DETERMINATION OF GENOTOXIC ALKYL PARATOLUENE SULFONATES IN CABAZITAXEL USING LC-MS/MS METHOD

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ABSTRACT

The present paper aimed to develop a suitable LC-MS method for the determination of genotoxic impurities such as alkyl paratoluene sulfonates (APTSs) in Cabazitaxel using LC-MS/MS method. APTSs were determined by LC-MS/MS method using Waters Symmetry C18 (75×4.6mm), 3.5µ column as stationary phase. Column temperature maintained 40°C, Injection volume 10µL, Flow rate was 0.8mL/min, sample cooler temperature 25°C and run time was 25mintues. Ammonium acetate buffer is used. The mixture of buffer and acetonitrile in 70:30 (v/v) was used as mobile phase. The method validation has been carried as per International Conference on Harmonization guidelines. Linearity was found to be 2.66-37 µg/mL, 2.80-150 µg/mL, 2.60-150 µg/mL for MPTS, EPTS and IPTS respectively. Correlation coefficient values were found to be 1.000 for MPTS and 0.999 for EPTS and IPTS respectively. Limit of quantitation (LOQ) was found to be for MPTS 2.66 µg/mL, EPTS 2.75 µg/ mL and IPTS 2.55µg/mL for APTSs. There was no validated method offered a higher sensitivity and smaller cost for the quantification of genotoxic paratoulene sulfonates in Cabaitaxel human plasma.

Key words: Genotoxic impurities, Cabazitaxel, Liquid Chromatography Mass Spectrometry (LC-MS) method, validation and limit of quantitation.

Abbreviations: APTS= (MPTS=Methyl para toluene sulfonate, EPTS=Ethyl para toluene sulfonate and IPPTS=Isopropyl para toluene sulfonate)

1. INTRODUCTION

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose [1]. These limits generally fall at low µg/mL levels. HPLC, GC methods (or final drug substance methods) are suitable for their determination. Their applications are oriented towards the potential identification and quantitation of trace level of impurities in drug substances [2].

Cabazitaxel[CBZ] is (1S,2S,3R,4S,7R,9S,10S,12R,15S)-4-(Acetyloxy)-15-{[(2R,3S)-3-{[(tert-butoxy) carbonyl] amino} -2-hydroxy- 3-phenylpropanoyl]oxy} -1-hydroxy- 9,12 –dimethoxy-10,14,17,17-tetramethyl-11-oxo-6-oxatetracyclo[11.3.1.03,10.04,7]heptadec-13-en-2-ylbenzoate. It has an empirical formula $C_{45}H_{57}NO_{14}$ and having the molecular weight of 835.93 g/mol. Cabazitaxel (**Fig.1**) is a taxane type of chemotherapy drug which is a semi synthetic derivative of a natural taxoid used to treat advanced hormone refractory prostate cancer that is no longer responding to hormone therapy [3].

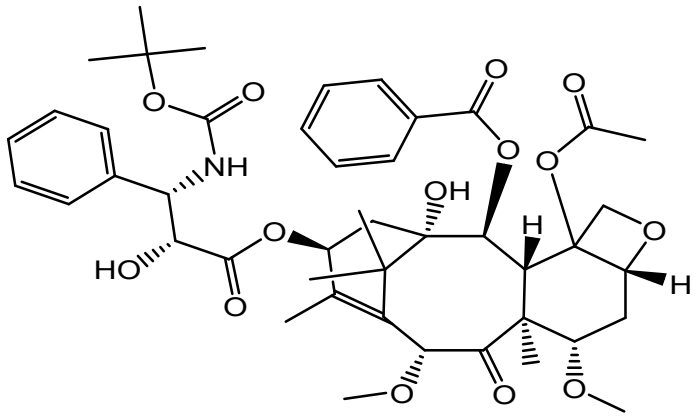

Fig.1. Chemical structure of Cabazitaxel.

Cabazitaxel is an anti neoplastic used with the steroid medicine prednisone. Cabazitaxel is used to treat people with prostate cancer that has progressed despite treatment with docetaxel. Cabazitaxel and prednisone combination is most suitable option for the treatment of hormone refractory prostate cancer. Cabazitaxel is a microtubule inhibitor. Cabazitaxel has been approved in the US by the Food and Drug Administration (FDA)[4-5] and by European Medicines Agency (EMA) in combination with prednisone for the treatment metastatic prostate cancer [6-8].

Impurities structures:

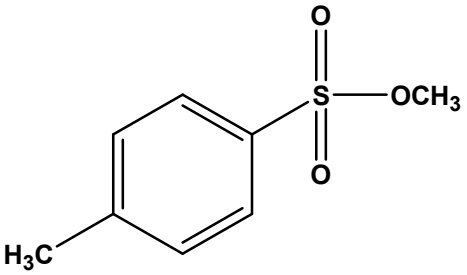

Fig.2. Chemical structure of Methyl para toluene sulfonate.

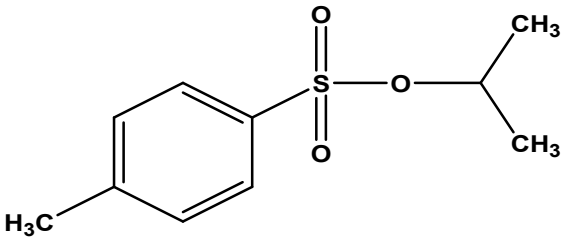

Fig.3. Chemical structure of Isopropyl para toluene sulfonate.

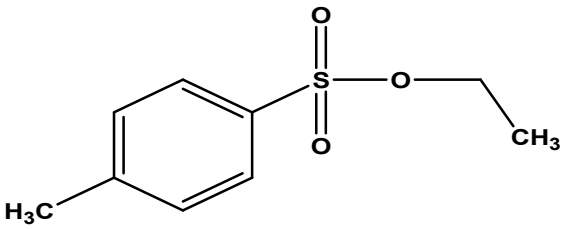

Fig.4. Chemical structure of Ethyl para toluene sulfonate.

In literature, very few analytical methods such as spectrophotometric methods including derivative and simultaneous spectrophotometric methods [9] and RP-HPLC methods [10-13], LC-MS/MS [14-16] were reported for the simultaneous determination of Cabazitaxel. Existing literature reported the analytical methods using hyphenated techniques for the determination of APTSs **(Fig.2-4)** [17-19]. However, no analytical method was reported for the determination of APTSs in CBZ. Hence the author was aimed towards the development of rapid, specific and robust methods for the determination of APTSs in CBZ at trace level concentration.

2. EXPERIMENTAL

2.1. Chemicals and reagents: Methyl para-toluene sulfonate (MPTS) (**Fig.2**), Ethyl para-toluene sulfonate (EPTS) (**Fig.3**) and Isopropyl para toluene sulfonate (IPPTS)(**Fig.4**) purchased from Aarti Drugs Ltd., Mumbai, India. Ammonium acetate and Methanol were procured from Merck, India. Pure samples of Cabazitaxel were obtained from synthetic division of Teva Czech Industries S.R.O. (R&D), Vadodara, and Gujarat, India.

2.2. Preparation of Standard solution: Methanol was used as diluent in the present method. MPTS, EPTS and IPPTS stock solutions were prepared by dissolving 10mg each individually in100mL of diluent. 1.0mL of the stock solution further diluted into 100mL with diluent. 12.5mL of this solution further diluted to 100ml with diluent.

2.3. Preparation of Test solution: Weighed accurately 50mg of test sample and transferred into a 10mL volumetric flask, dissolved and diluted up to mark of the flask with diluent. (Concentration 5mg/mL).

2.4. Preparation of Mobile phase-A:1.54g of ammonium acetate was transferred into a 1000mL beaker and was dissolved in 1000mL of water. Filtered through 0.22µ membrane filter paper and degassed.

2.5. Preparation of Mobile phase-B: Methanol

2.6. Diluent: Methanol.

Table 1. MS conditions

Instrument	ABSciex3200QTRAPwithLCAgilent1200.
Detector	Electro spray mass spectrometer operating in positive ion mode. The protonated species of Methyl tosylate (m/z 204.1), Ethyl tosylate (m/z 218.3) and Isopropyl tosylate (m/z 232.2) with ammonium aducts.
Scan	Q1 Multiple Ion
Mode	Selective Ion mode
Polarity	Positive
Ion Source	Turbo spray
Resolution QI	Unit

Table 2. Ql Multiple ions conditions for Methyltosylate

Methyl tosylate	204.100*m/z(parent) *±0.5units can be varied
Declustring potential	14
Entrance potential	4.16
Collision Exit potential	28.93
Ion source	5500

Table 3. Ql Multiple Ions Conditions for Ethyltosylate

Methyl tosylate	218.300* m/z(parent)*±0.5units can be varied
Declustring potential	29.35
Entrance potential	4.95
Collision Exit potential	27.13
Ion source	5500

Table 4. Ql Multiple Ions Conditions for Isopropyl tosylate

Methyl tosylate	232.200*m/z(parent) *±0.5units can be varied
Declustring potential	18.70
Entrance potential	4.69
Collision Exit potential	20.89
Ion source	5500

Table 5. Sou rce/Gas parameters

Curtain Gas	30
Temperature	500°C
Ion Source GS1	40
Ion Source GS2	40

Table 6. Valeo valve Condition

Valeo Valve	Diverter Total time(min)	Position
1	15	B
2	17	A

Position-A: HPLC flow to Mass spectrometer
Position-B: HPLC flow to Waste Collector

2.7. Chromatographic conditions: LC analysis was carried out on Agilent-1200 (Agilent Corporation, USA) Waters Symmetry C18 75×4.6mm, 3.5µ column was used as stationary phase. The mixture of ammonium acetate buffer and methanol was used as mobile phase. The flow rate of the mobile phase was kept at 0.8mL/min. The injection volume was set as 10µL. Column oven temperature and auto sampler temperature were set as 40°C and 25°C, respectively. Details of chromatographic conditions were incorporated in **Table 1-7**.

Table 7. Gradient Programme

Time(min)	Flow rate (mL/min)	Mobile phase-A	Mobile phase-B
0	0.8	70	30
5	0.8	70	30
10	0.8	50	50
13	0.8	20	80
18	0.8	20	80
20	0.8	70	30
25	0.8	70	30

3. RESULTS AND DISCUSSION

3.1. Method validation

The developed method was validated as per ICH guidelines in terms of specificity, limit of detection (LOD), limit of quantitation (LOQ), precision, linearity, accuracy, robustness and system suitability and the data are presented in **Table.8.**

The specificity of the developed LC-MS method was indicated by APTSs solutions (25µg/mL each) with respect to 5mg/mL of CBZ were injected separately and S/N ratios were recorded. These solutions were further diluted to achieve the signal-to-noise (S/N) ratios at about 3 and 10 for determining LOD and LOQ, respectively for both the methods. The precision of the methods was checked by injecting LOQ solutions for six times. The values of RSDs for areas of each APTS were calculated. The typical mass spectrograms of blank was shown in Figure 1.05.The Typical mass spectrograms of blank, MPTS,EPTS,IPTS and Cabazitaxel were given in **Fig. 5-9.**

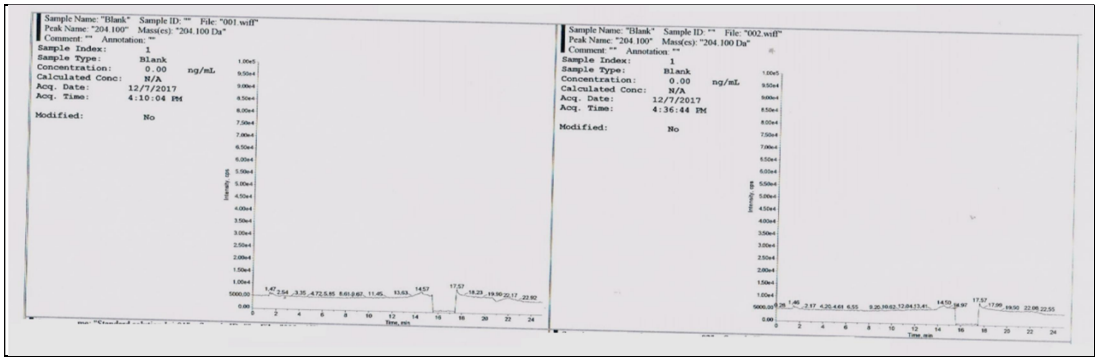

Fig. 5.Typical mass spectrograms of blank.

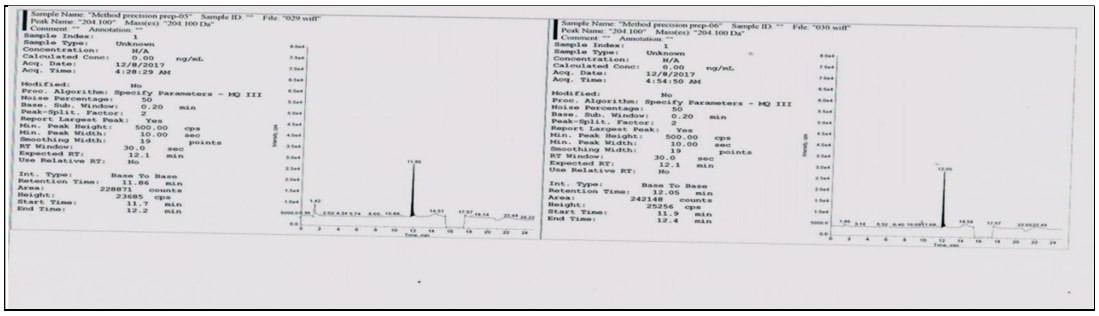

Fig.6. Typical mass spectrograms of Methyl para toluene sulfonate standard.

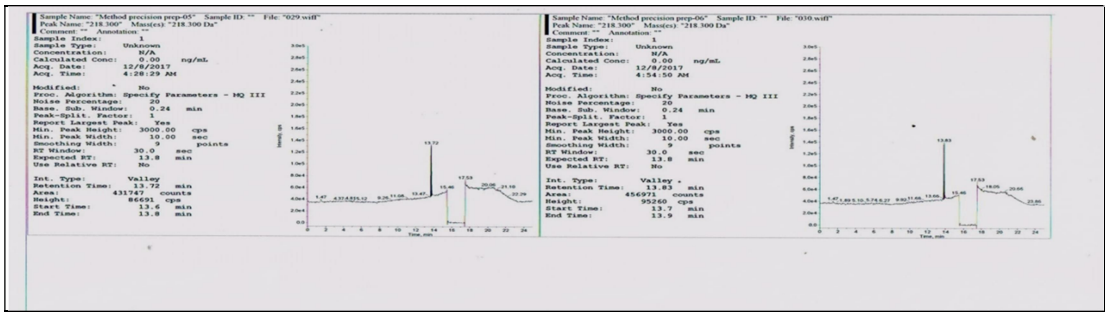

Fig.7. Typical mass spectrograms of Ethyl para toluene sulfonate standard.

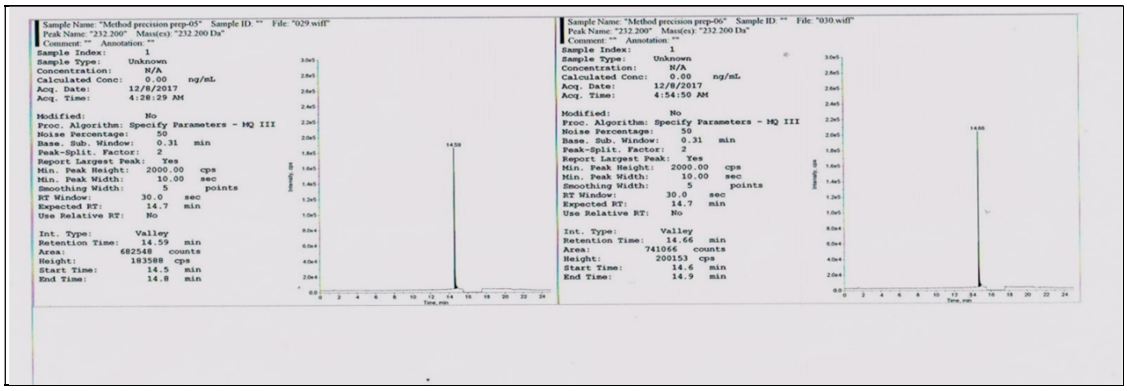

Fig.8. Typical mass spectrograms of Isopropyl para toluene sulfonate standard.

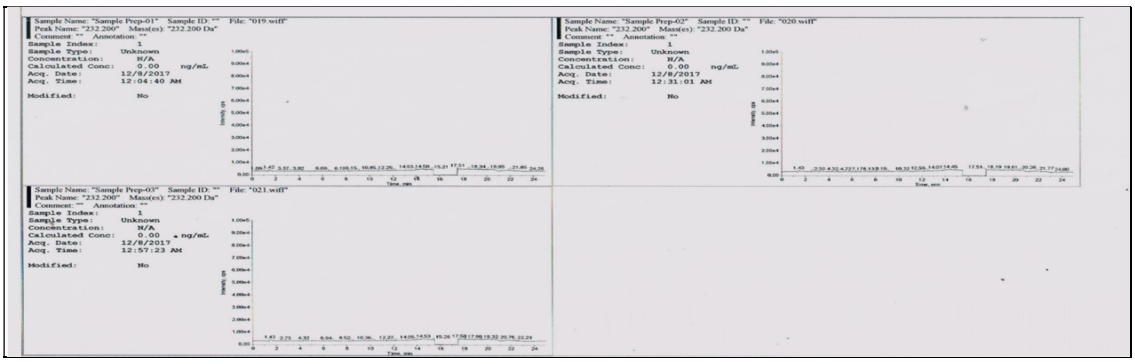

Fig.9. Typical mass spectrograms of Cabazitaxel sample.

Table 8. Validation data of CBZ for the determination of MPTS, EPTS and IPPTS.

Parameter	MPTS	EPTS	IPPTS
LOD (µg/mL)	0.80	0.83	0.77
LOQ (µg/mL)	2.66	2.75	2.55
Precision at LOQ level (RSD, %)	7.27	10.58	4.77
Precision at sixth level (RSD, %)	5.81	3.09	3.59
Linearity range (µg/mL)	2.66-37.5	2.80-150	2.60-150
Correlation coefficient	1.000	0.999	0.999
Slope	763	4501	7259
Intercept	0.000	3779	10199
Accuracy at LOQ (recovery, %)	97.0	101.0	98.2

The intermediate precision of the method was also verified on six different days in the same laboratory using the LOQ level solutions. The low RSD values ensured the precision of the developed method. Linearity test solutions for APTSs were prepared individually at six concentration levels in the range of LOQ to 150% of the specification level 25µg/mL. LOQ and sixth levels were injected six times and other four levels were injected thrice. The average peak areas versus concentrations were subjected to least-squares linear regression analysis. The derived correlation coefficients were above 0.9999 indicating the best fitness of the linearity curves of the developed method. Standard addition experiments were conducted in triplicate preparations to determine accuracy of the methods at LOQ level and recoveries of all the genotoxins were determined. The recoveries were found to be in the accepted range. The robustness of LC-MS method was ensure by getting the resolution between any two APTSs to be greater than 2.0, when mobile phase flow rate (1.0mL/min and 1.2mL/min), organic solvent ratio in mobile phase (90% and 110%) and column temperature (35°C and 45°C) were deliberately varied. The solution stability of APTSs in diluent in LC-MS method was determined by leaving

APTSs mixture solution at specification level in a tightly capped volumetric flask at room temperature for 48 hours and measuring the amounts of the APTSs for every 6 hours. All the APTSs were found to be stable up to 48 hours. The system suitability of the method was ensure by getting the %RSD less than 10.0 for six injections of all the APTSs in LC-MS method at specification level. Cabazitaxel at trace level concentration have been developed and validated as per ICH guidelines.

4. CONCLUSIONS

The proposed LC-MS/MS method that can quantify genotoxic alkyl para toluene sulfonates in Cabazitaxel at trace level concentration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensure by the specificity, precision, accuracy and robustness. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of Cabazitaxel into the market.

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