring period p.i. At the end of the study (6 days p.i.) a total of 37.35 ± 5.27% of product had cleared from the blood into urine meaning that around 63% remained in the body.

LONG ABSTRACT

Platinum agents continue to be the main chemotherapeutic agents used in first and second line treatment of cancer patients. It is important to fully understand the biological profile of these compounds in order to optimize the dose given to each patient. In a joint project with ANSTO and the Nuclear Medicine Department at Steve Biko Academic hospital, Necsa synthesized and supplied ^{195m}Pt-cisplatinum (commonly referred to as cisplatin) for a clinical pilot study on healthy volunteers. Enriched $^{194}PtCl_2$ was prepared by digestion of enriched ^{194}Pt metal (>95%) followed by thermal decomposition over a 3 h period. The $^{194}PtCl_2$ was then placed in a quartz ampoule and irradiated in *SAFARI-1* up to 200 h and cooled for a minimum of 34 h prior to synthesis. $^{195m}Pt(NH_3)_2I_2$, formed with the addition of KI and NH_4OH, was converted to the diaqua-species $[^{195m}Pt(NH_3)_2(H_2O)_2]^{2+}$ by reaction with $AgNO_3$. The conversion to ^{195m}Pt-cisplatinum was completed by the addition of concentrated HCl. Final product yield was 51.7 ± 5.2% (n=5). The chemical and radionuclidic purity in each case was >95%.

The use of a high flux reactor position affords a higher specific activity product (15.9 ± 2.5 MBq/mg at end of synthesis) than previously reported (5 MBq/mg) [10]. Volunteers received between 108 and 126 MBq of radioactivity which is equivalent to 6.8 to 10.0 mg of carrier cisplatinum. Such high specific activities afforded a significant reduction (~50%) in the chemical dose of carrier cisplatinum which represents less than 10% of a typical chemotherapeutic dose given to patients. A GMP compliant product was produced and administered to ten healthy volunteers as part of an ethical approval phase 0 clinical trial. The majority of the injected dose 27.53 ± 5.81% was excreted in the urine within 5 h post injection (p.i). Only 8.45 ± 3.07% of cisplatinum remained in blood pools at 5 h which gradually cleared over the six day monitoring period p.i. At the end of the study (6 days p.i.) a total of 37.35 ± 5.27% of product had cleared from the blood into urine, and approximately 63% remained in the body. The significantly lower concentration of carrier cisplatinum used for imaging resulted in a well-tolerated product.

INTRODUCTION

Cisplatinum (this terminology will be referred to in this paper as opposed to the commonly used term, cisplatin) is a heavy-metal alkylating agent that has proven successful, either alone or in combination with other chemotherapeutics, in the treatment of the majority of human solid malignancies [1]. However, due to the extensive side effects associated with cisplatinum it's use today is limited to the treatment of testicular, cervical, bladder and head- and neck cancers. The principal target organ for cisplatinum toxicity is the kidney. This toxicity is manifested by reduced renal function and leads to serum electrolyte changes and pathological changes in the urine analysis [2]. Other side effects are ototoxicity, nausea and vomiting [3]. Liver toxicity rarely occurs, but may be observed when the drug is administrated at high doses [4]. The toxicity observed following cisplatinum administration is currently believed to be due to distinct mechanisms in actively dividing tumor cells versus normal quiescent cells, for example renal proximal tubular epithelial cells, amongst others through the formation or metabolisation of a nephrotoxin , as well as to extensive (up to 65-80%) protein plasma binding [1, 5, 6, 7]. Consequently around twenty percent of patients reach the maximum tolerated dose and experience serious cytotoxic effects. This is because the dosage is often estimated using unreliable and indirect methods such as surface area and glomerular filtration rates. A process to determine more accurate dosage for each individual patient needs to be developed.

A radiolabelled form of cisplatinum that is imageable could potentially be a useful tool for determining the appropriate dosage for each patient as well as assist investigations into the mechanism of cisplatinum's action and its metabolism in humans. It may also play a role in determining intrinsic acquired resistance in humans [8].

The radionuclide, ^{195m}Pt (half life of 4.02 days which matches the biological half-life of many platinum agents) can be easily incorporated into the synthesis of platinum based cytotoxic agents. The advantages of ^{195m}Pt as a radiolabel, is that it emits penetrating γ radiation (66.8 keV (39%), 65.1 keV (22.5%), 75.7 keV (16.8%) 98.9 keV (11.4%)) which provides the capability of sensitive and direct detection of platinum at low concentrations in tissues and biochemical samples with little processing of sample and because it decays directly to stable ^{195}Pt which simplifies quantitation by gamma spectrometry or the more sensitive gamma counters [9].

The ability to produce platinum radiopharmaceuticals commercially has been limited by long and unreliable synthetic processes. ANSTO has developed a faster and reliable synthetic method for ^{195m}Pt-cisplatinum where the production times were dramatically reduced from up to 24 h to less than 3 h using a new process [10]. As ^{195m}Pt-cisplatinum was to be used in healthy individuals in the pilot study, it was important to ensure that a sterile product with the highest specific activity was produced. The synthesis procedure was further optimized by Necsa i.a. by using high flux positions in the SAFARI-1 reactor affording a higher specific activity product suitable for injection into humans.

MATERIALS AND METHODS

Irradiation of $^{194}PtCl_2$- metal

$^{195m}PtCl_2$ was produced at Necsa by a (n,γ) reaction, irradiating ~45 mg enriched $^{194}PtCl_2$ (>95%; ANSTO target material) for 200 h in SAFARI-1. Various parameters were investigated to enhance the specific activity of ^{195m}Pt-cisplatinum. These include using different irradiation positions in the reactor as well as limiting the cooling down period before synthesis.

Synthesis procedure for ^{195m}Pt-cisplatinum

The ANSTO patented synthesis procedure is schematically illustrated in Fig.1 and the synthesis for test, validation and patient runs was based there upon.

In more detail: The dark brown to black static powder received from the reactor was transferred into a centrifuge tube (S1) by adding 2 to 3 mL 0.1 M HCl in aliquots of 200 µl (step 1).
The suspension was sonicated for 3 min at 50 °C. To the mixture 400 µL 4 M KI was added to produce $[^{195m}PtI_4]^{2-}$ (step 2) followed by NH_3 and then reacted in the sonicator bath at 50 °C for 5 min (step 3). The mixture was centrifuged and the dark brown cis-$PtI_2(NH_3)_2$ precipitate (ppt1) was washed with 800 µl 0.01 M KI (step 7). After centrifuging the supernatant was discarded as waste into W1 (step 8). Aliquots of 100 µL 0.4 M $AgNO_3$ was added to the precipitate and vortexed between each addition (step 10). This mixture was centrifuged and the supernatant, a $[Pt(NH_3)_2(H_2O)_2]^{2+}$ solution and AgI precipitate were separated. The supernatant was transferred to a centrifuge tube labeled SN3 (step 11) while the remaining yellow precipitate (AgI) was washed with 800 µL 0.01 M sodium nitrate (step 12). After centrifugation the supernatant was added to tube SN3 (step 13). In order to remove the remaining silver from the supernatant 140 µL M HCl was added to form a AgCl precipitate, and then centrifuged (step 14). The supernatant in this step was transferred to tube SN5. Further precipitation was tested by adding 40 µL 0.1 M hydrochloric acid to tube SN5 (step 15). A white precipitate (AgCl) formed, which was centrifuged and the supernatant transferred to SN6. Concentrated HCl was added to SN6 or to SN5 if no further precipitation was found in step 15 (step 16). SN5/SN6 was placed in the ultrasonic bath for 5 min at 50 °C and transferred to an ice bath for 30 min to form ^{195m}Pt-cisplatinum precipitate.

The precipitate was filtered, washed with chilled ethanol and acetone, and dried under vacuum (step 17). The precipitate was weighed and dissolved in an appropriate amount of 0.9% saline to obtain a 1 mg/ml solution (step 18).

Radiochemical identification, chemical purity and chemical concentration

The radiochemical and chemical purity of the final products was determined using the Agilent 1200 series HPLC instrument with MWD UV and radioactive (Raytest Gabi Star) detectors and a Phenomonex Synergi 4u Hydro-RP 80A (250x4.6 mm 4 micron) column. Elution was carried out

with 0.9% saline as mobile phase, at room temperature for 20 min and a 0.5 ml/min flow rate. 20 µl aliquots of commercial cisplatinum standard (Platosin) and ^{195m}Pt-cisplatinum samples were injected during these analyses. Radiochemical identification was determined by comparing retention time and peak area of the cisplatinum standard (Platosin) and the product at 301 nm. The peak retention time of the HPLC-UV chromatogram and HPLC-radiometric chromatogram should correlate (5.2 ± 0.3 min) and the area under the peak should be >95% of the total area of the chromatogram. The chemical purity of the final product is also determined using the ratio of the HPLC-UV peak heights at absorbances of 301 and 365 nm (ratio $\lambda_{301}/\lambda_{365}$ = 5.2 ± 0.2 for 100% pure cisplatinum). The chemical concentration was determined at 301 nm against a standard calibration curve obtained using cisplatinum standards (Platosin 50-1000 ppm range) at the same wavelength.

Radionuclidic purity and radiochemical concentration

The radionuclidic impurities expected and analyzed for in the cisplatinum sample were ^{192}Ir, ^{194}Ir, ^{191}Pt, ^{197}Pt, ^{197m}Pt, ^{198}Au, ^{198m}Au, ^{199}Au, and ^{24}Na. A high purity germanium spectrometer Ortec Model GEM-10195 (10% relative efficiency; detector calibration with traceable europium-152 and barium-133 standards) was used. The spectrum for the final product was accumulated on the gamma spectrometer over an energy range of 60 to 1400 keV for 1000 seconds live time. Pulse processing was performed with a Canberra DSA1000 analyser and spectrum analyzed using Genie2000 suite of software.

Ethical considerations

The study was approved by the Ethics Committees of the University of Pretoria and Steve Biko Academic Hospital. Ten volunteer subjects participated after giving informed consent. The study was conducted in line with the Helsinki Declaration and the relevant guidelines of the University of Pretoria. All subjects were healthy according to medical history and physical examination as well as blood and urine analysis. They did not use any medication 14 days prior to nor during the study.

Urine and blood sampling

For all subjects, all voided urine from the time of injection until 144 h after injection was collected. The subjects were requested to collect urine before each emission scan and at home ad libitum. For each voiding, the urine was collected in a separate container, and the volume and time of voiding were recorded. For each void time, two 1 ml urine aliquots were sampled, and radioactivity was counted in a NaI (T1) counter (Cobra; Packard Instrument Co., Downers Grove, IL) after the counting efficiency of the system had been determined. The volume of the urine sample was determined by weight. The amount of radioactivity in the urine at each void time was expressed as percentage of the injected dose

(%ID). Blood samples (1 ml each) were taken at 5, 24, 72 and 144 h to determine the rate of clearance from the blood. All activities reported are decay corrected to the time of injection.

RESULTS AND DISCUSSION

Irradiation of $^{194}PtCl_2$- metal

The hydraulic position was originally viewed as the most suitable position for irradiation of a $^{194}PtCl_2$ target with a relative high flux of thermal neutrons while minimising fast neutrons and hence the activation of ^{35}Cl to ^{35}S, which is a non-preferred side reaction. However, according to the calculation model, a higher activity was expected in the core position with five times higher neutron flux capacity than in the hydraulic position. The measured $^{195m}PtCl_2$ starting activity doubled in the core position (see Table 1). The ^{35}S formed during irradiation was prevented from contaminating the end product since the synthesis procedure was developed to remove radionuclidic impurities, including the formed ^{35}S, during two phase reactions. The activated ^{35}Cl was also replaced by non-active chlorides with the addition of HCl. The advantage of a higher initial activity is a higher final product activity and therefore a better specific activity. The core position was hereby proved to be the optimal irradiation position to deliver the highest specific activity product for the clinical study.

Another aspect which was addressed in order to increase the specific activity was minimising the cooling down period after end of irradiation (Table 2). Specific activities were calculated in accordance to the specification of the radionuclidic purity which combines ^{195m}Pt and ^{197}Pt in the final product. The use of a high flux reactor position affords a higher specific activity product (15.9 ± 2.5 MBq/mg at end of synthesis) than previously reported (5 MBq/mg) [10]. The specific activity at end of bombardment is 21.4 ± 2.5 MBq/mg. Volunteers received between 108 and 126 MBq of radioactivity which is equivalent to 6.8 to 10.0 mg of carrier cisplatinum. Such high specific activities afforded a significant reduction (~50%) in the chemical dose of carrier cisplatinum which represents less than 10% of a typical chemotherapeutic dose given to patients.

Synthesis procedure for ^{195m}Pt-cisplatinum

Efficient stirring and accurate temperature control are essential for the formation of the intermediates and final product during this synthesis. More effective mixing and better temperature control for the completion of the first reactions were found to be important. The ultrasonic bath was used after adding $AgNO_3$ (step 10, Fig. 1) and transferring the supernatant to the centrifuge tube, SN3, for further reactions as described previously. The patented synthesis [10] of ^{195m}Pt-cisplatinum was

formulated for 60 mg $PtCl_2$ starting material. The ability to produce high specific activity $PtCl_2$ in the SAFARI-1 reactor enabled the use of less starting material in the range of 48 to 50 mg.

Radiochemical identification, chemical purity and chemical concentration

The patented method [10] used UV spectrometry to determine the chemical purity and concentration and HPLC only to confirm chemical purity retrospectively. These quality control (QC) methods were improved with the use of the HPLC-UV-radiometric detector combined system. This enabled the analyst to determine the chemical purity and concentration on one instrument setup and additionally the radiochemical identification could be done to strengthen quality control of the final product. The QC analyses was therefore more efficient, faster and accurate since one 20 µl HPLC injection could be used for multiple QC results. A further advantage was that the results of these samples were available at the time of product release.

The chemical purity and radiochemical purity of ^{195m}Pt-cisplatinum were determined by comparing the retention times and area under the peak of the HPLC-UV and radiometric peak of the cisplatinum reference standard, Platosin to the synthesized product.

For example in patient run P3, the same HPLC-UV retention time of 5.46 min was found for Platosin, the reference standard of cisplatinum, and the synthesized ^{195m}Pt-cisplatinum (Fig. 2), thus confirming that cisplatinum was synthesized. The corresponding radiometric peak at 5.56 min confirmed that cisplatinum was labeled with ^{195m}Pt (Fig.3). The 1.8% time delay in the retention time of the radiometric peak was due to the length of the tubing between the two detectors. The area under the peak was 100% for the UV chromatogram at 301 nm as well as the radiometric chromatogram indicating that the chemical and radiochemical purity according to the HPLC and radiometric detector system is within the specifications.

The chemical purity was also confirmed by determining the height ratio between cisplatinum detected at 301 nm and transplatinum detected at 365 nm (Fig. 2). The ratio of 5.4 was within the specification of $\lambda_{301nm}/\lambda_{365nm} = 5.2 \pm 0.2$ and therefore ensured the optimum performance of cisplatinum as an anticancer drug during treatment since transplatinum has no anticancer properties.

The concentration of the P3 solution was determined as 1.004 mg/ml (using the standard Platosin calibration curve – not shown) and corresponded to the specification of 1.0 ± 0.1 mg/ml. The concentration is used to determine the specific activity and the chemical dose of cisplatinum administered to the volunteer.

The chemical purity according to chemical ratio (cis vs trans) were within the specifications for all the production runs indicating a pure product.

Radionuclidic purity

Radionuclidic identification of the final product was done to determine the purity of the product and identify the impurities. The irradiation of $^{194}PtCl_2$ can produce several impurities including ^{192}Ir, ^{194}Ir, ^{197}Pt, ^{197m}Pt, ^{198}Au and ^{199}Au. Through the use of two phase reactions these impurities were mostly excluded in the end product having radionuclidic purity greater than 95% (Table 3).

The relative ratio of ^{195m}Pt: ^{197}Pt ($t_{½}$ = 20 h) decreased when the time of synthesis after irradiation was shorter (<46 h) as used for P3, P4 and P5 since ^{197}Pt, with a shorter half-life than ^{191}Pt and ^{192}Ir, would still be present at high levels. ^{192}Ir and ^{191}Pt are produced at low levels during irradiation as reported in Table 3. It was therefore not always quantifiable with the gamma spectrometer. Minimising the cooling down period to increase the specific activity was calculated in accordance to the radionuclidic purity specifications. The possible impurity levels of ^{192}Ir, ^{194}Ir, ^{191}Pt, ^{197m}Pt, ^{198}Au, ^{198m}Au, ^{199}Au, and ^{24}Na, did not influence the radionuclidic purity of the product with a minimum cooling of 34 h before synthesis started since the synthesis route include removal of these impurities. The only other isotope that showed significant levels was ^{197}Pt which increased to 7% with a shorter cooling down period. ^{197}Pt only emits low energy gammas (at low percentages) and therefore does not interfere with the imaging of ^{195m}Pt-195. Furthermore its half-life is 19.9 h which means it quickly decays after time of injection. The radionuclidic and radiochemical purity of the final product for all the experiments was within the specifications of more than 95% cisplatinum for the radiochemical purity and more than 95% [^{195m}Pt + ^{197}Pt] for the radionuclidic purity.

GMP compliance and human studies

According to normal GMP principles three consecutive validation runs were completed prior to patient production runs. Compliance was checked against the criteria listed in Table 4. Samples V4 to V7 met all criteria and based on this patient production runs were undertaken. The five production runs P1-P5 (for two volunteers each) were thereafter completed (also consecutive) and met all the release criteria of the product and were released by the responsible pharmacists for injection into the volunteers. Table 5 indicates the study protocol that was followed indicating the blood sampling and urine collection points. The 66.8 keV (39%) gamma emission was used in the SPECT scintigraphy but the results thereof do not from part of this publication and will be reported in a nuclear medicine journal. The significantly lower concentration of carrier cisplatinum (due to the high specific activity) resulted in a well-tolerated product and no adverse reactions were recorded in the trial.

Urine and blood sampling

From Fig. 4 it can be seen that little or no activity was found in volunteer P3-b and P5-b's 5 h urine sample but high values in the 24 h sample. This anomaly can best be described either by sample exchange or that voiding took place just after the 5 h period and both are considered outliers. Volunteer P-5a had overall low urine excretion, only 4.6% of the injected dose. This is also considered an outlier and most likely due to misplacement of the sample or not voiding in the designated container at first.

If the seven remaining volunteers are considered (see data in Table 6) there was an initial rapid excretion of ^{195m}Pt via urine. At 5 h after the injection on average 27.53 ± 5.27% (range 18-35%) of the injected ^{195m}Pt had been excreted. After 6 days post injection on average 37.35 ± 5.81% (range 29-43%) of the total injected dose was excreted. Two conclusions can be drawn from this. The two standard deviations are large, 19 and 16% of the averages respectively for the values at 5 h and the total over the 6 days period. These high standard deviations illustrate the great variance between the volunteers. The second conclusion is that the around 74% of the total dose that is excreted over the 6 day period is excreted in the first 5 h. This means that the collection for the first 5 h is representative of a volunteer's excretion profile. It is therefore imperative that patients are requested to void before and up to 5 h. The urine excretion found in this study compares well with that of literature values for patients where between 20 and 30% of the injected dose is found to be excreted in the first 4 h [11] while anything between 20 and 80% can be excreted in total [12].

The ^{195m}Pt-activity in the blood pool (Fig. 5 and Table 7) is the highest at 5 h viz. 8.45 ± 3.07% of the injected dose (range 5-14%) and then gradually decreases over the 6 day period. Therefore 86-95% of the activity (and therefore ^{195m}Pt-cisplatinum) had already cleared from the blood pool at 5 h, again indicating that the first five hours determine the fate of the cisplatinum in a particular volunteer. Furthermore, from the urinary excretion and blood pool activity data at 5 h a substantial variance (38% when compared to the average) in the ten volunteers is visible underlining the potential of the ^{195m}Pt-cisplatinum radiopharmaceutical to be used as a tool to optimize (individualize) the intended dose to be used for patients identified for cisplatinum treatment.

The significance of the five hour values is that for future use the patients can be injected in the morning and kept in the Nuclear Medicine ward where their urine collection can take place and be checked. A single SPECT scan and blood sampling at 5 h will conclude the study where after the patient can return home or to the refereeing oncology ward.

CONCLUSION

It is concluded that we were successful in producing a new high specific activity ^{195m}Pt-cisplatinum in the high flux region of SAFARI-1. The ^{195m}Pt-cisplatinum biodistibution and clearance was monitored in healthy volunteers in a phase 0 clinical trial. The product was well tolerated by all volunteers. The radioactivity associated with urine excretion and in blood plasma between volunteers varied significantly (over two standard deviations) indicating that individuals were metabolising the ^{195m}Pt-cisplatinum differently. The high rate of clearance of the agent (within five hours p.i) indicates the ^{195m}Pt-cisplatinum imaging agent could be used as a monitor to more accurately determine dosage for an individual. Such results support the applicability of the ^{195m}Pt-cisplatinum as companion diagnostic agent or theranostic imaging agent for patients undergoing platinum chemotherapy. Further, the information required to determine accurate dose for an individual, could be obtained within day one of the Nuclear Medicine procedure.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. Influence of the hydraulic and core irradiation position on ^{195m}Pt activity

Irradiation position	Mass $PtCl_2$ (mg)	^{195m}Pt activity (3 days decay, MBq)	
		Calculated	Measured*
Hydraulic	43.3	308.8	222.5
Core	44.1	583.0	581.0

Note: *An average 25% difference between calculated and measured activities after irradiation can be contributed to uncertainties and therefore over estimation of the values during calculations. These uncertainties could be due to the influence of the displacement of water in the reactor which was excluded in the calculation model and the higher total neutron flux used during the modeling.

Table 2: Irradiation conditions and Specific activity

Validation (V)/ Patient (P) runs	Actual irradiation time (h)	Cooling down period (h)	Specific activity EOS MBq/mg	Specific activity EOB MBq/mg
V2	199.1	42	13.5	21.5
V2-1	199.1	68	9.75	19.1
V3	198.9	55	14.4	19.3
V4	190.4	55	14.7	21.4
V5	191.4	55	13.4	19.7
V6	206.6	47	14.9	22.0
V7	198.1	55	13.5	18.8
P1	176.0	54	14.3	21.1
P2	195.4	52	12.7	18.7
P3	198.3	34	17.2	21.9
P4	204.3	46	16.1	22.4
P5	196.0	41	19.2	25.6

Table 3: Radionuclidic analyses results for ^{195m}Pt-cisplatinum

Runs	Radionuclidic purity (%)				
	^{195m}Pt	^{197}Pt	^{191}Pt	^{192}Ir	Reported values*
V2	95.4	4.5	0.1	<0.01	99.8
V2-1	97.3	2.6	0.1	<0.01	99.8
V3	95.0	4.8	0.2	<0.003	99.8
V4	95.6	4.2	0.2	0.001	99.8
V5	95.7	4.1	0.2	0.001	99.8
V6	93.5	6.3	0.2	ND	99.8
V7	94.0	5.8	0.2	ND	99.8
P1	94.6	5.2	0.2	ND	99.8
P2	95.5	4.3	0.2	ND	99.8
P3	92.3	7.6	ND	0.1	99.9
P4	94.3	5.6	ND	0.1	99.9
P5	93.5	6.4	ND	0.1	99.9

ND = not detectable

*^{195m}Pt+^{197}Pt ≥ 95% is defined as required for the product to meet specification

Table 4. Parameters checked on the batch sheet for release of GMP compliant ^{195m}Pt-cisplatinum

Property	Specification
Physical form	Greater than 10 ml of clear, colourless solution
Chemical form	A solution of [^{195m}Pt]-cisplatinum in an isotonic solution of sodium chloride
pH	4.5 - 8.5
Radiochemical purity	Greater than 95% as radioactive ^{195m}Pt
Radioactive concentration	Not more than 20 MBq/ml at activity reference time
Radionuclidic purity	Greater than 95% [^{195m}Pt + ^{197}Pt]
Chemical purity	Complies with $\lambda_{301nm} / \lambda_{365nm} = 5.2 \pm 0.3$
Chemical concentration	1.0 ± 0.1 mg/ml
Specific activity	≥3 MBq/mg at activity reference time
Residual Solvent	Acetone less than 100 ppm
Sterility	All aseptic fill parameters of the batch meet the validation acceptance criteria
Endotoxin	<175 EU
Expiry	5 days after activity reference time
Storage	Store below 30 °C

Table 5. Healthy volunteer study protocol.

Day_{-4} Friday	Day_0 Tuesday	Day_1 Wednesday	Day_3 Friday	Day_6 Monday
10h00: Volunteer examination and history; complete forms Consent + information forms Renal function; Tc-DTPA	$t_{0-2\ h}$: • Examination • Toxicity • FBC + diff • U&E/LFT	$t_{0+24\ h}$ = 11h00 • Urine (collected from $t_{0+5\ h}$ to $t_{0+24\ h}$)	$t_{0+72\ h}$: = 11h00 • Urine (collected from $t_{0+24hrs}$ to $t_{0+72hrs}$) • Blood	$t_{0+144\ h}$: • Examination • Toxicity • FBC + diff • U&E/LFT • Blood
	t_0 = 11h00 • [^{195m}Pt] cisplatinum injection • 30 min dynamic scan post injection with static scans at 90 min	$t_{0+24\ h}$ • Whole body scan • Statics scans	$t_{0+72\ h}$ SPECT • Whole body scan	$t_{0+144\ h}$ • Whole body scan • Statics scans
	$t_{0+5\ h}$: • SPECT-(shoulders to pelvis) • Whole body scan • Static scans • Urine • Blood			

Table 6. % of injected dose excreted in the urine for seven volunteers, the average and standard deviation between them.

Volunteer	P1-a	P1-b	P2-a	P2-b	P3-a	P4-a	P4-b	**Average**	**Std dev**	**%SD**
5 h	24.2	28.76	18.11	35.12	27.56	29.81	29.14	27.53	5.27	19
24 h	1.57	4.08	5.35	4.47	2.95	2.58	1.43	3.20	1.49	46
48 h	1.83	4.88	3.55	2.28	3.98	3.95	3.6	3.44	1.05	31
72 h	1.64	4.91	1.6	0.53	3.02	0.23	3.75	2.24	1.72	77
96 h			1.01	0.41	1.42	0.21	3.52	1.31	1.32	101
Total	29.24	42.63	29.62	42.81	38.93	36.78	41.44	37.35	5.81	16

Table 7. Percentage of injected dose in the blood pool at various time points for all volunteers, the average and standard deviation between them.

Volunteer	P1-a	P1-b	P2-a	P2-b	P3-a	P3-b	P4-a	P4-b	P5-a	P5-b	Average	Std dev
5 h	5.31	5.71	10.84	8.34	13.79	13.68	7.41	7.39	5.3	6.74	8.45	3.07
24 h			3.25	6.15	5.72	5.93	3.83	4.44	4.24	4.81	4.79	0.98
72 h	4.57	4.21	6.84	4.7	5.03	4.58	3.96	4.21	2.24	4.19	4.45	1.07
144 h	4.47	4.29	4.37	2.54	3.91	3.79	2.32	2.51	2.29	3.74	3.42	0.86

Captions to Figures

Figure 1. Flow diagram for synthesis of [^{195m}Pt]-cisplatinum

Figure 2: HPLC-UV chromatogram of ^{195m}Pt-cisplatinum (sample P3) for chemical purity

Figure 3: HPLC-Radiometric chromatogram of ^{195m}Pt-cisplatinum (sample P3) for radiochemical purity

Figure 4. % of injected dose excreted in the urine for all volunteers

Figure 5. % of injected dose in the blood pool at various time points for all volunteers

Fig 1

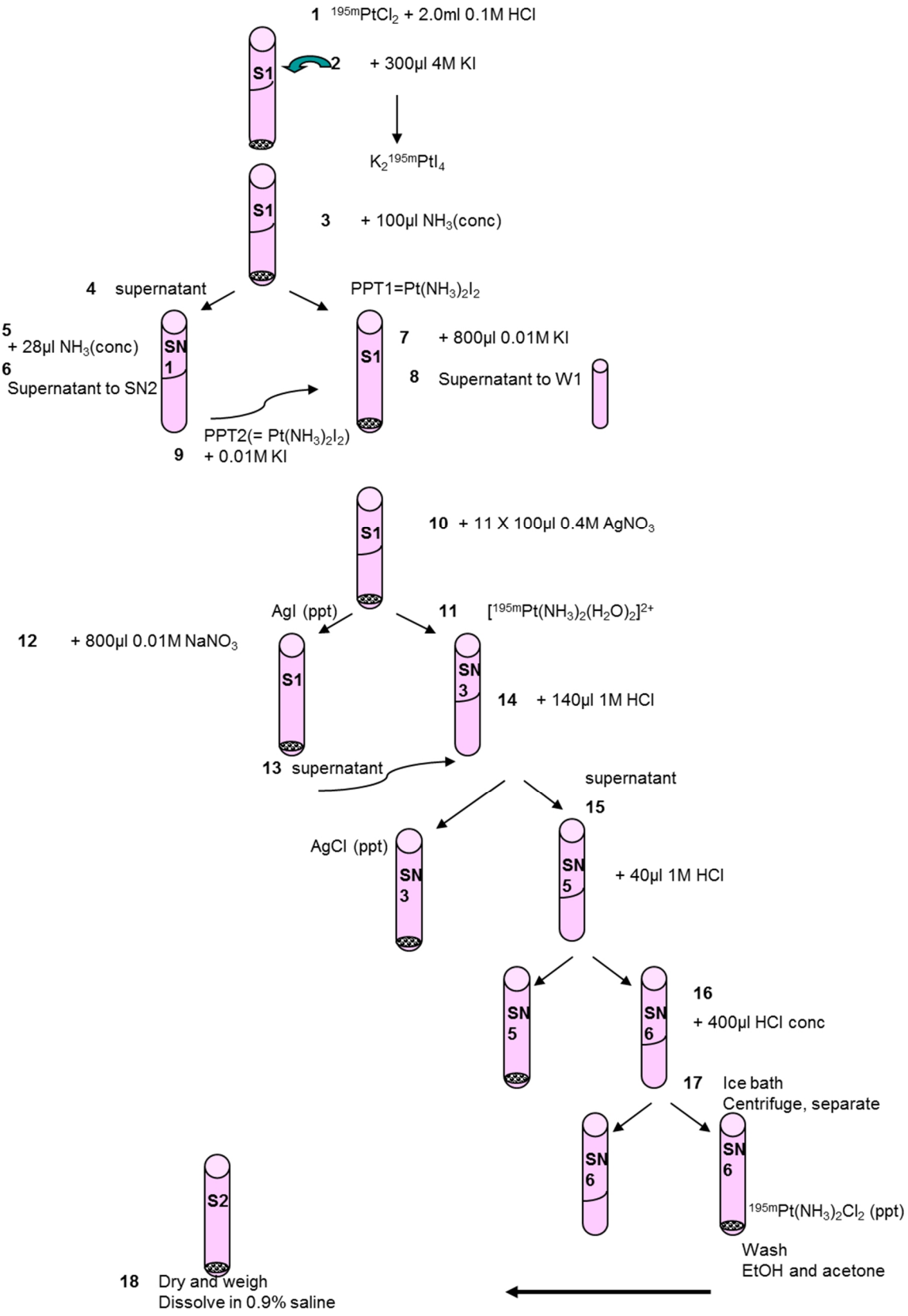
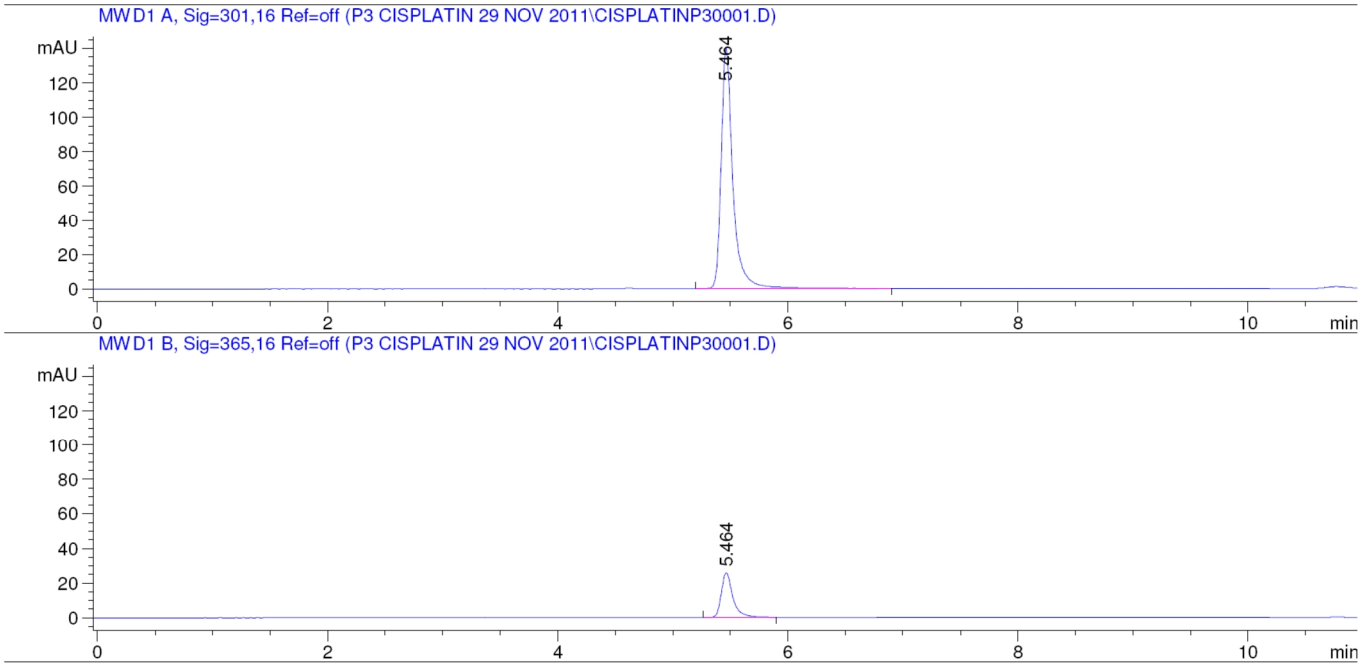

1 $^{195m}PtCl_2$ + 2.0ml 0.1M HCl
S1
2 + 300µl 4M KI
$K_2{}^{195m}PtI_4$
S1
3 + 100µl NH_3(conc)
4 supernatant
PPT1=$Pt(NH_3)_2I_2$
5
+ 28µl NH_3(conc)
6
Supernatant to SN2
SN 1
S1
7 + 800µl 0.01M KI
8 Supernatant to W1
PPT2(= $Pt(NH_3)_2I_2$)
9 + 0.01M KI
S1
10 + 11 X 100µl 0.4M $AgNO_3$
AgI (ppt)
11 $[^{195m}Pt(NH_3)_2(H_2O)_2]^{2+}$
12 + 800µl 0.01M $NaNO_3$
S1
SN 3
14 + 140µl 1M HCl
13 supernatant
supernatant
15
AgCl (ppt)
SN 3
SN 5
+ 40µl 1M HCl
SN 5
SN 6
16
+ 400µl HCl conc
17 Ice bath
Centrifuge, separate
SN 6
SN 6
$^{195m}Pt(NH_3)_2Cl_2$ (ppt)
Wash
EtOH and acetone
S2
18 Dry and weigh
Dissolve in 0.9% saline

Fig 2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mAU
120
100
80
60
40
20
0
5.464
0
2
4
6
8
10
min
MWD1 B, Sig=365,16 Ref=off (P3 CISPLATIN 29 NOV 2011\CISPLATINP30001.D)
mAU
120
100
80
60
40
20
0
5.464
0
2
4
6
8
10
min

Fig 3

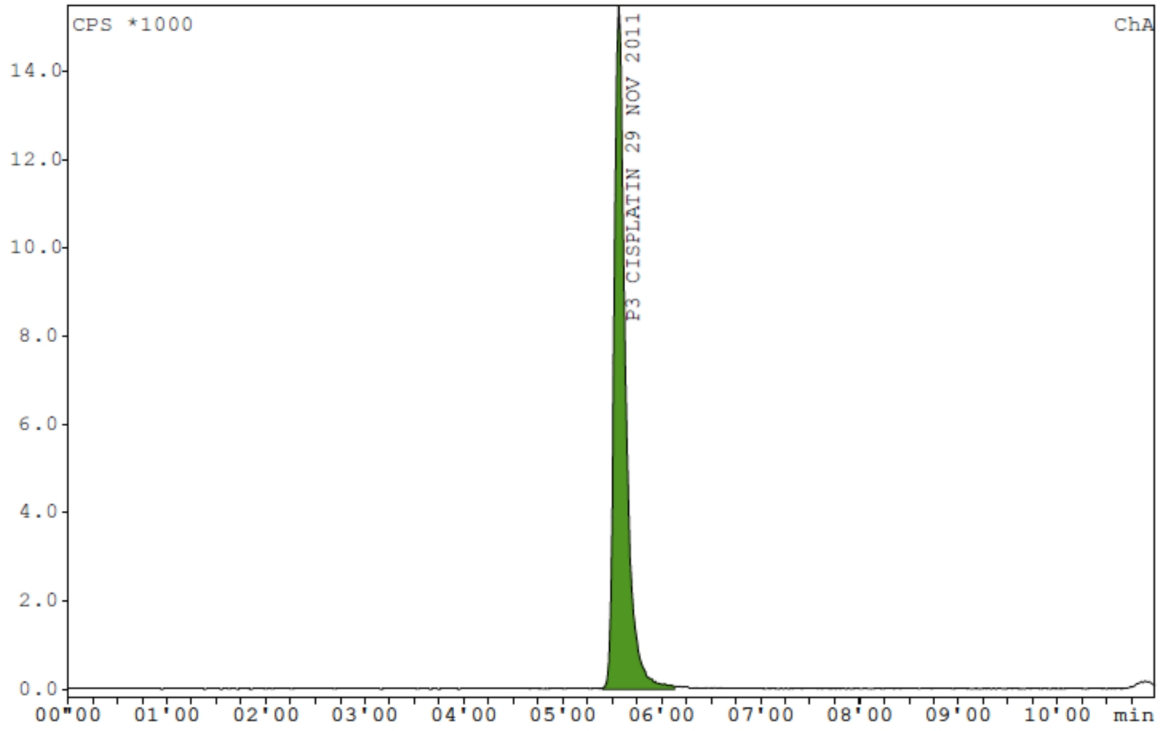

CPS *1000
ChA
P3 CISPLATIN 29 NOV 2011
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
00'00
01'00
02'00
03'00
04'00
05'00
06'00
07'00
08'00
09'00
10'00
min

Fig 4

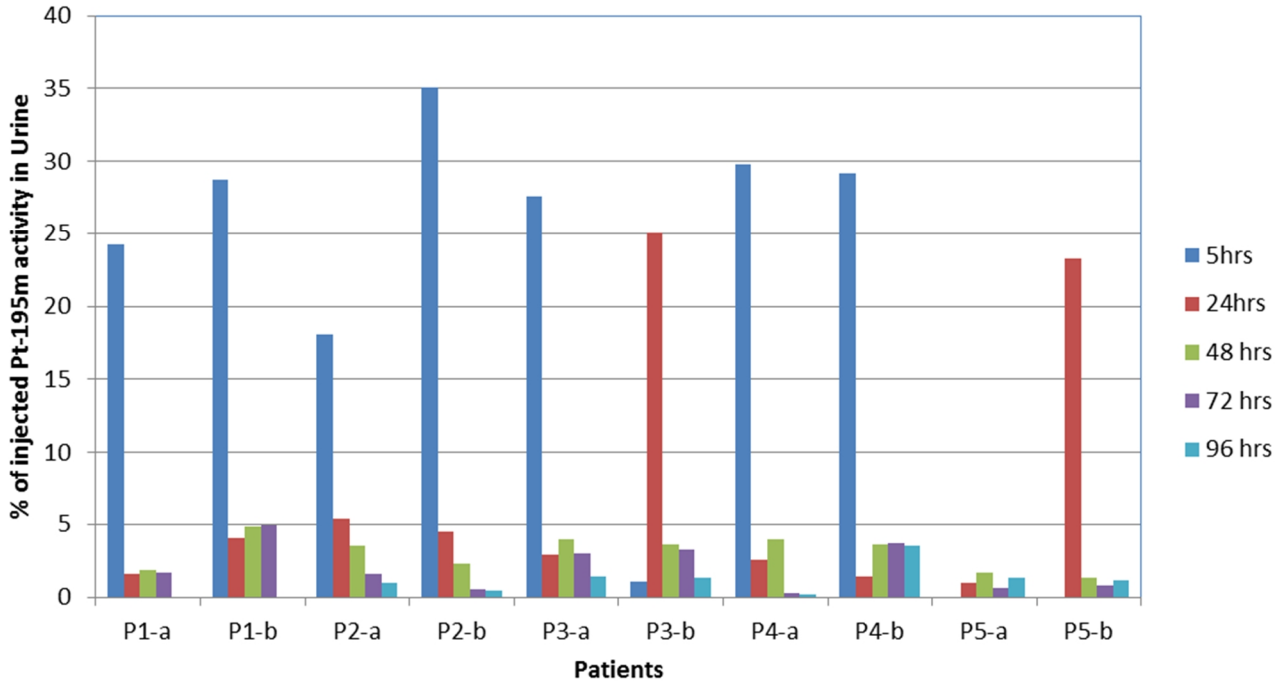
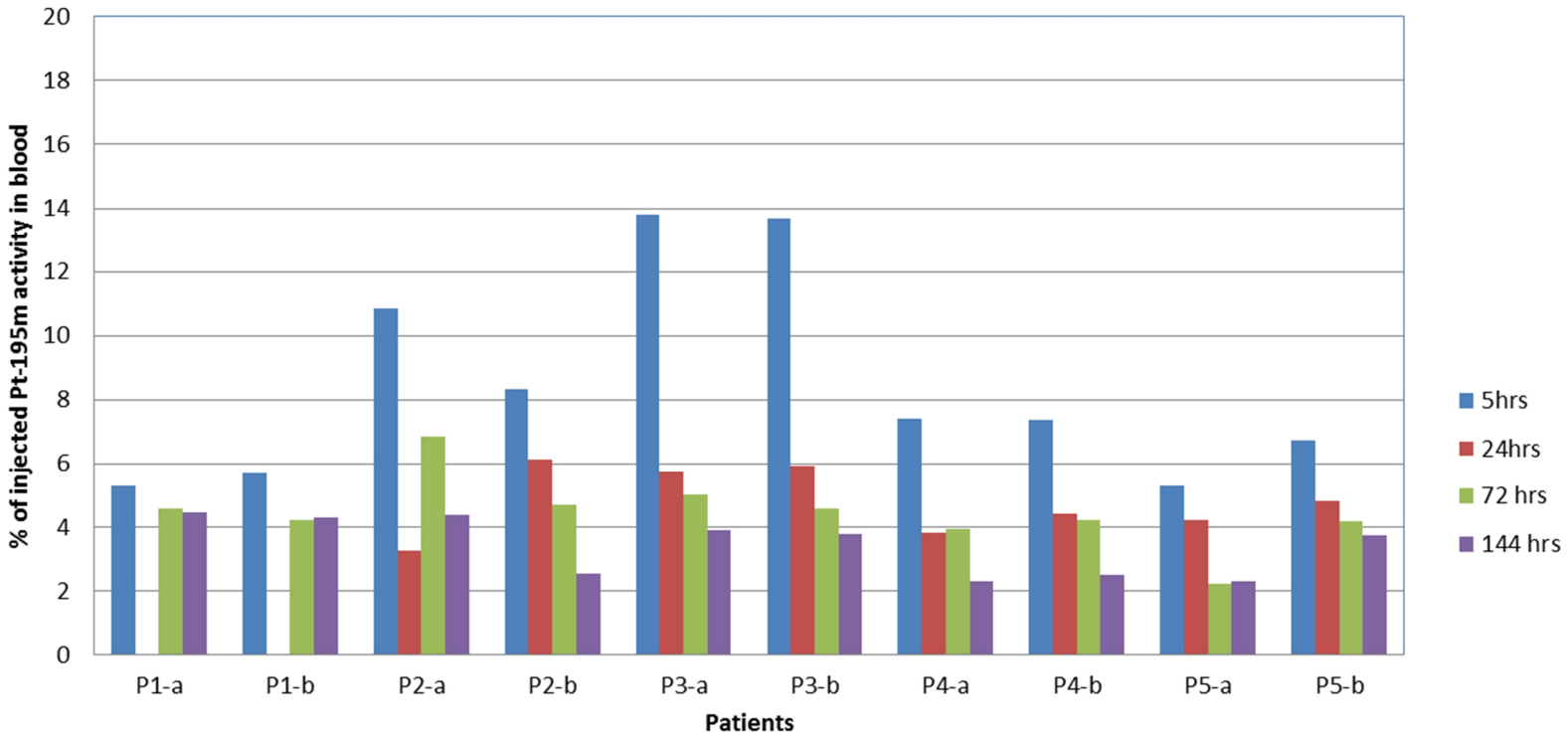

40
35
30
25
20
15
10
5
0
% of injected Pt-195m activity in Urine
P1-a
P1-b
P2-a
P2-b
P3-a
P3-b
P4-a
P4-b
P5-a
P5-b
Patients
5hrs
24hrs
48 hrs
72 hrs
96 hrs

Fig 5


20
18
16
14
12
10
8
6
4
2
0
% of injected Pt-195m activity in blood
P1-a
P1-b
P2-a
P2-b
P3-a
P3-b
P4-a
P4-b
P5-a
P5-b
Patients
5hrs
24hrs
72 hrs
144 hrs