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A NOVEL PACLITAXEL COATED DRUG ELUTING 316L STENTS

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ABSTRACT

LCMS/MS method was used (ESI $^{+}$Source) to determine the concentration of Paclitaxel coated drug eluting stents (316 LVM). LCMS/MS has proved to be a powerful research tool due to its sensitivity, high selectivity, and high throughput efficiency to determine drug concentration of the sample. The elution kinetics was carried out on flow through dissolution apparatus-USP-4 (Sotax) .The samples were collected according to study design.The extraction process of the elution samples were on Liquid –Liquid extraction procedure. Chloroform was used as extraction solvent. The evaporated samples were reconstituted with mobile phase (80:20+0.1% acetic acid). LCMS/MS, triple quadropole Separation was achieved using phenomenax C-18 column (250x4.60mm 5microns). The flow rate was set to 0.8ml/min. UV detection of paclitaxel was at 228nm.Total run time was 6.0 for each run. Drug release was observed in first hour of implantation of the stent. Percentage of drug release was increased 24 hours, later it was stable. The maximum of Paclitaxel was released in first two days. The cumulative percentage of drug release was 50% in 12 days .This was based on the polymers (PLA/PLGA) and drug interaction. Release was based on the composition of the drug and polymer composition. The studies of the paclitaxol coated drug eluted stents were preventing early thrombosis.

KEYWORDS

Paclitaxel, 316 L stents, L-L extraction, Release kinetics, LC-MS/MS.

INTRODUCTION

Paclitaxel (taxol, see Figure no: 1) is a natural product extracted from the bark of the Pacific Yew Tree, and takes its name from Latin, *Taxes brevifolia.* It blocks mitosis by stabilizing the microtubules in cancer cells. During normal cell division, the micro tubules are polymerized at the beginning of mitosis to be able to separate the daughter chromosomes. Then they depolymerise back to tubuline. Taxol stops this depolymerisation so that the cells become filled with microtubules and cannot divide again. Taxol active against a number of cancers e.g.

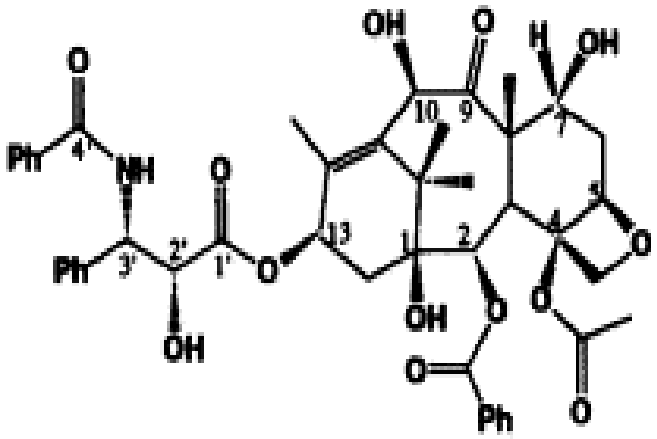

Figure no: 1
Paclitaxel structure

of the ovaries, breast, lung and stomach (http://www.oncolink.com). Suffer from relatively low sensitivity (typically LLOQ>10ng/mL; 1000µL; sample volume). With current developments of the taxanes there is a need for more sensitive assays and MS has become the detection method of many assays for the quantitative determination of paclitaxel and docetaxel described using LC-UV (Huizing et al., 1995; Rosing et al., 1997). LC-MS/MS assays have been described for the quantitative determination of paclitaxel and/or docetaxel in human plasma (Sottani et al., 1998; Esmaeli et al., 2002; Alexander et al., 2003; Basileo et al., 2003; Parise et al., 2003; Wang et al.,2003), human serum (Schellen et al., 2000), human tear flued (Esmaeli et al.,2002), dog plasma (Baldrey et al., 2002; Alexander et al., 2003), and mouse plasma and brain tissue (Guo et al., 2003). The first LC-MS/MS assay for the quantitative determination of a taxane was published by Sottani and Coworkers (1998) for paclitaxel in human plasma.

MATERIALS AND METHODS

Chemicals and Reagents: Paclitaxel was procured from Dr. Reedys Laboratories Limited Hyderabad. Paclitaxel Drug coated 18 mm 316 LVM stents samples were obtained our own made Relisys Medical Devices Ltd. Specific bio-degradable polymers PLA/PLGA(Poly latic acid , Poly L-glycolic acid) was procured from Germany.HPLC grade acetonitrile, chloropharm was from Merck & water from Milli –Q (S.G waters). All the reagents are used HPLC grade. Phosphate buffer saline (PBS P^H 7.2) was from Himedia. Flow through dissolution apparatus USP-4 was procured from Sotax (Switzerland).

Solutions and Buffers: Stock solution of Paclitaxel was prepared by dissolving 1mg of Paclitaxel in 1ml of acetonitrile which gives final concentration mg/ml. Standard solutions were obtained by diluting the solution with acetonitrile to give concentrations over the range of 10 – 1000 ng/ml for preparation of the standard curve. Mobile phase was 80:20(80% Acetonitrile,;20% water)+0.1% Acetic acid(100microliters in 100ml of mobile phase).Phosphate buffer saline (PBS) pH 7.2 was prepared 5.38gm in 500ml of water. The total amount of PBS was filtered by 0.1µ filtered without any particulate matter.

Flow through dissolution apparatus (USP-4) condition: For dissolution study, Flow through dissolution apparatus USP-4 was used. The method for elution study was created .Flow

rate 16ml/min, RPM 120 rounds per minute. Temperature of the flow cell was 37°c. All the 7 flow cells are filled with stents (6+1). Sample collection time points were given according to the study design.

Liquid chromatographic mass spectrometric conditions: LCMS/MS, Quattro micro API, triple quadropole. Mass lynx Soft ware version 4.1. LC consisted of a series of 2695 separation module and PDA (2996) detector all from Waters (Milford, MA, USA). Separation was achieved using phenomenax C-18 column (250x4.60mm 5microns). The mobile phase contains 0.1% Acetic acid (80:20+0.1%Acetic acid) was prepared and degassed. Chromatographic separations were performed at 30°C. The flow rate was set to 0.8ml/min. UV detection of paclitaxel was at 228nm.Total run time was 6.0 for each run.

ESI source was used for mass spectrometric analysis and detection. Mass spectrometric analysis was performed in the positive ion mode (ESI^+ **)** and set up in the multiple reaction monitoring(MRM).Nitrogen was used as sheath gas(100psi) .Argon used as collision gas (0.5psi) for fragmentation of the parent molecule. The capillary voltage was 40 v, cone voltage was 40 v. The product ions as *m/z* 876.20-286 for Paclitaxel.

Study design. Analysis of Paclitaxel coated drug eluting stents study was designed based on the drug release pattern of the drug coated stents. Elution study of Paclitaxel coated stents were carried out for 10 days. The time points for analysis at different time intervals are 1,6,24,48,72,96 hours, 5, 6,7,8,9 and 10 days. Samples from different intervals were collected from seven stents (6 Paclitaxel drug eluting stents +1 control) and stored at 4°c until analysis on LCMS/MS.

Sample preparation. Drug eluting stents were placed in 2ml PBS pH 7.2. Phosphate buffer saline (PBS) was collected at different time intervals according to the study design. PBS was taken out by using the micropipette. Same equal amount of PBS was replaced to each sample. The drug present in the PBS was vortex for 1 min extracted by using HPLC grade Chloroform. Then the chloroform was evaporated at 37^0C in 24 hours. The evaporated sample was reconstituted with mobile phase.

Extraction Procedure. Aliquots of 0.5ml of PBS PH7.2 (kinetics sample) was added with 1ml of chloroform and vortex for 1min. and kept the sample for 2min. A ring was formed between two solvents .The upper layer PBS was discarded with micro pipette remaining the chloroform was oven dried at 37^0C for overnight. The evaporated sample was reconstituted with 1ml of previously prepared mobile phase (80:20+0.1%acetic acid). This sample is vortexed for 1min.

Quantification: Calibration standards of paclitaxel were prepared standard solutions from 10-1000ng/ml. The sample preparation in PBS P^H $^{7.2}$ and extracted with 2mL of chloroform.Analysis was performed on LC-MS/MS.

RESULTS

In this study, ESI^+ was chosen as the ionization source. Signal intensity was high using ESI^+ source provided for the quantitation of elution samples(PK).Paclitaxel formed predominantly protonated molecules $[M+H]^+$ of *m/z* 876.20 in full scan spectra(Figure no:2).

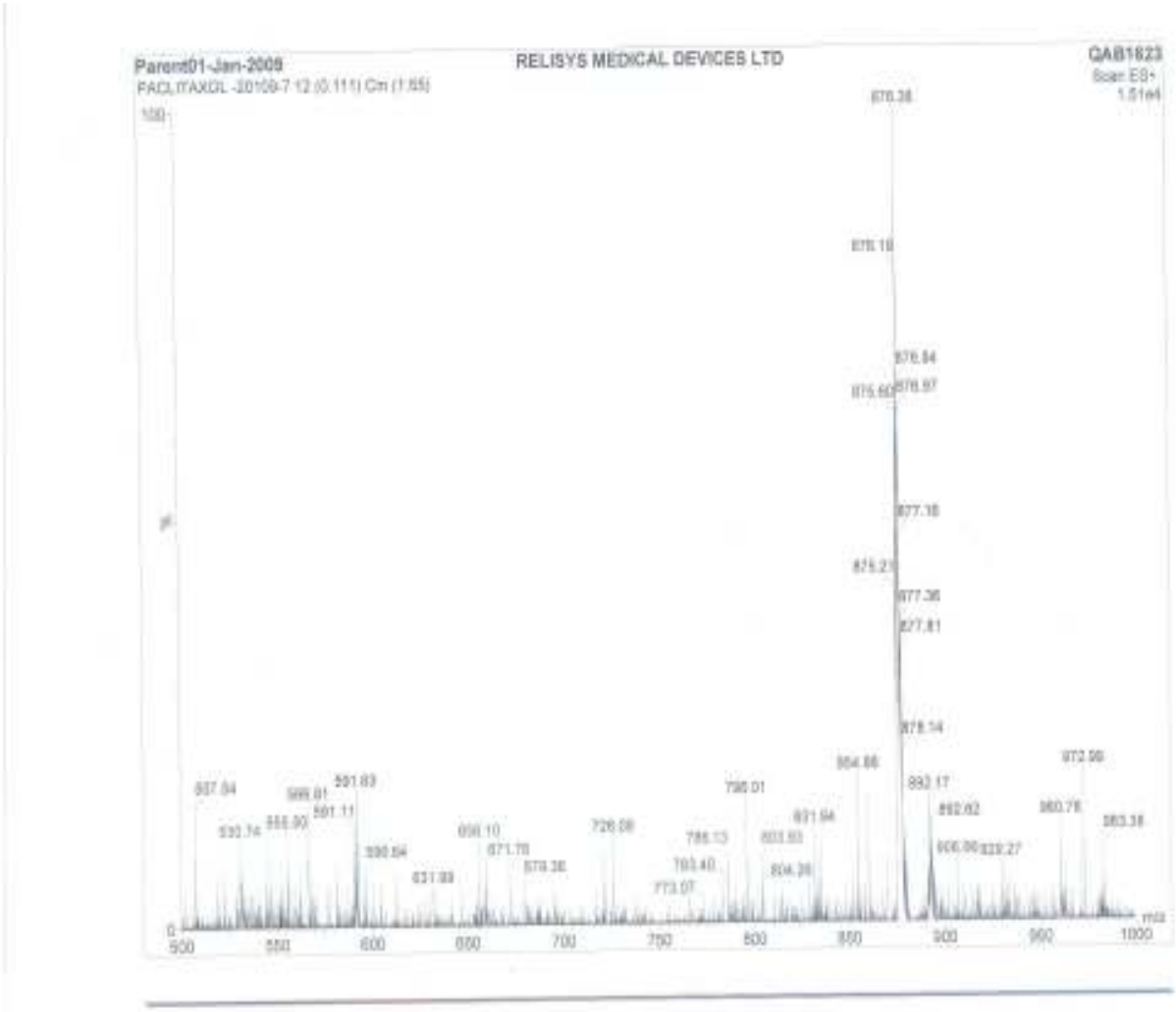

Figure no: 2

Paclitaxel parent molecular ion spectrum.

The most abundant ion in the product ion mass spectrum was at 308.00(Figure no: 3). The MRM transition of *m/z* 876.20-308.00 for paclitaxel was selected to obtain maximum sensitivity.

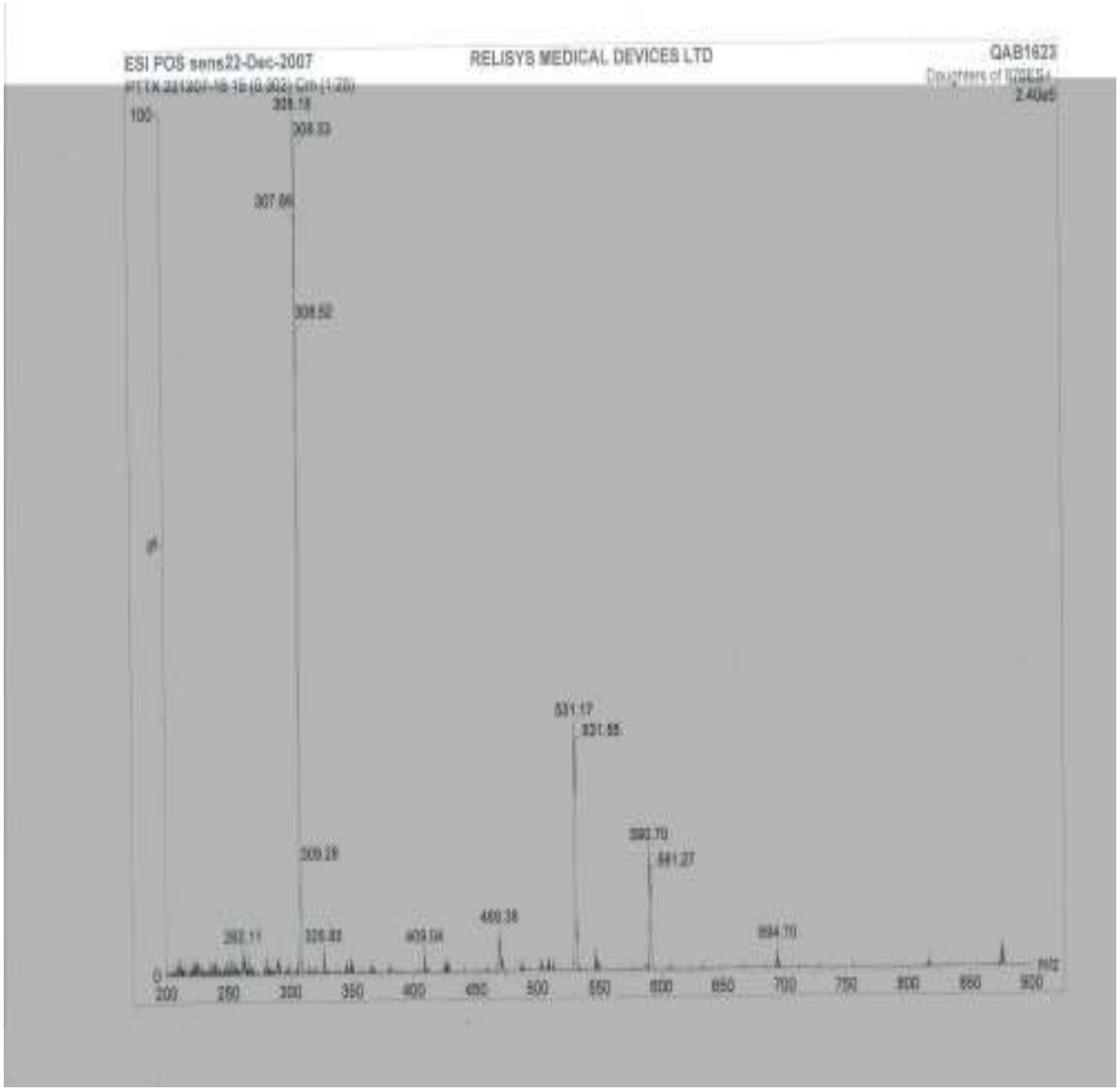

Figure no: 3
Paclitaxel product ion spectrum.

For quantification of paclitaxel coated drug eluting stents a new MRM method was created. For quantification of, the parent molecule was fragmented into the daughter ions through the argon as collision energy. A standard curve of Paclitaxel in different range of concentrations 10, 50,100.250, 500,1000ng/ml was prepared. The calibration curve displayed excellent linearity (r^2>0.99) (Figure no: 4) over the concentration range investigated.

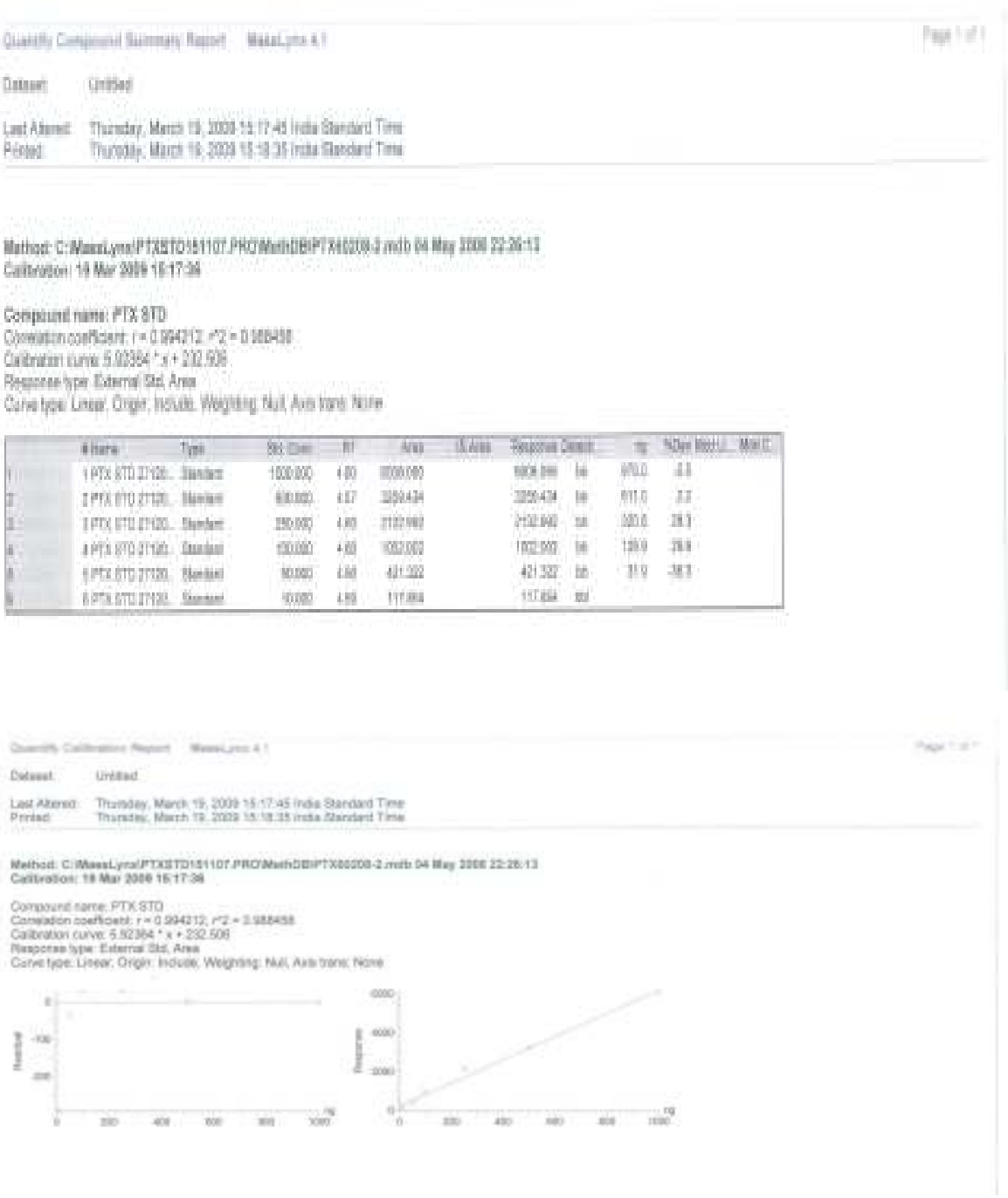

Figure no: 4
Paclitaxel standard calibration curve.

Paclitaxel coated drug eluting stents elution pattern was observed at different time intervals according to the study design. In the present study, a simple Liquid-Liquid extraction

procedure was used. The extraction efficiency was increased when liquid-liquid extraction using organic solvent as chloroform extraction procedure was used. The proposed chromatographic conditions of LCMS/MS analysis was carried out. Retention time of Paclitaxel approximately 4.6min. Representative Chromatograms were showed in Figure no: 5. Optimization was achieved by monitoring varying reversed phase column, mobile systems, flow rate and wavelength.

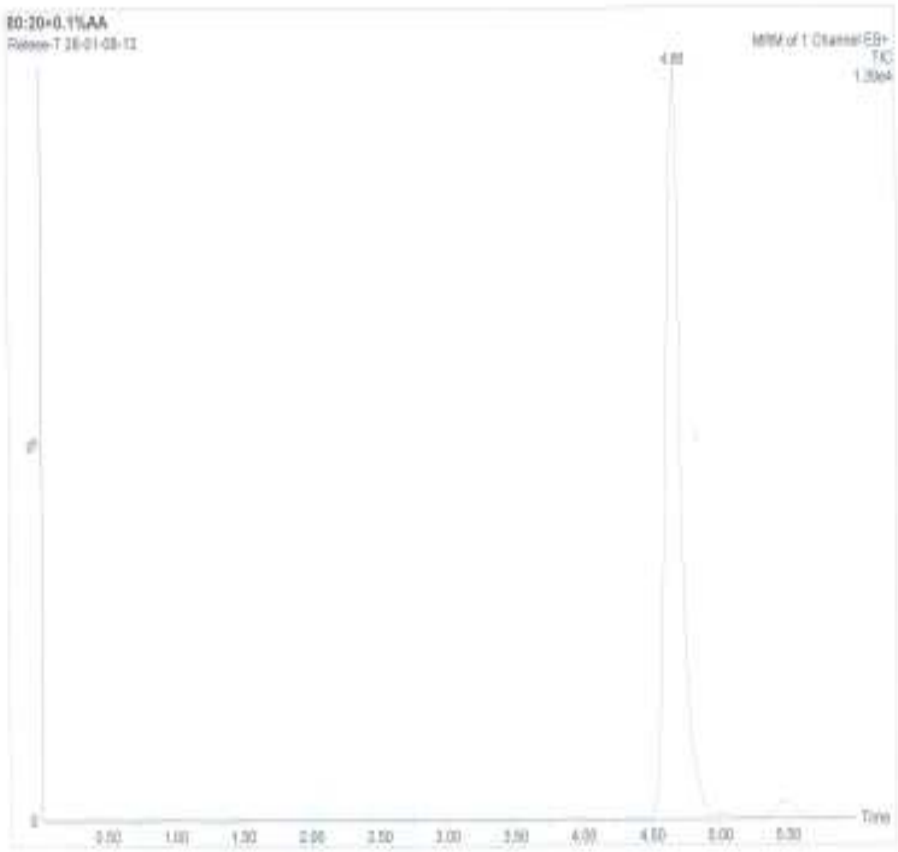

Figure no: 5
Total run time of Paclitaxel.

The results of drug coated stents were observed at 1st day of stent implantation. The release percentage of drug was observed in PBS P^H 7.2. The total samples before analysis and after analysis were weighed under sensitive balance (Table: 1) .The paclitaxel parent molecular contains adduct formation here, sodium (Na^+) was formed as adduct with paclitaxel.

Table no: 1

Paclitaxel coated stents weights before and after analysis.

S. No	Stent ID	Weight before analysis(mg)	Weight after analysis (mg)	Difference in Weight (mg)
1	RT-25180604	18.344	18.239	0.105
2	RT-25181604	17.245	17.148	0.097
3	RT-25184104	17.322	17.223	0.099
4	RT-25184404	17.849	17.765	0.084
5	RT-R27518080	17.298	17.214	0.084
6	S-1213(Cont)	17.915	17.914	0.001

(RT= Release Taxol)

DISCUSSION

In the present study the most important LCMS/MS technique for determination of Paclitaxel in biological fluids were studied. This technique is rapid and reliable and simple extraction method. LCMS/MS method was developed and validated. Several extraction methods have been used to accomplish extraction of drug from PBS P ^{H} 7.2.

To prepare matrix on the stent PLA/PLGA bio-degradable polymers were used .These polymers prepare matrix on the stent to adhesive drug on the stent. In this study a two step Liquid-Liquid extraction procedure is used chloroform as organic extracting solvent. In control samples (Non drug coated stents), there was no drug release. Mobile phase contains acetic acid as 0.1% to enhance ions in the drug sample. While extraction with organic solvent (Chloroform) a ring was formed between the organic and aqueous medium. The drug sample was extracted into organic solvent ($CHCl_3$) and then evaporated at 37^0 C in hot air oven. These evaporated samples were reconstituted with previously prepared mobile phase contains 0.1% Acetic acid. The recovery of the drug from the sample is 99 percentage. There was no drug in controlled stents. Variation in stents weight was found before and after analysis.

Drug release pattern was observed in first hour of implanted stent and the release pattern was started at the first hour of the implantation. And the drug release percentage was increased between 6-24hours (Figure no: 6).At the time of 48hours to 7 days uniform sample drug release was found. The highest percentage of Paclitaxel was released in first

two days of implantation of the stent. The cumulative percentage of drug was released in 12 days after implantation of stent was about 51% (Figure no: 7).

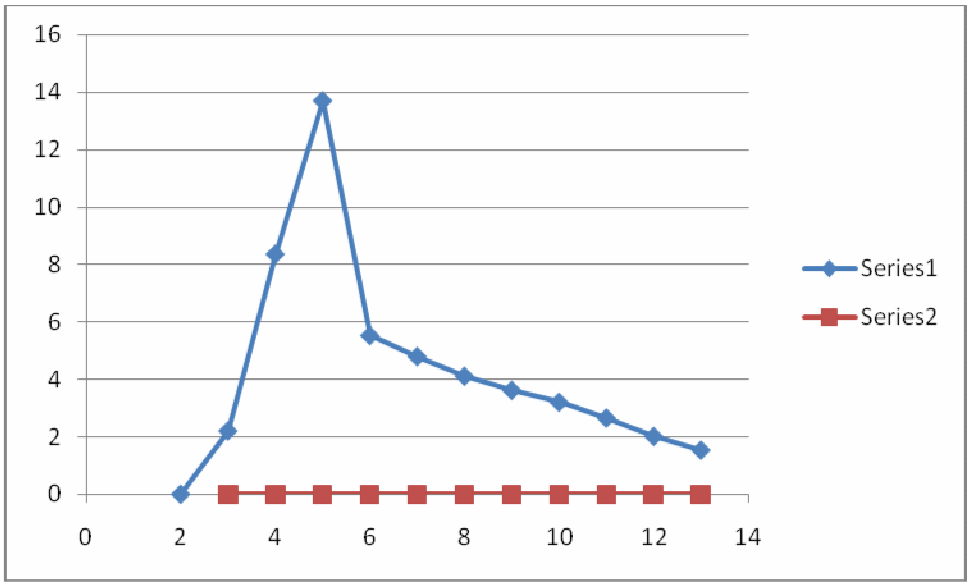

Figure no: 6

Paclitaxel coated stents release profile.

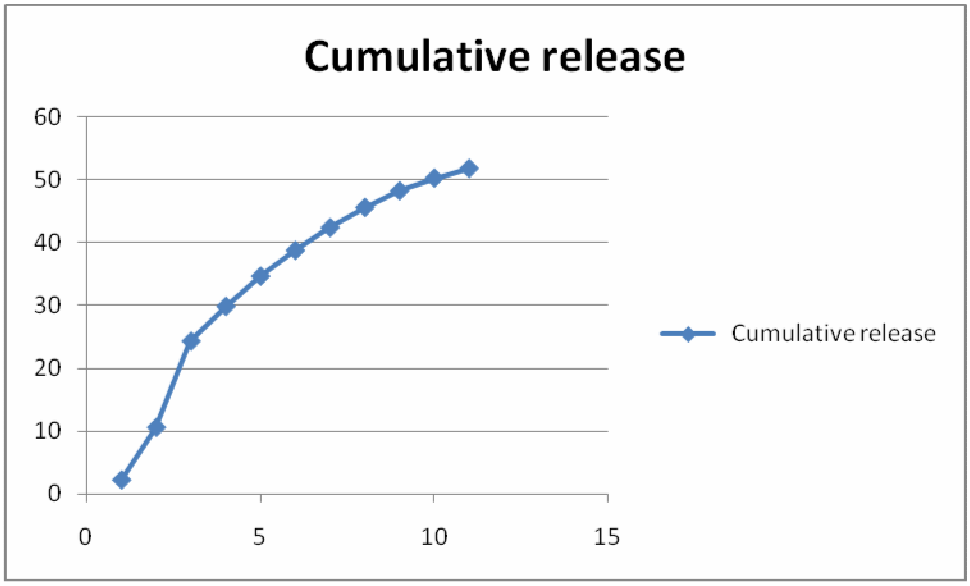

Figure no: 7

Cumulative release profile.

CONCLUSION

LCMS/MS has proved to be a powerful research tool due to its sensitivity, high selectivity, and high throughput efficiency. Derivatization techniques to improve the detect ability for LCMS/MS have been successfully used in the elution kinetic analysis. The study of elution kinetics shows a parametric drug release was observed. The drug release was found at the first day of implantation of the stent, however the highest percentage of release was observed at the time of 24 hrs of implantation of the stent. And the drug release comes down after implantation of the 48 Hrs. Drug release was stable with minimum of 2% after 8th day. The cumulative percentage of drug was released 51% of drug in 12 days of implantation of the stent.

After first week release was declined. This was based on the formulation of polymer (PLA/PLGA) and drug interaction. Release pattern was based on the composition of the drug and polymer composition. The study of the paclitaxel coated drug eluted stents was preventing early thrombosis while releasing the anti cancer drug.

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