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A high-performance liquid chromatography–tandem mass spectrometry method for the clinical combination study of carboplatin and anti-tumor agent eribulin mesylate (E7389) in human plasma

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ABSTRACT

An LC/MS/MS method was developed to quantify carboplatin and eribulin mesylate (E7389) in human plasma and urine. For carboplatin, sample clean-up by protein precipitation and supernatant injection into a Waters Spherisorb® S5 SCX column was used. Liquid-phase extraction and reverse-phase chromatography on a Polaris® C18 column were used for eribulin. Quantitation involved LC/MS/MS with positive electrospray ionization. Accuracy, precision, linearity, range, specificity, recovery and stability were also evaluated. Both compounds were stable in human plasma (≥80 days at −80 °C), at room temperature (≥4 h), following three freeze–thaw cycles and in 50/50 methanol/H_2O (<4 °C for ≥252 days).

1. Introduction

Eribulin mesylate (E7389) exhibits sub-nM growth inhibitory activities *in vitro* against numerous human cancer cell lines as well as marked *in vivo* activities at 0.1–1.0 mg/kg against human xenografts [1]. In order to fully explore the potential of eribulin for advanced solid tumors, a Phase Ib study of eribulin in combination with carboplatin in patients with advanced solid tumors has been initiated. To support this study, bioanalytical methods were established to quantify eribulin at concentrations of 0.2–100 ng/mL in plasma and 0.5–100 ng/mL in urine, and carboplatin at concentrations of 2–500 ng/mL in plasma and 20–10,000 ng/mL in urine. This is the first time a method sensitive to carboplatin has been reported.

Although published bioanalytical methods with suitable sensitivity and specificity for carboplatin are limited, they can be divided into non-specific (i.e., only determine the platinum element of carboplatin) and specific (i.e., selectively detect the intact molecule) methods. Detection of intact carboplatin is usually required for pharmacokinetic investigations. High-performance liquid chromatography (HPLC) appears to be the method of choice for carboplatin determination in biological samples, with assays using either normal-phase [2–5] or reverse-phase liquid chromatography (LC) [6–17] with UV [2,3,8–11,14,15], electrochemical detection [6], LC/mass spectrometry (LC/MS) [12,16] or LC–inductively coupled plasma-MS (LC–ICP-MS) [13,17]. UV detection is affected by non-specificity due to the lack of absorbance by carboplatin and the interference of either endogenous compounds or biotransformation products. Efforts to improve the sensitivity of carboplatin detection using a column-switching technique [9] resulted in an improved detection limit but did not alter this non-specific detection. Applicability of electrochemical detection for determination of platinum in biological samples is limited due to the reproducibility and the reliance on HPLC separation of carboplatin from possible interferences. In contrast, LC/MS with selected-ion recording mode offers some degree of detection specificity [12,16]. However, high background noise, inadequate evaporation and ionization efficiency, and possible matrix effects, constrain the limit of detection to 35–70 ng/mL. LC–ICP-MS [13,17] is extremely sensitive but requires chromatographic separation to attain specificity.

Recent advances in MS technology have improved the sensitivity and specificity of techniques, with a triple quadrupole instrument becoming the gold standard for bioanalytical applications. This report describes, for the first time, the development and validation of LC/MS/MS methods for the quantitation of both eribulin and carboplatin in human plasma and urine.

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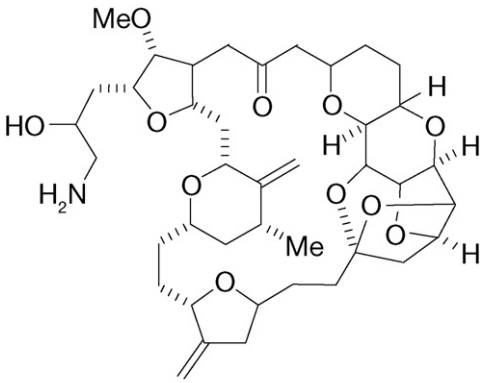
2. Experimental

2.1. Chemicals and reagents

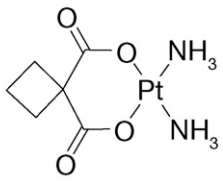
Carboplatin and its internal standard tolbutamide were purchased from Sigma (St. Louis, MO, USA). Eribulin and its internal standard, an analog of eribulin, were synthesized at the Eisai Research Institute (Andover, MA, USA) (Fig. 1). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from EMD Chemicals (Gibbstown, NJ, USA). House deionized water, further purified with a Milli-Q® (Millipore) water purifying system, Millipore gradient A10 (Millipore Corp., Bedford, MA, USA), was used. Formic acid (FA) was obtained from EMD Chemicals (Gibbstown, NJ, USA). Ammonium formate (97%) was purchased from Sigma (St. Louis, MO, USA). Blank human plasma, containing heparin as the anti-coagulant, and blank urine were purchased from Biological Specialty Corp. (Colmar, PA, USA).

2.2. Preparation of spiking solutions for standards and quality control (QC) samples

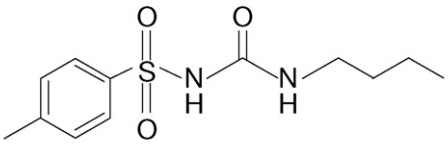
Standard master stock solutions containing carboplatin or eribulin (1.0 mg/mL) were produced by dissolving an appropriate amount of carboplatin in 50/50 MeOH/H_2O or eribulin in MeOH. QC master stock solution was prepared in the same way. Standard or QC spiking solutions were prepared by serial dilution of the standard master stock in 50/50 MeOH/H_2O. All solutions were stored in clear glass vials at 4 °C.



Eribulin



Carboplatin



Tolbutamide

Fig. 1. Chemical structures of eribulin, carboplatin, and tolbutamide.

2.3. Preparation of calibration standards and QC samples

Calibration standards and QCs were prepared by adding standard spiking solutions to human plasma or urine blanks to give nominal concentrations of 0.2–100 ng/mL (eribulin) or 2–10,000 ng/mL (carboplatin). The lower limits of quantitation (LLOQ) for eribulin and carboplatin in this assay were 0.2 and 2 ng/mL, respectively. For each validation run, the calibration standards and QCs were freshly made from spiking solutions.

2.4. Sample extraction

Carboplatin was extracted from human plasma or urine using the following method. For plasma, 90 μL of plasma was added to a 1.5 mL polypropylene tube followed by 10 μL of working spiked standard or QC solution. For urine, 50 μL of plasma and 45 μL of urine were added to a 1.5 mL polypropylene tube followed by 5 μL of working standard or QC solutions. 200 μL of the internal standard (IS) solution (1000 ng/mL tolbutamide in MeOH with 0.1% formic acid) was then added to each tube except blanks without IS. Tubes were vortex-mixed for 30 s, then centrifuged for 5 min at 14,000 rpm. Samples were filtered into a spin-x filter (0.22 μm) microfuge tube by centrifugation for 30 s at 14,000 rpm. Thirty microliters was then injected onto the triple quadrupole mass spectrometer (API4000™, Applied Biosystems/MDS SCIEX).

For eribulin, 50 μL of eribulin standard solutions was added to 450 μL of plasma or urine on the day of analysis. Twenty microliters of the IS (500 ng/mL in 50:50 of MeOH:H_2O) solution, 125 μL of 0.1N sodium hydroxide, and 500 μL of water was added to each tube. Tubes were vortex-mixed for 10 s and 5 mL of 90% ethyl acetate/5% MeOH/5% ethanol was then added to the plasma or urine. Tubes were shaken for 10 min at high speed, and then spun in a centrifuge for 5 min at 3000 rpm. The aqueous layer was frozen in a dry ice/isopropanol bath and the organic layer was dried down under nitrogen in a 37 °C water bath. The plasma extracts were reconstituted with 200 μL of a mixture of MeOH and H_2O (50:50) with 0.1% formic acid. The reconstituted samples were filtered into a spin-x filter (0.22 μm) microfuge tube. Thirty microliters were injected onto the mass spectrometer for plasma extracts and 20 μL for urine extracts.

2.5. Equipment and LC/MS/MS conditions

Sample analysis was performed using an LC/MS/MS system. A Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD, USA), composed of an autosampler, a column oven (set at 30 °C), a degasser and two pumps, and a switching valve for carboplatin samples was used. Separation of analytes and IS was achieved using a Symmetry Shield™ RP18 (Waters Corp.) column for carboplatin or Polaris® (MetaChem) C18 (3 μm, 2.0 mm × 30 mm) for eribulin.

For carboplatin, the gradient profile started with 100% mobile phase A (90/10 ACN/H_2O) at a flow rate of 0.5 mL/min and held for 1.0 min, was then switched to 100% mobile phase B (50/25/25 ACN/MeOH/H_2O plus 10 mM ammonium formate) between 1.01 and 5 min, and finally switched back to 100% mobile phase A at 5.1 min, with a switching valve going to waste from 0 to 0.75 min and 6 to 10 min. For eribulin, the gradient profile started with 100% mobile phase A (13% ACN in H_2O containing 0.1% formic acid) and held for 1.5 min, was then switched to 60% mobile phase B (30% tetrahydrofuran in ACN containing 0.1% formic acid) between

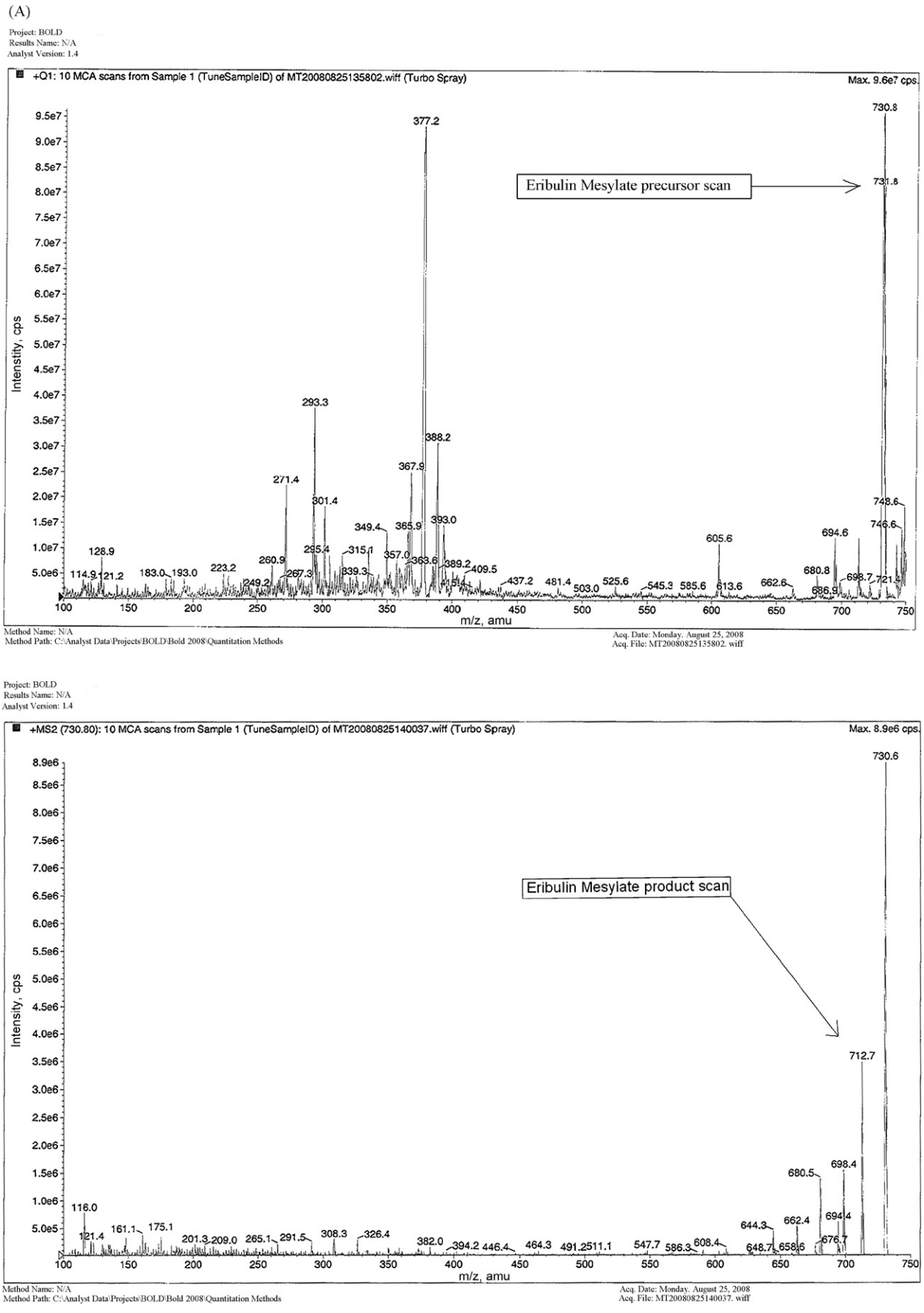
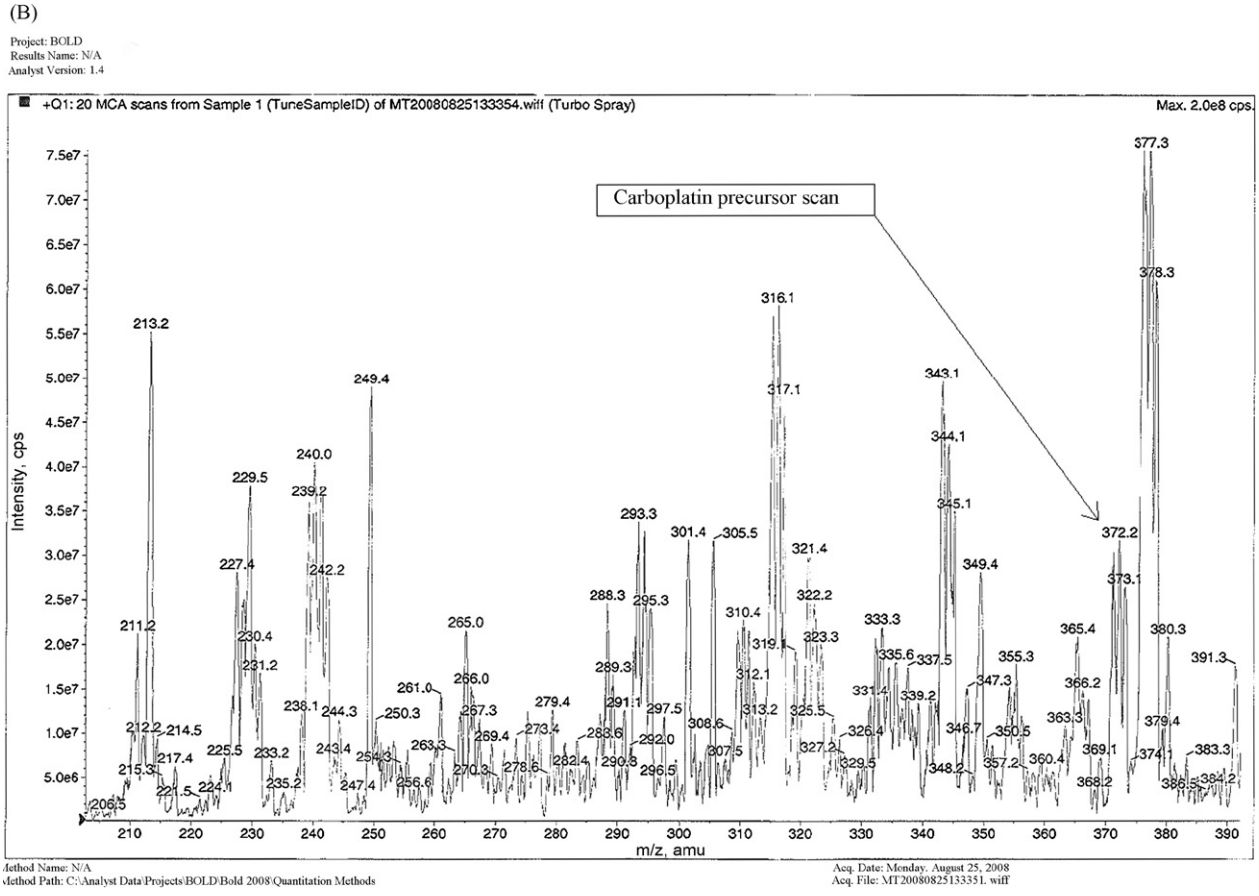


Fig. 2. Full scan mass spectra of (A) eribulin, (B) carboplatin, and (C) tolbutamide.

(B)



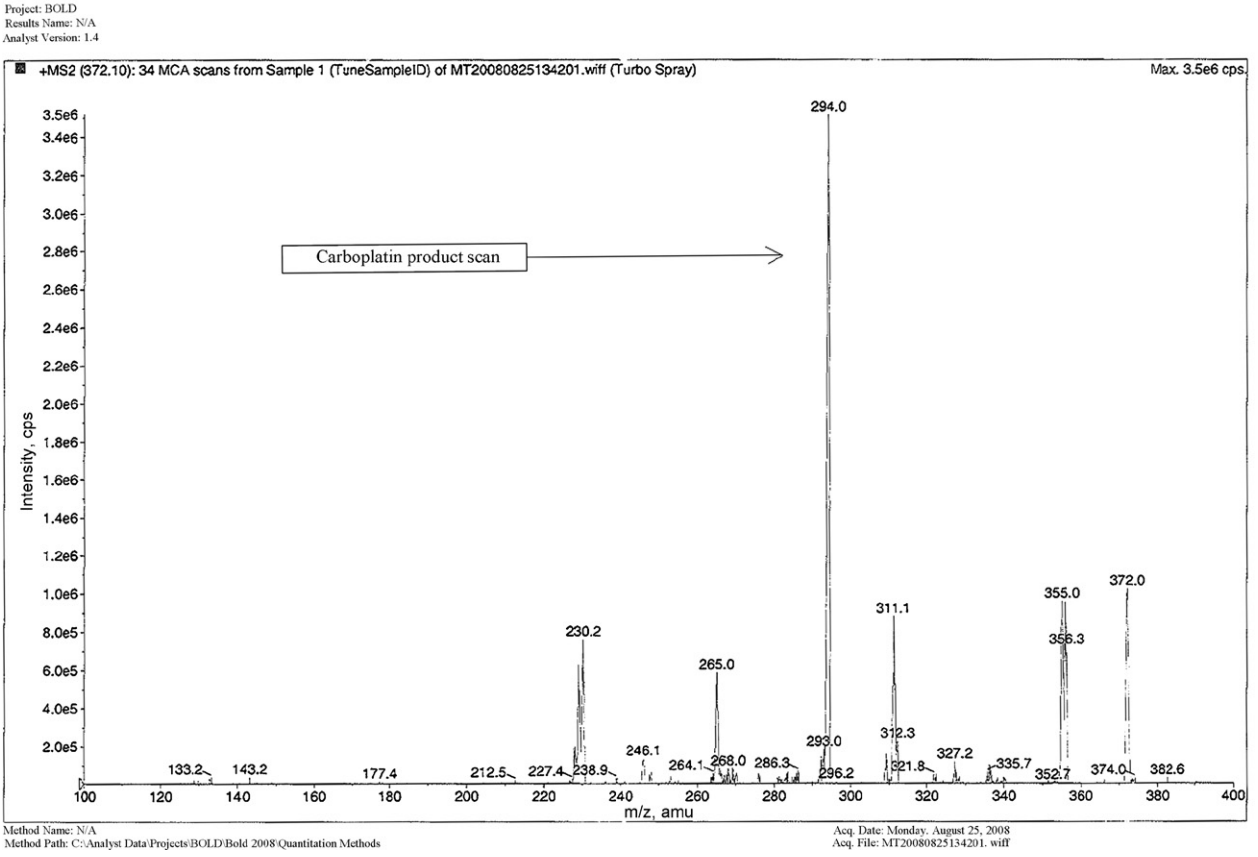
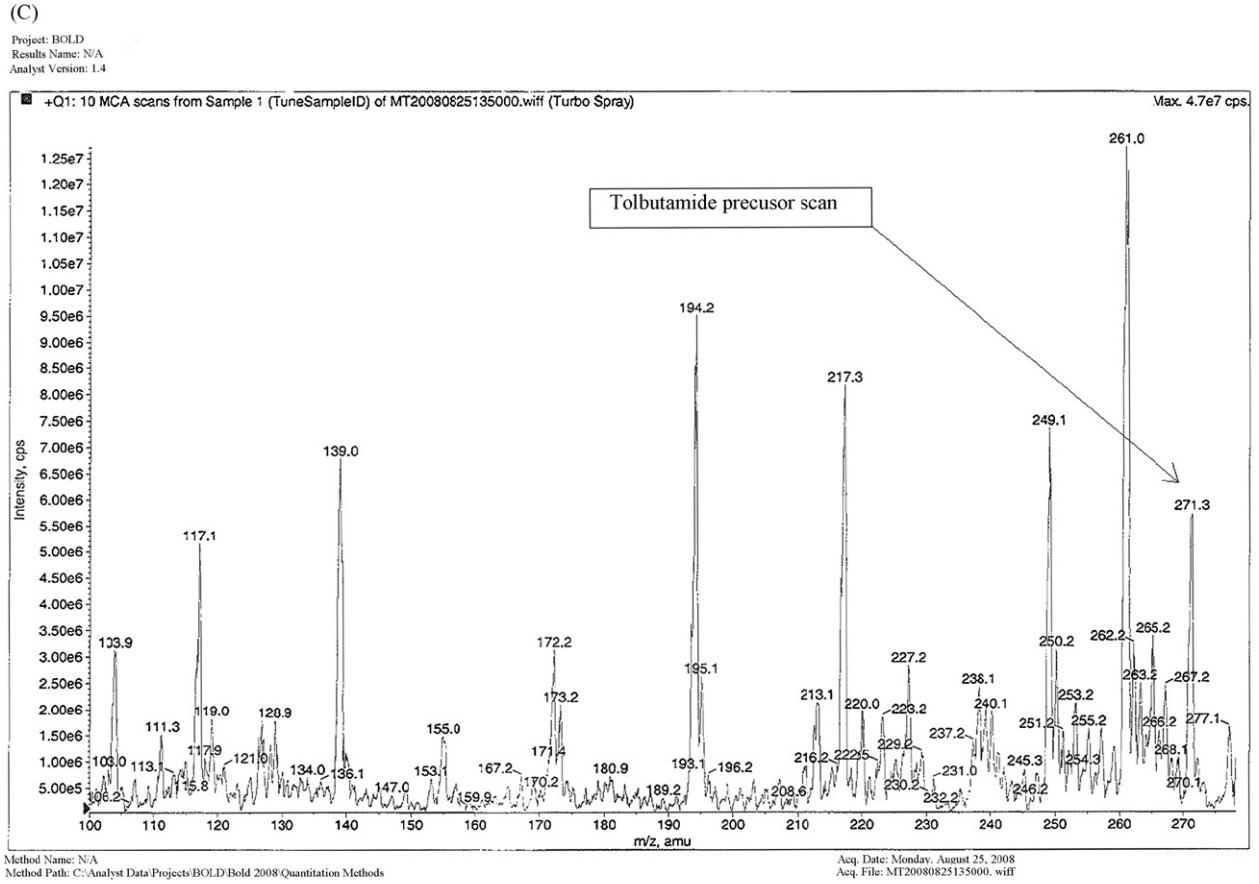


Fig. 2. (*Continued*)

(C)



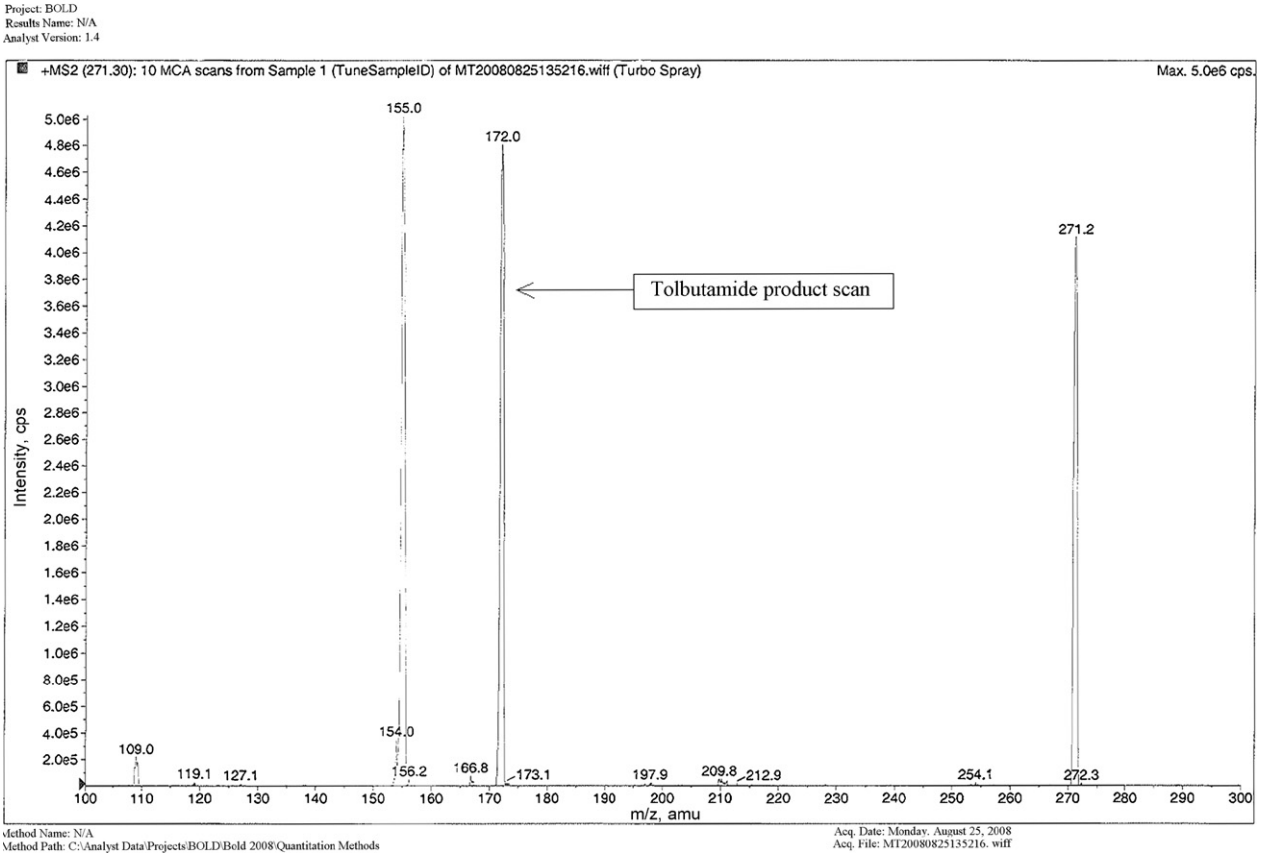


Fig. 2. (*Continued*).

1.5 and 5.5 min, and finally switched back to 100% mobile phase A at 7 min and stopped at 10 min.

The detection system was a Sciex™ 4000 (PerkinElmer), equipped with a turbo ionspray interface operated in triple quadrupole mode. The mass spectrometer was set at positive ion multiple reaction monitoring mode. The selected reaction monitoring transitions for analytes and IS were as follows: carboplatin, 372.1 → 294.1; eribulin, 730.5 → 712.5; IS for eribulin, 731.5 → 681.5; tolbutamide, 271.1 → 172.0. Full scan (precursor ion) and product ion mass spectra of carboplatin, eribulin, tolbutamide is shown in Fig. 2.

Ultra-high pure nitrogen was used as the nebulizer gas, curtain gas, turbo gas and collision gas, with the settings of 60, 20, 80 and 9, respectively. Other settings were as follows: dwell time, 200 ms with 0.62-s cycle time; source temperature, 450 °C; ion-spray voltage, 5500 V. The declustering potential, entrance potential, collision energy and collision-cell exit potential for carboplatin were 38, 10, 26 and 17, respectively; for eribulin, these values were 53, 15, 35 and 4.2, respectively. Analyst™ 1.4 (Sciex, Concord, Canada) was used for instrument control, data acquisition and data processing.

2.6. Method validation

The method was validated according to US Food and Drug administration (FDA) published guidelines for bioanalytical method validation [18].

2.6.1. Linearity and range

Carboplatin and eribulin were evaluated for linearity from 2 to 500 ng/mL and 0.2 to 100 ng/mL for plasma, and 20 to 10,000 ng/mL and 0.5 to 100 ng/mL for urine, respectively.

2.6.2. Accuracy and precision

The accuracy and precision of the method were assessed with three validation runs, one intraday assay and two interday assays. The intraday and interday assays both included calibration curve standards at eight concentration levels, QCs at four concentration levels (including LLOQ [intraday: $n = 5$, interday: $n = 2$, at each concentration level]), one blank plasma sample with IS, and one blank plasma sample without IS. In addition, the intraday assay included two dilution QCs at five replicates (1:2 and 1:100 dilutions), and the interday assay included any validation samples bracketed by two sets of QCs. The interday accuracy and precision was determined for 3 days (any set of QCs from intraday assay and any set of QCs from two interday assays) at four concentration levels.

2.6.3. Specificity

Six independent of human blank plasma containing sodium heparin as the anti-coagulant purchased from Biological Specialties Corp. (Colmar, PA) and six lots of control urine, purchased from Bioreclamation Inc. (Hicksville, NY) were evaluated for specificity. The peak areas at the retention times of E7389 and carboplatin were examined and compared with those of the lowest quantifiable standard for each matrix. The interference peak from each matrix should be equal to or less than 20% of the signal from the lowest quantifiable concentration.

2.6.4. Extraction recovery and matrix effect

Duplicate recovery of carboplatin and eribulin in human plasma samples was evaluated at three concentration levels by comparing the signals from extracted samples with the signals from unextracted samples. The extracted samples were prepared as stated in Section 2.4. The unextracted samples were prepared by adding 10 μL of the respective spiking QC solutions and 90 μL of water to 200 μL of protein-precipitation solvent for carboplatin, and 50 μL of QC solutions, 20 μL IS solution and 130 μL reconstituted solution for eribulin. The matrix effect of eribulin extraction was assessed by adding 50 μL of QC solutions, 20 μL IS solution and 130 μL reconstituted solution to plasma extracts.

2.6.5. Stability studies

The stability of carboplatin and eribulin in human plasma under various conditions was examined. For all assays, compounds were considered stable if the deviation was not greater than 15% from the mean of Day 0 values.

- For frozen stability, aliquots of plasma samples were prepared at 5, 100 and 500 ng/mL. One aliquot for each concentration level was analyzed in triplicate on Day 0, at two middle time points and at an end time point to cover the overall sample storage time period. The means of back-calculated values for middle and end time points were compared with Day 0 results.
- The short-term stability was evaluated from the same aliquot in triplicate at two concentration levels (5 and 500 ng/mL). Aliquots were frozen for at least 12 h, thawed and stored at room temperature for 2–24 h, based on the expected duration those samples would be maintained at room temperature during the extraction procedure. Mean back-calculated concentrations were compared with Day 0 samples.
- The influence of freeze/thaw cycles on carboplatin and eribulin in human plasma was also evaluated by storing QC aliquotes at −80 °C for at least 24 h, thawed unassisted at room temperature, and refrozen for at least 12 h under the same conditions. The freeze/thaw cycle was repeated three cycles. The aliquot was analyzed after the third freeze/thaw cycle and the results compared with Day 0 samples.
- Stability of processed samples in the autosampler was assessed by comparing the concentrations of the first set of carboplatin and eribulin QCs with the original injection and reinjection after storage at three concentration levels (5, 100 and 500 ng/mL). The stability of carboplatin and eribulin in whole blood during the sample collection was also evaluated in triplicate at these concentrations. One set of whole blood stability samples were spun down immediately after the spiking of QCs into whole blood (time 0). The remaining stability samples were evaluated 1 and 2 h after spiking on wet ice. The plasma samples from 0, 1 and 2 h were assayed within the established short-term stability period.
- To evaluate the stability of carboplatin and eribulin in stock solution stored at 4 °C, the mean absolute area count ($n = 3$) of a 100 ng/mL solution in 50/50 MeOH/H_2O prepared from a solution that was at least 252 days old was compared with that of a freshly prepared solution. A mean difference of less than 10% in the responses established the stability of the stored compounds.

3. Results and discussion

3.1. Chromatography

The different physicochemical properties of carboplatin and eribulin make the development of a single HPLC method unrealistic. Carboplatin is relatively polar and has little retention on most reverse-phase columns, whereas eribulin is relatively hydrophobic and has a very strong retention on ODS columns, which results in a lot of carry over. Normal phase columns (e.g., NH_2 column) have been tested without success, therefore, a strong cation-exchange column has been considered based on the structures of the two compounds.

Carboplatin has two ammonium groups coordinated with platinum that can interact with propyl sulfonic acid anion, the

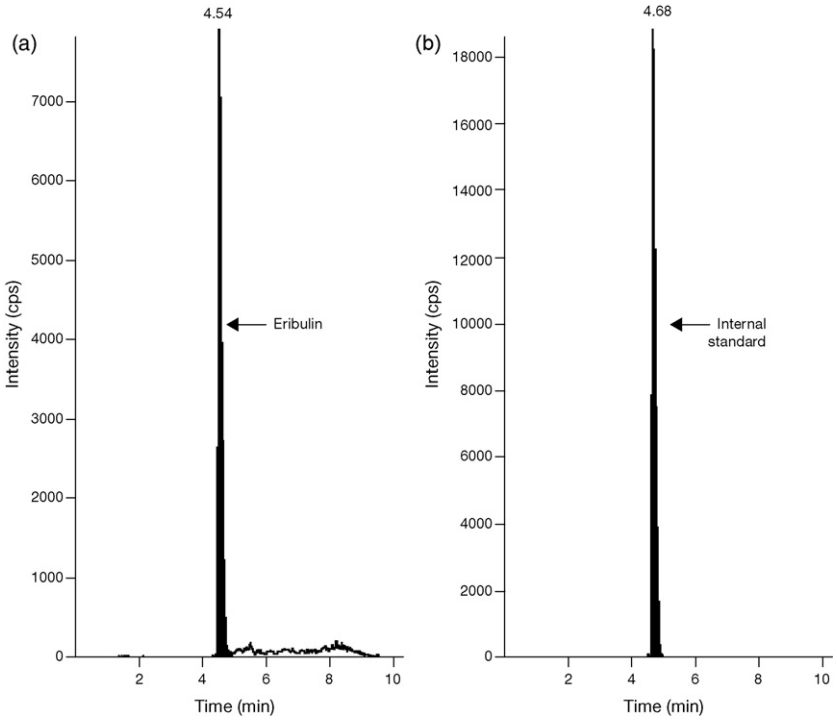


Fig. 3. Representative chromatograms for eribulin 10 ng/mL in human plasma.

strong-cation exchange group on the Spherisorb® (Waters) SCX column. Eribulin has one primary amine group that can interact with the propyl sulfonic acid anion on the Spherisorb® SCX column under acidic conditions. However, because the peak shape was not very sharp, a Polaris® (MetaChem) C18 was chosen for eribulin (Fig. 3). An additional benefit of the SCX column is that it has only a small amount of hydrophobic interaction. In order to achieve better retention of carboplatin in the SCX column, mobile phase A was started with 90% ACN and 10% H_2O for 1 min and then switched to mobile phase B (50/25/25/ACN/MeOH/water plus 10 mM ammonium formate) and held for 5 min. The retention time of carboplatin was approximately 4.6 min, which was sufficiently distant from any endogenous peaks in plasma and urine during the first few minutes (Fig. 4).

Several extraction methods have attempted to extract carboplatin from urine and plasma. We found that adding plasma protein to the urine samples stabilized the signals of LC/MS/MS. Therefore, a simple protein-precipitation method was used for extracting

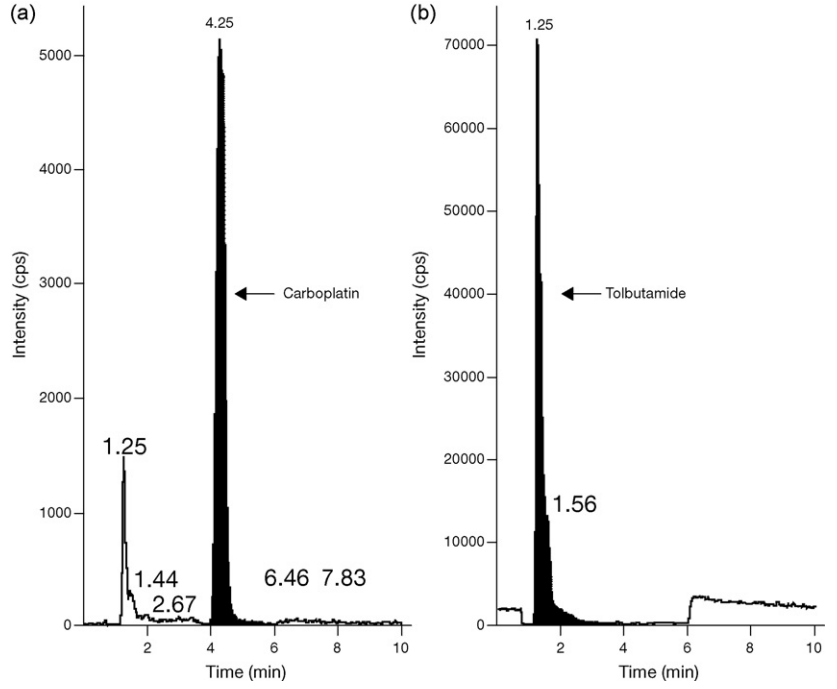


Fig. 4. Representative chromatograms for carboplatin 5000 ng/mL in human urine.

carboplatin from human plasma and urine, and the recovery was excellent (70–90%).

3.2. Mass spectrometry

The full-scan spectrum of carboplatin showed a cluster of molecular ions (MH^+) (371, 372 and 373) corresponding to the major platinum isotopes ^{194}Pt, ^{195}Pt and ^{196}Pt with natural abundances of 32.9, 33.8 and 25.3%, respectively. The MH^+ of 372.1 had the highest intensity, and thus was chosen as the precursor ion for carboplatin.

For eribulin, the energy and temperature settings are crucial. Eribulin is a relatively large molecule: however, the only fragment detected was a loss of water (730.5–712.5). Lowering the collision gas/energy would result in no fragmentation of eribulin, whereas adding a little more would cause the molecule to split apart.

3.3. Regression, accuracy and precision

The peak area ratios of carboplatin and eribulin to their respective internal standards were correlated with the standard concentration samples (2–500 and 0.2–100 ng/mL for plasma, and 20–10,000 and 0.5–100 ng/mL for urine, respectively). The $1/x^2$ weighted quadratic and linear regression had the best fit for carboplatin and eribulin, respectively. The correlation coefficients (r) of carboplatin and eribulin were $\geq$0.9966 and $\geq$0.9950, respectively.

The performance of the assay was assessed by intraday and interday precision (% CV) as shown in Table 1. Current FDA guidelines for precision and accuracy are ±15% except for LLOQ (±20%) [18]. For intraday validation in plasma, precision (% CV) for carboplatin and eribulin ranged from 3.4 to 10.2% and 3.2 to 12.6%, with interday precision (% CV) ranging from 5.7 to 11.9% and 5.0 to 10.8%, respectively. For intraday validation in urine, precision (% CV) for carboplatin and eribulin ranged from 3.7 to 10.4% and 0.8 to 13.0%, respectively, and interday precision (% CV) ranged from 4.3 to 14.6% and 6.1 to 20.0%, respectively. Intraday accuracy (% diff) in plasma ranged from 2.3 to 10.9% for carboplatin, and 1.0 to 7.3% for eribulin; interday was 6.9–13.3% for carboplatin, and 0.1–6.4% for eribulin. In urine, intraday accuracy ranged from 0.6 to 11.6% for carboplatin, and 1.0 to 12.7% for eribulin; interday accuracy was 0.2–10.3% for carboplatin and 0.3–19.2% for eribulin (Table 1). These results indicate that both methods were precise and accurate.

3.4. Recovery

Recoveries for carboplatin and eribulin were examined in duplicate at three (5, 100 and 500 ng/mL) concentration levels. The mean recovery for carboplatin was 90.0% for 5 ng/mL, 77.2% for 100 ng/mL and 92.0% for 500 ng/mL. The mean recovery for eribulin was 33.0% for 5 ng/mL, 43.9% for 100 ng/mL and 45.3% for 500 ng/mL. These numbers were lower compared to those from spiking QC solutions directly to plasma extracts (>70%), indicating

Table 1
Precision and accuracy for quality control samples in plasma (A) and urine (B)

Nominal (ng/mL)	(A) Plasma					
	Intraday			Interday		
	Mean ± SD	% CV	% Diff	Mean ± SD	% CV	% Diff
Carboplatin						
2	1.942 ± 0.198	10.2	−2.9	2.181 ± 0.216	9.9	9.1
5	4.781 ± 0.462	9.7	−4.4	4.310 ± 0.443	10.3	−13.8
10	9.255 ± 0.455	4.9	−7.4	8.844 ± 0.681	7.7	−11.6
200	195.382 ± 8.745	4.5	−2.3	186.124 ± 10.638	5.7	−6.9
500	518.062 ± 36.203	7.0	3.6	452.001 ± 53.602	11.9	−9.6
50 (1:10 dilution)	554.667 ± 19.005	3.4	10.9	NA	NA	NA
250 (1:2 dilution)	548.048 ± 25.407	4.6	9.6	NA	NA	NA
Eribulin						
0.2	0.198 ± 0.025	12.6	−1.0	0.203 ± 0.022	10.8	1.5
0.5	0.480 ± 0.058	12.1	−4.0	0.468 ± 0.043	9.2	−6.4
5	5.207 ± 0.259	5.0	4.1	5.005 ± 0.313	6.3	0.1
100	100.833 ± 3.222	3.2	0.8	100.798 ± 5.083	5.0	0.8
100 (1:50 dilution)	104.844 ± 9.891	9.4	4.8	NA	NA	NA
100 (1:2 dilution)	107.270 ± 6.679	6.2	7.3	NA	NA	NA
Nominal (ng/mL)	(B) Urine					
	Intraday			Interday		
	Mean ± SD	% CV	% Diff	Mean ± SD	% CV	% Diff
Carboplatin						
20	20.773 ± 1.403	6.8	3.9	21.369 ± 2.374	11.1	6.8
50	48.532 ± 2.831	5.8	−2.9	49.879 ± 6.699	13.4	−0.2
100	88.422 ± 9.200	10.4	−11.6	99.646 ± 4.254	4.3	−0.4
5000	5307.891 ± 351.571	6.6	6.2	4549.922 ± 662.146	14.6	−9.0
10000	9349.889 ± 661.939	7.1	−6.5	8972.140 ± 382.262	4.3	−10.3
100 (1:100 dilution)	9941.813 ± 780.611	7.9	−0.6	NA	NA	NA
5000 (1:2 dilution)	9981.098 ± 339.693	3.7	−8.2	NA	NA	NA
Eribulin						
0.5	0.495 ± 0.046	9.3	−1.0	0.404 ± 0.081	20.0	−19.2
1	0.941 ± 0.117	12.4	−5.9	1.064 ± 0.099	9.3	6.4
50	44.133 ± 1.613	3.7	−11.7	51.742 ± 6.674	12.9	3.5
100	93.783 ± 12.216	13.0	−6.2	99.733 ± 6.043	6.1	−0.3
20 (1:10 dilution)	17.464 ± 0.754	4.3	−12.7	NA	NA	NA
100 (1:2 dilution)	90.990 ± 0.750	0.8	−9.0	NA	NA	NA

Precision: % CV = (S.D./mean) × 100; accuracy: % diff = (mean found − nominal/nominal) × 100; NA, not applicable; level not needed for assay validation.

Table 2
Summary of stability

	Eribulin (5–500 ng/mL)		Carboplatin (5–500 ng/mL)	
	Plasma[a]	Urine[a]	Plasma[a]	Urine[a]
Room temperature 4 h	−9.9 to −9.0%	−8.4 to 2.8%	−4.9 to 0.5%	−0.5 to 1.3%
−80 °C Long-term 80 days	−9.2 to 7.2%	−9.0 to −1.8%	−11.9 to 7.2%	
4 °C Autosampler 20 h	−12.1 to 4.1%	−6.8 to 5.0%	−14.4 to 8.9%	−3.6 to 10.8%
4 °C Stock solution 252 days	−5.50%		3.40%	
3 Cycles freeze/thaw	−11.2 to 0.9%	−13.4 to −5.3%	−2.7 to 6.6%	−0.8 to 5.8%

[a] % Diff = (mean at *x* time − mean at 0 h)/mean at 0 h × 100.

that there was some matrix effect to quantifying eribulin, although the consistency of recovery at different concentration levels for eribulin and carboplatin demonstrated that the bioanalytical method was reproducible.

3.5. Stability results

Carboplatin has been reported to have limited stability in human plasma at −25 °C [2]. In our experiments, the half-life values for carboplatin in plasma at concentrations of about 6 and 38 μg/mL were 49 and 54 days, respectively. After 18 days of storage, only around 70% of each of the original concentrations was present as carboplatin. Since this method is to be used to support clinical sample analysis, short-term frozen stability may be an issue.

For this reason, samples were stored at −80 °C and, after 3, 32 and 80 days, the percentage difference from the nominal concentration for 500 ng/mL carboplatin was −5.7, 9.8 and −8.5%, respectively; for 500 ng/mL eribulin, the difference was 2.2, −9.0 and −1.4%, respectively. The stability of the other concentrations tested was similar.

Experiments designed to measure short-term stability at room temperature for 4 h showed a difference of −4.9 and −0.5% for carboplatin at 5 and 500 ng/mL, respectively, and −9.0 and −9.9% for eribulin at 5 and 500 ng/mL, respectively. Likewise, the percentage differences in concentration following the freeze–thaw stability tests were less than 15% (−2.7, 6.6 and −0.6% for 5, 100 and 500 ng/mL carboplatin, respectively, and −11.2, 0.9 and −0.2% for 5, 100 and 500 ng/mL eribulin, respectively), therefore, the drugs were considered stable under these conditions. Based on the criteria stated in Section 2.6, carboplatin and eribulin were stable in plasma for at least 80 days at −80 °C, 4 h at room temperature, and after three freeze–thaw cycles. In addition, extracted carboplatin and eribulin were also stable in the autosampler (4 °C) for at least 20 h. The stability data are summarized in Table 2.

Due to higher distribution of carboplatin in plasma compared with blood cells, stability tests in plasma showed a higher concentration of carboplatin than in the original spiked whole blood sample at all three carboplatin concentrations (5, 100 and 500 ng/mL) (Table 3). However, when data from 1 and 2 h were compared with time 0, the differences were not greater than 15%, indicating that carboplatin was stable during the sample collection period for at least 2 h. Data from eribulin also demonstrated sufficient room temperature stability.

Studies examining the stability of carboplatin in water at −25 °C [2] have suggested that 1 mg/mL carboplatin in water is stable for no longer than 1 week when stored at −25 °C. Here, stock solution stability of carboplatin and eribulin was tested as described in Section 2.6. The percentage difference of mean absolute area count ($n = 3$) of a 100 ng/mL solution in 50/50 MeOH/H_2O prepared from a solution at least 252 days old and a freshly prepared solution is 3.4% for carboplatin and −5.5% for eribulin. This demonstrates that both carboplatin and eribulin were stable in 50/50 MeOH/H_2O at 4 °C for at least 252 days.

Table 3
Sample collection stability

Spiked concentration in blood (ng/mL)	Carboplatin in plasma		Eribulin in plasma	
	Mean (ng/mL)	% Diff[a]	Mean (ng/mL)	% Diff[a]
Hour 0				
5	8.726	NA	5.373	NA
100	153.613	NA	101.692	NA
500	758.369	NA	596.733	NA
Hour 1				
5	7.509	−13.9	4.727	−12.0
100	139.583	−9.1	96.143	−5.5
500	745.719	−1.7	537.942	−9.9
Hour 2				
5	7.815	−10.4	4.642	−13.6
100	132.805	−13.5	95.699	−5.9
500	731.325	−3.6	507.509	−15.0

NA, not applicable.
[a] % Diff = (mean at *x* h − mean at 0 h)/mean at 0 h × 100.

4. Conclusion

Eribulin, a non-taxane microtubule dynamics inhibitor with promising anti-cancer activity, is currently being evaluated in combination with carboplatin for the treatment of solid tumors. As currently available techniques for the analysis of carboplatin in human plasma were not sufficiently sensitive to meet the requirements of this trial, sensitive and specific LC/MS/MS methods were developed and validated for the determination of carboplatin and eribulin in human plasma. The method met regulatory requirements for accuracy, precision, sensitivity, selectivity and analyte stability, and calibration curves for both carboplatin and eribulin showed goodness of fit over the concentration range studied using a $1/x^2$ weighted linear regression.

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References

[1] M.J. Towle, K.A. Salvato, J. Budrow, B.F. Wels, G. Kuznetsov, K.K. Aalfs, S. Welsh, W. Zheng, B.M. Seletsky, M.H. Palme, G.J. Habgood, L.A. Singer, L.V. Dipietro, Y. Wang, J.J. Chen, D.A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M.J. Yu, B.A. Littlefield, Cancer Res. 61 (2001) 1013.
[2] R.C. Gaver, G. Deeb, Cancer Chemother. Pharmacol. 16 (1986) 201.
[3] G.F. Duncan, H.C. Faulkner III, R.H. Farmen, K.A. Pittman, J. Pharm. Sci. 77 (1988) 273.
[4] R.C. Gaver, N. Colombo, M.D. Green, A.M. George, G. Deeb, A.D. Morris, R.M. Canetta, J.L. Speyer, R.H. Farmen, F.M. Muggia, Cancer Chemother. Pharmacol. 22 (1988) 263.
[5] P.A. Reece, J.F. Bishop, I.N. Olver, I. Stafford, B.L. Hillcoat, G. Morstyn, Cancer Chemother. Pharmacol. 19 (1987) 326.
[6] F. Elferink, W.J. van der Vijgh, H.M. Pinedo, Anal. Chem. 58 (1986) 2293.
[7] R.C. Gaver, A.M. George, G. Deeb, Cancer Chemother. Pharmacol. 20 (1987) 271.
[8] K. Tyczkowska, R.L. Page, J.E. Riviere, J. Chromatogr. 527 (1990) 447.

[9] M.A. Allsopp, G.J. Sewell, C.G. Rowland, J. Pharm. Biomed. Anal. 10 (1992) 375.
[10] J.A. Hadfield, A.T. McGown, M.J. Dawson, N. Thatcher, B.W. Fox, J. Pharm. Biomed. Anal. 11 (1993) 723.
[11] J. Prat, M. Pujol, V. Girona, M. Munoz, L.A. Sole, J. Pharm. Biomed. Anal. 12 (1994) 81.
[12] R.B. Burns, R.W. Burton, S.P. Albon, L. Embree, J. Pharm. Biomed. Anal. 14 (1996) 367.
[13] R. Falter, R.D. Wilken, Sci. Tot. Environ. 225 (1999) 167.
[14] R.B. Burns, L. Embree, J. Chromatogr. B: Biomed. Sci. Appl. 744 (2000) 367.
[15] L. Zufia, A. Aldaz, C. Castellanos, J. Giraldez, J. Chromatogr. B: Biomed. Sci. Appl. 764 (2001) 457.
[16] P. Guo, S. Li, J.M. Gallo, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 783 (2003) 43.
[17] S. Hann, Z. Stefanka, K. Lenz, G. Stingeder, Anal. Bioanal. Chem. 381 (2005) 405.
[18] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation, Research, Center for Veterinary Medicine, Federal Register 66 (2001) 28526.