Mo, J., Eggers, P., Raston, C. L., & Lim, L. Y. (2014). Development and validation of a LC/TOF MS method for the determination of carboplatin and paclitaxel in nanovesicles. Analytical and Bioanalytical Chemistry, 406(11), 2659-2667.

Development and validation of a LC/TOF MS method for the determination of carboplatin and paclitaxel in nanovesicles

Jingxin Mo[1], Paul K. Eggers [2], Colin L. Raston [3] and Lee Yong Lim[1*]

[1]Pharmacy, School of Medicine and Pharmacology, [2]Centre for Strategic Nano-Fabrication, School of Chemistry and Biochemistry, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia. [3]School of Chemical and Physical Sciences, Flinders University, Sturt Rd, Bedford Park, SA, 5042, Australia.

Keywords: Carboplatin, paclitaxel, co-loaded nanovesicles, LC/TOF MS, fetal bovine serum, simultaneous determination.

*** Corresponding author:** Tel: +61-8-64884413, Fax: +61-8-64887532, Email: lee.lim@uwa.edu.au

Abstract

Carboplatin and paclitaxel co-loaded nanovesicles (CPT-PTX-CLV), a novel intravenous formulation void of Cremophor EL, may have significant advantages over conventional carboplatin and paclitaxel formulations with respect to tumor targeting, sustained drug release, reduced toxicity and synergistic efficacy profiles. The aim of this study was to develop and validate a rapid, specific, sensitive, and reliable liquid chromatography-time of flight-mass spectrometry (LC/TOF MS)-based bioanalytical method for the simultaneous quantification of CPT and PTX in a fetal bovine serum (FBS) vehicle containing the dispersed nanovesicles. The analytes were extracted from FBS by simple protein precipitation, with subsequent separation of CPT and PTX on a WATERS HPLC SUNFIRE C_{18} column at a flow rate of 0.25 ml/min using gradient elution mode. The total analytical time was only 12 min. Detection and quantitation were performed by electrospray ionization (ESI) in the positive ionization mode with Selective Ion Monitoring (SIM) at *m*/*z* 310.0152 for CPT and 876.3224 for PTX. The calibration curves were linear over the concentration range of 10-4000 ng/ml for CPT and 5-2000 ng/ml for PTX ($r^2 > 0.99$), with the respective lower limit of quantification (LLOQ) at 10 and 5 ng/ml. The intra- and inter-day precision and accuracy of analysis of the quality control samples at low, medium, and high concentration levels were ≤13.6% relative standard deviation (RSD) and ≤14.6% relative errors (RE). The rapid, sensitive and reproducible LC/TOF MS method may be used to support preclinical and clinical pharmacological studies of the CPT-PTX-CLV administered by injection in animal and human cancer models.

1. Introduction

For certain types of cancer, especially cancers at an advanced stage, combinations of antineoplastic agents may be administered to improve treatment outcomes. Synergistic combinations and rational sequences of drug administration are devised by utilizing drugs that are effective when given alone, yet have different mechanisms of action and non-overlapping toxicities. Paclitaxel (PTX) co-administered with carboplatin (CPT) is one such FDA- approved combination therapeutic strategy for the treatment of patients with advanced and recurrent cervical carcinoma, non-small cell lung cancer (NSCLC) and gynecological cancers. [1]

PTX, a major anticancer drug with a molecular weight of 853.90 Da that was isolated from the bark of *Taxus brevifolia,* is a natural taxane found to be effective against the carcinomas of the breast, ovary and lung.[2] PTX has a unique mechanism of action in that it alters the normal equilibrium between tubulin dimers and microtubules by stabilizing the intracellular microtubules, thereby disrupting the G2 and M phases of the cell cycle and other associated cellular activities.[3] PTX has been reported to induce bcl2 phosphorylation followed by apoptosis.[4] It is a BCS class IV drug with an extremely low water solubility of 4 μg/ml.[5]

CPT (molecular weight of 371.3 Da) is a second generation platinum compound with a wide range of antineoplastic properties similar to cisplatin, yet possessing an improved toxicity profile that has been attributed to its greater chemical stability.[6] CPT has been extensively prescribed for the treatment of ovarian, head, neck, lung, bladder, and testicular cancer. It forms reactive intracellular platinum complexes that bind to nucleophilic groups in the DNA, producing both inter- and intra-strand crosslinks that inhibit DNA replication, RNA transcription and protein synthesis, and results in apoptosis and cell growth inhibition.[7] Unlike PTX, CPT is relatively soluble in water (solubility of 10 mg/ml).

To date, there is no commercial product that incorporates both PTX and CPT as actives. To meet this need, we have developed a novel vesicular nanocarrier system (CPT-PTX-CLV) designed to deliver PTX and CPT simultaneously without harmful adjuvant for the enhancement of therapeutic

effectiveness. Analytically, however, the combination chemotherapeutic product presents significant challenges, as the different physiochemical properties of PTX and CPT typically require separate methods of assay.

CPT is a polar, weakly basic molecule that is stable when stored in the dark at room temperature.[8] A number of methods, including high performance liquid chromatography[9], atomic absorption spectrometry[10], inductively coupled plasma-mass spectrometry [11], X-ray [12] and nuclear magnetic resonance [13], have been used to assay CPT. More recently, a LC/MS method with a sensitivity (LLOQ of 2 ng/mL) approaching that of atomic absorption spectrometry has been developed for the quantification of CPT in human plasma ultrafiltrates.[14] PTX, on the other hand, is analytically a non-polar molecule. There are several LC methods published for the determination of PTX by UV detection[15,16] and MS detection[17,18]. Compared to CPT, the extraction of PTX from biological samples is less challenging due to its relative lipophilicity, and many protocols have been proposed, including liquid–liquid extraction, solid phase extraction and protein precipitation. Most of these have again been carried out off-line with high recoveries, typically 91–92%.[19,20]

The aim of this study was to develop and validate a rapid, specific, sensitive, and reliable liquid chromatography-time of flight-mass spectrometric (LC/TOF MS) method for the simultaneous quantification of CPT and PTX in biological samples. Fetal bovine serum (FBS) was used as the surrogate plasma vehicle. The analytes were extracted by precipitation of FBS with acetonitrile, and subsequently separated on a HPLC column. We were able to demonstrate the reproducible quantification of CPT and PTX using the LC/TOF MS method. To the best of our knowledge, this is the first report of a method that can simultaneously extract, separate, detect and quantify two chemotherapeutic agents of vastly different physical properties in a serum sample.

2. Experimental

2.1. Chemicals and reagents

Carboplatin (purity >99%) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Paclitaxel (purity >99%) was purchased from Shanghai 21CEC Pharma (Shanghai, China).

Phosphonato calixarene (P4C6, purity > 95%) was synthesized in our laboratory according to published methods with some minor modifications[21]. HPLC-grade methanol and acetonitrile (ACN) were procured from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Analytical grade sodium acetate was from Sigma. FBS was obtained from Life Technologies Australia Pty Ltd. (Mulgrave, Australia). Ultrapure water (0.22 μm), deionized and further purified by means of a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was used throughout the study. All other reagents were of commercially available analytical grade.

2.2. LC/TOF MS conditions

Chromatography was performed using a WATERS Prominence instrument (Waters MS Technologies, Manchester, UK) equipped with a 100 × 3.0 mm Symmetry C_{18}, 3.5-μm column (Sunfire, Waters Corp, Milford, USA). The gradient elution mobile phase comprised of ACN (A) and 1 mM aqueous sodium acetate (B) mixture delivered at a flow rate of 0.25 ml/min. The gradient was controlled as follows: 0-3.0 min, 20% A, 3.0-6.0 min, linear increase to 80% A, 6.0-9.0 min, 80% A, 9.0-12.0 min, linear decrease to 20% A. The column temperature was maintained at 40 °C and the autosampler was thermostatted at 4 °C. A 10.0-μl aliquot was injected into the LC/TOF MS system, and the total analytical run time was 12.0 min.

Mass spectrometry was performed on a Micromass LCT Premier system (Waters MS Technologies, Manchester, UK) operating in positive ion mode. The nebulization gas was set to 300 L/h at a temperature of 350 °C, the cone gas was 10 L/min and the source temperature was 80 °C. The capillary voltage and cone voltage were 3000 V and 60 V, respectively. The LCT-Premier was operated in the W optics mode with 12 000 resolution using the dynamic range extension (DRE). Data acquisition rate was set to 0.1 s, with 0.1 s interscan delay. All analyses were acquired using the lock spray to ensure accuracy and reproducibility; leucine-enkephalin was used as the lock mass (m/z 556.2771) at a concentration of 50 fmol/μL and flow rate of 5 μL/min. Data were collected in centroid mode, the lock spray frequency was set at 5 s, and data were averaged over 10 scans.

2.3. Preparation of calibration and quality control standards

Stock solution containing 500 µg/ml of PTX was prepared by dissolving accurately weighed PTX in ACN in a 50-ml volumetric flask, while stock solution containing 1000 µg/ml of CPT was similarly prepared by dissolving accurately weighed CPT in water. The two stock solutions were mixed (50:50 v/v), then diluted with ACN:water (50:50 v/v) to give a series of diluted working solutions.

Calibration and quality control standards were prepared using a P4C6 in FBS dispersion as vehicle. The commercial FBS samples were thawed, allowed to equilibrate to room temperature, and then mixed with P4C6 under vortex to give transparent dispersions containing 1 mg/ml of P4C6. Calibration standards containing CPT and PTX in the concentrations (CPT/PTX, ng/ml) of 10/5, 200/100, 500/250, 1000/500, 2000/1000, and 4000/2000 were prepared by vortex-mixing 100 µl of the appropriate working solution with 400 µl of P4C6-FBS vehicle for 30 s in 2 ml pre-labeled microcentrifuge tubes. Blank samples were similarly prepared by spiking 400 µl of the P4C6-FBS vehicle with 100 µl of ACN:water (1:1 v/v). Quality control (QC) standards were prepared by vortex-mixing 100 µl of the working solutions containing CPT/PTX concentrations of 50/25, 1000/500, 10000/5000 and 20000/10000 ng/ml with 400 µl of the P4C6-FBS vehicle for 30 s. The final nominal concentrations of CPT/PTX (ng/ml) for the QC samples were 10/5 for the LLOQ, 200/100 for low QC, 2000/1000 for middle QC, and 4000/2000 for high QC (also known as ULOQ).

Prior to analysis, the FBS in the calibration and QC standard solutions was precipitated by vortex-mixing 500 µl of each solution with 2.5 ml of ACN for 5 min, followed by centrifugation at 4000 rpm (5418 centrifuge, Eppendorf) for 10 min. The supernatant was immediately transferred into a 15-ml centrifuge tube and freeze dried (Sentry 2.0, SP Industries, PA, US). The residue was reconstituted by vortex mixing with 500 µl of ACN:water (50:50 v/v) and, after centrifugation at 12,000 rpm for 10 min, the supernatant was transferred into glass autosampler vials and a 10 µl-aliquot sample was injected into the LC/TOF MS system for analysis.

2.4. Assay validation

Assay validation to demonstrate the reliability of the bioanalytical method for the intended applications was performed according to the USA Food and Drug Administration (FDA) guidelines

on bioanalytical method validation.[22] Validation involved the evaluation of selectivity and specificity, linearity, precision and accuracy, lower limit of quantification, carry-over effect, recovery, matrix effects (ion suppression/enhancement), stability and dilution integrity.

2.4.1. Selectivity and specificity

To determine whether the P4C6-FBS matrix interfered with the ion mass chosen for CPT and PTX, the selectivity and specificity of assay were investigated by analysing six replicates of the LLOQ QC samples (CPT/PTX concentration of 10/ 5 ng/ml).

2.4.2. Precision and accuracy

Intra- and inter-day precision and accuracy were determined by analysing the LLOQ, low, medium and high QC samples (CPT/PTX concentrations of 10/5, 200/100, 2000/1000 and 4000/2000 ng/ml, respectively). Precision (as relative standard deviation, RSD%) and accuracy (as relative error, RE%) were evaluated based on the assay of 6 replicate QC samples on three different days. The deviation of precision and accuracy from the nominal concentration was required to be < ±15% except for the LLOQ, where it should not exceed 20%.

2.4.3. Calibration curve and lower limit of quantification

The calibration curve was constructed using 6 non-zero calibration standard samples containing 10/5, 200/100, 500/250, 1000/500, 2000/1000, and 4000/2000 ng/ml of CPT/PXT. The linearity of the relationship between concentration and peak area response was assessed by weighted ($1/x^2$) least-squares linear regression analysis of the calibration curves obtained on three consecutive days. A correlation coefficient (r^2) of 0.99 or better was desirable for all calibration curves. The back-calculated standard concentration was less than ±15% deviation from the nominal value except at the LLOQ, which was set at ±20%. The LLOQ, defined as the lowest concentration on the calibration curve that could be measured with acceptable precision and accuracy, was determined in six replicates on three consecutive days.

2.4.4. Recovery and matrix effect

A post-extraction spiking experiment was performed to determine the recovery of CPT and PTX following FBS precipitation with ACN. Recovery was measured by comparing the peak areas obtained from P4C6-FBS samples spiked with 200/100, 2000/1000 or 4000/2000 ng/ml of CPT /PTX before FBS precipitation (A) with those from P4C6-FBS samples to which the equivalent amounts of CPT/ PTX were added after FBS precipitation (B). The ratio ($A/B \times 100$) % was used to evaluate recovery. The matrix effect was quantitatively assessed by comparing the peak areas of P4C6-FBS samples spiked with CPT/ PTX after FBS precipitation (B) with that of neat solution (1:1 ACN:water as vehicle) containing equivalent amounts of CPT/PTX (C). The ratio ($B/C \times 100$) % was defined as the matrix effect.

2.4.5. Stability

Stability studies were conducted for 6 replicates of stock and QC samples under several storage conditions. Samples were stored in closed containers at room temperature for 4 h and at −20 °C for one month to probe the short- and long-term stability, respectively. Freeze and thaw stability (comprising three sequential cycles, −80 °C to room temperature), stock solution stability (−4 °C for one week), and post-preparation stability (in the autosampler at 4 °C for 24 h) were also assessed. QC samples were prepared from freshly made stock solutions of CPT and PXT for stability determinations. Samples were considered stable if the deviation from nominal values was < ±15%. To meet acceptance levels, concentrations in at least 2/3 of the QC samples should remain within ±15% of initial, with accuracy in the range of ±15%.

2.4.6. Carry-over effect

Carry-over effect was studied by injecting three processed blank P4C6-FBS samples subsequent to the injection of a QC sample containing CPT/PXT at the upper limit of quantification (ULOQ) in three independent runs. The responses were evaluated at the respective retention times of CPT and PTX.

2.4.7. Dilution integrity

To verify the dilution carried out on high analyte concentrations, dilution integrity evaluations were carried out with the ULOQ sample (CPT/PTX of 4000/ 2000 ng/ml) in six replicates. Samples were diluted 20 and 200 times with drug-free P4C6-FBS, and their measured concentrations were calculated by applying the dilution factors of 20 and 200, respectively, against the freshly prepared calibration curve.

3. Results and discussion

3.1. Method development

3.1.1. Chromatography

Adding a low concentration of sodium acetate as modifier in the mobile phase was demonstrated to improve the ionization efficiency, and to increase the signal response and peak symmetry, thus enabling increased sensitivity with minimized matrix effects. When the mobile phase of 1:1 v/v ACN:water containing 0.1 mM sodium acetate was applied in the isocratic mode, the retention times for CPT and PTX were 1.89 min and 16.84 min, respectively. Gradient elution of the same mobile phase was found to narrow the peak width, improve the sensitivity of detection, and ensure complete peak resolution. It also changed the elution time of CPT to 2.52 min and that of PTX to 10.52 min, rendering the total run time of a single determination procedure to only 12 min. No significant interference from endogenous substances in the P4C6-FBS vehicle was observed at the retention times of CPT and PTX. During the development phase, two solvent systems were evaluated for the reconstitution of the freeze dried supernatant obtained following FBS precipitation from the standard solutions. Between methanol:water (1:1, v/v) and ACN:water (1:1, v/v), the latter was the preferred reconstitution solvent as it yielded better peak shape and lower noise level for the SIM chromatograms.

3.1.2. Mass spectrometry

Under the electrospray ionization conditions chosen, CPT and PTX existed predominantly as protonated molecules ($[M\text{-}CO_2\text{-}NH_3 + H]^+$ and $[M+Na]^+$) in the mobile phase. Thus, the MS

parameters were optimized to maximize the response for the production of *m*/*z* 310.0152 for CPT and 876.3224 for PTX. Optimization was performed using the positive ionization mode as it was found superior to the negative ionization mode. This results in the most intense peaks being used as the quantifiers for CPT and PTX (Fig. 1).

3.2. Assay validation

3.2.1. Selectivity and specificity

Both the UV and mass detectors were used for monitoring CPT and PTX, which greatly increased the specificity of the assay. Since the two detectors were connected in series, the mass peaks always lagged the UV peaks by about 0.2 s, which helped in the identification of the peaks of interest. Regardless of which detector was used, there was a clear separation of the CPT (retention time of 2.52 min) and PTX (10.52 min) peaks from the endogenous peaks originating from the blank matrix. Typical SIMchromatograms for the middle QCand LLOQ samples are presented in Fig. 2. The resolution of peaks and the apparent lack of background interference in the CPT peak and PTX peak windows indicate adequate selectivity and specificity of the assay method.

3.2.2. Precision and accuracy

The intra- (n = 6) and inter-day (n = 6) precisions for the assay of QC samples were <13.4% and <13.6%, respectively, with accuracy <±14.6% for both (Table 1). Precision and accuracy were therefore satisfactory, suggesting the reproducibility of analysis from day to day.

3.2.3. Calibration curve and lower limit of quantification

Calibration curves of CPT and PTX exhibited excellent linearity over the concentration ranges of 10-4000 ng/ml and 5-2000 ng/ml, respectively, with reproducible coefficients of correlation (r^2) larger than 0.99. The respective LLOQ values for CPT and PTX were 10 ng/ml and 5 ng/ml, with a signal-to-noise ratio [S/N] ≥ 10 compared to blank response. At this concentration, the deviations from accuracy and precision were less than 7.4% (RE %) and 17.8% (RSD %), respectively. The response

was distinct and reproducible; a stable baseline was maintained throughout the assay with the [S/N] ≥ 10-fold that of the baseline.

3.2.4. Recovery and matrix effect

In this study, a simpler, cleaner and more economical sample preparation method was employed, whereby proteins in the sample were precipitated simply by the addition of an organic solvent. The respective analyte extraction recoveries (mean ± RSD) for the low, medium and high QC samples were 64.82 ± 4.1%, 65.85 ± 1.6%, and 65.00 ± 1.1% for CPT, and 79.77 ± 1.9%, 78.92 ± 4.7%, and 82.34± 3.2% for PTX. These values suggest that the extraction recovery of CPT and PTX from the QC samples may be regarded as having met the requirements for persistence, precision and reproducibility.

The matrix effect, i.e. the potential ion suppression or enhancement of CPT and PTX due to the presence of other molecules, was determined in the low, medium, and high QC samples as the area responses ratio of the analyte with and without the presence of matrix ions (P4C6, FBS). The range of values obtained were 97.72% -101.35% for CPT and 98.28%-102.08% for PTX, with RSD % values <8.6%. The collective results reflected a negligible matrix effect, suggesting that any co-eluting matrix components, impurities or degradation products did not affect the ionization of CPT and PTX. Furthermore, ion suppression or enhancement from the FBS matrix was consistent under the assay conditions.

3.2.5. Stability

The stability data for the CPT/PTX stock and standard solutions under different storage conditions are presented in Table 2. Both drugs were stable when subjected to three freeze-thaw cycles, and to post-extraction storage for 24 h at 4 °C in the autosampler. They were also stable for 4 h at ambient temperature, while the stock solutions were stable when stored for one week at -4 °C, and for one month at −20 °C. Taking into account the inherent analytical variability, these results indicate that the stability of the processed samples was acceptable, and that the prescribed storage conditions under normal laboratory conditions did not incur a significant loss of analyte detection.

3.2.6. Carry-over effect

The chromatogram of blank P4C6-FBS samples analysed immediately after the injection of the ULOQ QC sample did not show any apparent response peaks at the same retention time of CPT and PTX. Therefore, any carry-over effects from previous concentrated samples may be regarded as negligible.

3.2.7. Dilution integrity

The deviation from accuracy of mean back-calculated concentrations for the 1/20 and 1/200 dilutions of the QC samples were <±12.5% of their nominal concentration, while the precision levels were 14.2% and 14.6%, respectively. This suggests the preservation of dilution integrity for the highly concentrated samples.

4. Conclusion

A LC/TOF MS method for the simultaneous determination of CPT and PTX in a P4C6-FBS matrix has been developed. The validated linear ranges were 10-4000 ng/ml for CPT and 5-2000 ng/ml for PTX, with LLOQ of 10 ng/ml for CPT, and 5 ng/ml for PTX. Compared with published assays [9,24], the relative ease of sample preparation, coupled with a shorter chromatographic run time of only 12 min, provide the method with the capacity for high sample throughput for *in vivo* pharmacokinetic analysis. The collective validation data demonstrate a high sensitivity with acceptable accuracy and precision, allowing this method to be applied to support the *in vivo* pharmacologic studies of P4C6 nanovesicles co-loaded with CPT and PTX.

ACKNOWLEDGMENTS

The authors acknowledge the facilities, and the scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy, Characterisation &

Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments.

This work is supported by a grant (P1028339) from the Australian NHMRC (National Health and Medical Research Council).

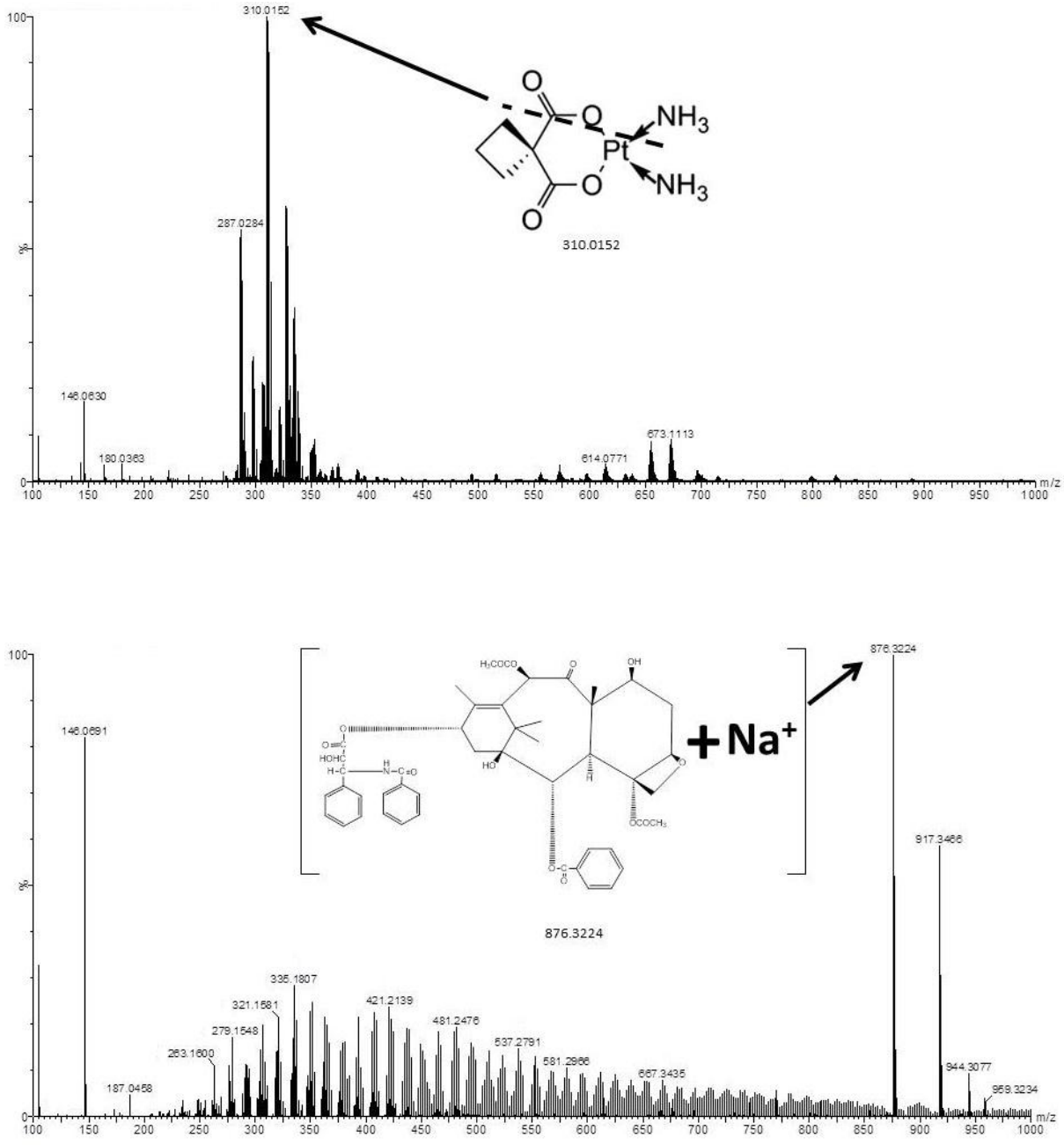
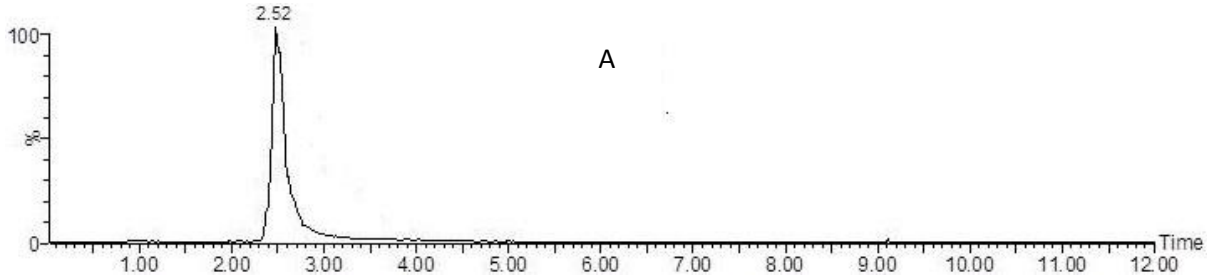


Figure 1. The chemical structures and mass spectra of carboplatin (CPT) (upper) and paclitaxel (PTX) (lower).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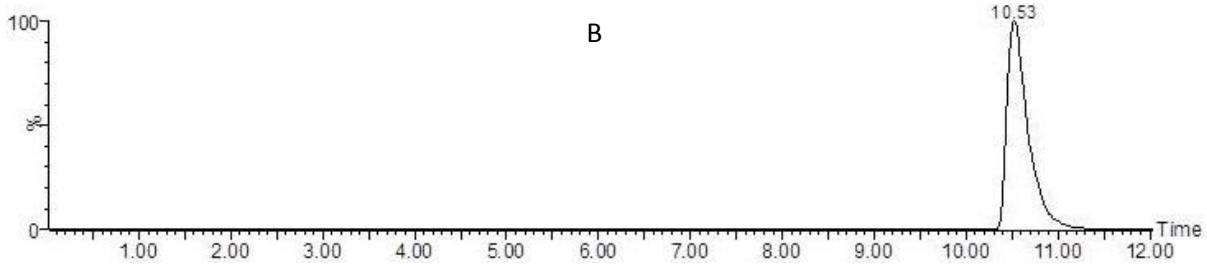
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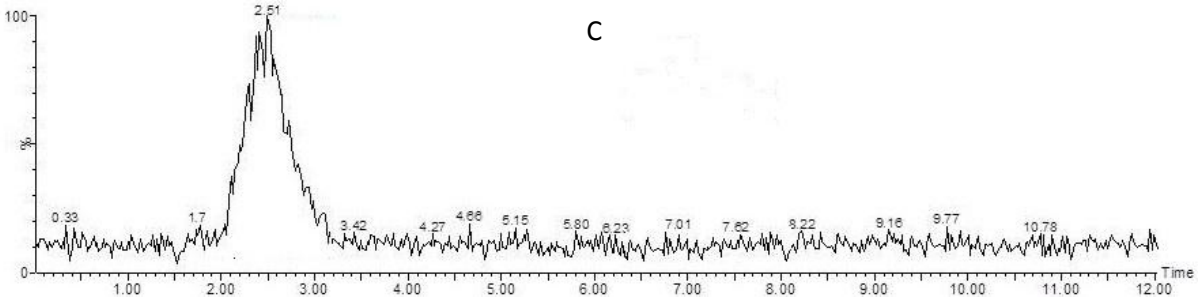
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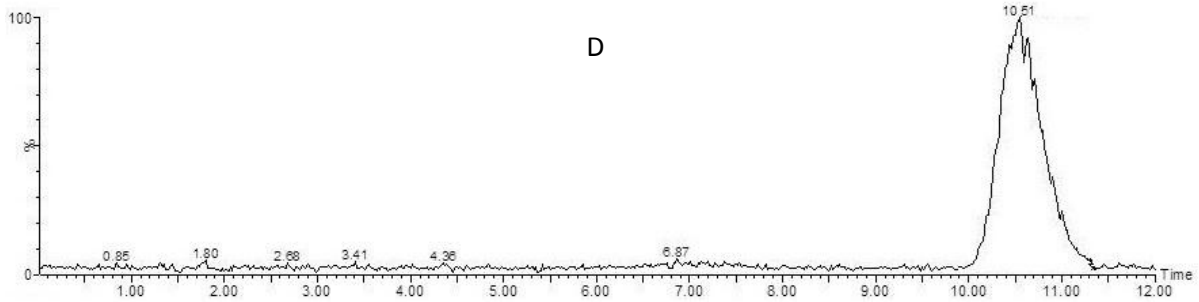
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Figure 2. Typical chromatograms based on selective ion monitoring (SIM) for selected samples. (A) SIM chromatogram for QC sample at m/z 310.0152; (B) SIM chromatogram for QC sample at m/z 876.3224; (C) SIM chromatogram for QC LLOQ at m/z 310.0152; (D) SIM chromatogram for QC LLOQ at m/z 876.3224;

Table 1

Intra- and inter-day precision and accuracy for the determination of carboplatin (CPT) and paclitaxel (PTX) in phosphonato calixarene-fetal bovine serum (P4C6-FBS) vehicle (mean ± SD, six replicates for each concentration).

Analyte	Nominal concentration (ng/ml)	Intra-day (n=6)			Inter-day (n=6)		
		Measured concentration (ng/ml)	RSD (%)	RE(%)	Measured concentration (ng/ml)	RSD (%)	RE (%)
carboplatin	200	226.58±30.37	13.4	13.3	223.61±30.52	13.6	11.8
	2000	2206.48±144.88	6.6	10.3	2105.30±192.32	9.1	5.3
	4000	3571.33±202.58	5.7	-10.7	3645.50±308.63	8.5	-8.9
paclitaxel	100	114.64±15.34	13.4	14.6	111.46±14.56	13.1	11.5
	1000	1110.04±80.24	7.2	11.0	1111.30±90.16	8.1	11.1
	2000	1834.54±121.48	6.6	-8.3	1849.66±167.39	9.0	-7.5

Table 2

Stability of carboplatin and paclitaxel in phosphonato calixarene-fetal bovine serum (P4C6-FBS) vehicle under various storage conditions (mean ± SD, six replicates for each concentration).

carboplatin	Nominal concentration (ng/ml)	200			2000			4000		
		Measured (ng/ml) (% of nominal)	RSD (%)	RE (%)	Measured (ng/ml) (% of nominal)	RSD (%)	RE (%)	Measured (ng/ml) (% of nominal)	RSD (%)	RE (%)
	Short-term	223.24±26.34 (111.62)	11.8	11.6	2113.53±50.27 (105.68)	2.4	5.7	3567.87±217.83 (89.20)	6.1	-10.8
	Post-extraction	227.55±31.84 (113.78)	14.0	13.8	2181.64±217.79 (109.08)	10.0	9.1	4069.61±370.04 (101.74)	9.1	1.7
	Long-term	218.35±27.36 (109.18)	12.5	9.2	1784.88±264.04 (89.24)	14.8	-10.7	3514.77±211.08 (87.87)	6.0	-12.1
	Three freeze-thaw cycles	204.45±29.16 (102.23)	14.3	2.2	1830.45±222.29 (91.52)	12.1	-8.5	4128.61±479.26 (103.22)	11.6	3.2
	Stock solution	203.96±15.85 (101.98)	7.8	2.0	1990.30±173.63 (99.52)	8.7	-0.5	3842.01±111.11 (96.05)	2.9	-4.0
paclitaxel	Nominal concentration (ng/ml)	100			1000			2000		
		Measured (ng/ml) (% of nominal)	RSD (%)	RE (%)	Measured (ng/ml) (% of nominal)	RSD (%)	RE (%)	Measured (ng/ml) (% of nominal)	RSD (%)	RE (%)
	Short-term	110.10±8.52 (110.10)	7.7	10.1	1081.44±26.34 (108.14)	6.0	8.1	1828.08±120.59 (91.40)	6.6	-8.6
	Post-extraction	113.06±14.77 (113.06)	13.1	13.1	1109.14±60.26 (110.91)	5.4	10.9	1759.48±86.11 (87.97)	4.9	-12.0
	Long-term	111.23±14.51 (111.23)	13.0	11.2	1073.68±37.52 (107.37)	3.5	7.4	2093.16±107.47 (104.66)	5.1	4.7
	Three freeze-thaw cycles	87.83±9.10 (87.83)	10.4	-12.2	1067.62±118.16 (106.76)	11.1	6.8	1845.72±94.15 (92.27)	5.1	-7.7
	Stock solution	113.05±11.63 (113.05)	10.3	13.0	925.25±127.92 (92.52)	13.8	-7.5	1852.91±111.84 (92.65)	6.0	-7.4

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