JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

DRUG ELUTION KINETICS OF PACLITAXOL COATED STAINLESS STEEL STENTS

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INTRODUCTION:

Paclitaxel (taxol) is a natural product extracted from the bark of the Pacific Yew Tree, and takes its name 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. of the ovaries, breast, lung and stomach [1] suffer from relatively low sensitivety (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(e.g., Huizing et al., 1995; Rosing et al., 1997). However, they all have been chocen as. 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.

Key words: Paclitaxel, Pacific Yew Tree, cancer cells, human plasma.

MATERIALS AND METHODS

Chemicals and Reagents. PacliTaxol was procured from Dr. Reedys Laboratories Limited Hyderabad. Drug coated stents18 mm 316 LVM stainless steel stents. (6+1) were obtained from Relisys Medical Devices. HPLC grade Acetonitrile & water and Chloropharm were from Merck(Germany). Phosphate buffer saline was from HIMEDIA. Orbital shaker incubator was procured from Cintex(Mumbai, India.)

Solutions and Buffers: Stock solution of Paclitaxol was prepared by dissolving 1mg Paclitaxol 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 1000ml of water which gives 0.1 molar.The total amount was filtered by 0.1µ filtered without any particulate matter.

Chromatographic Conditions: LCMS/MS, API Quattro micro triple quadropole. Consisted of a series of 2695 separation module and PDA (2996) detector all from Waters (Milford, MA, USA). Separation was achieved using phenamenax C-18 column (250x2.5microns). 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.

Orbital shaker incubator Condtions. Orbital shaker incubator maintained speed 120RPM and Temperature 37°C Throughout the study.

Study design. Analysis of drug eluting stents was designed for 10 days at different time intervals those 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 to be collected for analysis from 7stents.(6 Paclitaxol drug eluting stents ,1 control) for analysis.

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^{0}C in 24 hours. Then 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 keep 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 24 hours. The evaporated sample was reconstituted with 1ml of previously prepared

mobilephase (80:20+0.1%acetic acid). This sample is vortex for 1min.

Quantification: Calibration standards of paclitaxol were prepared standard solutions from 10-1000ng/ml. The sample preparation and extraction and LCMS analysis were performed as described above.

Results: Paclitaxol coated drug eluting stents elution pattern was observed at different time intervals. In this a simple two step extraction method was followed. Under the proposed chromatographic conditions the retension time of Paclitaxol approximately 4.6min. Representative Chromatograms are shown in Fig 1.Optimization was achieved by monitoring varying reversed phase columns, mobile systems, flow rate wavelength.

Figure:1

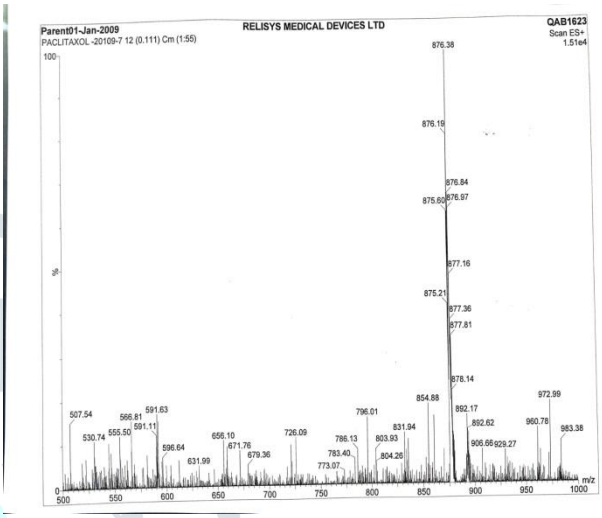
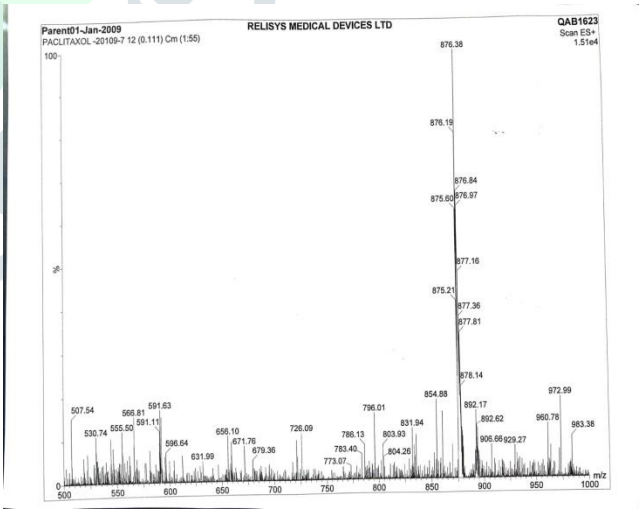
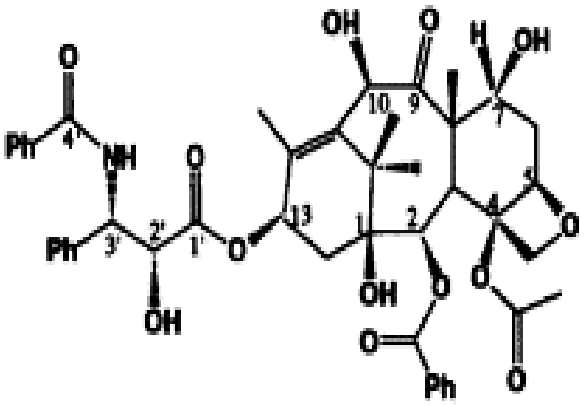


Figure:2





Structure of Paclitaxol

The results of drug coated stents was observed at 1st day there was a release percentage of drug was observed in PBS.

Calibration. A standard curve of Paclitaxol in different range of concentrations 10,50,100.250,500,1000ng/ml was prepared. The calibration curve displayed excellent linearity (r^2>0.994212)over the concentration range investigated.

Quantification: For the quantification of paclitaxel coated drug eluting stents a MRM method was created. For quantification the parent molecule was breaked into the daughter ions through the collision energy argon was used. (Figure no. 1)

The paclitaxel parent molecular weight was 854.70 but here, sodium (Na^+) was formed as a adopt ion. (Figure No. 2)

LOD / LOQ: Lower limit of detection / Lower limit of quantification for paclitaxel is 10 pico grams.

DISCUSSION.

In the present study the most important LCMS/MS technique for determination of Paclitaxol in biological fluids. 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.

In the present study a two step extraction procedure is described using chloroform as extracting solvent. In the control sample there was no drug was observed .In mobile phase while adding acetic acid to enhance ions in the sample. Extraction with organic solvent (Chloroform). A ring was formed between the organic and aqueous medium. The drug sample were extracted into organic solvent ($CHCl_3$) and evaporated at 37^0 C for one day. This evaporated sample was reconstituted previously prepared mobile phase contains 0.1% Acetic acid. The recovery of the drug from the sample is 95 percentage. There was no quantification in controlled stents.

Drug release pattern was observed in first hour of implanted and it was release percentage was increased between 6-48hours.At the time of 72hours to 5 days sample drug release profile

was stable at percentage of approximately 10. From day 5th to 8 a second release was observed. The cumulative percentage of drug release in this study approximately 70%In 10 days.

Conclusion:

LCMS/MS has proved to be a powerful research tool due to its sensitivity ,high selectivity, high throughput efficiency. Derivatization techniques to improve the detect ability for LCMS/MS have been successfully used in the analysis. The study of elution kinetics shows a parametric drug release was observed. The drug release was observed at the first day of implantation, however the highest percentage of release was observed at day 3-5.

After first week release was declined. This was based on the formulation of drug and polymer interaction.

The study of the paclitaxol coated drug eluted stents was preventing early thrombosis while releasing the drug.

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