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LC-MS/MS Determintion of Cabazitaxel in Rat Whole Blood on Dry Blood Spots

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Abstract

A, rapid sensitive and specific method based on high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) detection with electro spray ionization in positive mode has been developed for the determination of Cabazitaxel in rat whole blood on dry blood spots. Chromatographic separation was performed on Agilent Eclipse XDB column (C18 150 X 4.6 mm 5µ), with a mobile phase consisting of Acetonitrile and 1mM ammonium acetate at a flow rate of 0.4 ml/min. The analytes were then detected by monitoring the transitions for Cabazitaxel m/z 836.400/433.200, 836.400/555.300, 836.400/523.200 836.400/541.200 and for Paclitaxel m/z 854.400/286.000 using API-4000 LC-MS/MS system. Calibration curve were linear within the range of 0.2 to 20.00 ng/mL. The lower limit of quantitation (LLOQ) was 0.2ng/mL. The method has been validated in rat whole blood on dry blood spots. This method can be successfully applied to the pharmacokinetic study sample analysis.

Keywords: Cabazitxel; LC-MS/MS; Dry Blood spots; Pharmacokinetic.

Introduction

Dried blood spot (DBS) screening has been used as far back as the 1960s for the early detection of metabolic disorders in infants such as phenylketonuria [1-3]. The technique is increasingly viewed as a viable alternative to venous blood sampling for large-scale pediatric clinical trials primarily because of the small sampling volume required (usually between 10 and 30µL). Other advantages of DBS include simplified sample collection, easy storage and less expensive sample shipment under ambient conditions [3-7]. These benefits also make DBS a practical alternative for pre-clinical toxicokinetic studies because it facilitates multiple sampling of the same animal, thus significantly reducing the number of animals per study [8-10]. In recent years, the DBS technique has extended beyond pediatric screening to monitoring concentrations of xenobiotic drugs in biological matrices [7,8,11-15].

The drug substance is cabazitaxel acetone solvate. Cabazitaxel (CABZ; Figure 1), (1S,2S,3R,4S,7R,9S,10S,12R,15S)-4-(Acetyloxy)-15-{[(2*R*,3*S*)3{[(tertbutoxy)carbonyl]amino}-2-hydroxy-3-phenylpropanoyl]oxy}-1-hydroxy-9,12-dimethoxy-10,14,17,17 tetramethyl11oxo-6 oxatetracyclo [11.3.1.$0^{3,10}$.$0^{4,7}$] heptadec-13-ene-2-yl benzoate, A semi-synthetic derivative of the natural taxoid 10-deacetylbaccatin III with potential antineoplastic activity. Cabazitaxel binds to and stabilizes tubulin, resulting in the inhibition of microtubule depolymerization and cell division, cell cycle arrest in the G2/M phase, and the inhibition of tumor cell proliferation. Unlike other taxane compounds, this agent is a poor substrate for the membrane-associated, multidrug resistance (MDR), P-glycoprotein (P-gp) efflux pump and may be useful for treating multidrug-resistant tumors for the treatment of hormone-refractory prostate cancer [16].

Cabazitaxel (Jevtana) is a new member of the Taxane family. Other compounds in this group include docetaxel and paclitaxel. Many of these low-aqueous solubility compounds can be solubilised for intravenous infusion by using a combination of alcohols and non-ionic surfactants, in this particular case ethanol and polysorbate 80. The product is supplied as a sterile non-aqueous concentrate for solution for infusion containing cabazitaxel 60mg/1.5ml packaged in a glass vial, and an additional solvent for dilution of the Concentrate the Solvent is a sterile, non pyrogenic 13% w/w aqueous solution of ethanol. The solvent is a clear colourless liquid and is packaged in a glass vial. The concentrate and the solvent are intended for the preparation of a premix solution of cabazitaxel at 10mg/ml prior to dilution with 0.9% saline or 5% dextrose solution in an infusion bag [17].

Very few methods have been developed and validated for detection and estimation of Cabazitaxel biological media using LC-MS/MS [18].The current work describes a novel DBS LC-MS/MS method which allows to monitor CABA concentrations in rodent whole blood samples. The method may be useful for routine drug monitoring in newborns and elderly patients, experimental research in small animals such as rats, and assays that require small sample volumes. The assay was validated on the basis of the latest Food and Drug Administration (FDA) guideline for bioanalytical method validation [19].

Materials and Methods

Chemicals and rat whole blood

Cabazitaxel (CABA; Figure 1) and Paclitaxel (PAC) were purchased from a local market in Hyderabad, India. HPLC-grade acetonitrile was purchased from J.T Baker. HPLC-grade water was obtained from a Waters. DMS blood spot cards or Dry Blood Spot cards (DBS) were supplied by Agilent, Sample tubes were obtained from Tarsons (Kolkata, India). The repeater multi pipette used for spotting blood was obtained from Brand (Germany), EDTA coated capillaries from Sarstedt, Rat whole blood from Dpt. from Zoology, HLB 1CC 10mg Cartridges from Waters were used.

Preparation of standard stock and working solutions

Primary stock solutions of CABA and PAC (internal standard)

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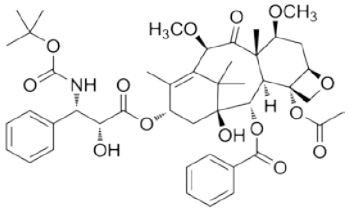

Figure 1: Cabazitaxel.

were prepared in acetonitrile (1.0 mg/mL). Working standard solutions of CABA in acetonitrile-water (50:50, %v/v) were prepared from the primary stocks. Internal standard working solutions (5.0 ng/mL) were prepared from the primary stock using acetonitrile–water (50:50%, v/v) for blood spot analysis. All the stock solutions and working standards were stored at 4°C.

Preparation of calibration standards and quality control samples

The calibration curve (range 0.2–20 ng/mL) standards were prepared in acetonitrile-water (50:50, v/v) by adding known amounts of CABA stock solutions. The whole blood calibration standards were prepared fresh on the day of analysis by diluting the appropriate working solutions with blank whole rat blood. The concentrations of calibrants were 0.2, 0.5, 2.0, 5.0, 10.0 and 20.0 ng/mL. Lower limit of quantification (LLOQ) QC and low-quality control (LQC) samples were obtained by spiking CABA in whole rat blood; the final concentrations were 0.2 and 0.6 ng/ml, respectively. Middle-quality control (MQC) and high-quality control (HQC) samples were obtained by spiking in whole rat blood with concentrations of 2.0 and 20.0 ng/ml, respectively. The concentrations were corrected for potency and amount weighed. The resulting peak area ratios were plotted against the concentrations. Spiked blood was kept on vortex shaker for 5.0 min for even distribution in the sample before spotting onto the DBS cards. The spotted DBS cards were allowed to dry at room temperature.

Blood spotting

Aliquots (15µL) of calibration standards and QC samples were spotted on DBS cards with a calibrated pipette and allowed to dry at room temperature for at 2 hour prior to analysis. When required, the QCs were stored at room temperature in sealed plastic bags containing desiccant until analysis.

Sample processing

A 3.0mm disk was punched from the center of the DBS into a clean tube (Figure: 2). It was then extracted by the addition of 500 mL extraction solution (acetonitrile) followed by vortex mixing for 5.0 min. The tube was centrifuged for 10.0 min at 4000 rpm, and the supernatant was transferred to a clean tube and filtered through HLB Solid phase cartridges before LC-MS/MS analysis.

Instrumentation

The HPLC system consist of a Shimadzu Prominence model equipped with prominence binary pump, SIL HTC auto injector, CTO oven coupled with Mass detector API-4000 (Applied Biosystems) triple Quadra pole system. Separation was achieved by using Agilent Eclipse XDB C18 150 X 4.6 mm; 5µ column. The mobile phase contains 1mM Ammonium acetate and Acetonitrile The flow rate was set to 0.4mL/min with gradient mobile phase starting 90% aqueous till 2.0 mins and from 2.01 mins increase the organic to 70% till 4.0mins and after 4.01 mins increased the Aqueous to 90% until the end of chromatogram. The total run time was 5.0mins.

The sample injection volume was 15uL and the column temperature was maintained at ambient conditions. The electrospray ionization (ESI) positive mode of ion detection was used for mass spectrometric analysis and detection. Mass spectrometric analysis was performed in positive ion mode and set up in multiple reaction monitoring (MRM). Nitrogen was used as a current gas (40 psi).Zero air used as collision gas for fragmentation of the parent molecule. The product ions for Cabazitaxel m/z 836.400/433.200, 836.400/555.300,836.400/523.200 836.400/541.200 and for Paclitaxel m/z Mass (es): 854.400/286.000 .

Validation procedures

The validity of the analytical procedure was established through a study of specificity, linearity, precision and accuracy according to the compliance criteria of Food and Drug Administration (FDA) guideline for bioanalytical method validation.

Results and Discussions

Method validation

Linearity: Linearity was evaluated by analysis of calibration samples containing six different concentrations (0.2, 0.5, 2, 5, 10 and 20 ng/mL). Calibration plots of the analyte peak area ratio compared with the internal standard vs the nominal concentration in blood were constructed.

Selectivity: Selectivity was performed, by analyzing the rat whole blood from four different animals to test for interference at the retention time of Cabazitaxel and internal standard Paclitaxel. No interference was observed, at the retention times of analyte as well as the internal standard. The % RSD of the area ratios of the six replicates of DBS LLOQ samples was less than 15% for cabazitaxel, confirming that interference does not affect the quantification at LLOQ level.

Matrix effect: To assess the matrix effects of the LC-MS/MS detector response, from four independent sources of matrix were spiked and extracted with analyte at the concentrations 0.2, 0.6, 2.0 and 20 ng/mL and internal standard at the concentration used in method .The peak areas of the analyte and internal standard in extracts of replicate DBS QC samples were compared with those of the same concentration of analyte and internal standard spiked directly into acetonitrile- water (50:50, %v/v). No unacceptable interferences, with peak areas of >20% were observed for the lower limit of quantification, at the retention times of analyte or its internal standard.

A representative mass chromatograms of a control DBS blank sample, LLOQ and IS were shown in Figure 3a, 3b, 3c. The suppression

Figure 2:

of the response for the internal standard when analyzing DBS extracts by LC-MS/MS was negligible (less than 5%)

Accuracy and Precision: The intra- and inter- assay accuracy and precision of the method were determined by assaying six replicates of each of the three concentrations (0.6, 2.0, 20.0 ng/mL) of validation QC samples on two consecutive days. Concentrations were determined from the appropriate calibration plot within each analytical run. Accuracy was assessed for each concentration by calculating the percentage deviation from the theoretical (nominal) concentration. The precision was determined for each concentration by calculating the percentage coefficient of variation (%CV; relative standard deviation) for each set of replicates. The intra- and inter-run performance data are summarized in Table 1.

Recovery: Six sets of aqueous recovery comparison samples were prepared and injected. The recovery comparison samples of analyte were compared against extracted samples of LQC, MQC and HQC samples. The percentage recovery of the analyte and the internal standard was calculated and it was 45.6% and 63.5 %.

Stability: Stability experiments were performed on dried blood spots samples under different storage (ambient temperature under vacuum desiccator and refrigerator at 2-8°C) and processing conditions. The stability of the analyte in dried blood spots were assessed at 2-8°C for 15 days. The accuracy, precision and sensitivity of processed DBS validation samples were found to be acceptable (accuracy and precision were well within the 15% pre-defined limits) on re-injection with freshly prepared calibration standards, after storage at ambient temperature under vacuum desiccator and refrigerator at 2-8°C for 6 hrs. The stability experiments were executed at two concentration levels (LQC and HQC). All experiments were performed in triplicate. The stability data for analyte are presented in Table 2.

Conclusions

A simple and rapid assay was developed and validated for the determination of CABA in DBS samples by LC-MS/MS. All validated parameters were within accepted limits. The developed sample pretreatment procedure has a small, negligible matrix-effect and good extraction recovery that is, more importantly, consistent. The within-

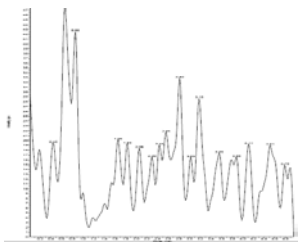

Figure: 3a

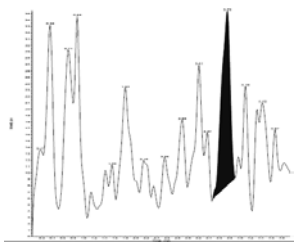

Figure: 3b

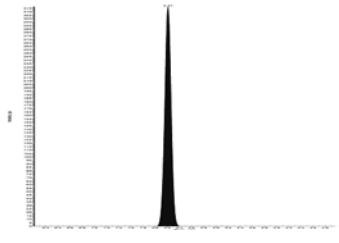

Figure: 3c

Figure 3:

Within batch precision (%RSD) n=6	Precision and Accuracy -1	1.23% - 3.65%
	Precision and Accuracy -2	0.99% - 3.33%
	Precision and Accuracy -3	1.34% - 2.19%
Within bath accuracy(%nominal) n=6	Precision and Accuracy -1	90.63% - 100.03%
	Precision and Accuracy -2	89.64% - 100.89%
	Precision and Accuracy -3	91.49% - 100.15%
Intraday batch precision(%RSD) n=6	Day-1	1.49% - 2.68%
	Day-2	1.34% - 2.19%
Intraday batch Accuracy (%nominal) n=6	Day-1	90.14%- 100.46%
	Day-2	90.20% - 100.02%
Between batch precision(%RSD)n=6	NA	1.48% - 3.19%
Between batch accuracy (%nominal)n=6	NA	91.49% - 100.15%

Table 1: Precision and Accuracy of assay at three QC concentration levels of Analyte.

Experiment	QC level	Change %	Stability Duration
Bench Top Stability (ambient temperature under vacuum desiccator)	LQC HQC	-6.5 -5.0	6.0 Hr
Refrigerator at 2-8°C	LQC HQC	-4.3 -2.7	6.0 Hr
Re-injection reproducibility	LQC HQC	-3.8 -2.9	6.0 Hr
Long term stability on Dry blood spots at 2-8 °C	LQC HQC	-7.5 -6.7	15 Days

Table 2: Stability Data.

and between-run accuracy was sufficient for LOQQC, LQC, MQC and HQC. The within- and between run precisions were well within limits for all quality controls. The blood spots proved to be stable during a period of at least 15 days at ambient temperature and at least 15 days at 4°C. The method is matrix-effect free, accurate and precise; furthermore, blood spots proved to be stable for an appropriate period of time.

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