ejpmr, 2017,4(6), 510-514

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

VALIDATION OF A UPLC METHOD WITH DIODE ARRAY DETECTION FOR THE DETERMINATION OF NOSCAPINE IN SYRUP DOSAGE FORM

Anas Rasheed*[1] and Dr. Osman Ahmed[2]

[1]Research Scholar, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur.
[2]Research Supervisor, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur.

***Corresponding Author: Anas Rasheed**

Research Scholar, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur.

Article Received on 04/04/2017 **Article Revised on 24/04/2017** **Article Accepted on 14/05/2017**

ABSTRACT

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of Noscapine in bulk drug and syrup dosage form. The method employed, with Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) in a gradient mode, with mobile phase of Octane sulphonic acid buffer : acetonitrile 35:65 %v/v. The flow rate was 1.0 ml/min and effluent was monitored at 260 nm. Retention time was found to be 2.050±0.880 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100 µg/ml respectively. The LOD and LOQ values for were found to be 2.098(µg/ml) and 6.3597(µg/ml) respectively. No chromatographic interference from syrup excipients and degradants were found. The proposed method was successfully used for estimation of Noscapine in syrup dosage form.

KEYWORDS: Noscapine, syrup dosage form, UPLC method estimation.

1. INTRODUCTION

Noscapine, also referred to as narcotine chemically it is, (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[1,2&11] (Fig. 1). Noscapine is a benzylisoquinoline alkaloid from plants of the poppy family, without analgesic properties. Noscapine is practically used for its antitussive i.e. cough-suppressing effects. The analytical data are a prerequisite for correct interpretation of any dosage form.[4,5] The objective of UPLC method development and validation of Noscapine in syrup dosage form procedure is to provide information about potency.[6-9] The validation of a specific method must be demonstrated through laboratory experiments by routinely analysing samples.[10]

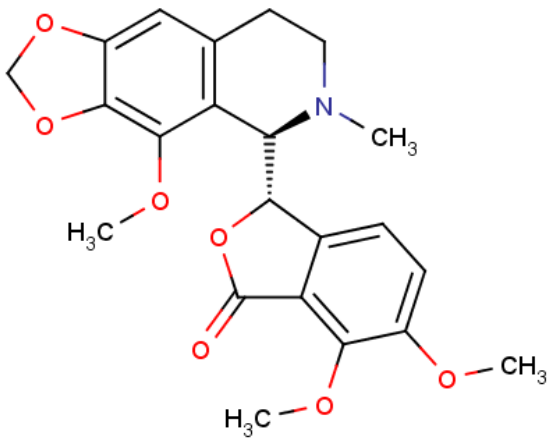

Fig.1: Molecular Structure of Noscapine, (3S)-6,7-dimethoxy-3-[(5R)-4-methoxy-6-methyl-7,8-dihydro-5H- [1,3] dioxolo[4,5–g]isoquinolin-5-yl]-3H-2-benzofuran-1-one hydrochloride[2,11]

2. EXPERIMENTAL

Materials

Noscapine (99.50% purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad). UPLC grade methanol, Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai,

India. Distilled, 0.45 µm filtered water used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade. The syrup - dosage (Coscopin Linctus Syrup 15mg per 10 ml) labelled to contain 7.5 mg per 5 mL of container for Noscapine. All chemicals used were of pharmaceutical or special analytical grade.

Instrumentation

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Bandline sonerex sonicator was used for pH adjustment.

Chromatographic Conditions

Table 1: Chromatographic Conditions of the validating method

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm)
Mobile Phase	Octane sulphonic acid buffer : acetonitrile 35:65 %v/v
Flow rate	1mL/min
Run time	8 Min.
Column Temperature	Maintained at 25°C
Injection volume	20 µL
Detection wavelength	260 nm
Diluent	Mobile Phase

Preparation of Standard Stock Solution

Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.0 mL min-1 shows good resolution. The PDA detector response of Noscapine was studied and the best wavelength was found to be 260 nm showing highest sensitivity.

The mixture of two solutions Octane sulphonic acid buffer: acetonitrile 35:65 %v/v. The buffer used is 100 mg of anhydrous octane sulphonic acid sodium salt was weighed and transferred to 100 ml of water and sonicated well. The pH of the solution was adjusted to 3 with orthophosphoric acid solution. Gradient programming was employed to mobile phase at 1.0 mL/min flow rate was found to be an appropriate mobile phase for separation of Noscapine. The column was maintained at 25°C temperature.

Preparation of internal standard solution

Weighed accurately about 10 mg of papaverine into a clean and dry 100 mL volumetric flask, dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Noscapine standard solution

Transfer accurately about 10 mg of Noscapine into 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Linearity was determined in the range of 20-100 µg mL-1.

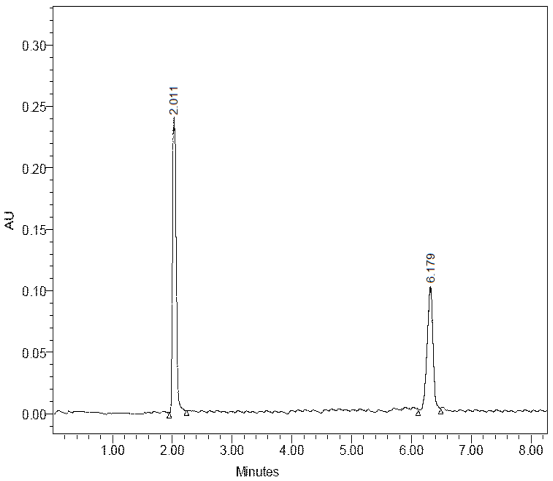

Fig. 2: Optimized chromatogram of Noscapine and internal standard using mobile phase of Octane sulphonic acid buffer: acetonitrile 35:65 %v/v

3. RESULTS AND DISCUSSIONS

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

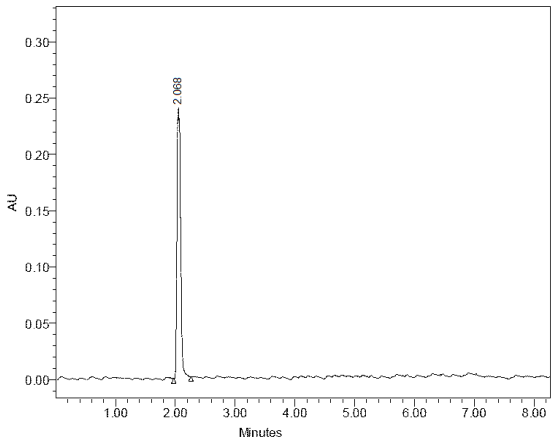

Fig. 3: Standard Chromatogram of Noscapine using mobile phase of Octane sulphonic acid buffer: acetonitrile 35:65 %v/v

Linearity and Range

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The calibration curve showed good linearity in the range of 20-100 µg/mL, for Noscapine (API) with correlation coefficient of 0.9971. A typical calibration curve has the regression equation of y = 344.01x +1435.0858 for Noscapine. Results are given in Table 2.

Table 2: Summary of validation parameters for the proposed method

Noscapine		
Linearity level	**Concentration in µg/mL**	**Area**
1	20 µg/mL	2119.645
2	40 µg/mL	2737.159
3	60 µg/mL	3569.198
4	80 µg/mL	4282.409
5	100 µg/mL	4787.021
Correlation co-efficient	0.9971	
Slope	344.01	
Intercept	1435.0858	

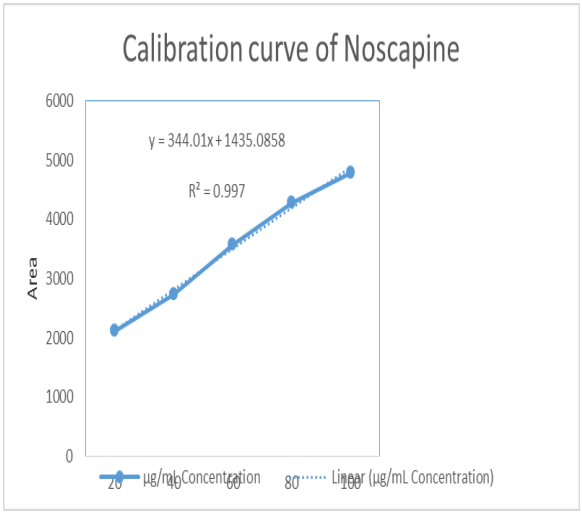

Fig. 4: Calibration curve of Noscapine

Precision

The Precision of the method was studied in terms of intraday and interday precision of sample injections (20 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.06%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.02% Low % RSD values indicate that the method is precise. The results are given in table 3.

Robustness

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed UPLC method for the analysis of Chlophedianol hydrochloride. The results are given in table 5.

Accuracy

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

Table: 3, Results of Precision Studies

Replicate	*Noscapine*		
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	20	2118.211	99.93%
2		2119.821	100.08%
3		2118.332	99.93%
4		2119.241	99.98%
5		2118.899	99.96%
6		2118.947	99.96%
Average			99.97%
Std.Dev			0.0557
% RSD			0.06%
Standard weight			20 mcg
Standard potency			99.50 %

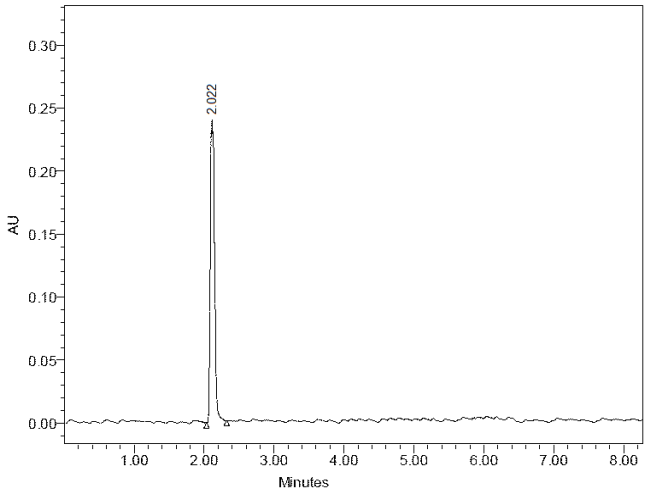

Fig. 5: Chromatogram Showing accuracy results

Results of accuracy study

Table: 4, Results of accuracy study

Noscapine						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	07.81	07.64	97.82	98.93	0.9634	0.97%
100	15.62	15.55	99.55			
150	23.43	22.30	99.42			

Robustness Studies

Table: 5, Results of Robustness Studies

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	Low	2118.621	0.05%
	Actual	2120.427	
	Plus	2120.638	
Temperature	Low	2118.932	0.04%
	Actual	2119.484	
	Plus	2120.691	
Wavelength	Low	2118.883	0.02%
	Actual	2119.476	
	Plus	2119.862	

ANALYSIS OF FORMULATION

Assay studies for the analysis of spray- dosage formulation of Noscapine. Fixed chromatographic conditions were made use for the analysis of formulation and was found to be 100.21%.

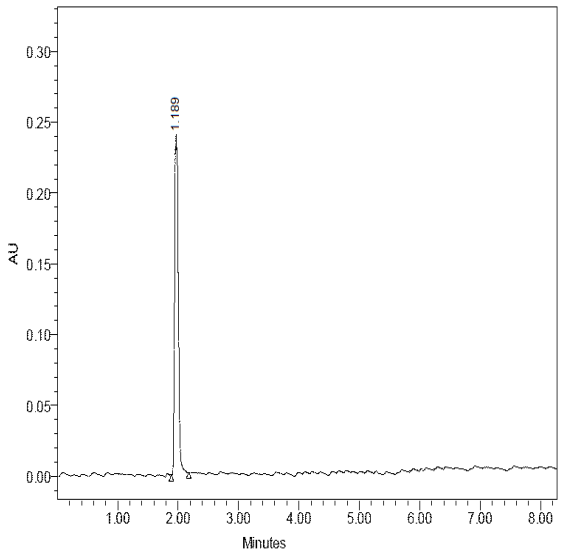

Fig. 6: Chromatogram of Assay Studies

4. CONCLUSION

The method provides selective quantification of Noscapine without interference from blank affirming precise method. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters.

The developed method was robust in the separation and quantification of Noscapine in syrup dose. This method can be used for the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies. The current method is validated for the assay study of the formulation and was found to be satisfactory.

5. REFERENCES

1. Senthilkumar GP et al., Development and Validation of Noscapine In Bulk and Pharmaceutical Formulations by RP-HPLC Method. American Journal of Pharm Tech Research, 2017.
2. http://pubchem.ncbi.nlm.nih.gov/compound/9933439#section.
3. Acevska J, Dimitrovska A, Stefkov G, Brezovska K, Karapandzova M, Kulevanova. Development and validation of a reversed-phase HPLC method for determination of alkaloids from *Papaver somniferum* L. (Papaveraceae). J AOAC Int., 2012; 95(2): 399-405.
4. International Conference on Harmonization Guideline on Validation of Analytical Procedures (2005) Text and Methodology: Q2 (R1).
5. Rania N, El-Shatieny, Fathalla F.B. Journal of Chemistry, 2015; 1-9.
6. Nguyen D.T., Guillarme D, Rudaz S, Veuthey J. L., Fast Analysis In Liquid Chromatography Using Small Particle Size And High Pressure. J Sep Sci., Aug, 2006; 29(12): 1836-48.
7. Katharina Sterz, Gerhard Scherer, Josef Ecker. A Simple And Robust UPLC-SRM/MS Method To Quantify Urinary Eicosanoids.J Lipid Res., 2013; 1-28.
8. Ashok kumar, UPLC: A preeminent technique in pharmaceutical analysis. Acta poloniae pharmaceutica– drug research, 2012; 69(30): 371-380.
9. Michael E Swartz, Ultra performance liquid chromatography UPLC: an introduction. Separation science redefined, 2005; 1: 8-14.
10. Rasheed A *et al.*, Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel Bisulphate and Atorvastatin Calcium in Tablet Dosage Form. American Journal of Pharm Tech Research, 2014.
11. David A. Williams, William O. Foye, Thomas L. Lemke; Foye's principles of medicinal chemistry; 6th Ed Wolters Kluwer Health, 2008; 698-728.