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Comparison of Absorption Pattern of Paclitaxel and Vehicle Castrol Oil in Human Plasma under Different Physiological Solution by LCMS

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Abstract: Paclitaxel represent important antineoplastic agents with broad spectra of antitumor activity. The authors developed and validated an Ultra Flow liquid chromatography-tandem mass spectrometry (UFLC-MS/MS) method for quantifying both Paclitaxel and Castrol oil in human plasma. Paclitaxel and Castrol oil were extracted from human plasma using a Liquid Liquid extraction procedure. The separation was achieved by YMC Pack C18, 250 X 4.6mm, 3µ (Buffer: Acetonitrile:: 20:80 v/v) as mobile phase. Detection was made at m/z 854.5/286.2 for Paclitaxel, using ESI Negative ion spray ionization mode and for Castrol oil 732.8/307.4 using ESI Positive ion spray ionization mode. Analyst 1.5.1 software was used for the quantification. The method was subsequently used to measure concentrations of Paclitaxel and Castrol oil in plasma samples in support of a formulation and development and to assess the influence of Castrol oil concentrations on the disposition and toxicity profile of Paclitaxel. The reference and test products absorption pattern are similar.
Keywords: Human plasma; Paclitaxel, Castrol oil; Liquid Liquid extraction; LCMS/MS.

Introduction:

Paclitaxel Injection is a clear, colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Paclitaxel is a natural product with antitumor activity. Paclitaxel is obtained via a semi-synthetic process from Taxus baccata. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine. Paclitaxel has the following structural formula:

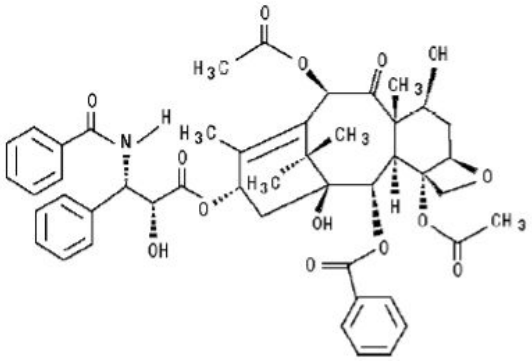

Figure No 1: Paclitaxel Molecular structure

Paclitaxel is a white to off-white crystalline powder with the empirical formula C47H51NO14 and a molecular weight of 853.9. It is highly lipophilic, insoluble in water, and melts at around 216–217° C.

Detailed assessment of paclitaxel revealed several methods based on techniques viz. HPLC [1-9] and liquid chromatography/ tandem mass spectrometry (LC-MS/MS) (10-12) for its determination in pharmaceutical dosage form and in human plasma. Hence, the aim of the present study is to develop a simpler, rapid and cost effective analytical method development and simultaneous estimation of paclitaxel & castrol oil in human plasma under various physiological solution. The method was subsequently used to estimate the concentrations of Paclitaxel and Castrol oil (test and reference product) in plasma samples, to support newly developed and to assess the influence of castrol oil concentrations on the disposition profile of paclitaxel.

Experimental

Instrumentation and chromatographic conditions
Ultra flow liquid chromatography Tandem Mass Spectrometry was used method development and validation. Mass Spectrometry Model API 4000, UFLC model is UFLC XR equipped with a model LC-20ADXR a binary pump, SIL-20ACXR auto sampler used to keep temperature at 10°C, CTO-20AC column oven used to keep temperature at 35° C and CBM-20Alite system controller. Detection was made at m/z 854.5/286.2 for paclitaxel, using ESI Negative ion spray ionization mode and for Castor Oil 732.8/307.4 using ESI Positive ion spray ionization mode. Analyst 1.5.1 software was used for the quantification. The separation was achieved by YMC Pack C18, 250 X 4.6mm, 3µ (Buffer: Acetonitrile :: 20:80 v/v) as mobile phase.

Chemicals and Reagents
Acetonitrile of HPLC grade was procured from JT Becker. Water HPLC grade was obtained from a Milli-Q water purification system. Ammonium Acetate was procured from CDH. Ethanol was procured from Merck. A reference standard of paclitaxel& castrol oil was provided by Strides Arcolab Bangalore, India. A commercial sample of lyophilized injection of paclitaxel (Taxol; 6 mg / ml Bristol-Myers Squibb) used in this study was purchased from the local market.

Stock and working solution preparation
Stock standard solutions of 30 mg of paclitaxel & castrol oil were weighed individually and transferred into a 10 mL volumetric flask and dissolved in 5 mL of the ethanol. The solution was sonicated for 20 min and then the volume made up with a further quantity of the ethanol to get approximately 3.0 mg/mL solution. These solutions were stored at 2-8ºC.

Method optimization and chromatographic conditions
The LCMS procedures were optimized with a view to develop a method for paclitaxel & castrol oil. The standard stock solution was diluted in methanol to a concentration containing 100 µg/mL of paclitaxel & castrol oil. Then, the stock solution is injected into the YMC Pack C18, 250 X 4.6mm, 3µ (Buffer: Acetonitrile :: 20:80 v/v) as mobile phase. Different ratio of acetonitrile and 2mM ammonium acetate buffer was tried. The optimum mobile phase was found to be acetonitrile: 2mM ammonium formate (80:20, v/v). The separation was carried out at ambient temperature with a flow rate of 0.5 mL. The injection volume was 5 µl and run time was 2 minutes.

Sample Extraction
10 µL of the drug (paclitaxel and castrol oil) was spiked into 190 µl of plasma and vortexed. To this 2.5 ml of Ethyl acetate was added and the mixture kept on the shaker at 2500 RPM for 5 mins. The mixture was then centrifuged for 2 min at 4000 rpm. The supernatant liquid (2 ml) was transferred in to glass test tube and evaporated at 40°C until dryness and reconstitute with 1 ml of dilution solvent (Water: Acetonitrile :: 20:80 v/v) and these sample was transferred to the auto sampler vial and 5 µl was injected into the chromatographic system.

System suitability
The system suitability was performed before starting each day's activity according to in-house and it was within acceptance criteria 4 %.

Linearity
Standard curves were constructed at concentrations 0.012, 0.015, 0.020, 0.037, 0.068 and 0.077 µg/mL of paclitaxel and castrol oil. The standard calibration curves were shown to be linear in the above mentioned range in human plasma. Curves were obtained by plotting the peak area against concentrations of these drugs. Linear calibration curves were generated by linear regression analysis and obtained over the respective standard concentrations ranges. The suitability of the calibration models were confirmed by back-calculating the concentrations of the calibration standards. The standard curve, slope, intercept and the correlation coefficient were determined.

Precision and Accuracy
The precision of the assay was measured by the percent coefficient of variation over the concentration

range of QCL, QCM and QCH samples respectively during the course of sample analysis. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the low, middle and high quality control samples to their respective nominal values, expressed in percentage.

Sample Preparation:

Test and Reference Samples:

0.25 mL of paclitaxel injection (Test and Reference) spiked in to 5 mL volumetric flask and diluted to the mark with 5% dextrose (physiological solution) to make a solution of 0.3 mg / mL. 40 µL of the above solution was spiked in to 160 µL of human plasma sample, vortexed and extracted as per the sample extraction procedure.

1 mL of paclitaxel injection (Test and Reference) spiked in to 5 mL volumetric flask and diluted to the mark with 5% dextrose (physiological solution) to make a solution of 1.2 mg / mL. 10 µL of the above solution was spiked in to 190 µL of human plasma sample, vortexed and extracted as per the sample extraction procedure.

0.25 mL of paclitaxel injection (Test and Reference) spiked in to 5 mL volumetric flask and diluted to the mark with 0.9% sodium chloride (physiological solution) to make a solution of 0.3 mg / mL. 40 µL of the above solution was spiked in to 160 µL of human plasma sample, vortexed and extracted as per the sample extraction procedure.

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0.25 mL of paclitaxel injection (Test and Reference) spiked in to 5 mL volumetric flask and diluted to the mark with 5% dextrose in Ringers injection (physiological solution) to make a solution of 0.3 mg / mL. 40 µL of the above solution was spiked in to 160 µL of human plasma sample, vortexed and extracted as per the sample extraction procedure.

1 mL of paclitaxel injection (Test and Reference) spiked in to 5 mL volumetric flask and diluted to the mark with 5% dextrose in Ringers injection (physiological solution) to make a solution of 1.2 mg / mL. 10 µL of the above solution was spiked in to 190 µL of human plasma sample, vortexed and extracted as per the sample extraction procedure.

All the above samples were run along with calibration cure standards with interspersed quality control samples.

Chromatography

Representative chromatograms of calibration curve of paclitaxel and castrol oil are given in Figure No: 2 to 3.

Data Processing

The chromatograms were acquired and were processed by peak area ratio method using the Analyst Version 1.5.1 Software. The concentration of the unknown was calculated from the following equation using regression analysis of spiked standard with the reciprocal of the ratio of the (drug concentration)^2 to internal standard concentration as a weighing factor ($1/x^2$):

$y = mx + c$

Where,

y = peak area ratio of Pravastatin to internal standard
m = slope of calibration curve
x = concentration of Pravastatin
c = y-axis intercept of the calibration curve

Figure No 2: A Representative Calibration Curve for Paclitaxel

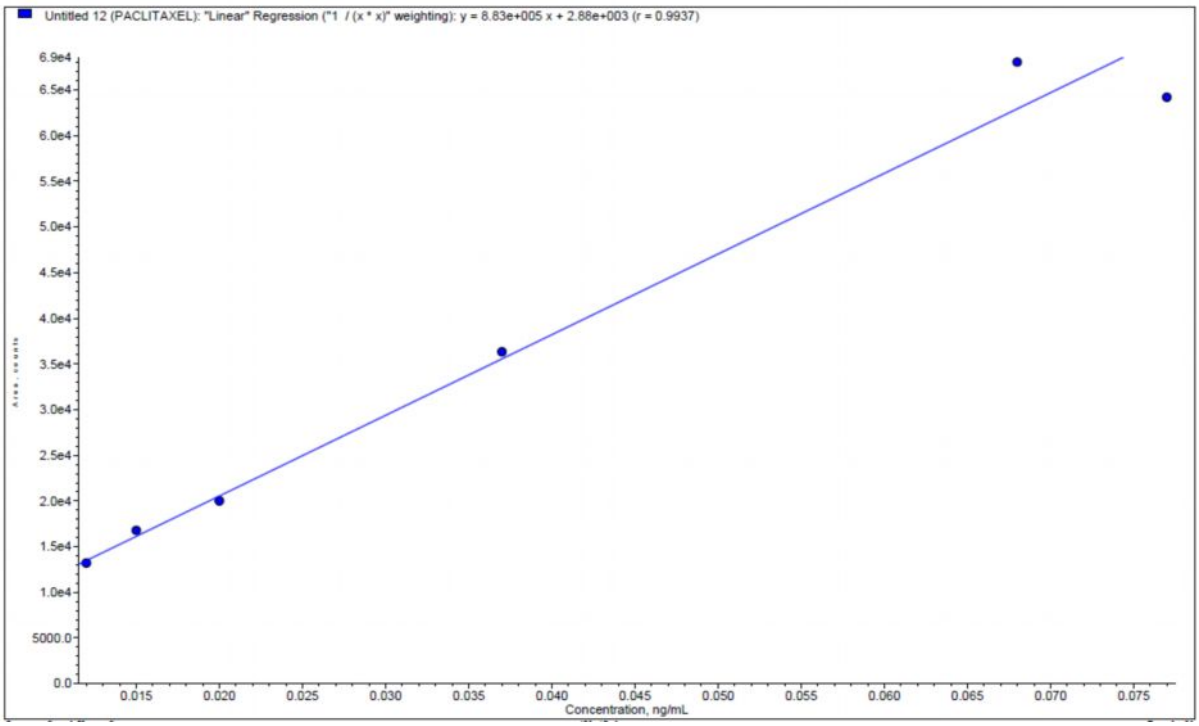

Figure No 3: A Representative Calibration Curve for Castrol Oil

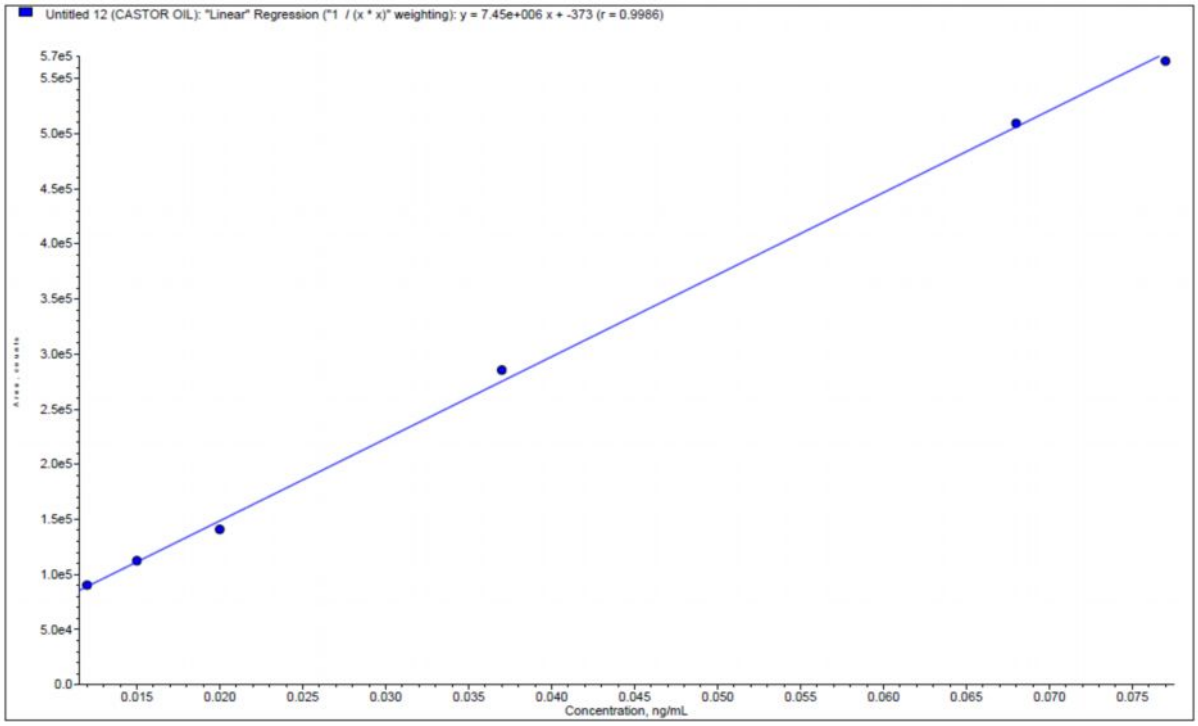

Table No 1: Concentration of Test and Reference Product in Human Plasma

Sample Name	Concentration (µg/mL)
Test 0.3 mg 5% Dextrose	0.061
Test 0.12 mg 5% Dextrose	0.065
Test 0.3 mg 0.9% NaCl	0.068
Test 0.12 mg 0.9% NaCl	0.068
Test 0.3 mg 0.9% sodium chloride and 5% dextrose	0.067
Test 0.12 mg 0.9% sodium chloride and 5% dextrose	0.070
Test 0.3 mg 5% dextrose in Ringers injection	0.068
Test 0.12 mg 5% dextrose in Ringers injection	0.069
Reference 0.3 mg 5% Dextrose	0.062
Reference 0.12 mg 5% Dextrose	0.065
Reference 0.3 mg 0.9% NaCl	0.067
Reference 0.12 mg 0.9% NaCl	0.065
Reference 0.3 mg 0.9% sodium chloride and 5% dextrose	0.064
Reference 0.12 mg 0.9% sodium chloride and 5% dextrose	0.062
Reference 0.3 mg 5% dextrose in Ringers injection	0.066
Reference 0.12 mg 5% dextrose in Ringers injection	0.066

Results and Discussion

This work was designed to develop an LCMS method based for the estimation of paclitaxel and castrol oil in human plasma. The goal of this study was to develop a rapid, more accurate, precise, reliable, least time consuming LCMS method for the simultaneous estimation of paclitaxel & castrol oil and to compare absorption profile in human plasma under physiological solution. This analytical method was developed taking in account the therapeutic concentration range, has been validated and holds well for the determination of drugs in raw materials, dosage formulations and especially in human plasma.

The concentration of the test and reference products distributed into plasma is given below Table No: 1.

Conclusion

In short, our method is specific, sensitive, rapid and easy to perform for simultaneous determination of paclitaxel and castrol oil. The sample volume and short chromatographic time of this method makes it advantageous for adaptation to routine assay requirements and enables simultaneous determination of paclitaxel and castrol oil because of good separation and resolution of the chromatographic peaks. The obtained results are in good concord with the reference formulations. Test and reference products have similar distribution profile in human plasma under physiological solution.

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