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Development and Validation of Noscapine In Bulk and Pharmaceutical Formulations by RP-HPLC Method

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ABSTRACT

A simple, specific, quick, isocratic Reversed Phase High Performance Liquid Chromatographic method was developed and validated for the analysis of Noscapine. RP-HPLC method was developed on a Symmetry C-8 (4.6 × 150 mm), 3.5 µm particle, reversed-phase column. The mobile phase was 0.1% octane sulphonic acid (pH- 3): acetonitrile, 40:60 (v/v) at a flow rate of 0.8 ml/min. and the eluate was monitored at 260 nm. The retention time of the drug was found to be 2.314 min. The method was linear over the range of 4-8 µg/ml with a regression coefficient of 0.999 and validated with respect to accuracy, precision, linearity, and specificity, limit of detection and limit of quantization as per the guidelines of International Conference for Harmonization (ICH). This method can be used in the industries for determination of Noscapine to analyze the quality of formulation without interference of the excipients.

Keywords: Noscapine, RP-HPLC, validation, ICH.

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INTRODUCTION

Noscapine is chemically (3S)-6,7-dimethoxy-3-[(5R)-4-methoxy-6-methyl-7,8-dihydro-5H- [1,3]dioxolo[4,5-g]isoquinolin-5-yl]-3H-2-benzofuran-1-one hydrochloride (Figure 1). Noscapine Hydrochloride is the orally available hydrochloride salt of the opioid agonist noscapine, a phthalideisoquinoline alkaloid derived from the opium poppy *Papaver somniferum*, with mild analgesic, antitussive, and potential antineoplastic activities. Noscapine binds to tubulin and alters its conformation, resulting in a disruption of the dynamics of microtubule assembly (by increasing the time that microtubules spend idle in a paused state) and subsequently, the inhibition of mitosis and tumor cell death. Unlike other tubulin inhibitors such as the taxanes and vinca alkaloids, noscapine does not affect microtubule polymerization[1].

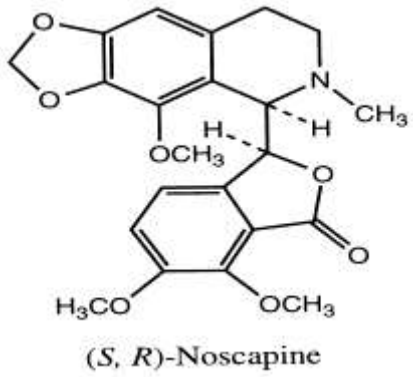

Figure 1: Chemical Structure of Noscaine

Literature survey reveals that few High performance liquid chromatographic (HPLC) methods have been described for the estimation of Noscapine hydrochloride individually and combination with other drugs. base-deactivated stationary phase[2,] dosage forms with an aqueous-organic mobile phase[3], in Human Plasma[4,] determination of alkaloids from *Papaver somniferum* L. (Papaveraceae)[5,] dosage forms with an aqueous-organic mobile phase[6], determination of degradation impurity[7], in plasma by liquid chromatography[8.] The main objective of the present study is to develop simple, sensitive, accurate and precise RP-HPLC method for estimation of Noscapine hydrochloride in bulk and liquid oral dosage forms. The validation has been carried out as per ICH guidelines.

MATERIALS AND METHOD

Instruments

Chromatographic separation was performed, under ambient conditions, with Waters alliance 2695 module (Waters Corporation, Milford, USA) equipment comprising a

variable wavelength Waters 2487 dual λ absorbance UV detector with empower-2 software was used for the analysis. Samples (20 µl) were injected by means of a Rheodyne injector fitted with a 20µl loop. Compounds were separated on a Symmetry C-8 (4.6×150mm), 3.5 µm particle, reversed-phase column. The mobile phase was 0.1% octane sulphonic acid (pH- 3): acetonitrile, 40:60 (v/v) at a flow rate of 0.8 ml/min.

Chemicals

An analytically pure sample of Noscapine hydrochloride was procured as gift sample from Hyderabad. Pharmaceutical Formulation [NOSCOTUSS^{TM}-suspension, Sirmour Remedies (P) Ltd., Sirmour] were procured from a local pharmacy with labeled amount 15 mg. HPLC-grade Acetonitrile and octane sulphonic acid was purchased from Qualigens fine chemicals, India. High-purity water was prepared using Millipore purification system. Other chemicals and reagents were of AR-grade.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, 0.1% octane sulphonic acid buffer p^{H}-3: acetonitrile [40:60 v/v] was chosen with detection wavelength 260 nm, since it gave sharp peak with good symmetry within limits.

Preparation of (0.1%) octane sulphonic acid Buffer pH-3:

1.0 gm of octane sulphonic acid sodium salt (anhydrous) was weighed and transferred to 1000 ml of water and mixed well. The P^{H} of the solution was adjusted to 3 with *o*-phosphoric acid solution.

Preparation of mobile phase:

Mix a mixture of 400 ml of above buffer 400 ml (40%) and 600 ml of HPLC acetonitrile (60%) filtered through 0.45 µ filter under vacuum filtration degas in ultrasonic water bath for 15 minutes.

Preparation of diluents

0.1N HCl

Measure accurately 8.5 ml of concentrated hydrochloric acid was dissolved in 1000 ml of HPLC water.

Chromatographic conditions

The optimized parameters which were used as a final method for the estimation of represented in the Table 1.

Table 1: Optimized chromatographic conditions

Mobile phase	0.1% octane sulphonic acid buffer pH-3 : acetonitrile [40:60 v/v]
Stationary phase	X-Bridge, C_{18} (4.6 x 150mm, 3.5µm partical size)
Wavelength	260 nm
Run time	7 min
pH mobile phase	3
Flow rate	0.8 ml per min
Injection volume	10 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Preparation of working standard stock solution:

Accurately weigh and transfer 10 mg of Noscapine HCl working standard into a 10 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the diluents (0.1N HCl) To obtain standard stock solution of 1000 µg/ml (stock solution-1). From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluents to obtain 100 µg/ml (stock solution-2).

Preparation of standard solution preparation:

Aliquots of 0.4, 0.5, 0.6, 0.7 and 0.8 ml was transferred to 10 ml of volumetric flasks and made up to the mark with diluent 0.1N HCl to get concentration of 4.0, 5.0, 6.0, 7.0 and 8.0 µg/ml. An aliquot (10 µl) of each solution was injected under the operating chromatographic conditions and responses were recorded. Calibration curve was constructed by plotting the peak areas versus the concentration and the regression equation was calculated.

Preparation of Sample Solution Preparation:

Accurately weigh and transfer equivalent to 10 mg of Noscapine HCl sample into a 10ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution). Further, pipette 0.6 ml of Noscapine HCl of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject 2 µL of the standard, sample into the chromatographic system and measure the areas for the Noscapine HCl peaks and calculate the % Assay by using the formula.

Method Validation [9, 10]

The optimized spectrophotometric and chromatographic methods were completely validated according to the procedures described in ICH guidelines Q2 (R1) and Q1 A (R2) for the validation of analytical methods and Stability Testing of New Drug, respectively.

Linearity

Appropriate aliquots of standard Noscapine HCl stock solutions (100 µg/ml) were taken in different 10 ml volumetric flasks and resultant solution was diluted up to mark with diluents to obtain final concentration of drug solution. Calibration curve of Noscapine HCl was constructed by plotting peak area *vs*. applied concentration of Noscapine HCl. The slope, intercept and correlation coefficient were also determined.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, twenty tablets of formulation were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by standard addition method by adding known amount of standard drug solution (50, 100 and 150%) to the sample solution and % Recovery was calculated.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peaks was observed. From the data obtained, the developed method was found to be precise.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of PRG was determined by calculating the signal-tonoise (S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines.

Robustness

The samples were analyzed separately by slightly changes in the analytical methods such as by changing flow rate of mobile phase ±0.1 ml and by changing ratio of organic composition of the mobile phase *i.e.* acetonitrile: buffer from ± 10%, the chromatograms were recorded. The retention time values were observed.

System-Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. 10 µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Table 2: System suitability studies by RP-HPLC method

System suitability parameters	Results
Retention time	2.314
Area	234536
Theoretical plate number	2559.08
Tailing factor	1.65

RESULTS AND DISCUSSION

To develop simple and economical RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with X-Bridge, C_{18} (4.6 x 150 mm, 3.5 µm particle size) column and mobile phase comprising of 0.1% octane sulphonic acid buffer p^H-3: acetonitrile [40:60 v/v] at a flow rate of 0.8 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 260 nm based on peak area. The retention time was found to be 2.314 min. The optimized method was validated as per ICH guidelines.

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, degradants or excipients. A volume of 10µl of working sample solution was injected and the chromatogram was recorded. Peak was found at retention time of 2.314 min. Hence, the proposed method was specific for Noscapine hydrochloride (Figure 2).

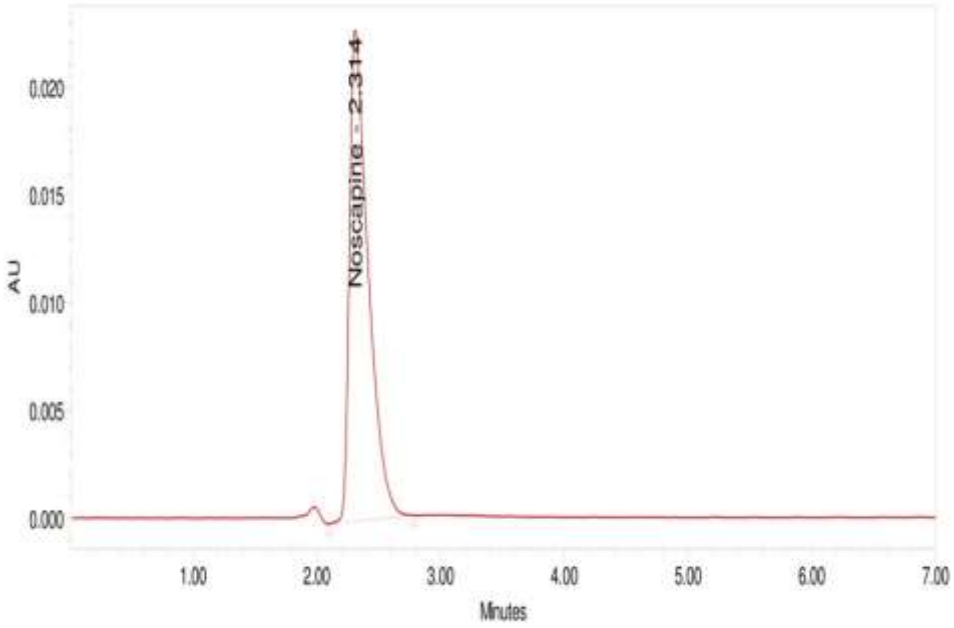

Figure 2: Chromatogram of Noscapine hydrochloride at 260 nm

Linearity

Under the optimum experimental conditions, the concentration vs peak area plot for the proposed method was found to be linear over the range of 4-8 µg/ml. The parameters for the regression analysis are given in Table 3. Linearity graph of different concentration of PRG shown in Figure. 3.

Table 3: Summary of optical and regression analysis of the calibration curve for PRG

Statistical Parameters	Pregabalin
Linearity range (µg/ml)	4-8
Regression equation	y = mx + c = Y=38340X+1116
Slope (m)	38340
Intercept	1116
Standard deviation of slope	0.025
Correlation coefficient (r)	0.999
Limit of Detection (LOD), µg/ml	1.57
Limit of Quantification (LOQ), µg/ml	4.83

[a]with respect to y = mx + c, where x is the concentration in µg/ml, Y is the peak area.

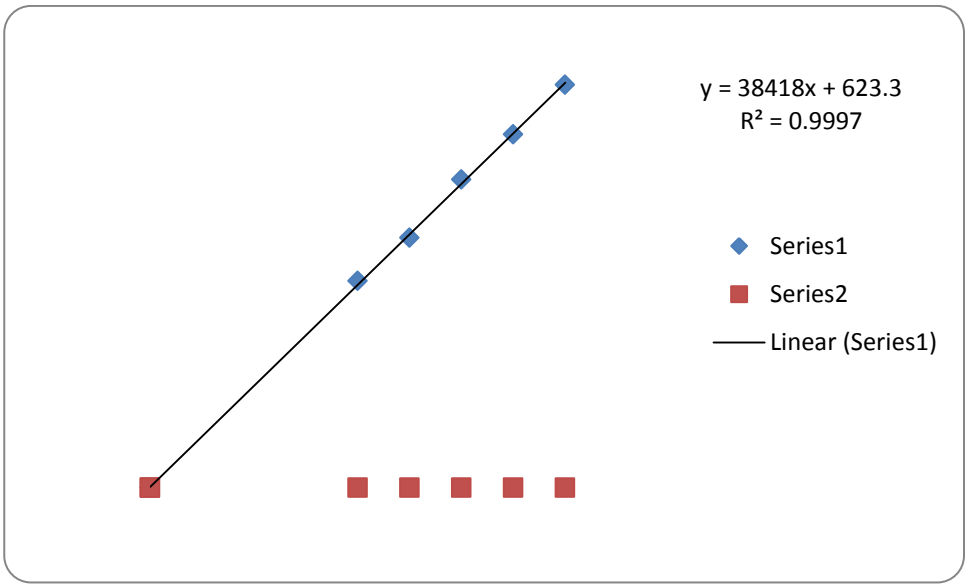

Figure 3: Calibration curve of Noscapine hydrochloride by RP-HPLC

Precision

The precision of the analytical method was determined by intraday and interday precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times in the same day and in interday precision, injecting six solutions of same concentration for six different days in a week. The precision of the proposed method was carried in terms of the inter-day and intra-day time periods. The average and standard deviation of mean area were taken and % RSD was calculated and reported. The results of precision were tabulated in table-4. The low % RSD values of intra-day (0.9 %) and inter-day (0.94 %) variations revealed that the proposed method was precise.

Table 4: Results of Precision studies

Concentration (6 µg/ml)				
Precision	**Intraday**	**Interday**		
		Day 1	**Day 2**	**Day 3**
Mean area*	1717626.1	1717576	1717573	1717508
Standard deviation	16169.8	16171.81	16172.18	16172.69
% RSD	0.9	0.94	0.941	0.941

*indicates average of six determinations, RSD indicates relative standard deviation

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (50%, 100% and 150% of final concentration). The results are reported in Table 5 (pure drug) and the recoveries ranged from 100.52 to

101.51% for pure drug. The proposed method results are satisfactorily accurate and precise.

Table 5: Results of Accuracy studies

Drug name	Levels	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean	Standard deviation	% RSD
Noscapine HCl	50%	3	3.04	101.51	858648	4792.4	0.5
	100%	6	6.03	100.52	1691672.3	8938.5	0.5
	150%	9	9.12	101.40	2573164.2	13240.1	0.5

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the Flow rate (± 0.1), organic solvent content (± 10%), None of these alterations caused a significant change in peak area RSD, tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible. The results were represented in Table 6.

Table 6: Results of Robustness studies

Parameters	Flow rate		Mobile phase	
	0.7 ml/min	0.9 ml/min	50:50	70:30
Mean area	2245583	1775329	1857895	1777209
Standard deviation	28294.51	13535.77	11469.32	12123.73
% RSD	1.26	0.76	0.61	0.68

[a] indicates three factors were slightly changed at three levels,

*indicates average of six determinations and RSD indicate relative standard deviation.

Limit of detection and Limit of quantification

LOD and LOQ was found to be 1.57 and 4.83 µg/ml, respectively. The results were represented in Table 7.

Table7: Results of LOD and LOQ

Parameters	Results
LOD (µg/ml)	1.57
LOQ (µg/ml)	4.83

CONCLUSION

A specific, precise, accurate, rapid and reliable RP-HPLC method has been developed and validated. It has short runtime 7 min and retention time 2.314 allows analysis of large number of samples in a short period of time. Finally, since no pharmacopoeial method for determination of Noscapine in bulk and pharmaceutical formulations have been reported

yet, the proposed method could be useful and suitable for the determination of Noscapine in bulk and pharmaceutical formulations.

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