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Bioanalytical Method Development and Validation of Tadalafil in Human Plasma by LC-UV Method

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ABSTRACT:
Tadalafil is classified as potent and highly selective phosphodiesterase type5 (PDE5) inhibitor that is used for treating erectile dysfunction (impotence) and pulmonary arterial hypertension. Present study was aimed to develop simple method for isolation and estimation of tadalafil from human plasma. A simple bioanalytical LC-UV method for estimation of tadalafil in human plasma by protein precipitation technique. Analyte was recovered from plasma by acetonitrile as a protein precipitating agent and subsequently separated on Phenomenex Luna C18 column (150X4.6 mm, 5µ) with security guard cartilage C18 using acetonitrile : water (45:55) as mobile phase at flow rate 1.0 ml/min at 286 nm. Tadalafil was eluted at 6.7 min. The calibration curve was linear (R2=0.999) over concentrations range of 0.25-5µg/ml in human plasma. The intraday and inter-day precision value were <15% and accuracy all within 15% (for LLOQ20%).
Keyword: Tadalafil, LC-UV, human plasma, Bioanalytical Method.

INTRODUCTION:

Tadalafil is a potent and highly selective phosphodiesterase type 5 inhibitor. It is used clinically, for the treatment of erectile dysfunction [1]. In the corporal smooth muscles, it's efficacy is based on its ability to avoid the breakdown of cGMP, which is produced by the nitric oxide (NO)-dependent activation of guanylyl cyclase [2, 3].

Several UV- spectrophotometric [4, 5] and high performance liquid chromatographic methods (HPLC) [6, 7 8] reported for estimation tadalafil. Bioanalytical method is particular method used for isolation quantitative measurements of analyt in a matrix such as blood, plasma, serum and urine. Quantification of tadalafil have been reported on LC-MS, LC-MS/MS, ultra performance liquid chromatography (UPLC) coupled with quadrupole-time-of-flight mass spectrometry (Q-TOF MS) [9, 10]. But these methods are costly and complex. So it is necessary to develop and validate simple, accurate, economic and quick LC-UV bioanalytical method for tadalafil from human plasma. In this study, we have developed a

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LC-UV method with a protein precipitation extraction for the determination of tadalafil in plasma and the developed method is validated as per regulatory requirements.

MATERIALS AND METHODS:

Tadalafil gift sample supplied by Kopran Pharma Pvt. Ltd. Khopoli. HPLC Grade Solvents (Acetonitrile, Methanol, and Water) all were purchased from Merck Specialties Pvt. Ltd., Mumbai. Younglings Acme 9000 LC system equipped with a UV detector. Chromatographic separations were performed using the Phenomenex Luna C18 (150x4.6 mm, 5µ) column with security guard cartilage C18(4.6x 3 mm), analyzed by LC software Autochro-3000 and Cool Micro Centrifuge (REMI-120). The mobile phase consisted of Acetonitrile: water (45:55) at a flow rate of 1ml/min. total run time for each sample analysis was 10 min.

Preparation of Standards and Quality Control Samples:

A stock solution of 1 mg/ml of tadalafil was prepared in methanol. Standard working solutions for spiking (12.5, 25, 50,100,150,200,250µg/ml) were prepared by dilution of stock solution with methanol. Standard plasma solution for calibration and quality control samples (0.25, 0.50,1,2,3,4,5 µg/ml) and 1µg/ml (LQC), 3µg/ml (MQC) and 5µg/ml (HQC) were prepared by spiking 10µl of standard working solution in 490µl of plasma.

Sample preparation and extraction:

500µl of Acetonitrile was added to standard plasma solution and vortexed for 5 min then the solution was centrifuged at 8˚C, 15000rpm for 10 min. 20µl aliquot was injected into the HPLC system.

HPLC method:

Method validation

The proposed method was validated according on the Guidance for Industry: Bioanalytical Method Validation' by the US FDA [11] for different parameters as linearity, accuracy, precision, recovery and stability studies.

Selectivity and Linearity

On the spiking plasma samples with seven non-zero calibration working solutions covering the total range of quantification (0.25-5µg/ml concentration in plasma) along with blank samples. The calibration curve was generated by plotting the ratio of AUC of analyte vs. concentration of analyte on linear regression method following the equation=14.18x+2.151 where Y denote analyte area and x is denote concentration of analyte. The LLOQ as the lowest concentration yielding a signal to noise ratio of at least 5 with a coefficient of variation (CV) <20% and accuracy of 80-120%. The acceptance criteria for other standard concentration were within a 15% deviation from the nominal values.

Recovery

Recovery tests were performed in replicate at three different quality control samples (1,3 and 5µg/ml). Recovery calculated by comparing the responses of plasma quality control samples spiked with analyte with response of analyte in equivalent to methanolic solution.

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Precision and accuracy

Both repeatability (within –run and between- run) were determined by replicate analysis of 5 sets of quality control samples that were spiked with 3 different QC concentrations. The precision was determined in the term of coefficient of variation (CV %) and accuracy was expressed as the absolute percent bias (APB (%). The acceptance criteria should not exceed 15% (for LOQ not exceed 20%).

Stability studies

Tadalafil stability samples compared with freshly prepared tadalafil solutions. For the stability study, two different QC sample concentrations levels i.e. HQC (5µg/ml) and LQC (1 µg/ml) in plasma was used. For determination of freeze thaw stability for tadalafil, the plasma samples were frozen for 24 hr and thawed unassisted at room temperature when sample thawed then again refrozen for 24 hours. On repeating cycle for 3 times.

RESULTS AND DISCUSSION:

Chromatographic Optimization:

Chromatographic conditions, particularly the composition of the mobile phase was optimized by several trials for improve the peak shape of analyte for achieving appreciable HPLC separation. The best signal for the analyte was achieved by using acetonitrile: water (45:55) using a Phenomenex Luna C18 (150x4.6 mm, 5µ) column at flow rate 1ml/min and detection wavelength is 286nm.

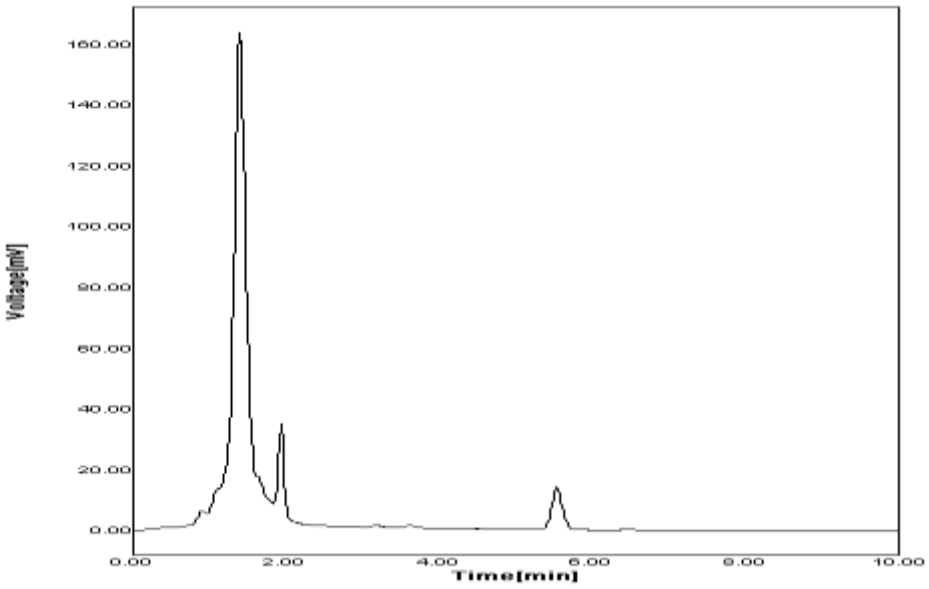

Fig 1: Chromatogram of Blank plasma in Optimized chromatographic conditions

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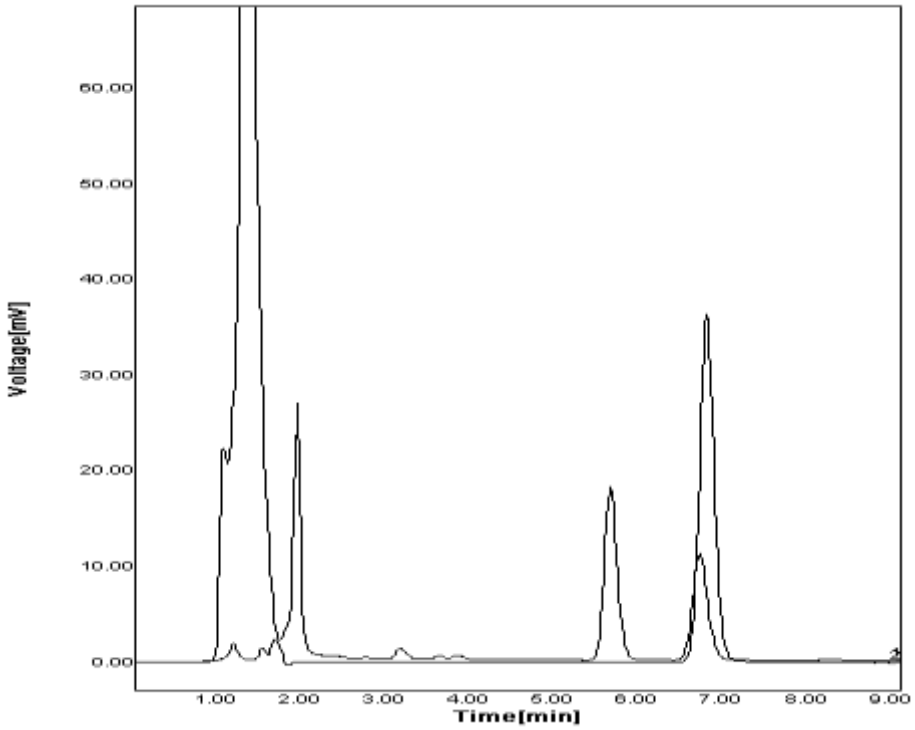

Fig 2: Overlain chromatograph of spiked plasma (5 µg/ml of tadalafil) and methanolic solution of Tadalafil (Rt 6.8 min)

Selectivity:

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Prepared method used LC-UV separation of tadalafil samples showed no interference peak in plasma at the retention time of tadalafil. Fig.1 overlay chromatogram of blank plasma and fig. 2 spiked plasma and methanolic solution of tadalafil respectively.

Linearity:

The seven points standard standard curve (fig 3) was linear over the wide range of calibration 0.25-5µg/ml. The mean correlation coefficient for the linearity equation was calculated to be 0.999 using regression equation=14.18x+2.151 where Y denote analyte area and x is denote concentration of analyte.

Accuracy and precision:

The intraday and interday accuracy (within –run and between- run) for tadalafil were 95-97 the intra and interday precision (%CV) for tadalafil were 1.68-1.21, Table 1 provide summary of the intraday and interday precision for the tadalafil. (1,3 and 5 µg/ml).

Table1. Within batch and between batch precision and accuracy of Tadalafil

	Concentration (µg/ml)	Accuracy (% mean)	Precision(%CV)
Intra-day (within-run)	1(LQC)	103.31	4.45
	3(MQC)	98.88	1.68
	5(HQC)	99.63	0.47
Inter-day	1(LQC)	100.95	3.9

(Between-run)	3(MQC)	101.1	1.29
	5(HQC)	97.94	0.72

Recovery:

Recovery of tadalafil was determined by comparing peak areas from non-extracted (methanol sample) and extracted sample (quality control samples). There were three different quality control samples used in three replicates for recovery study i.e. low, medium and high (1,3and 5 µg/ml) samples. The percentage recovery was calculated for Tadalafil in plasma and it's ranged from 89.0 to 93.22% for the low, medium and high quality control samples represented in table 2.

Table 2. Recovery result of Tadalafil from plasma.

Concentration (µg/ml)	% Mean recovery
1(LQC)	89.01
3(MQC)	86.09
5(HQC)	93.22

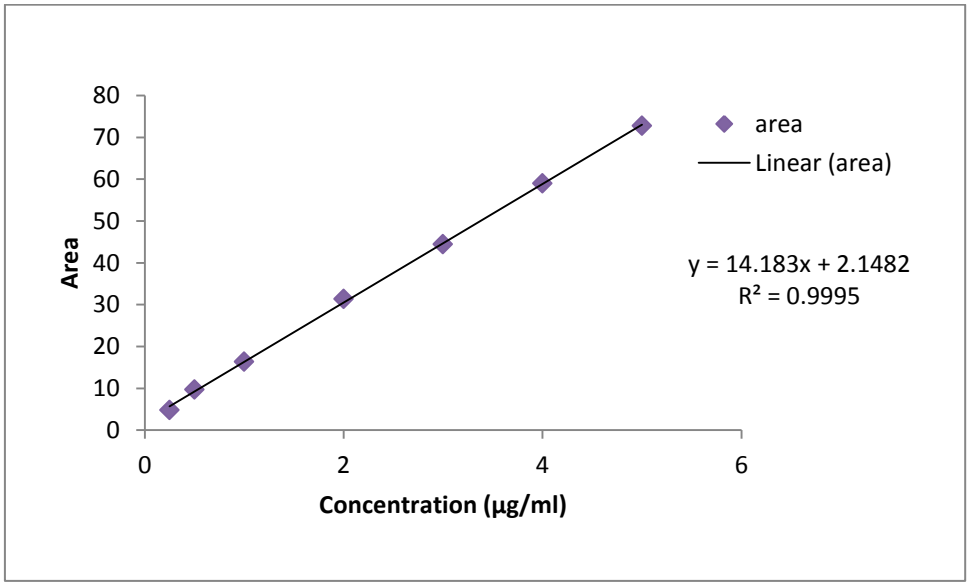

Fig 3: Calibration curve of Tadalafil in plasma

Stability studies:

The stability studies done on low medium and high concentrations of QC samples were tested in freeze thaw stability (3cycle). Bench top stability for 6 hrs, short term stability for 4 hrs at room temperature had no effect on quantification. Long term stability of samples was found 6 days at -4^0 C.

Table 3. Validation parameters of Tadalafil by HPLC method.

S. no	Parameter	Result
01	Selectivity	Pass
02	System suitability	Pass

03	Linearity	R^2=0.999
04	Range	0.25-5 µg/ml
05	Recovery	Pass
06	Accuracy and precision	Pass
07	Bench top stability	6 hrs
08	Freeze thaw stability	Pass
9	Short term stability	4 hrs
10	Long term stability	6 days

CONCLUSION:

In the present work, a rapid, sensitive, specific, precise and accurate bioanalytical method for Tadalafil in human plasma has been developed and validated with a larger calibration curve range (i.e. 0.25 to 5µg/ml) using high performance liquid chromatography which can be used for routine drug analysis and bioanalysis.

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