Original Article

Development and validation of RP-HPLC method for estimation of ethacridine lactate in bulk and in pharmaceutical formulation

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Access this article online

Website: www.phmethods.org

DOI: 10.4103/2229-4708.84453

Quick response code

Abstract

A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for estimation of Ethacridine lactate in pharmaceutical formulation. The method was carried out on a Qualisil RP C-18 (250 mm x 4.6 mm, 5 µm) column with a mobile phase consisting of methanol: water (60:40 *v/v*), pH adjusted to 2.8 with *ortho*-phosphoric acid and flow rate of 1.0 mL/min. Detection was carried out at 271 nm. The retention time for ethacridine lactate was found to be 4.41 min. The ethacridine lactate followed linearity in the concentration range of 2- 12 µg/mL (r^2= 0.9980). The amount of the drug estimated by proposed method was found to be in good agreement with label claim. The developed method was validated for sensitivity, accuracy, precision, ruggedness and robustness. The LOD and LOQ were found to be 0.11 and 0.33 µg. The proposed method can be used for routine analysis of ethacridine lactate in bulk drug and pharmaceutical formulation.

Key words: Ethacridine lactate, methanol, RP-HPLC validation

INTRODUCTION

Ethacridine lactate (EL) [Figure 1], 2- ethoxy-6, 9- diaminoacridine monolactate monohydrate (British Pharmacopoeia, 2005) also known as rivanol is employed as a potent anti- microbial agent from the penicillin era and in various other tests on antigens too. In addition, it is a drug commonly used for second trimester termination of pregnancy that has associated with the lowest rate of complication. Ethacridine lactate as an abortifacient is found to be safer and better tolerated than 20% hypertonic saline. The drug is official in British Pharmacopeia[1] and Martindale.[2] Literature survey revealed that one HPLC method is reported for ethacridine lactate in human plasma[3] and one SP-HPLC method is developed.[4] Therefore, the main objective of this work is to develop the simple and economical RP- HPLC method for ethacridine lactate. The second objective is to validate the method as per the ICH guidelines.[5-7]

MATERIALS AND METHODS

Chemicals

Ethacridine lactate is obtained from Venus Remedies Ltd., Haryana, India as a gift sample. Methanol (HPLC grade) was purchased from Merck (India) Ltd., Worli, Mumbai, India. Ethacridine lactate solution infusion was purchased from Indian market, containing ethacridine lactate 1mg per 100mL.

Instrumentation and chromatographic conditions

Analysis was performed on chromatographic system of Agilent liquid chromatograph comprising G 1311A solvent delivery system (pump), G1315 diode array detector, UV detector and a Rheodyne injector with 20 µL loop. EZChrome

Elite was used as a data processer. A Qualisil BDS C-18 column (250 × 4.6 mm i.d., 5µm) was used for chromatographic separation under suitable conditions. The mobile phase consists of methanol: water (60: 40 *v/v*), pH adjusted to 2.8 with *ortho*-phosphoric acid at a flow rate of 1.0mL/min and the run time was 7 min. Before analysis, both the mobile phase and sample solution was filtered through a 0.45 µm membrane filter and degassed for 15 min in an ultrasonicator. The detection of the drug was carried out at 271 nm. The UV-spectra of the drug in methanol is shown in Figure 2.

Preparation of stock standard solution and the calibration graph

Stock standard solution was prepared by dissolving 10 mg of EL in 10 mL methanol that gives concentration of 1000 µg/mL from the stock standard solution, aliquots of 20, 40, 60, 80, 100, and 120µL was taken in 10mL volumetric flasks with the help of micropipette and diluted up to the mark with mobile phase previously filtered and sonicated such that to obtained concentration of EL in the range 2-12µg/mL. Each sample of 20 µL volume was injected with the help of the Hamilton syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *versus* the drug concentration.

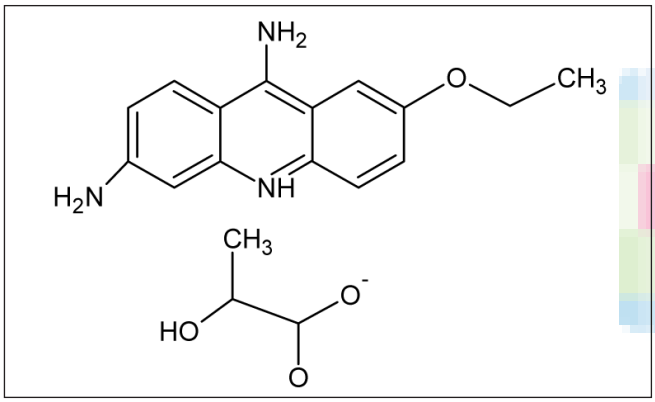

Figure 1: Chemical structure of ethacridine lactate

Analysis of marketed formulation

Ethacridine lactate infusion contained 1 mg /mL of ethacridine lactate in 100 ml of the infusion. From this 10 ml of the solution was taken in the10 ml volumetric flask to give 1000 µg/mL concentration. From this 60 µL was taken with micropipette and was further diluted with the mobile phase to get final concentration of 6 µg/mL. This was analyzed by the proposed method and amount of EL was determined.

Method validation

The HPLC method was validated in accordance with ICH guidelines.[5-7]

Precision

The precision of the method was studied as intra-day, inter-day, and repeatability of sample injections. Intra-day precision was determined by analysis of the solution three times on the same day. Inter-day precision was assessed by analysis of the solution on three different days over a period of 1 week. Repeatability of sample injections was performed by injecting same concentration of the drugs for six times and effects on peak areas were examined.

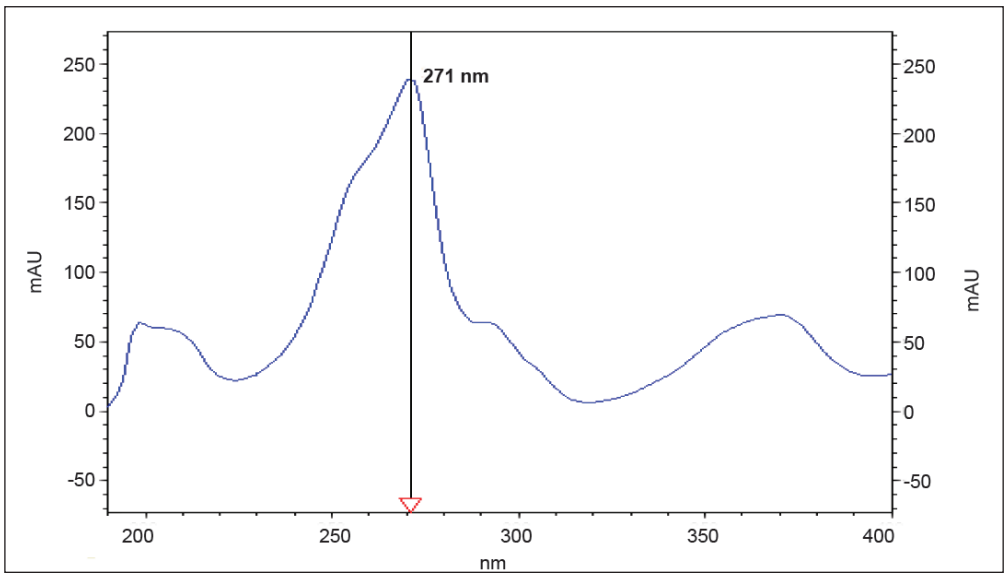

Figure 2: UV spectra of ethacridine lactate at 271 nm

Specificity and selectivity

Specificity of the method was ascertained by analyzing drug standard and sample. The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in the presence of components that may be expected to be present in the sample matrix.

The method is quite selective. There was no other interfering peak around the retention time of ethacridine lactate; also, the base line did not show any significant noise.

Accuracy

The accuracy of the method was studied by the recovery study. To the preanlaysed sample solution, (6µg/mL of EL) a known quantity of EL was added at the 80%, 100%, and 120% level and analyzed by the proposed RP-HPLC method.

Sensitivity

Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ). LOD = 3.3 ×ASD/S and LOQ = 10 × ASD/S, where ASD is the average standard deviation and S is the slope of the line.

Robustness

Robustness of the method was studied by making deliberate changes in few parameters, namely variation of flow rate, mobile phase composition, and change in pH. The effects on the results were studied by injecting 6 µg/mL of EL.

Ruggedness

From the stock solution, sample solution of EL (6 µg/mL) was prepared and analyzed by two different analysts using similar operational and environmental conditions. The peak area was measured for the same concentration solutions, six times.

RESULTS AND DISCUSSION

Selection of chromatographic condition and optimization of the mobile phase

After trying columns containing different stationary phases, the final choice giving satisfactory resolution and run time was Qualisil BDS RP C-18 column (250 × 4.6 mm i.d., 5 µm). The mobile phase was chosen after several trials with methanol and water in various proportions. A mobile phase consisted of methanol: water (60:40 *v*/*v*) resolved peak with tailing. It was overcome by adjusting the pH of the mobile phase to 2.8 with the *ortho*-phosphoric acid. Finally, methanol:water (60:40 *v*/*v*), pH 2.8 was selected to achieve symmetrical peak. The effects of flow rates in the ranges of 0.7 to 1.1mL/min were examined. A flow rate of 1.0mL/min gave good results, system suitability parameter, and reasonable retention time. The retention time of EL was observed 4.47 min at 271 nm wavelengths. The total time of analysis was less than 10 min. A typical chromatogram of the drug is shown in Figure 3.

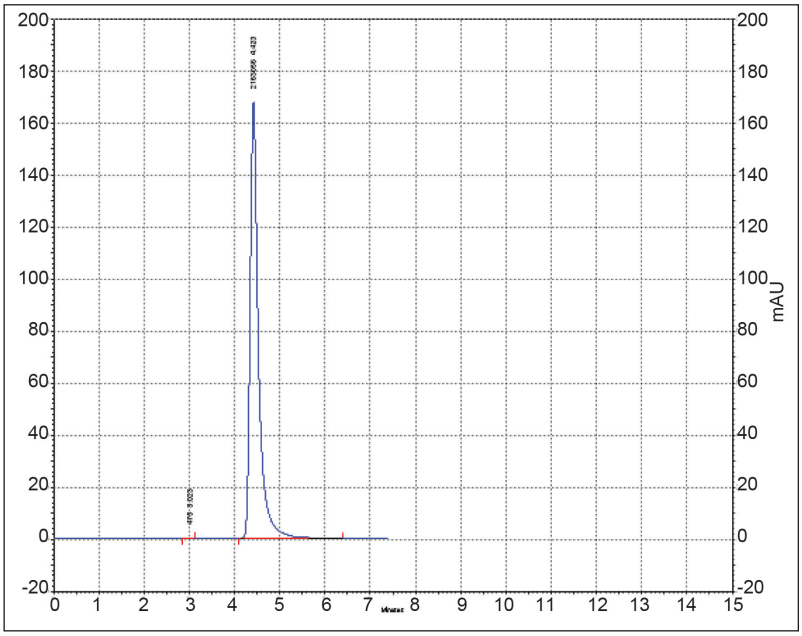

Figure 3: Chromatogram of standard ethacridine lactate

Linearity

The linearity was determined for ethacridine lactate. Solution of the drug at six different concentrations was analyzed and calibration curve was constructed by plotting the mean response factor against the respective concentration. The method was evaluated by determination of the correlation coefficient and the intercept value. Ethacridine lactate follows linearity in the concentration range of 2-12µg/mL; respectively. The result is shown in Table 1.

Precision

The precision study was evaluated on the basis of the % RSD value. The intra-day precision for ethacridine lactate was found to be in the range 0.33-0.69 % and 0.51-0.79 %, respectively. The low values of % R.S.D. indicate high precision of the method. Results of precision study are shown in Table 2.

Specificity and selectivity

Specificity of the method was ascertained by comparing the chromatogram obtained from formulation and standard drug. The retention time of the standard drug and the drug from formulation was same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the formulation. The method is quite selective. There was no other interfering peak around the retention time of ethacridine lactate; also, the base line did not show any significant noise.

Accuracy

The accuracy of the method studied at three different concentration levels, that is, 80%, 100%, and 120% showed affordable % recoveries in the range of 98.90-100.15 % for ethacridine lactate. The results are shown in Table 3. The low value of % R.S.D. indicates accuracy of the method.

Sensitivity

The LOD for ethacridine lactate was found to be 0.11 µg and the LOQ for ethacridine lactate was found to be 0.33µg. The low values of LOD and LOQ indicates adequate sensitivity of the method.

Robustness and ruggedness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The content of the drugs were not adversely affected by these changes as evident from the low values of % relative standard deviation (less than 2%) indicating robustness of the method. When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged.

Analysis of marketed formulationSix replicates of the sample solution (20µL) were injected for quantitative analysis. The amount of ethacridine lactate estimated was found to be 99.52%, respectively. A good separation and resolution of the drug indicates

Table 1: Linearity study of EL

Concentration of EL (µg/mL)	Mean peak area*	R.S.D.(%)
2	1313383 ± 8884.6	0.68
4	2153063 ± 17300.9	0.80
6	2990790.2 ± 23599.1	0.78
8	3678820.6 ± 13434.1	0.36
10	4449574.8 ± 47752.28	1.07
12	5353228.8 ± 44730.76	0.83

*Average of six determinations

Table 2: Precision studies (intra-day and inter-day)

Drug	Concentartion (µg/mL)	Intra-day Amount found* (µg/mL)		Inter-day Amount found* [µg/mL]	
		Mean ± S.D.	% R.S.D. n=3	Mean ± S.D.	% R.S.D. n=3
EL	4	3.96 ± 14814.3	0.87	10.06 ± 0.05	1.26
	6	5.99 ± 9760.8	0.33	15.07 ± 0.08	0.63
	10	9.82 ±16625.3	0.37	19.84 ± 0.06	0.94

*Average of three determinations

Table 3: Recovery studies

Drug	Initial amount (µg/mL)	Amount added (µg/mL)	Amount recovered* ± S.D. (µg/mL)	Recovery (%)	R.S.D. (%)
EL	6	4.8	10.68 ± 0.04	98.90	0.41
	6	6	12.01 ± 0.05	100.15	0.46
	6	7.2	13.09 ± 0.05	99.18	0.45

* Average of three determinations at each level

Table 4: Summary of the validation parameter and system suitability study

Parameter	Ethacridine lactate
Linearity range (µg/mL)	2-12
Correlation coefficient	0.9980
LOD (µg)	0.11
LOQ (µg)	0.33
% Recovery (*n* = 9)	98.88
Analyst I (*n* = 6)	99.78
Analyst II (*n* =6)	99.76
Precision (% R.S.D.)	
Repeatability of injection (*n* = 6)	0.29
Intra-day (*n* = 3)	0.33–0.69
Inter-day (*n* = 3)	0.51–0.79
Robustness	Robust
Specificity	Specific
Retention time (t_R)	4.443
Theoretical plates (N)	4279.4
Capacity factor (k')	0.79
Asymmetry (T)	1.31

that there was no interference from the excipients commonly present in pharmaceutical formulation.

System suitability test

According to USP, system suitability test is an integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. An earlier prepared solution for chromatographic conditions was tested for the system suitability testing. The results obtained from validation of the method and system suitability studies are summarized in Table 4.

CONCLUSIONS

The developed RP-HPLC method is simple, precise, accurate, selective, and reproducible. The method has been found to be adequately rugged and robust and can be used for the determination of ethacridine lactate in pharmaceutical formulation. The method was validated as per ICH guidelines.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and management, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur (M.S.), India, for providing the required facilities to carry out this research work.

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How to cite this article: Jain PS, Jivani HN, Khatal RN, Surana SJ. Development and validation of RP-HPLC method for estimation of ethacridine lactate in bulk and in pharmaceutical formulation. Pharm Methods 2011;2:112-6.
Source of Support: Nil, **Conflict of Interest:** None declared.