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ESTIMATION OF ALOSETRON HYDROCHLORIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT:

A simple, precise, rapid and accurate RP- HPLC method was developed for the estimation of Alosetron Hydrochloride (AST) in tablet dosage forms. Waters Spherisorb® 5µm CN, 250x4.6 mm, column with 5 µm particle size and the Mobile Phase consisting of 0.01M Ammonium Acetate in water adjusting the pH-3.2 with Methanol & Tetra Hydro Furan in ratio of 700:240:60 v/v & mobile phase used as a diluent in the gradient mode. The flow rate was 1.0 ml/min and the effluents were monitored at 295 nm. The retention time was 11.570 min and the detector response was linear in the concentration range of 56.1- 673.2 µg/mL for AST successively. The respective linear regression equation being Y= 27228.755x + 221036.4041 for AST. The Limit of Detection (LOD) & The Limit of Quantification (LOQ) was found to be 0.1 & 0.3 µg/mL for AST. The percentage assay of AST was 99.93%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of AST in bulk drug and in its pharmaceutical dosage forms.

KEY WORDS: *Alosetron Hydrochloride (AST), RP-HPLC, Estimation, and Tablets.*

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INTRODUCTION: Alosetron HCl (AST), chemically is designated as 2, 3, 4, 5-tetrahydro-5-methyl - 2 - [(5-methyl - 1H-imidazol-4-yl) methyl] - 1H-pyrido [4, 3-b] indol-1-one, Monohydrochloride. Alosetron is achiral [1,2,3] and has the empirical formula: $C_{17}H_{18}N_4O.HCl$, representing a molecular weight: 330.8 gms/mol (Figure: 1). It is a potent and selective antagonist of the serotonin 5-HT_3 receptor type. Activation of these receptors affects the regulation of visceral pain, colonic transit, and GI secretions. By blocking the action of serotonin on the intestinal system, the receptors are able to effectively control Irritable Bowel Syndrome (IBS) [4-8]. This reduces the cramping, stomach pain, stomach discomfort, urgency, and diarrhea caused by IBS. 5-HT_3 receptors are nonselective cation channels that are extensively distributed on enteric neurons in the human gastrointestinal tract, as well as other peripheral and central locations. AST inhibit activation of non-selective cation channels, results in the modulation of the enteric nervous system in neuronal depolarization affect. Pertinent Literature survey reveals a few chromatographic methods [9] to determine the AST in tablet dosage form and also in biological fluids. So far, no assay methods by liquid chromatography were reported for the estimation of AST in pharmaceutical dosage forms at the time of commencement of these investigations. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of AST in pharmaceutical formulations. A detailed account of all analytical methods existing for the drug is made to avoid duplication of the method developed. The authors had made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developing analytical methods, by using HPLC System. The results of this labor of love are set forth by developing a simple, precise and accurate reverse-phase HPLC method for the estimation of AST in bulk drug samples and in pharmaceutical dosage forms.

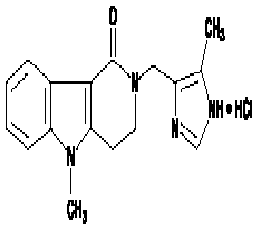


Fig 1: Alosetron Hydrochloride

EXPERIMENTAL:

Materials / Chemicals and Reagents: Methanol & Water used were of HPLC grade (Qualigens). Ammonium Acetate & Tetra Hydro Furan (THF) were obtained from SDFCL, Mumbai. Commercially available tablets (Lotronex®) were procured from local market and used within their shelf life period.

Chromatography Instrument: Quantitative HPLC was performed on liquid Chromatograph, Waters Spherisorb® 5µm CN, 250x4.6 mm; column with 5 µm particle size with injection volume 20 µL was used. The HPLC system was equipped with Empower 2 Software and the data integration was carried out. The column was maintained at ambient temperature and eluted under isocratic conditions over 20.0 min at a flow rate of 1.0 ml/min. A LeelaSonic -60 degassing sonicator was used for dissolution of the sample. Chemiline Digital pH meter CL110 was used for pH adjustments.

HPLC Conditions: The contents of the Mobile Phase consisting of 0.01M Ammonium Acetate in water adjusting the pH-3.2 with Methanol & Tetra Hydro Furan in ratio of 700:240:60 v/v & mobile phase used as a diluent in the gradient mode. They were filtered before use, through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 20.0 min and the column temperature was ambient. Prior to the injection (20 µL) of the drug solution, the column was equilibrated for at least 20 min with the mobile phase flowing through the system. The eluents were monitored at 295 nm.

***Preparation of the Primary Standard/Stock Drug Solution*:** A standard stock solution of the drug was prepared by dissolving 561.0 mg of AST in 100 ml volumetric flask by using A grade bulb pipettes containing 30 ml of diluent {$NH_4C_2H_3O_2$: CH_3OH: $(CH_2)_4O$}, sonicated for about 15 min and then made up to 50 ml with diluent to get standard stock solution of 0.561 mg/mL of AST.

***Preparation of the Working Standard Drug Solution*:** 5ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent{$NH_4C_2H_3O_2$: CH_3OH: $(CH_2)_4O$}(700:240:60 v/v) to get a concentration of each 561.0 µg/mL of AST respectively.

***Quantification/Preparation of Sample solution*:** Twenty tablets (Lotronex® - Prometheus Laboratories Inc) [10,11] were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture (77200 mg) containing concentration of each 0.561 mg/mL of AST active ingredients, was mixed with 30 ml of $NH_4C_2H_3O_2$: CH_3OH: $(CH_2)_4O$}(700:240:60 v/v) as diluent in 50 ml volumetric flask. The mixture was allowed to

stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding methanol up to 100 ml to obtain a stock solution each of 0.561 mg/ml of AST. 5ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of each 561.0 µg/mL of AST respectively. The peak areas were measured at 295 nm.

Method Validation: The method was validated in accordance with ICH guidelines [13]. The parameters assessed were Linearity, Accuracy, Limit of detection (LOD), Limit of Quantification (LOQ), Precision, Reproducibility, Robustness and System Suitability tests.

Linearity: Aliquots of standard AST stock solution was taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of AST was in the range of 56.1-841.5 µg/mL respectively. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 295 nm and the calibration graph was obtained by plotting peak area versus concentration of AST. The plot of peak area of each sample against respective concentration of AST was found to be linear in the range of 112.2-673.2 µg/mL with correlation coefficient of 0.999. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective linear regression equation being Y= 27228.755x + 221036.4041 for AST. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

Accuracy: Accuracy was evaluated in triplicate by addition of three different amounts of AST to a previously analyzed sample and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 80, 100, and 120% of the amount originally present. %Recovery and RSD (%) were calculated for amount added. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in Table: 2. The accuracy was expressed as percent analyte recovered by the proposed method.

Precision: The precision of the method was ascertained, separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation.

The HPLC systems were set up, describing chromatographic conditions, mentioned as above and following the system equilibration of the working standard solution containing 561.0 µg/mL of AST was injected six times and the response peak areas were recorded. The precision was repeated with the formulated sample for the same concentrations by injecting the working sample solutions containing 561.0 µg/mL of AST. The sample was processed six times for the response of peak area. The % Relative Standard Deviation (RSD) and % range of error (at 0.05 and 0.01 confidence levels) were calculated and presented in Tables: 3 & 4 respectively.

Limits of Detection and Quantitation: Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

Method Applicability & System Suitability: The present developed method was evaluated by applying to Pharmaceutical dosage forms for the estimation of AST by our research group. To ensure the validity of the analytical procedure, a system suitability tests were established. The following parameters like theoretical plate number (N), tailing factor, retention time, resolution, area and % peak area were analyzed by using 20 µL of the working standard solution containing AST (561 µg/mL) injecting six times into HPLC system.

Assay: 20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 11.570 min for AST successively. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Recovery Studies: Accuracy was determined by recovery studies of AST; known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of recovery studies are shown in Table 2. The study was done at three different concentration levels.

RESULTS AND DISCUSSIONS:

HPLC Method Development and Optimization[12]: In response to lack of simple, reliable and easy-to-use method for the determination of AST concentrations in pharmaceutical matrices, an isocratic RP- HPLC method was developed for quantification of above mentioned, API. Nevertheless, there is need to consider the successive steps for the development of the method. In fact, the problems relating to the standardization of sample preparation and selection of mobile phase needs to be emphasized. The authors examined several HPLC method variables with respect to their corresponding effects on the

result of analysis. To optimize the chromatographic conditions, different combinations of Methanol-Water, Acetonitrile-Water, and Acetonitrile-Ammonium acetate buffer with THF & Acetonitrile-Methanol were tested. 0.01M Ammonium Acetate in water adjusting the pH-3.2 with Methanol & THF was promisingly preferred, because it resulted in greater resolution of API after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were temperature, flow rate, detection wavelength and volume of injection. Buffer molarity was changed and optimum buffer strength was selected as 0.01M on the basis of theoretical plate number. At 295 nm, UV responses of all three active pharmaceutical analytes were good and free form interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram of AST (Standard and Working Sample) has been shown in Figure: 2 & 3. The system suitability tests were carried out on freshly prepared standard stock solutions of AST. Parameters that were studied to evaluate the suitability of the system were discussed and presented in Table 5.

Method Validation Tests: Recommended method validation characteristics including method precision (RSD, %), method accuracy (Recovery % and RSD, %), linear range (Correlation Coefficient), and LOD & LOQ, were investigated systematically.

Linearity: The plot of peak areas of each sample against respective concentrations were found to be linear, in the range of 112.2-673.2 µg/mL for AST with correlation coefficient of 0.999 (Table: 1). Linear regression least square fit data obtained from the measurements are given in Table: 1. The respective linear regression equation being Y= 27228.755x + 221036.4041 for AST. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1. These results show that there was an excellent correlation between peak areas and analyte concentration.

Accuracy: Recovery of the individual substances at 80%, 100%, and 120% of specified concentrations were between 94.91-104.50%, which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (Table: 2)

Precision: The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (Table: 3).

Robustness: Robustness was studied out to evaluate the effect of small but deliberate variations in the chromatographic conditions at three different levels, i.e. –2, 0, +2. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated by another analyst using same instrument and same laboratory. One factor at a time was changed to study the effect. Variation of different columns (Inertsil & Hypersil), mobile phase flow rate by 0.8 and 1.2 mL min−1 and column temperature by 40°C & 50°C had no significant effect on the retention time, % RSD and chromatographic response of the method, indicating that the method was robust and reproducible. So therefore, the method was robust as minor changes in the chromatographic parameters did not alter any significant changes in the peak area and retention time. The results are shown in Table: 5.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The Limit of Detection (LOD) & The Limit of Quantification (LOQ) analyzed were found to be 0.1 & 0.3 µg/mL for AST respectively. These values reflect the high sensitivity of the method, which is of great importance in most studies and also indicating the method can be used for detection and quantification of analytes in a very wide concentration range.

Specificity: No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical dosage formulations was tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms.

CONCLUSION:

A simple and easily available HPLC method was developed in this study for the quantification of AST in pharmaceutical matrices. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of AST can be used for routine analysis in pharmaceutical quality control within a short time.

Table 1: Linear Regression Data of Calibration Curve & Proposed Method

Parameter	Alosetron HCl (AST)
Concentration range (µg/mL)	112.2-673.2
Slope (m)	27228.755
Intercept (Y)	112888.7521
Standard error of estimate (c)	221036.4041
Correlation coefficient (r)	0.999
Linear regression coefficient (r^2)	0.999
%RSD	0.4

Table 2: Assay & Accuracy Recovery Studies of Alosetron Hcl (AST) In Tablet Dosage Forms

Tablet formulation	Amount claim (mg/tablet)	Amount Obtained (mg)* by proposed method	** % Recovery by the Proposed method
	AST	AST	AST
1) 120%	1	0.87	94.91
2) 100%	1	0.94	98.08
3) 80%	1	1.05	104.50
Average Mean	1	0.95	99.16

*Average of three determinations ** After spiking the sample

ACCURACY PARAMETER	Alosetron HCl (AST)	
Assay (120%)	131.30%	
Assay (100%)	111.70%	
Assay (80%)	92.48%	
	Standard	Spiked
% RSD (120%)	0.6	0.5
% RSD (100%)	0.1	0.1
% RSD (80%)	0.2	0.0
	Area	
Standard Deviation (120%)	103738.0	102997.1
Standard Deviation (100%)	21593.9	9410.1
Standard Deviation (80%)	24631.3	788.2

Table: 3: Precision of Recommended Procedure Using API & Sample (Lotronex®): Alosetron HCl (AST)

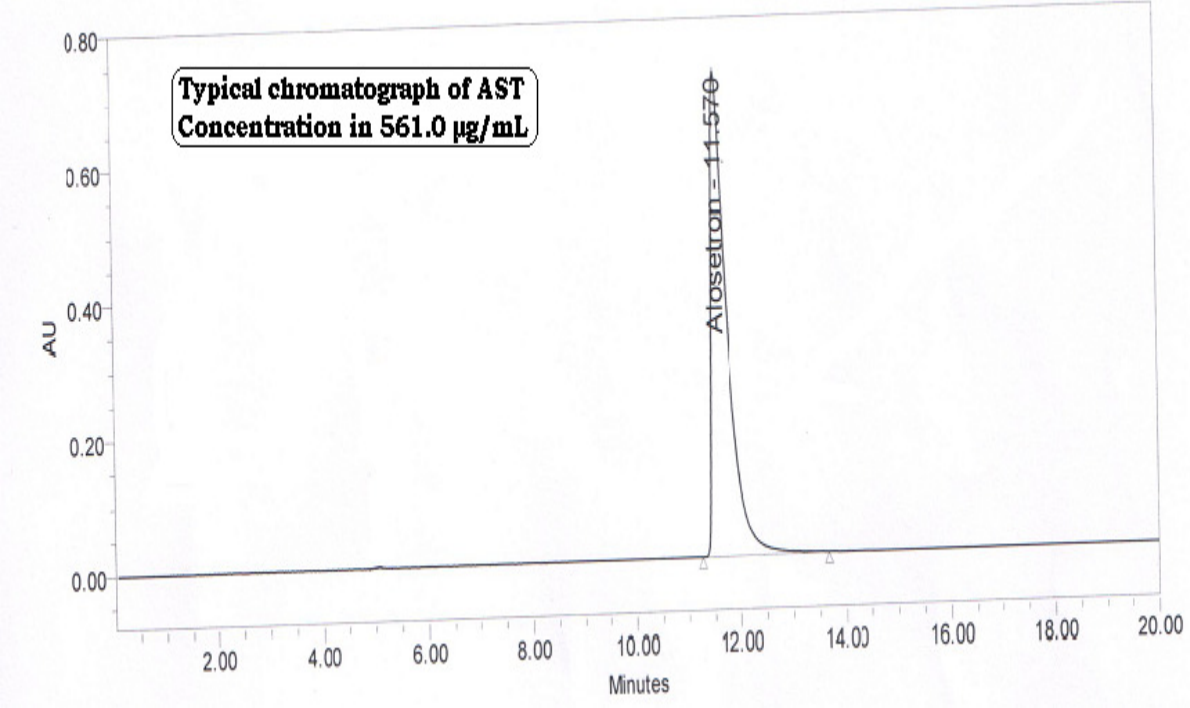
Sr. No	Inj. No	Name of the Drug & Conc. (561.0 µg/mL)	Standard Drug		Sample Drug	
			Retention time in minutes	Peak Area	Retention time in minutes	Peak Area
1	1	AST	11.597	15208123	11.640	15203608
2	2	AST	11.605	15159650	11.653	15274472
3	3	AST	11.616	15343636	11.670	15271256
4	4	AST	11.622	15216780	11.664	15252420
5	5	AST	11.630	15167117	11.669	15234690
6	6	AST	11.637	15220342	11.678	15302519
7		Mean	11.618	15219274.6	11.662	15256494.2
8		Standard Deviation	0.015	66140.6	0.013	34491.7
9		% RSD	0.13	0.4	0.12	0.2

Table 4: Validation Summary / System Suitability:

PARAMETER	ALOSETRON HCl (AST)
Theoretical Plates(N)	7244.46
Tailing factor	2.55
Retention time(min)	11.570
Resolution	15.66
Area	15451077
% Peak Area	99.85%
LOD (µg/mL)	0.1
LOQ (µg/mL)	0.3

Table 5: Robustness testing results of the method (n=3, 100% of the Working Standard Solution & Sample solution contains: 561.0 µg/mL of Alosetron HCl (AST)

Condition Studied in Robustness	Modification In OFAT analysis	Parameter Fixation	Mean Peak Area ± S.D	% RSD (Peak Area)	Mean Retention Time (in min) ± S.D	% RSD (Retention time)
			AST	AST	AST	AST
Column(s) (Waters Spherisorb® 5µm CN)	Inertsil & Hypersil	Standard	15348319.8 ± 22760.7	0.1	11.834 ± 0.004	0.04
		Sample	15292447.5 ± 24633.4	0.2	11.846 ± 0.003	0.03
Flow rate (1.0 ml/min)	0.8 ml/min & 1.2 ml/min	Standard - Increase	13999972.2 ± 61760.6	0.4	9.978 ± 0.002	0.02
		Standard - Decrease	17083238.7 ± 51251.8	0.3	12.148 ± 0.003	0.02
		Sample - Increase	13988401.3 ± 19981.9	0.1	9.980 ± 0.003	0.03
		Sample - Decrease	17084606.0 ± 62252.0	0.4	12.154 ± 0.003	0.03
Column temperature (45°C)	40°C & 50°C	Standard - Increase	15205000.6 ± 33026.0	0.2	9.876 ± 0.009	0.09
		Standard - Decrease	15211707.5 ± 12461.1	0.1	12.335 ± 0.016	0.13
		Sample - Increase	15186791.8 ± 29947.7	0.2	9.873 ± 0.004	0.05
		Sample - Decrease	15393603.2 ± 28171.0	0.2	12.321 ± 0.006	0.05



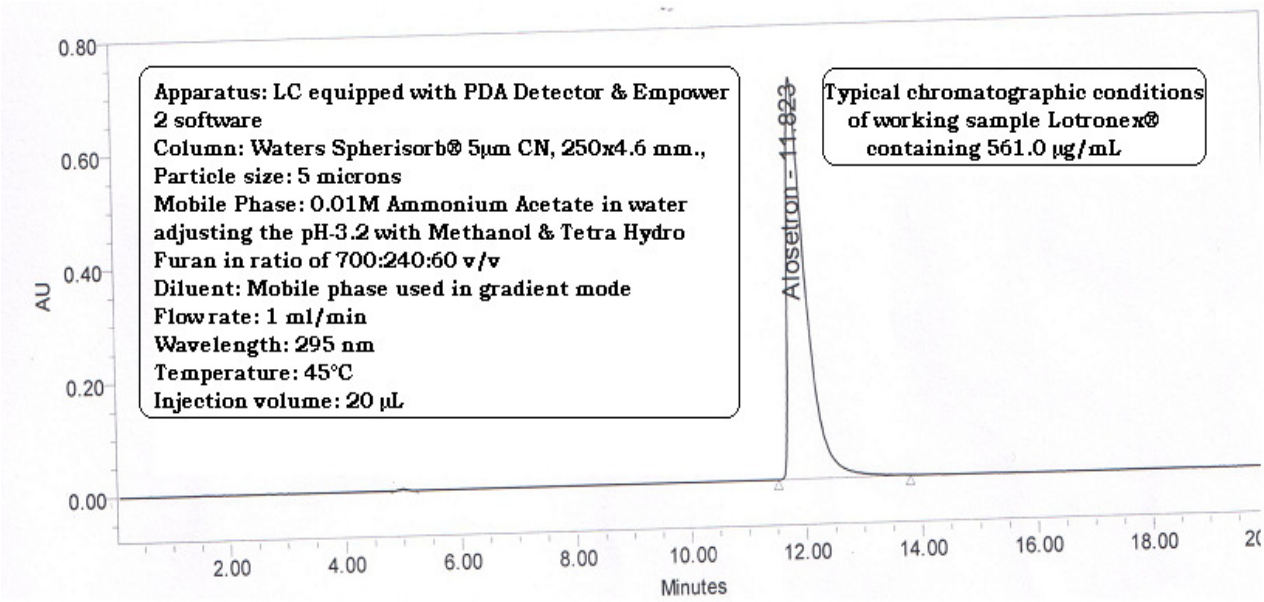


Fig 2 & 3: Typical Chromatogram of Alosetron HCl (AST) {(Standard & Working Sample)} by RP-HPLC

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