Original Article

# Spectrophotometric determination of ethacridine lactate in infusion

*Abstract*

**Aim:** A simple, rapid, selective, accurate, and precise UV spectrophotometric method has been developed for the estimation of ethacridine lactate from bulk and pharmaceutical formulation. **Materials and Methods:** Appropriate aliquot portions of stock standard solution of ethacridine lactate were transferred into five separate 10 ml volumetric flasks, and the volume was adjusted to the mark with double distilled water to obtain concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 μg/ml. The $\lambda_{max}$ of ethacridine lactate in double distill water was found to be 271 nm with an apparent molar absorptivity of $59.781 \times 10^3$ l/mol cm. The drug follows linearity in the concentration range 2–12 μg/ml with a correlation coefficient value of 0.998. **Results:** The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 99.71% was found to be in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels, i.e., 80%, 100%, and 120%. The % recovery was found to be in the range 99.26–100.25%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as intraday, interday variations and repeatability. The % RSD value less than 2 indicates that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. **Conclusion:** The results indicated that the method could be used for the routine estimation of ethacridine lactate from tablet formulations.

**Key words:** Double distilled water, ethacridine lactate, method development, spectrophotometric, validation

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## INTRODUCTION

Ethacridine lactate [Figure 1], 2-ethoxy-6,9-diaminoacridine monolactate monohydrate (British Pharmacopoeia, 2005), also known as rivanol has been 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 which 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] The literature survey revealed that one HPLC method is reported for ethacridine lactate in human plasma[3] and one SP-HPLC method is developed.[4] There are no spectrophotometric methods reported on ethacridine lactate in pharmaceutical dosage form. Therefore, the main objective of this work is to develop a simple, selective, accurate, and precise spectrophotometric 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 1 mg per 100 ml.

### Instrumentation conditions

Analysis was performed on UV-visible spectrophotometer Schimazdu 2450. Before analysis, the 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 [Figure 2].

### Preparation of stock standard solution and calibration graph

Accurately weighed 10 mg of ethacridine lactate transferred to 100 ml volumetric flasks containing 40 ml double distilled water, and volume was made up to the mark using the same solvent to obtain a concentration of 100 μg/ml. Appropriate aliquot portions of stock standard solution of ethacridine lactate were transferred into five separate 10 ml volumetric flasks, volume was adjusted to the mark with double distilled water to obtain concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 μg/ml. Absorbance of these solutions were recorded at 271 nm and the calibration curve was plotted, absorbance *vs.* concentration [Figure 3]. All measurements were repeated five times for each concentration, and the calibration curve was constructed by plotting the peak area *vs.* the drug concentration.

### Analysis of marketed formulation

For quantitation of commercial formulation, 10 ml of an accurately weighed ethacridine lactate was transferred into a 100 ml volumetric flask containing 30 ml double distilled water shaken manually for 10 min, volume was adjusted to the mark with the same solvent and filtered through Whatman filter paper no. 41.

### Method validation

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

#### *Precision*

Precision of the method is studied as repeatability, intraday and interday precision. Repeatability was determined by analyzing ethacridine lactate (6 μg/ml) for six times. Intraday precision was determined by analyzing the 6 μg/ml of ethacridine lactate for three times in the same day. Interday precision was determined by analyzing the same concentration solution daily for 3 days.

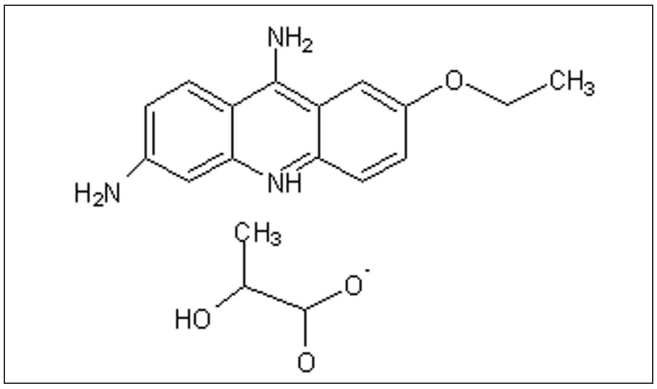


**Figure 1:** Chemical structure of ethacridine lactate

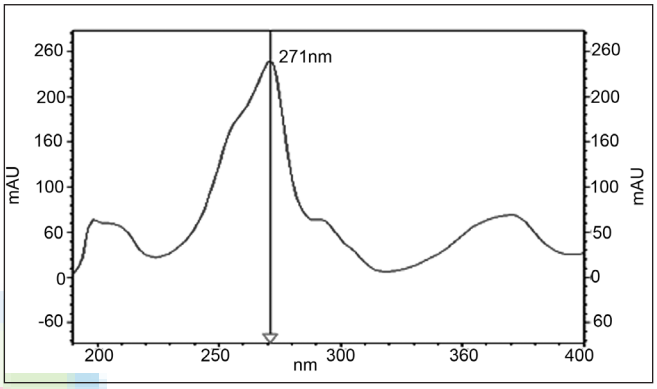


**Figure 2:** UV spectra of ethacridine lactate at 271 nm

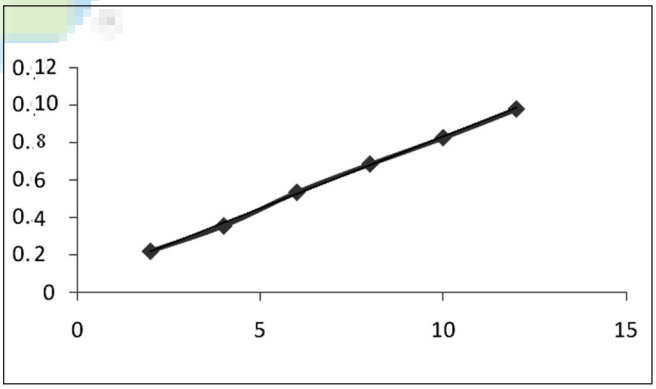


**Figure 3:** Calibration curve of EL at 271 nm

#### *Specificity and selectivity*

Specificity of the method was ascertained by analyzing the drug standard and the 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 components 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.

*Accuracy*

To assess the accuracy of the proposed method, recovery studies were carried out three different levels, i.e. 80%, 100%, and 120%. To the preanalyzed sample solution, a known amount standard drug solution was added at three different levels, and absorbance was recorded. The % recovery is shown in Table 1.

*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.

*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 same concentration solutions, six times.

Analysis of the bulk sample by the proposed method

Accurately weighed quantities of 10 mg of ethacridine lactate was transferred into a 100 ml volumetric flask containing 40 ml RO water (50% v/v), and volume was made up to the mark using the same solvent. An appropriate aliquot portions were further diluted with the solvent to get a final concentration of 6 µg/ml of ethacridin lactate, and the absorbance was measured at 271 nm against the solvent as blank.

## RESULTS AND DISCUSSION

### Linearity study

The linearity was determined for ethacridine lactate. Solution of the drug at six different concentrations was analyzed, and the calibration curve was constructed by plotting absorbance against the respective concentration. The method was evaluated by determination of the correlation coefficient and intercept value. Ethacridine lactate follows linearity in the concentration range of 2–12 µg/ml. The linear regression of absorbance on concentration gave the equation $Y = 0.016795X + 0.00768$ with a correlation coefficient of 0.998 [Table 2].

### Analysis of marketed formulation

The spectrum was recorded at 271 nm. The percentage content of ethacridine lactate was determined in infusion and was found to be 99.71 ± 0.47 with % RSD was 0.47 [Table 3].

### Precision

The precision study was evaluated on the basis of the % RSD value. Its value for intraday and interday precision for ethacridine lactate was found to be in the range of 0.77–1.12 and 0.50–1.27%, respectively. The low values of % RSD indicate high precision of the method [Table 4].

### Specificity and selectivity

Specificity of the method was ascertained by comparing the chromatogram obtained from formulation and the standard drug. The absorbance spectra of the standard drug and the drug from formulation were 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.

### Accuracy

The accuracy of the method studied at three different

**Table 1: Recovery studies**

| Drug | Initial amount (µg/ml) | Amount added (µg/ml) | Amount recovered* ± SD (µg/ml) | % Recovery | % RSD |
|---|---|---|---|---|---|
| EL | 6 | 4.8 | 3.98 ± 0.04 | 99.26 | 0.98 |
| | 6 | 6 | 5.98 ± 0.05 | 99.37 | 0.40 |
| | 6 | 7.2 | 7.22 ± 0.05 | 100.25 | 0.53 |

*Average of three determinations at each level

**Table 2: Linearity study of EL**

| Concentration of EL (µg/ml) | Absorbance ± SD | %RSD |
|---|---|---|
| 2 | 0.211± 0.003 | 1.61 |
| 4 | 0.385 ± 0.002 | 0.74 |
| 6 | 0.483 ± 0.002 | 0.53 |
| 8 | 0.652 ± 0.006 | 0.92 |
| 10 | 0.812 ± 0.007 | 0.94 |
| 12 | 0.995 ± 0.007 | 0.70 |

*Average of six determinations

**Table 3: Application of proposed method for analysis of infusion**

| Label claimed | % Label found ± SD | % RSD |
|---|---|---|
| 100 mg/100 ml | 99.71 ± 0.47 | 0.47 |

**Table 4: Precision studies (intraday and interday)**

| Drug | Concentration (µg/ml) | Intraday (*n*= 3) Abs. | % RSD | Interday (*n*= 3) Abs. | % RSD |
|---|---|---|---|---|---|
| Ethacridine | | 0.357 | 1.12 | 0.358 | 1.27 |
| Lactate | 6 | 0.508 | 0.79 | 0.509 | 0.50 |
| | | 0.663 | 0.77 | 0.668 | 0.99 |

*Average of three determinations

**Table 5: Results of ruggedness studies**

| Drug | Analyst I Amount found % ± S.D. (*n* = 3) | %RSD | Analyst II Amount found % ± SD [*n* = 3] | %RSD |
|---|---|---|---|---|
| EL | 99.76 | 0.80 | 99.30 | 0.68 |

**Table 6: Analysis of ethacridine lactate in bulk**

| Amount taken (µg/ml) | Amount found | % Amount found ± S.D. | % RSD |
|---|---|---|---|
| 6 | 5.83 | 96.76 ± 0.33 | 0.89 |

**Table 7: Summary of validation parameters**

| Parameter | Ethacridine lactate |
|---|---|
| Linearity range (µg/ml) | 2–12 |
| Correlation coefficient | 0.998 |
| LOD (µg) | 0.11 |
| LOQ (µg) | 0.43 |
| % Recovery (*n* = 9) | 99.26–100.25 |
| Analyst I (*n* = 6) | 99.76 |
| Analyst II (*n* =6) | 99.30 |
| Precision (% RSD) | |
| Precision | Precise |
| Intraday (*n* = 3) | 0.77–1.12 |
| Interday (*n* = 3) | 0.50–1.27 |
| Specificity | Specific |
| Molar absorptivity | 59.781 × $10^3$ l/mol cm |

concentration levels, i.e., 80%, 100%, and 120% showed affordable % recoveries in the range of 99.26–100.25% for ethacridine lactate. The results are summarized in Table 1. The low value of % RSD 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 0.43 µg. The low values of LOD and LOQ indicates an adequate sensitivity of the method.

### Ruggedness study

When the method was performed by two different analysts under the same experimental and environmental conditions, it was found to be rugged. 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 the ruggedness of the method [Table 5].

### Analysis of bulk drug

The amount of ethacridine lactate estimated was found to be 96.77% with less than 2% RSD [Tables 6 and 7].

## CONCLUSION

The developed spectrophotometric 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.

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