RP-HPLC based method for the determination of Tamsulosin HCl in API, Prepared dosage form and spiked plasma

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Abstract: An analytical method for measurement of tamsulosin HCl from its different life cycle stages was developed using RP-HPLC technique. The elution of tamsulsoin was studied using Mediterranea^{TM} Sea 18 column (dimensions 250×4.6 mm and pore size 5µm) and mobile phase comprising of buffer (pH 5.4): Acetonitrile (ACN) at ratio 60:40. The tamsolusin HCl elution was also studied by varying the operating conditions including the composition and flow rate of mobile phase, stationary phase temperature and scanning wavelength. The optimal elution conditions include; a) mobile phase flow rate; 1ml/min, b) wavelength; 224nm and stationary phase temperature 30°C. Linearity was recorded in the absorption data over tamsulosin concentration range of 0.007 to 40µg/ml. The values of paramters LOD and LOQ noted for tamsulosin dissolved in mobile phase were 0.0014 and 0.042µg/ml respectively, while for the counterpart in spiked plasma were 0.0017 and 0.051µg/ml respectively. The analytical method was prompt, accurate, reproducible and suitable for analysis of tamsulosin HCl in different samples of interest at formulation, regulation and clinical evaluations.

Keywords: Tamsulosin HCl, spiked plasma, acetonitrile.

INTRODUCTION

Tamsulosin HCl is commonly prescribed for the treatment of benign prostrate hypertrophy and urinary obstruction ensuing from lower urinary tract infections (Franco-Salinas *et al*., 2010). This drug remained a subject of interest for the formulation scientists as its rapid rate of release in the gastrointestinal tract poses adverse effects. Efforts have been paid to design drug delivery systems associated with relatively lower side effects (Shaikh *et al*., 2018). As a prerequisite, a sensitive, robust and validated analytical method is needed to determine drug substance in raw material verify of claims of a formulation, confirming the drug contents in the dosage forms and in the measurement of drug in the plasma or other physiologic fluids (Bari *et al*., 2011; Mhamunkar *et al*., 2012). An increasing level of method sensitivity is needed as one proceed from raw material quantification to the measurement of plasma levels. The situation is even more challenging if the daily dose of the drug like tamsulosin HCl is less than 1mg (Rao *et al*., 2008). The existing method of analysis developed for UV-visible spectroscopy (Kumar *et al*., 2012) are less sensitive and acceptable for the measurement of drug substance in the raw material or for in vitro assessments (Abbas *et al*., 2017; Amanlou *et al*., 2014). Nevertheless, plasma samples with low drug contents require a HPLC method (Monir *et al*., 2019). The methods already reported in literature require a complex mobile phase comprising of methanol (0.02 mole per Liter) and ammonium acetate buffer with triethylamine (Patel *et al*., 2010). Another feature of these methods is a longer retention time in excess of 8 minutes which add to the running cost of analysis by consuming more volume of expansive solvents and protracted machine run time. There is a margin for the improvement of efficiency.

This study aims the development of an analytical method for the determination of tamsulosin HCl using a reverse phase HPLC techniques. The elution behavior of tamsulosin HCl was studies using different proportion of acetonitrile in the mobile phase, various flow rates of the mobile phase as well as altering the column temperatures and scanning wavelengths.

MATERIALS AND METHODS

Chemicals and reagents

Tamsulosin HCl purity 99.69 % was received as a kind gift from Consolidated Chemicals Limited (CCL) Lahore. Acetonitrile (ACN) was purchased from Fisher Scientific UK. Monobasic potassium phosphate, citric acid and sodium hydroxide was procured from Sigma-Aldrich (Germany). Purified reverse osmosis grade water prepared using an in house installed facility i.e. Millipore ultra-pure water system (Milford, USA) was used. All other chemicals and reagents of HPLC grade were used during this study.

Methods

Instrumentations

A water (USA) HPLC alliance e 2695 separation module equipped with a injection plunger (0.1-100µL) and autosampler was used for the chromatographic separation. Mediterranea^{TM} Sea 18 column having dimensions 250 mm × 4.6 mm, and average pore size 5µm connected to temperature regulator -4 to 65°C (Torrance, CA, USA) was employed for the analysis. The detection was carried

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out with the PDA detector. Empower software version 3 was used for the interpretation of the results.

Chromatographic operating conditions

During the screening studies, the HPLC system was operated at following operational settings: a) mobile phase composition was changed in terms of percentage of acetonitrile (30 to 60% by volume with an increment of 5%), b) flow rate was varied from 0.8, 1.0, 1.25, 1.5 to 2.0 $ml.min^{-1}$, c) column temperature was raised from 20-40 °C with an increment of 5°C, and d) absorption of UV light was recorded at 215, 220, 224 and 230 nm. For each analysis 20µl of the test solution (10µg/ml) was injected. After each sample run, the column was washed for 2h by repeated flushing of mobile phase, ethanol and water. The chromatographic profiles were investigated for suitability testing to ensure acceptable operating conditions.

Composition of the Mobile phase

A binary solution consisting of acetonitrile (ACN) and water was prepared using different volume fractions of each constituent. The optimized mixture showing best solubility of tamsulosin HCl includes ACN and water at ratio 20:70 with 1ml triethylamine (TEA) at pH adjusted at 5.9±0.1. A binary solution of ACN and buffer at ratio 40:60 was used in this study. The buffer solution was prepared by adding TEA (1 ml) in 1liter and pH of 5.4 was maintained by adding citric acid and/or sodium hydroxide.

Tamsulosin HCl stock solution and its dilutions

A stock solution of tamsulosin HCl (100µg/ml) was prepared by mixing 10mg of the former in 100ml of the solvent (mobile phase). In the next stage, different dilutions containing tamsulosin HCl 0.625, 1.25, 2.5, 5 and 10µg/ml were prepared by diluting different volume fractions of the above stated stock solution in the mobile phase. These dilutions and other working solutions of tamsulosin HCl were labeled appropriately and stored in refrigerator until further analysis. The drug concentration determined from absorbance peak at 224nm.

Pharmaceutical dosage form

The integrity of a pharmaceutical preparation containing tamsulosin HCl was also proved by analyzing the contents for active pharmaceutical ingredients. For this purpose, the components of a capsule were dissolved in the mobile phase and its dilution (10µg) was eluted through the HPLC system. The area under curve of absorbance peak was used to calculate the drug contents and a comparison was made with the label claims.

Spiked plasma samples

In order to simulate the quantification of tamsulosin HCl from the blood, the spiked drug plasma samples were prepared. Briefly, the blood samples from the retro-orbital plexus of aneasthized rats were collected in 3ml vacutainer tubes. These samples were subjected to centrifugation (4000 g for 10 min) to extract plasma as clear supernatant layer. The plasma was spiked with tamsulosin HCl solution (10µg/ml) 200µl and the final contents of tamsulosin were adjusted to 1µg/ml. This solution was deprotienated by adding 200µl of acetonitrile and the mixture was agitated using a vortex mixer for 2 minutes to ensure a prompt precipitation of the plasma proteins. From the clear supernatant layer, a volume fraction of 200µl was separated and an aliquot of 20µl was injected for chromatographic analysis.

Analytical method validation

Linearity

Two calibration curves were prepared from the elution of tamsulosin HCl dissolved in a) mobile phase and b) spiked plasma. The area of tamsulosin HCl absorption peaks measured from different concentrations (0.625, 1.25, 2.5, 5 and 10µg/ml) in the relevant solvents i.e. mobile phase and spiked plasma was fitted with linear regression equation. The value of the model fit results such as correlation coefficient (R^2) was calculated to register the reliability of the linear equation in translating the absorption data. The regression functions were used to determine the slope (m), intercept (b) of standard curve. The concentration ranges of tamsulosin HCl following the linear regression was also determined.

Specificity

This validation parameter was evaluated by recording the output responses in terms of area and position of chromatographic peaks of tamsulosin HCl in samples containing an internal standard diclofenac sodium (each analyte 0.012mg/ml). The chromatograms of drug solutions were compared with different control solutions e.g. blank solvent, blank pharmaceutical excipients and blank plasma.

Accuracy

Accuracy measures the closeness of experimentally determined concentration values to the known counterparts or a reference value. For this study, it was caluclated form the percentage recovery of tamsulosin obtained by the following expression:

$\%\ \text{Recovery} = [C_t] / [C_s] \times 100$

Where $[C_t]$ state the concentration of the tamsulosin HCl measured by using the test method and $[C_s]$ is the known concentration in the mobile phase.

Precision

In this step, the closeness of the responses was recorded following the injection of a tamsulosin HCl solution of known strength. Precision is described in terms of repeatability and reproducibility. The repeatability studies were performed by injecting a drug solution (10 injections) under the same conditions and comparing the amounts recovered from each injection were recorded as %RSD.

Reproducibility of the outputs from this analytical method was determined in terms of interday, intraday and by varying the operator and machine or facility. Here again, the covariance of the output was considered as a benchmark of precision.

Sensitivity

Limit of detection (LOD) refers to the lowest concentration for reliable detection of an analyte from a background. While the limit of quantification (LOQ) states the lowest concentration at which the analyte is accurately measured. These parameters are calculated by using the following equations.

LOD = 3.3 σ / S

LOQ = 10 σ / S

Where σ is the symbol of standard deviation and S is the symbol of slope for the standard curve.

Robustness

The validation parameter of analytical method was tested by recording the responses following a minor deliberate variation in operating conditions such as temperature of column by ±1°C, pH and flow rate of the mobile phase by ±1% and ±0.2 ml/min, respectively. Furthermore, the chromatograms were recorded from above state drug solutions using different batches of column and different instrument and variability in response was analysed.

Tamsulosin HCl stability

The stability of aqueous solution tamsulosin HCl was studied to indicate the interference which could be happened due to any possible degradation at room temperature during analysis period. Stability of sample was done at bench-top room temperature for 24 and 48 hours.

STATISTICAL ANALYSIS

The results of different parameters of this study are described as mean ± standard deviation. The statistical analysis was carried out using Microsoft Excel 2010.

RESULTS

Effect of mobile phase

Different chromatograms obtained as a function of composition of the mobile phase are presented in fig. 1. In general, two events are evident from each chromatogram. A small noisy peak at ~2.5-3 minutes was recorded due to mobile phase while the second well differentiated peak registers the elution of tamsulosin HCl. Retention time decreases exponentially with an increase in the volume fraction of acetonitrile in the mobile phase (fig. 1 b, c). Furthermore, the value of area under the curve and number of theoretical plates increases with the acetonitrile fractions. It is postulated that by increasing the polarity of mobile phase the drug elutes faster resulting a reduction in retention time. Due to the reason, mobile phase with higher volume fraction of acetonitrile is selected for the drug elution (i.e. ACN 40 %).

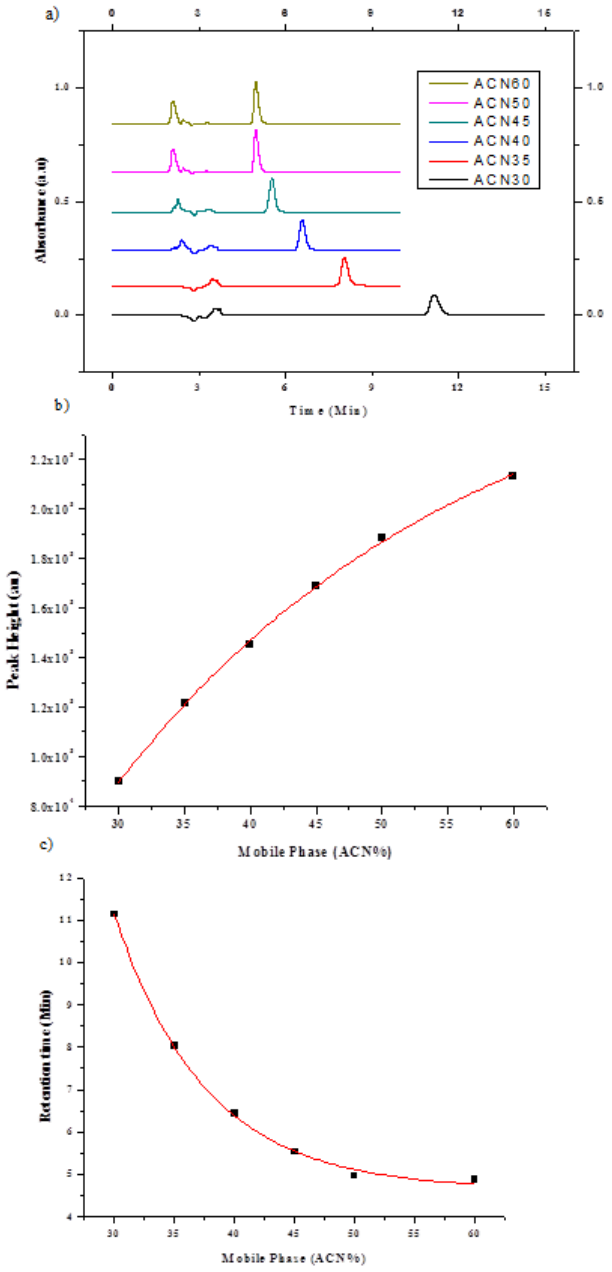

Fig. 1: Tamsulosin HCl chromatograms as; (a) a function of mobile phase composition (b) peak height vs mobile phase composition; (c) retention time (Rt) vs mobile phase composition.

Effect of Flow rate of the mobile phase

Once the composition of mobile phase ascribed with optimal drug elution is known, another parameter of interest would be its flow rate in order to optimize the drug elution pattern. The results shown in fig. 2 suggest a decrease in the retention time (from 8 to 4.5 minutes) as the flow rate increases from 0.8 to 1.5ml/min (fig. 2a). In

Table 1: Optimization of operating parameters for system suitability parameters

	Variable values	RT (min)	Peak Height (au)	Area (au)	Tailing Factor	USP Plate count
Tempe-rature °C	20	6.740	1.30E+05	2.07E+06	1.260	4417.6
	30	6.428	1.45E+05	2.07E+06	1.241	4917.4
	35	6.338	1.54E+05	2.09E+06	1.223	5240.0
	40	6.286	1.59E+05	2.10E+06	1.179	5373.9
Flow rate ml/ min	0.8	8.256	1.40E+05	2.56E+06	1.216	4909.0
	1	6.568	1.35E+05	2.05E+06	1.247	4526.0
	1.25	5.323	1.30E+05	1.63E+06	1.201	4364.0
	1.5	4.432	1.29E+05	1.36E+06	1.190	4148.0
% Acetonitrile	30	11.133	8.99E+04	2.11E+06	1.297	5529.0
	35	8.029	1.22E+05	2.11E+06	1.299	5240.0
	40	6.428	1.40E+05	2.11E+06	1.303	4679.0
	45	5.525	1.49E+05	2.12E+06	1.080	3569.0
	50	4.960	1.89E+05	2.13E+06	1.420	4808.0
	60	4.880	2.13E+05	2.15E+06	1.367	4908.0
Wave-length nm	215	6.590	1.14E+05	1.68E+06	1.214	4573.0
	220	6.569	1.20E+05	1.78E+06	1.227	4552.5
	224	6.569	1.35E+05	2.05E+06	1.247	4528.0
	230	4.432	1.16E+05	1.74E+06	1.236	4545.0

Table 2: Precision and accuracy of method for determination of tamsulosin HCl

Sample detail		Interday			Intraday		
	Added concentration (µg/ml)	Interday	% Recovery	%RSD	Intraday	% Recovery	%RSD
Tamsulosin Pure	5	5.022±0.08	100.44	1.646	4.97 ±0.06	99.44	1.26
	10	10.06±0.11	100.6	1.172	9.99±0.09	99.93	0.95
	20	19.63±0.30	98.17	1.549	19.75±0.24	98.75	1.22
Dosage form (Capsule)	5	5.024±0.08	100.48	1.712	4.9±0.05	99.21	1.17
	10	9.99 ±0.11	99.94	1.116	9.99±0.11	99.94	1.11
	20	19.73±0.25	98.67	1.314	19.73±0.25	98.67	1.31
Spiked Plasma	5	4.90±0.07	98.03	1.599	4.93±0.06	98.76	1.3
	10	9.97±1.11	99.73	1.164	9.98±0.11	99.8	1.12
	20	19.90±0.30	99.5	1.511	19.8±0.26	99.33	1.32

Table 3: Stability of the tamsulosin HCl solution in mobile phase at room temperature

Sample detail	24 hours		48 hours	
Concentration (µg/ml)	Recovered Concentration	%RSD	Recovered Concentration	%RSD
2.5	2.455±0.03	1.548	2.448±0.03	1.452
5.0	4.873±0.06	1.246	4.880±0.06	1.375
7.5	7.406±0.11	1.506	7.423±0.10	1.437

addition, peak height was slightly decreased at higher flow rates. Both of these parameters decrease exponentially with the flow rates (figs. 2b and c). A possible explanation to this effect is reduced interaction of the elutant i.e. tamsulosin HCl with the stationary phase i.e. C18 due to a higher flow rate.

There are different justification regarding the selection of a flow rate; slower rates may provide better resolution but these are time consuming, energy exhaustive and expansive. Likewise, a faster flow rate is linked with a compromise in the resolution but a shorter retention time. Nevertheless, the intermediate to higher rates with acceptable resolution limit are favored as it reduces machine run time and operating costs. A flow rate of 1 ml/min was selected for further analysis as it provides an acceptable resolution.

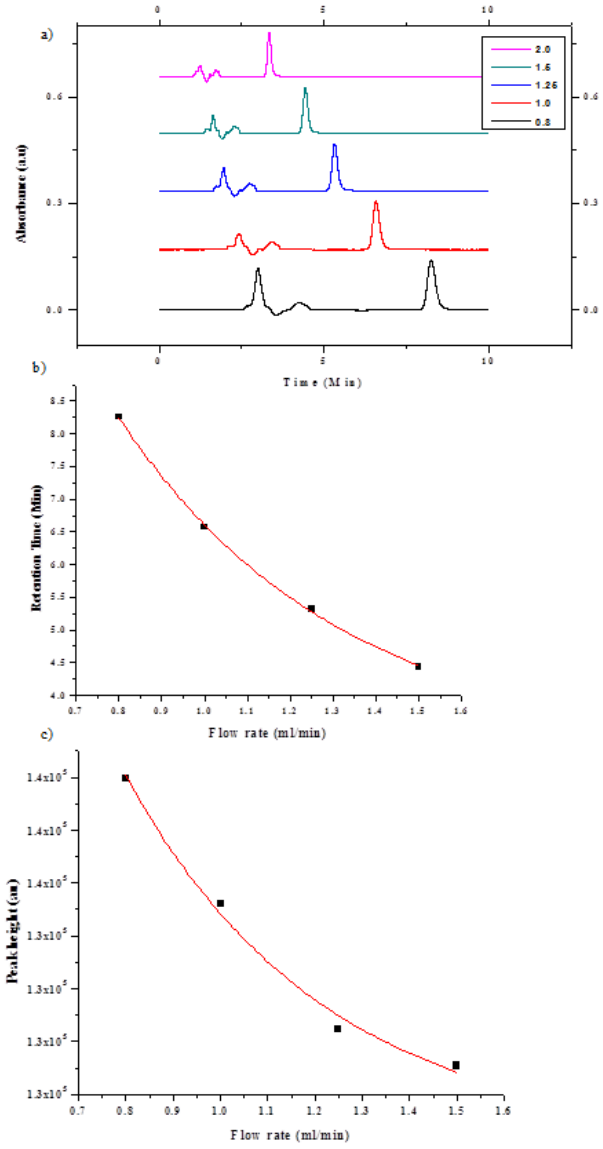

Fig. 2: Chromatogram of tamsolusion HCl (a) as a function of flow rate mobile phase, (b) flow rate Vs retention time and (c) flow rate Vs height of the elution peak

Effect of temperature

Temperature of the mobile phase is another parameter of interest impacting the drug elution. For the purpose, tamsulosin HCl elution patterns were recorded at temperatures 20-40°C (fig. 3a). The results indicate that the peak height decreases exponentially with the column temperature (fig. 3b). Higher temperatures decrease viscosity of solvents which result in reduced friction and improved interaction with the stationary phase. This effect is considered to improve the peak resolution observed in terms of peak height and theoretical plates. The optimized temperature was considered as 30 °C.

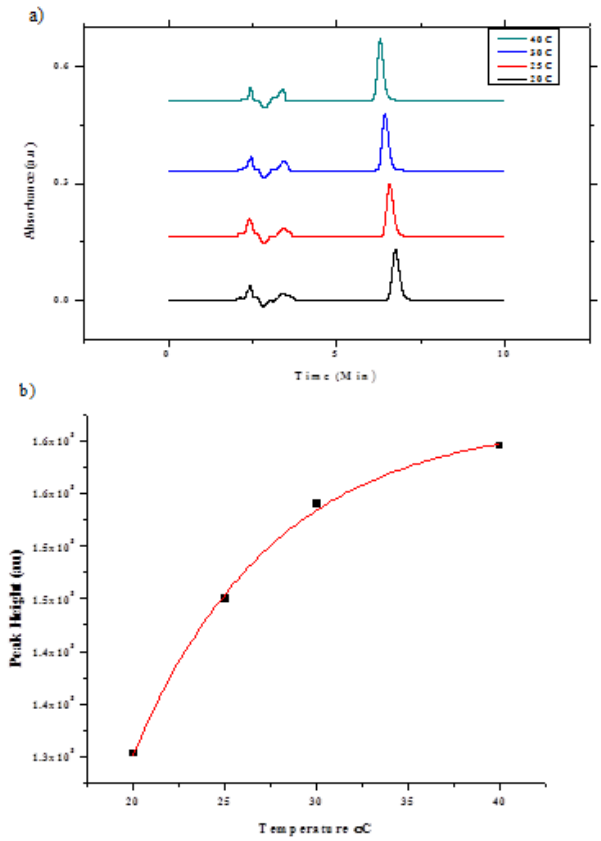

Fig. 3: Chromatogram of tamsulosin HCl; (a) as a function of elution temperature, (b) temperature vs peak height.

Effect of wavelength

This study was performed to confirm that λ_{max} of spectrophotometer is reproduced in the course of HPLC analysis. three dimensional plot describing wavelength scan as function retention time depict maximal absorption at 224 nm (fig. 4a). Likewise, the height and area of drug elution peak remained maximum at 224 nm (fig. 4b and c). Therefore, this wavelength was selected for further analysis.

System suitability

The results described the variability of tamsulosin HCl peak area and the retention time in terms % RSD value < 2% (table 1) denotes the suitability of the system. Furthermore, the results of parameters number of theoretical plates and tailing factor recorded from injections of drug solutions (n=6) were >2000 ±1% and >2 -±0.2% respectively, which also affirms the suitability of the technique in quantification of tamsulosin HCl.

Tamsulosin HCl being a Sulphur containing polar compound with water solubility 215mg.L^{-1} can be quantified using a reverse Phase HPLC method. The drug was screened for solubility in different mobile phase solutions. The drug is suitably detected by UV visible

spectrophotometer, the spectrum with a peak at 224 nm. This wavelength was therefore used for the quantification of drug using HPLC. Optimized parameters after above investigations were mobile phase containing acetonitrile 40% flow rate 1.0 ml/min Column temperature 30 °C and absorption wavelength 224 nm.

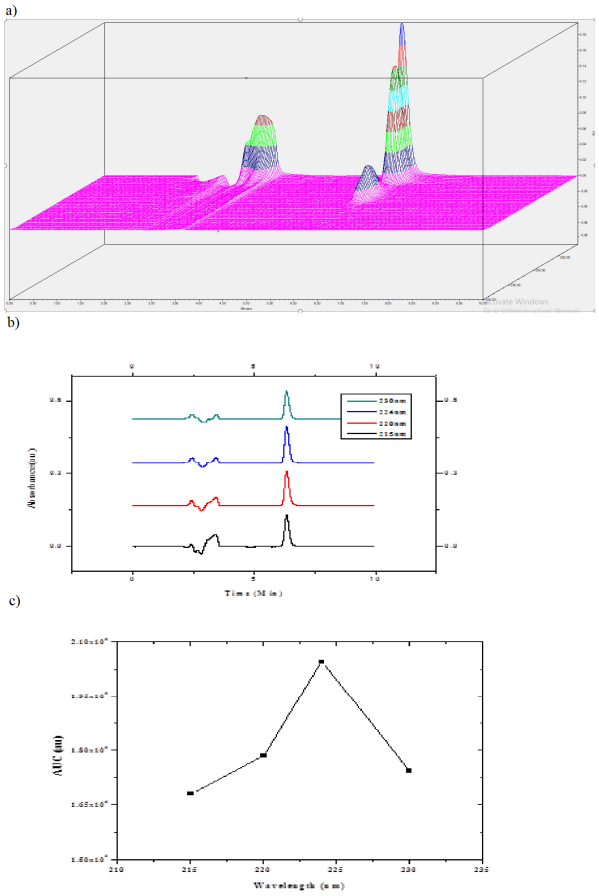

Fig. 4: Elution pattern of tamsulosin HCl showing (a) 3D view of AUC as a function of wavelength (b) selected chromatograms recorded at different wavelengths and (c) AUC vs wavelength.

Validation of optimized analytical method

Linearity for tamsulosin HCl from different solutions

The primary peaks recorded from the elution of different dilutions of stock solution are shown in fig. 5a. The results shown in fig. 5a indicate that both the height and area under the curve of absorbance peak increases proportionally with the drug concentration. The area under the curve of primary peaks plotted as function of tamsulosin HCl concentration in standard solutions as well as spiked plasma was modelled with a linear regression equation (y=197051x-7265) and (y=215740x-14893) respectively (figs. 5b and c). The results of model fit parameter, correlation coefficient (R^2 = 0.999) for both standard curves indicate that the model equation can be reliably used to calculate the drug concentration in an unknown sample if its absorbance is known. Linearity range for tamsulosin HCl in mobile phase and in spiked plasma was 0.07 to 40 µg/ml.

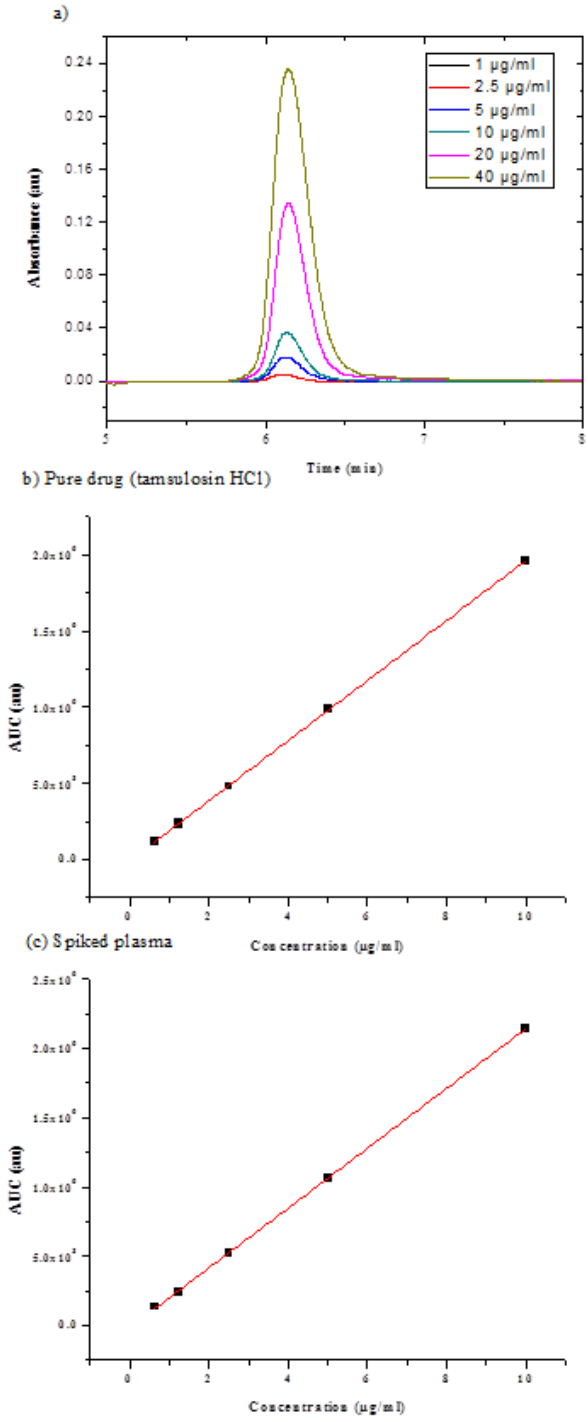

Fig. 5: Elution profile of tamsulosin HCl; (a) primary peaks plotted as function of tamsulosin concentration; (b) calibration curve in mobile phase; (c) calibration curve in spiked plasma

Accuracy

Accuracy refers to the recovery of the analyte following the injection of know concentrations. The % RSD

following the injection of 2.5, 5 and 7.5µg/ml of tamsulosin HCl for pure drug, active pharmaceutical ingredient and spiked plasma was less than 2%. The results of %RSD confirms the acceptability of present method presented for the measurement of tamsulosin HCl.

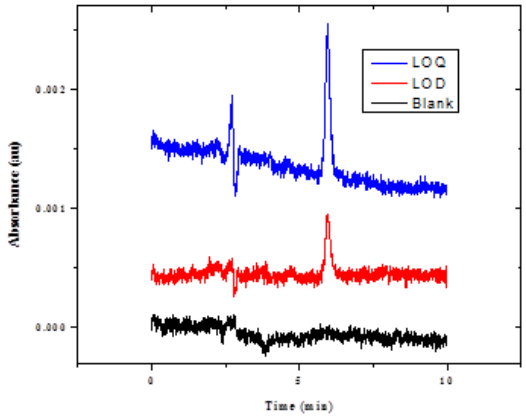

Fig. 6: HPLC chromatogram of tamsulosin HCl at LOQ and LOD.

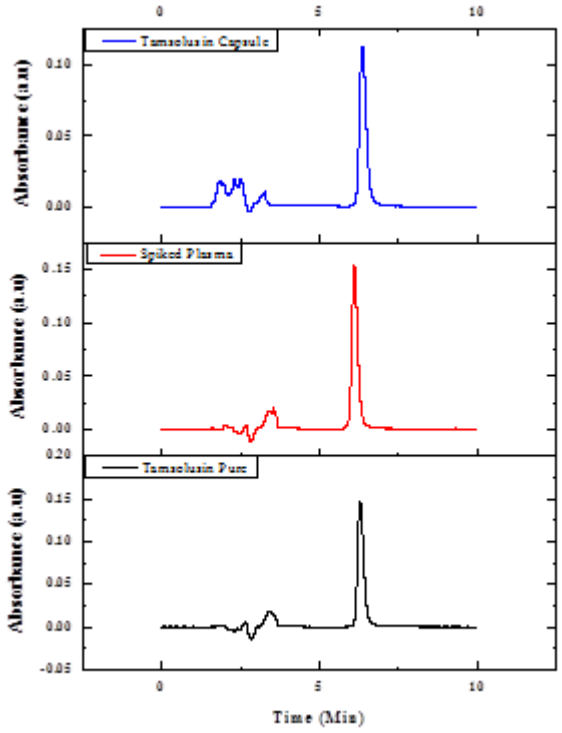

Fig. 7: Chromatogram of tamsulosin HCl (top) pharmaceutical dosage form; (middle) spiked plasma; and (bottom) pure tamsulosin HCl

Precision

The precision parameter records variation in the output following a change in machine, operator, or facility. The precision data for the analytical method are given in table 2. An Intra-day and inter-day precision for pure drug, active pharmaceutical ingredient and spiked plasma was less than 2%.

LOD and LOQ

For pure tamsulosin HCl quantification, the values of the parameters LOD and LOQ were observed as 0.0014 and 0.042µg/ml respectively. However, these values were slightly higher for spiked plasma; LODs and LOQs were 0.0017 and 0.051µg/ml, respectively (fig. 6).

Specificity

The chromatograms recorded from blank solvent, blank pharmaceutical excipients dissolved in the mobile phase and the blank plasma did not show any elution peak after 4 minutes. A stable baseline with S/N ratio ~1 confirms no absorption at this wavelength. It was therefore confirmed that the elution peak recorded from the drug solution was specific to the tamsulosin HCl. Following the injection of tamsulosin solution, pharmaceutical preparation and spiked plasma the R_t of the absorbance peak was persistent (fig. 7). Furthermore, the peak resolution complies with the literature guidelines (ICH, 1996). It was therefore conferred that the present method has an acceptable specify of the tamsulosin absorbance peak.

Robustness

Following deliberate changes in the column temperature ±0.5°C, the tamsulosin absorbance peak was recorded without significant changes in its attributes such as shape height area. Likewise, the features of the tamsulosin absorbance peak at 224 nm resides with in the acceptable limit following slight variation in the pH of the mobile phase (±0.5 units), confirming the ability of the analytical method to withstand minor operational changes.

The results from drug analysis performed using different batch of the column and different HPLC equipment (Perkin Elmer USA), in both the cases, the resultant chromatograms were found with an acceptable limit i.e. RSD ≤2%. The results confirm that the method is robust to the operational variations.

Stability of tamsulosin HCl

The results for sample stability at bench top for 24 and 48 hours are presented in table 3. The results indicated that samples were stable during analysis period.

DISCUSSION

Development of RP-HPLC based analytical method that explores the effect of composition elution medium as well as the equipment settings is described in this study. The proportion of ACN in the mobile phase showed a positive impact on the peak area however, it negatively affects the retention time. The flow rate of the mobile phase inversely affects the retention time as well as the area of the tamsulosin HCl elution peak. In contrast, the column temperature improves the tamsulosin HCl elution

phenomenon. Based on the above mentioned correlation between the input and output parameters, a precise adjustment of the operational settings was made to develop a methodology to determine the concentration of tamsulosin HCl from different solutions. Furthermore, the proposed analytical method was validated according to the ICH guidelines. The results confirm the application of this method the quantification of tamsulosin HCl in different samples intended for the standardization of raw materials, pharmaceutical preparations as well as in different bio-relevant media.

CONCLUSION

The bespoke validated analytical method is fast, adaptable and reliable for the quantification of tamsulosin HCl. This method being simple, and adaptable for the determination of tamsulosin HCl in its solutions at different product life cycle will serve the pharmaceutical analysis at research and development as well as the clinical settings.

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