8. Development and application of an optimised and validated LC-MS/MS method

8.1. Introduction

LC with tandem MS/MS detection is a powerful technique that affords the quantitation of low picogram (on-column) amounts of several compounds in parallel. It is considered the method of choice for quantitation of compounds within diverse biological matrices. [150] LC-MS/MS detection in multiple reaction monitoring (MRM) mode gives the appearance of perfect selectivity because only the specific transitions of the analytes of interest are monitored (Q1>Q3). Viewing such an extracted ion chromatogram may give one the false sense that sample clean-up is not required; however co-eluting, undetected matrix components may suppress or enhance the ion intensity of analytes and thereby have a detrimental effect on the accuracy and precision of an assay. [151] It is therefore of importance to routinely evaluate the effect of the sample matrix on assay validity. Excipients, particularly surfactants used in drug formulation may themselves be a source of these matrix effects.

Pre-clinical development of drugs requires that a reliable bioanalytical method be available with sufficient sensitivity to quantitate drug concentrations within small samples facilitating pharmacokinetic studies in mice. In addition, the same analytical method may serve to determine compound stability at various storage conditions as well as to quantitate drug loading within various dosage forms.

Linearity, accuracy and precision, recovery as well as matrix effects were investigated as validation parameters. Matrix effects from several tissue samples were investigated quantitatively through comparison of the calibration standard slopes and mean peak areas (at equivalent nominal concentrations) of matrix matched and solvent based standard sets. Initial method development was supported by post-column, constant analyte infusion experiments that were used to qualitatively observe the matrix effects on individual analytes.

The objective was to develop a general sample clean-up method applicable to diverse sample matrices and to quantitate both PTX and B663 using a simple, rapid and validated LC-MS/MS method. This method was applied to the biopharmaceutical analysis of Riminocelles and Taxol so as to assess the influence of formulation upon the disposition of PTX.

8.2. Materials

Deionised water (~18MΩ) was produced from the municipal water supply after processing by an ELGA purification unit (ELGA, Wycombe, UK). Ammonium hydroxide solution was purchased from Merck (Darmstadt, Germany). Mass spectrometry grade methanol (MeOH) and formic acid (HCO_2H) were purchased from Fluka Analytical (Buchs, Switzerland). Methyl *tert* butyl ether (MtBE) was purchased from Sigma Aldrich (St Louis, MO, USA).

Paclitaxel (PTX) and its internal standard Docetaxel (DTX) were purchase from Sigma Aldrich (St Louis, MO, USA). Clofazimine (B663) was supplied by Dr J.F. O' Sullivan, (Laboratories of the Medical Research Council of Ireland, Trinity College, Dublin, Republic of Ireland).

DSPE PEG 2000 and LIPOID S 75-3 samples were generously provided by Lipoid GmbH (Ludwigshafen, Germany) and were used to co-formulate PTX and B663 within mixed lipopolymeric micelles (Riminocelles) at a final concentration of 1 mg/ml and 2.5 mg/ml respectively. Concentrated Taxol 6 mg/ml was prepared in house with 50:50 (v/v) Cremophor EL: ethanol. Prior to administration, Taxol was diluted to 1 mg/ml with sterile saline 0.9% (w/v).

8.3. Methodology

8.3.1. Stock solutions, calibration standards, quality control and recovery samples

Numerous 0.5 mg/ml stock solutions of the individual analytes (PTX and B663) and 0.2 mg/ml stock solutions of the internal standard (DTX) were prepared in MeOH. The solutions were aliquoted and stored in microreaction vials at -20°C.

Working solutions of each analyte were separately prepared on each day of experimentation from stock solutions to 5000 ng/ml before the two analytes were mixed in equal volumes and further diluted with MeOH to produce a concentration of 1250 ng/ml each for both analytes. Further working solutions were prepared through appropriate serial dilution into six clean tubes.

These six working solutions were diluted 4:1 with 1000 ng/ml DTX to prepare appropriate volumes of the following final (analyte equivalent) MeOH calibration standards: 1.95, 7.81, 31.25, 125, 500, 1000 ng/ml (all with a final constant DTX concentration of 200 ng/ml).

Matrix matched calibration standards for each tissue (liver, kidney, fat, spleen and plasma) were prepared by reconstituting (with the aid of sonication) dried drug naïve control tissue sample extracts within 500 µl of the various calibration solutions.

Three independent quality control (QC_{100}) samples of 100 ng/ml in MeOH were prepared on each day of experimentation. In addition, recovery samples (10, 250 and 500 ng in extraction solvent (final volume in vial 500 µl , therefore 20, 500 and 1000 ng/ml) were spiked into each of the blank tissue matrices (from drug naïve control mice) prior to extraction and comparing recovered analyte concentration versus expected analyte concentration and reported as a percent recovery.

Each day of analysis therefore included: triplicate runs of newly prepared blank standards; triplicate runs of matrix matched standards; triplicate quality control

(QC_{100}) runs and triplicate runs of three recovery samples from each of the respective matrices at three nominal concentrations (20, 500, 1000 ng/ml) spanning the calibration range.

Replicate sets of known concentrations were run before and after the analysis of the unknown samples thereby allowing the assessment of analyte stability over the time required for sample processing.

8.3.2. Sample preparation

Liquid-liquid extraction with MtBE was used for mouse plasma sample preparation. In brief, 50 µl of each plasma sample was aliquoted into a clean 2 ml microcentrifuge tube. For recovery samples, known amounts of analytes (in MeOH) were spiked pre-extraction into control (drug naïve) plasma to facilitate recovery assessment. Half a millilitre of MtBE was added and the mixture thoroughly vortex mixed, then centrifuged (Heraeus Instruments, Megafuge 1.0R) at 15 000 x *g* for 10 min at 10°C and finally placed in -70°C to freeze the aqueous layer. The upper, fluid ether layer was decanted into clean microcentrifuge tubes. This procedure was repeated thrice, each time pooling the organic layer that was subsequently dried using a Centrivap vacuum centrifuge concentrator with cold trap (Labconco) and reconstituted with the aid of sonication bath in 400 µl MeOH (with or without standards depending on whether the sample was an unknown or a matrix matched standard) and 100 µl IS solution.

Concerning different mouse tissues, representative sections (target 20 mg, range 15-25 mg) were accurately weighed into clean microcentrifuge tubes. After adding 1 ml of a 95% MeOH in 1% HCO_2H solution (with addition of analyte spikes for recovery samples), the mixtures were homogenized using an ultrasonicator (Biologics, Inc. Model 3000) with a power output of ~ 150 watts at 20 kHz (80% pulse - to ensure that the samples did not overheat) for 2 min using a stepped titanium micro tip (3.81 mm diameter). The samples were centrifuged at 15 000 x *g* for 10 min at 10°C, the supernatant was carefully removed, transferred to new tubes and dried in a Centrivap vacuum centrifuge to approximately 200 µl before

being diluted 1:3 with 1% HCO_2H in water to ensure full analyte ionization and loaded onto a SPE cartridge (BondElut Plexa 60 mg, 1 ml) that had been sequentially conditioned and equilibrated with 1 ml of MeOH and H_2O respectively. The cartridges were washed with 1 ml of 40% MeOH before the analytes were eluted with 95% MeOH in 1% HCO_2H followed by a 50 µl MeOH slug to clean the frit. Similar to the plasma procedure the cleaned extracts were dried using a Centrivap vacuum centrifuge concentrator and reconstituted in 400 µl MeOH and 100 µl IS solution. Thereafter all samples were transferred to clean, clear 2 ml vials with snap caps (Chromacol, Trumbull, USA) before injection of 10 µl into the LC-MS/MS system for analysis.

Various matrix blanks from different tissue taken from drug naïve control mice were likewise processed to facilitate qualitative evaluation of matrix effects over the course of the chromatographic run through post column, direct infusion experiments. Through such means, zones of matrix interferences with the chromatographic run were identified.

8.3.3. Chromatographic conditions

An Agilent 1100 series HPLC consisting of a binary pump, vacuum degasser and an autosampler was used. Baseline chromatographic separation was achieved with an Apollo C18 (150 mm x 4.6 mm), 5 µm column protected by a C18 Security Guard cartridge. As isocratic mobile phase of 95% MeOH in 0.1% HCO_2H (pH adjusted with ammonia hydroxide to 3.5) was used at a flow rate of 1 ml/min and column temperature of 40°C. The total run time was 5 min.

8.3.4. Mass spectrometric conditions

An AB Sciex 4000 QTrap mass spectrometer (Applied Biosystems/ MDS Sciex) with a Turbo “V” electrospray ionization (ESI) source was operated in positive ion mode using Multiple Reaction Monitoring (MRM).

Compound dependent ionization parameters were quantitatively optimized for each drug (in mobile phase) through direct injection into the ESI source using a Harvard syringe pump at a constant flow rate of 10µl/min.

Compound independent parameters that remained constant were as follows: Curtain gas, 25 psi; Ion spray voltage, 5500 V; Ion source temperature, 450℃; Ion source gas 1, 35 psi; Ion source gas 2, 40 psi; Collision gas, medium; Entrance potential, 10 V.; Collision cell exit potential, 12 V.

All modules of the complete LC-MS/MS system were centrally operated by Analyst software, version 1.5.2 (Applied Biosystems/MDS Sciex) facilitating data acquisition and processing.

8.3.5. Validation procedures

Validation of the bioassay was performed with due consideration to FDA guidelines for Bioanalytical Methods Validation [152].

Linearity was assessed in various sets of MeOH and various matrix matched standards over the concentration range of 1.95-1000 ng/ml. Intra-day precision and accuracy were determined for both MeOH standards and for each specific tissue matrix by analysing replicates of three nominal concentrations (1.95, 31.25 and 500 ng/ml). Inter-day precision and accuracy was determined for three nominal concentrations in MeOH on 5 separate days. Percent coefficient of variance (% CV) was used as the measure of precision .The percent accuracy (% Accuracy) was determined by comparison of the measured concentration with known nominal concentration. Deviations greater than ±15% away from the expected nominal concentration indicate unacceptable accuracy and precision.

Extraction recovery (%) of the two analytes was assessed at three levels (20, 500, 1000 ng/ml) in triplicate by spiking blank matrix prior to sample processing and expressing recovery as mean percentage ± SD of the expected concentration.

Matrix effects were evaluated in three ways: firstly the slope of calibration curves for a particular matrix were compared with the slope of a blank matrix (solvent) calibration curve and expressed as the slope factor; secondly through determination of percentage matrix effect (% ME) by comparing the mean peak area of matrix matched standards with the peak area of blank matrix standards at various nominal concentrations; thirdly, matrix effects were qualitatively observed through direct post-column infusion of the analytes whilst injecting relevant reconstituted drug naïve (blank matrix) extracts using the LC autosampler.

8.3.6. Application in pharmacokinetic study

The optimized LC-MS/MS analytical method was used to assess the pharmacokinetics and tissue distribution of the novel co-formulation in parallel with an equivalent PTX dose (10 mg/kg) of Taxol. Fifty five female BALB/c mice were acclimatized to laboratory setting for a week prior to experimentation. The animals were fed standard rodent feed and water *ad libitum.* Prior to experimentation, mice were randomly assigned to eleven cages of five mice each. The two PTX formulations were administered to five mice at each time point (30 min, 1, 3, 6 and 24 h). Before IV administration of the formulation via the lateral tail vein, each animal was weighed and an appropriate dose calculated. Precise timing of when the dose was given and when the animal was euthanised was documented in study monitoring sheets. Saline was administered to 5 mice who were euthanised after 24 h serving as the source of blank drug naïve matrix.

At euthanisation, blood samples were drawn via cardiac puncture from isoflurane anaesthetized mice into heparin treated paediatric blood collection tubes. Blood samples (~500 µl) were immediately centrifuged, plasma harvested, appropriately labelled with group, time and dose and stored at -70℃. Organs (liver, spleen, kidney and adipose tissue) were dissected out, labelled and stored at -70ºC until analysed.

This study complied with the SANS 10386:2008 guidelines for research animals with ethical approval given by the Animal Use and Care Committee (AUCC) of the University of Pretoria (Project # H10-09).

The study schedule followed is summarised in Appendix C.

8.4. Results and discussion

8.4.1. Chromatography and Mass spectrometry

Infusion based quantitative optimization experiments were conducted to achieve the greatest possible signal. Optimized MRM transition parameters for each of the compounds attained in positive ion mode are presented in Table 8.1.

Baseline chromatographic separation of the two analytes was achieved using a simple isocratic mobile phase in 5 min. Due to the similarity in structure, the internal standard, DTX and PTX were shown to co-elute with a retention time (RT) of 1.76 min. The isocratic method favouring a fast run time (without the necessity of column re-equilibration) was seen as favourable over achieving resolution between the taxanes having different molecular masses. No fragment cross talk (Q3) was evident between the co-eluting taxane (PTX and DTX) fragments due to the different characteristic fragment ions selected. A MRM dwell time of 50 ms allowed for sufficient data points to be acquired across the respective ~30 second peak widths of PTX and DTX without any loss in sensitivity.

B663 eluted with a retention time of 3.61 min. A representative MRM chromatogram is shown in Figure 8.1. Numerous studies have reported MS detection of PTX. This is the first study using MS for the detection of B663.

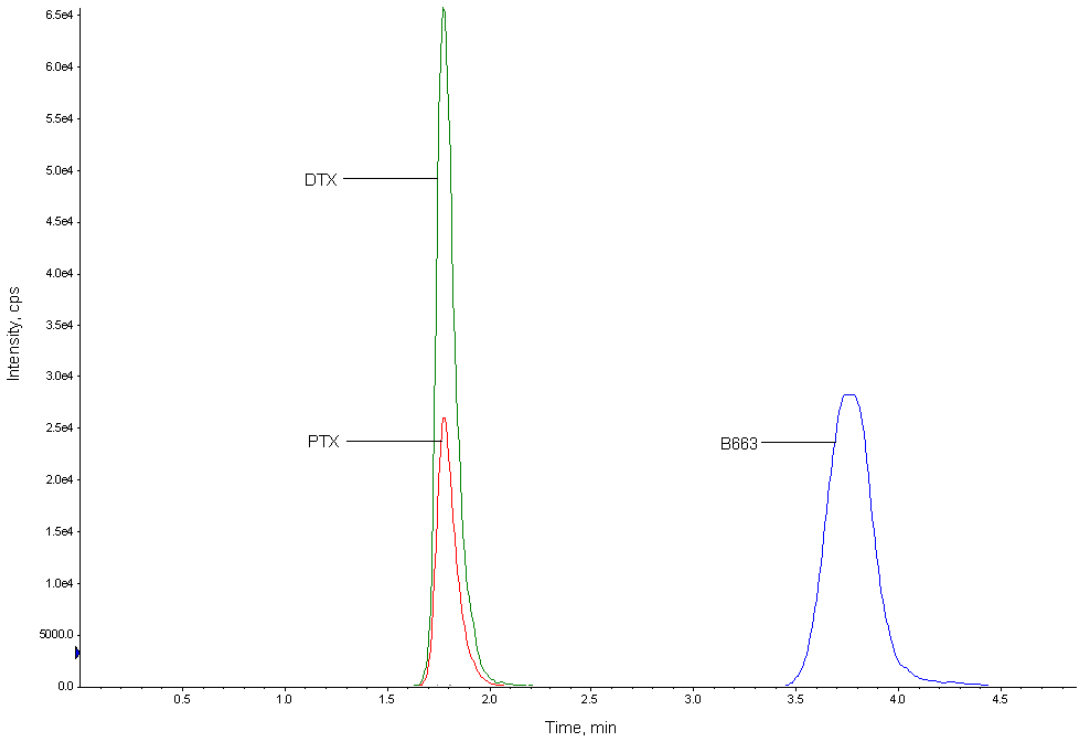

Figure 8.1. Representative Extracted Ion Chromatogram (XIC) of paclitaxel (PTX), docetaxel (DTX) and clofazimine (B663). Mobile phase: 95% MeOH in 0.1% HCO_2H (pH 3.5 with ammonium hydroxide). Green - DTX; Red - PTX; Blue - B663

Table 8.1. Quantitatively optimised MRM transitions determined for the analytes and IS after direct infusion into the ESI source

Compound	MRM transition (m/z)	DP (V)	CE (V)	CXP
Paclitaxel	854.3 > 569.4	51	17	8
Clofazimine	474.0 > 431.1	76	51	12
Docetaxel	808.3 > 527.2	46	15	4

8.4.2. Assay validation parameters

Specificity was determined using blank matrix of drug naïve mice where no peaks were detected using the specified MRM transitions. A standard curve for PTX was established by plotting concentration verses the ratio of the peak area of PTX to that of chemically similar DTX (IS) to increase the accuracy and precision of the assay by corrected for small amounts of variation including matrix effects. The calibration curve for B663 used external standardization alone considering the large peak area and matrix elution zone differences between B663 and DTX.

A reproducible linear relationship between concentration and detector response was observed for both analytes over the concentration range of 1.95-1000 ng/ml with average correlation coefficients being above 0.99 for most sample matrices using a weighting factor of $1/x^2$ due to the use of serial dilution sequence. The linear equations describing the calibration curves in various matrices are shown in Table 8.2. The LOQs were established as being the lowest points of the standard curves, i.e. 1.95 ng/ml as the variability in quantitation was less than ±15%.

Results of intra-day and inter-day accuracy and precision of the assay in MeOH are shown in Table 8.3. Both the accuracy and the precision on any of the given 5 days for the selected standards and independent quality control (QC_{100}) samples were within the defined ±15% acceptance criteria. The results of assay performance for each respective matrix are shown in Table 8.4. In nearly all cases the precision of the assay declined with decreasing concentrations. Selected nominal standard concentrations of analysis were within 15% reflecting the suitability of assay.

Recovery using liquid-liquid extraction with MtBE was found to extract 100.93% ± 15.79 of PTX and 103.65% ± 4.95 of B663 from plasma at a spiked concentration of 100 ng/ml. Ultrasonic extraction of various tissue sections into an acidic buffer followed by C18 SPE (following a generic method) was shown to effectively extract and efficiently clean-up the analytes of interest (Table 8.5). Generally, the extraction efficacy was found to be concentration-independent regardless of the

matrix. The variability and in certain cases low extraction yield (B663) attained by using a generic sample preparation protocol for all tissues attests to the diversity each respective tissue matrix.

This slope of the lines of the respective matrix calibration standards was compared to the slope constructed for solvent calibrators to produce a slope factor designating the overall extent of the matrix effect over the entire calibration range (Table 8.2). The effect of matrix was largely mitigated through adequate sample preparation as shown through post-column, constant infusion experiments and did not greatly influence the assay outcomes (Figure 8.2). Both B663 and PTX were shown to have similar matrix effect-time profiles (degree of suppression) suggesting the presence of easily ionizable compounds from tissue matrix competing successfully for charge in the ESI source (Figure 8.2.A).

Table 8.2. Mean standard calibration lines of paclitaxel (PTX) and clofazimine (B663) in blank solvent and various matrices comparing the slope as an expression of matrix interference over the full calibration range

Compound	Calibration range	Equation	Weighting factor	*R*	Slope factor
		MeOH			
Paclitaxel	1.95-1000 ng/ml	$y=2.34X + 0.0255$	$1/x^2$	0.99	1
Clofazimine	1.95-1000 ng/ml	$y=1.41x10^4X + 2.12x10^4$	$1/x^2$	0.99	1
		Plasma			
Paclitaxel	1.95-1000 ng/ml	$y=2.63X + 0.00128$	$1/x^2$	0.99	1.12
Clofazimine	1.95-1000 ng/ml	$y=1.53x10^4X + 9.44x10^4$	$1/x^2$	0.99	1.09
		Liver			
Paclitaxel	1.95-1000 ng/ml	$y=4.44X + 0.0023$	$1/x^2$	0.99	1.90
Clofazimine	1.95-1000 ng/ml	$y=2.61x10^4X + 3.59x10^4$	$1/x^2$	0.99	1.85
		Spleen			
Paclitaxel	1.95-1000 ng/ml	$y=2.42X + 0.00675$	$1/x^2$	0.99	1.03
Clofazimine	1.95-1000 ng/ml	$y=2.77x10^4X + 5.1x10^4$	$1/x^2$	0.97	1.96
		Kidney			
Paclitaxel	1.95-1000 ng/ml	$y=2.27X + 0.00493$	$1/x^2$	0.99	0.97
Clofazimine	1.95-1000 ng/ml	$y=2.9x10^4X + 4.47x10^4$	$1/x^2$	0.98	2.06
		Fat			
Paclitaxel	1.95-1000 ng/ml	$y=2.42X + 0.00675$	$1/x^2$	0.99	1.03
Clofazimine	1.95-1000 ng/ml	$y=2.28x10^4X + 2.1x10^4$	$1/x^2$	0.99	1.62

Table 8.3. Inter- (n = 3) and intra- (n = 5) day precision and accuracy of the analytes in MeOH and QC of the assay

Nominal [ ] (ng/ml)	PTX			B663		
	Calculated mean (ng/ml)	% CV	% Accuracy	Calculated mean (ng/ml)	% CV	% Accuracy
Intra-day						
1.95	1.86	9.14	97.83	1.83	8.75	96.54
31.25	33.37	2.04	106.62	34.39	1.46	109.89
500	462.95	12.30	92.60	457.19	5.13	91.44
QC_{100}	106.67	4.30	106.67	114.00	1.10	114.00
Inter-day						
1.95	1.89	13.18	96.67	1.82	19.75	93.48
31.25	29.13	13.06	93.07	29.72	14.32	94.96
500	427.64	5.08	85.53	493.76	14.52	98.75
QC_{100}	96.75	10.96	96.75	90.45	6.09	90.45

Table 8.4. Performance of the assay and matrix effects. Intraday accuracy and precision (n = 3) of analytes at various nominal concentrations from plasma and various tissues

Nominal [ ] (ng/ml)	PTX				B663			
	Calculated mean (ng/ml)	% CV	% Accuracy	% ME*	Calculated mean (ng/ml)	% CV	% Accuracy	% ME
Liver								
1.95	1.95	6.55	99.88	90.60	1.91	11.53	97.93	114.10
31.25	32.39	5.25	103.47	38.50	34.91	3.95	111.55	86.93
500	488.42	3.26	97.68	46.10	463.87	3.05	92.77	91.00
Spleen								
1.95	1.96	4.66	100.10	65.60	1.89	15.60	96.97	140.50
31.25	31.20	6.18	99.68	61.10	34.17	14.70	104.62	105.80
500	501.57	3.34	100.31	65.60	474.14	13.70	94.83	109.20
Kidney								
1.95	1.93	14.65	99.12	48.90	1.83	8.90	94.02	132.70
31.25	30.30	2.71	96.79	43.70	34.85	10.70	111.34	112.40
500	509.26	3.77	101.85	49.00	455.98	8.47	91.20	110.00
Fat								
1.95	1.97	14.22	101.28	56.60	1.92	12.98	98.41	88.00
31.25	32.05	4.17	102.39	59.30	34.55	14.00	110.38	86.24
500	482.82	3.82	96.56	64.10	472.81	12.69	94.56	89.70
Plasma								
1.95	1.94	1.60	99.30	69.46	1.85	10.36	94.89	134.34
31.25	33.88	1.55	108.25	85.15	31.51	3.54	100.66	98.4
500	477.43	1.32	95.49	96.34	493.81	4.98	98.76	107.38

* % ME = percentage change due to matrix effects

Table 8.5. Recovery (extraction efficacy % ±SD) of paclitaxel and clofazimine form various tissue matrices, (n=3)

Nominal	Liver		Kidney		Spleen		Fat	
[ ] (ng/ml)	PTX	B663	PTX	B663	PTX	B663	PTX	B663
20	97.6 ± 22.4	33.5 ± 1.4	86.2 ± 2.6	59.5 ± 7.8	98.7 ± 8.5	95.8 ± 17	75.3 ± 2.6	60.4 ± 11.5
500	104.5 ± 2	45.2 ± 20.9	93.3 ± 3.9	60 ± 7.6	89.1 ± 1.5	47.8 ± 7.5	80.9 ± 3.5	65 ± 12.5
1000	102 ± 6	42 ± 9.2	92.4 ± 3.9	52.4 ± 6	91.6 ± 7.2	60.5 ± 7.6	69 ± 1.8	50.1 ± 14.4

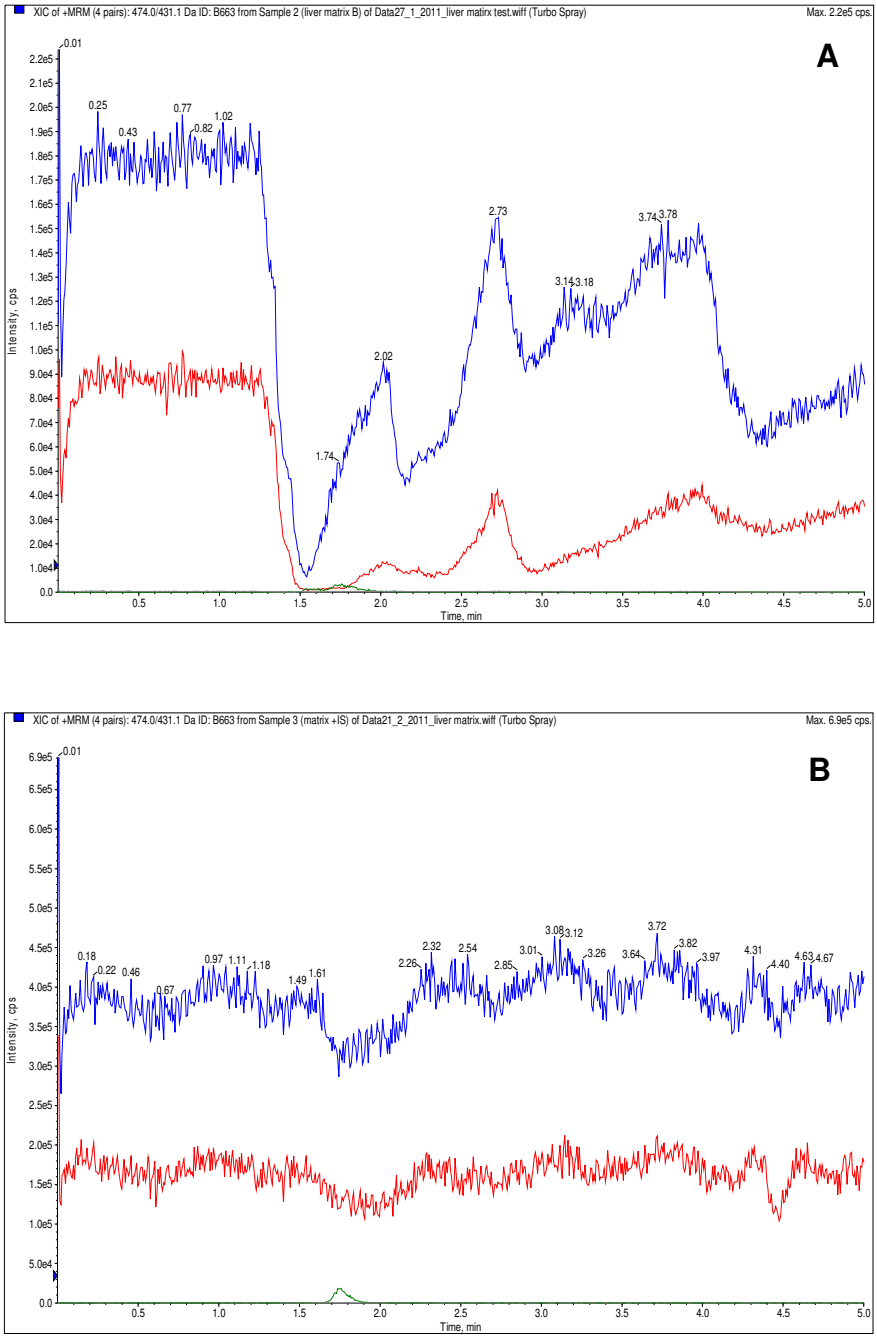

Figure 8.2. Qualitative matrix monitoring: Direct post-column infusion of an analyte mixture into the ESI source at constant flow rate whilst separating a blank tissue extract (Liver matrix) by the LC-MS/MS method. Red - PTX; Blue - B663

A) Prior to SPE B) After SPE clean up.

8.4.3. Method application

Despite the low recoveries attained for B663 from various tissue matrices using the generic extraction and SPE sample preparation procedures, the simple isocratic method proved robust and validation parameters suggested the suitability of the method to reproducibly quantitate the analytes accurately from diverse matrices. The developed and optimized sample clean up and LC-MS/MS methods were applied to assess the pharmacokinetics and tissue disposition of the PTX-B663 nanoparticulate co-formulation (Riminocelles) in comparison to Taxol at an equivalent PTX dose of 10 mg/kg.

Statistically significant differences ($P<0.05$) were found for the PTX concentration (between the two formulations) within plasma after 30 min and 1 hr. (Figure 8.3.) and within the liver after 30 min (Figure 8.3.) indicating that the micellular nanoparticulate delivery system appears to accumulate preferentially within the liver. This same effect has been reported for paclitaxel-loaded gelatine nanoparticles [150]. The highest concentration of PTX for both formulations was found in the liver which is to be expected and in general a similar tissue disposition time profile was shown for fat, kidney and spleen independent of which formulation was administered (Figure 8.3.). After initial distribution primarily to the liver, B663 was seen to slowly re-enter the plasma compartment reaching a C_{max} at 3 h and to ultimately accumulate in (and stain) fat tissue for prolonged periods as is a well-established characteristic of B663. [154]

Of importance is that the tissue concentration-time profiles demonstrate that the *in vitro* optimized fixed ratio (PTX:B663) is not maintained in circulation for longer than the first 30 min. This is suggestive of rapid micelle dissociation due to *in vivo* thermodynamic instability owing to the abundant presence of plasma proteins, especially albumin [155]. This lack of micelle integrity has a direct impact on the efficacy of the developed nanoparticulate co-formulation. The specified (*in vitro* optimised) synergistic FRDC is not maintained *in vivo* nor are the drugs (now free) selectively delivered to the tumour site.

The success of ratio dependent synergistic drug interactions is dependent upon the development of delivery systems that are stable after intravenous administration and that avoid immune recognition permitting prolonged systemic circulation facilitating passive tumour accumulation. Without such delivery systems the full potential of synergistic FRDC cannot be realised and clinically utilized against disseminated cancer.

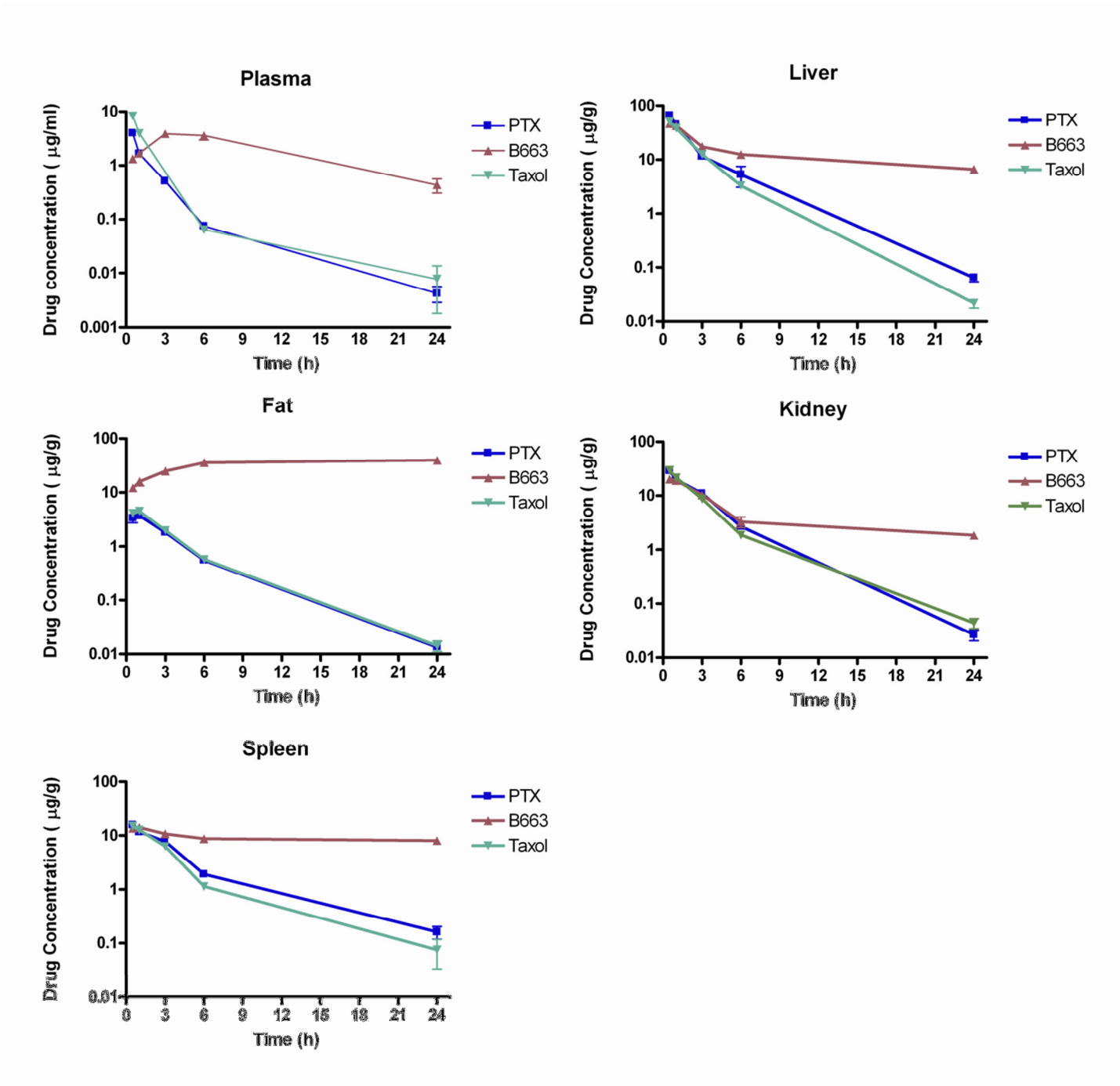

Figure 8.3. Plasma and various tissue concentration time profiles after an IV bolus administration of Taxol (10 mg/kg PTX) and a novel co-formulation (10 mg/kg PTX and 25 mg/kg B663)

8.5. Conclusion

In conclusion, an optimized and validated LC-MS/MS method has been developed for the quantitation of paclitaxel and clofazimine from diverse tissue sources. Good linearity with r>0.99 was attained over a concentration range of 1.95 ng/ml - 1000 ng/ml. The sensitivity of this method is demonstrated by the fact that only 50 µl of mouse plasma and 20 mg of tissue were required for analysis. The LOQ of the method was established as 1.95 ng/ml for both PTX and B663. Acceptable recoveries and mitigation of matrix effects through sample clean up suggests that this method is suitable for a wide range of applications. The method could be successfully applied in a pharmacokinetic study of a novel nanoparticulate co-formulation of PTX and B663 following IV administration.

The large differential change of the ratio of PTX to B663 in various tissue types is thought indicative of micelle disassembly due to the *in vivo* thermodynamic instability of lipopolymeric micelles constructed from DSPE-PEG 2000 and lecithin. The pharmacokinetics and tissue disposition of PTX was very similar for both Taxol and Riminocelles. Although Riminocelles did not positively alter (control) the pharmacokinetics of PTX, Riminocelles is a well-tolerated, Cremophor-free IV formulation and will therefore avoid the need for premedication clinically to prevent the allergic reactions associated with this excipient.

The results of this study are in strong support of improving the *in vivo* integrity of such simple lipopolymeric micelles so as to maintain the optimised fixed-ratio of the drugs and support tumour targeting.