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Development and Validation of New UV Spectrophotometric Method for Determination Ethacridine Lactate in Solution during the Period of Use.

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ABSTRACT

In this paper, the content of ethacridine lactate in extemporaneous preparations was determined during the period of use. For this purpose a new rapid, accurate, repeatable, reproducible and acceptable UV spectrophotometric method has been developed. The proposed method was linear over the selected range 1-10 µg/mL with a correlation coefficient of 0.9999. The accuracy was between 99.29% - 100.23%. The LOD and LOQ were 0,0454µg/mL and 0.1512 µg/mL, respectively. The results obtained using the proposed method have shown that the content of the ethacridine lactate in the tested samples decreases with the duration of use and according to this results it is recommended to use the extemporaneous prepared ethacridine lactate solution within 30 days after preparation and opening.

Keywords: ethacridine lactate, method development, UV spectroscopy

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INTRODUCTION

Acridine derivatives are slow-acting antiseptics and they are used for the treatment of infected wounds and burns, as well as for disinfection of the skin.They can be used topically in the treatment of eye infections, throat or urogenital tract infections. Some acridine derivatives aretested as anticancer drugs because they have the ability to interclock to the DNA of DNA and topoisomerase enzyme.Ethacridine lactate (2-ethoxy-6,9-diaminoacridine monolactate monohydrate)(Figure 1) is used as dermoantiseptic as 0.05% or 0,1% solution and 0.1% ointment[1, 2].It isslow acting antiseptic, effective against many Gram positive bacteria, but weakly effective against Gram negative bacteria and bacterial spores[3].Ethacridine lactate can also used, in diarrhea treatment [4]and as a drug for second trimester termination of pregnancy.

It is considered safer and more tolerable than hypertonic (20%) NaCl solution[5]. A solution of ethacridine lactate is very often compounded in a pharmacy as extemporaneous medicine as aqueous solution without preservatives and has a limited period of use.

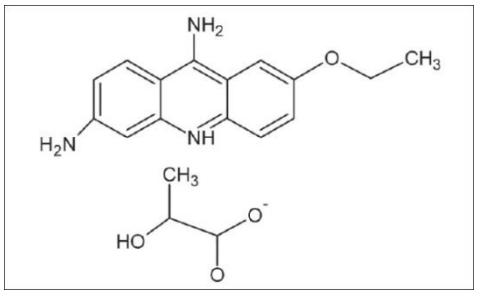

Fig 1: Chemical structure of ethacridine lactate [2]

However, patients often use ethacridine lactate solutions longer than the recommended time of use, so we considered that it necessary to examine the stability of the aqueous solution of ethacridine lactate during and after the recommended use. For analysis were taken five ex tempore prepared 0,1% solutions of ethacridine lactate.

For each of the prepared solutions, the content of ethacridine lactate was determined immediately after preparation, and one, two and three months after preparation. During the experiment, all samples were kept at room temperature in dark glass bottles.Several methods including catalytic photokinetic method[6], electroanalytical method[7,8], spectrofluorimetric [9] UV/Vis spectrophotometric analysis [5, 10, 11]and HPLC[12]have been used to determine ethacridine lactate in biological samples or pharmaceutical formulations.In the present investigation we developed and validated a new UV spectrophometric method for ethacridine lactate determination in solution.

EXPERIMENTAL

Apparatus

For absorbance measurements was used double beam UV-VIS spectrophotometer (Spectronic® Genesys™ 2) with 1.0 cm path length quartz cells.

Reagents and materials

Ethacridine lactate standard was purchased from Pharmamed, Travnik, Bosnia and Herzegovina. $NaH_2PO_4xH_2O$, Na_2HPO_4(Merck) and other chemicals were of analytical reagent grade. Distilled water was used.

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Method validation

The validation of this method was performed according to the ICH guidelines Q2A [13]**.**Following parameters were validated: selectivity, linearity, precision, accuracy, recovery, limit of detection (LOD) and limit of quantification (LOQ)**.**

Procedures

Phosphate buffer. The phosphate buffer (1M) used in this method was prepared according to the following procedure:

Solution 1: 69.0 g $NaH_2PO_4xH_2O$ was dissolved in 500 mL of distilled water (pH 7).
Solution 2: 71.0 g of Na_2HPO_4 was dissolved in 500 mL of distilled water (pH 7).
Solution 1 (211,5 mL)and Solution 2 (288,5 mL)were mixed and pH adjusted to 7.0 with 0,1 M NaOH.

***Preparation of standard stock solution*. Standard stock solution was prepared as follows:**

10 mg of ethacridine lactate was accurately weighed,transferred to a 100 mL volumetric flask and filled with distilled water to the mark. The concentration of the prepared standard stock solution was 100µg/mL.

Series of working solutions for calibration curve. The appropriate amount of the standard stock solution (0,2mL; 0,4 mL; 0,6 mL; 0,8 mL; 1 mL;) was diluted in the 10 mL volumetric flask with the phosphate buffer pH 7,0 to get working solutions concentrations 1-10 µg/mL. The solutions were mixed and left in a dark place at room temperature for 15 minutes. The absorbance of all prepared solutions was measured spectrophotometrically at 268nm with phosphate buffer as a blank.

Sample preparation

For the analysis was taken 0,4 mL of sample, transferred to a 100 mL volumetric flask and filled with phosphate buffer pH 7,0 to the mark.

The solution was mixed and left in a dark place at room temperature for 15 minutes. The absorbance was mesured at 268 nm with phosphate bufferas a blank.

RESULTS AND DISCUSSION

The first step in the development of the analytical method for the identification and quantification of ethacridine lactate was to determine the apsorption maximum. The spectrum was recorded in the wavelength range of 200-400 nm (Figure 1).Based on the spectrum, λ_{max} of 268 nm was selected for further analysis.

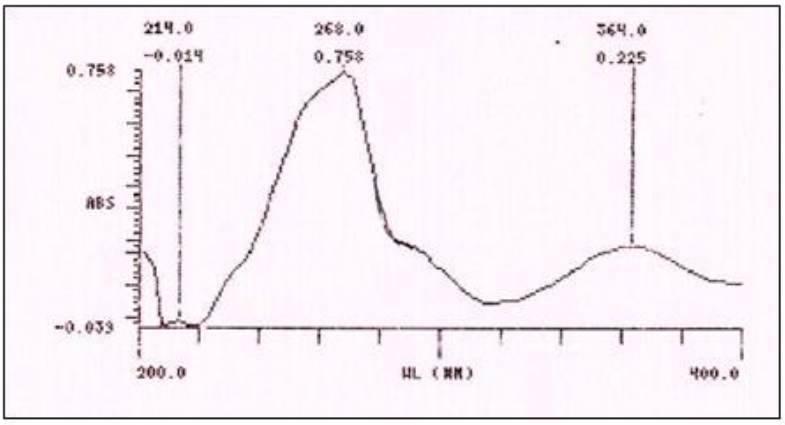

Fig 2: The absorption spectrum of etacridine lactate in phosphate buffer pH 7.0

Our preliminary investigations have shown that ethacridine lactate in aqueous solution (pH 6-7) is very unstable and it is difficult to measure the absorbance with satisfactory reproducibility.Ethacridine lactate dissolved in water has a pH about 6 and maximum absorption at 272 nm.

However, by repeated absorbance measurement in the repeatability test, we have found that the absorbance of the same concentration, drops after each repated measurement, and it is not possible to obtain repeatable absorbance under these conditions. We recorded the absorbance in time of one hour and noticed that the absorbance linear decrease in time. The absorbance measured in acidic or basic solution (Fig.3) increased in time and the repeatability also, was not satisfactory.

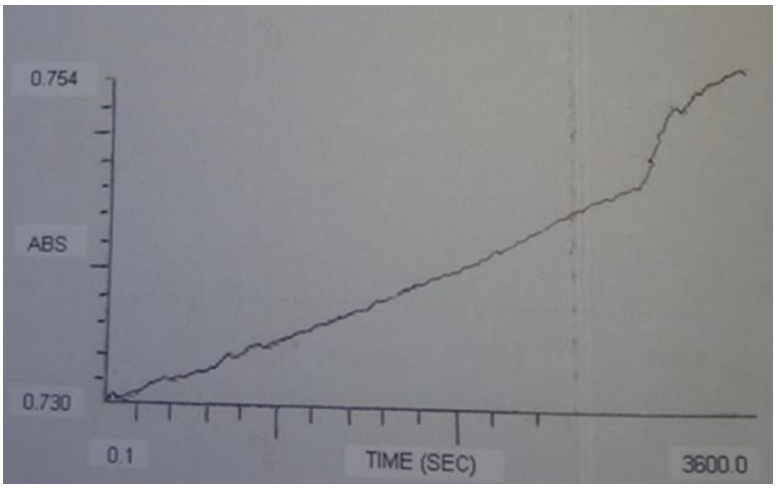

Fig 3: The absorbance of acidic ethacridine lactate solution measured in time

An explanation could be found in the structure of ethacridine lactate because ethacridine lactate can undergo keto-enol tautomerization. It has one keto group (that of the acid group) adjacent to one C bound to one -OH (-ol). This kind of compounds are extremely sensitive to pH. Although usually one -enol is one isomer of aldehydes or ketones. In this case we have one group -OH, and in the adjacent C one keto group and another -OH. Anyway, the stability of the -C=O is greater than that of the -C=C-. The enolization can be accomplished at acid or basic pH.

For avoiding the enolization we adjust the pH of solution to 7, to tray to slow down enolization. Therefore all measurements were made in phosphate buffer pH 7,0.

Selectivity, linearity, detection limit and quantification limit

Selectivity:

No interfering absorption peaks were observed at the maximum of the ethacridine lactate in phosphate buffer.

Linearity:

The linearity test was performed at six concentration levels of ethacridine lactate 1, 2, 4, 6, 8 and 10 µg/mL. All measurements were repeated six times against phosphate buffer pH 7,0 as blank. Linear calibration curve was plotted between the absorbance and the corresponding concentration of ethacridine lactate working solutions.

Correlation coeficient was calculated using regression equation. The linearity was in the range of 1-10 µg/mL with correlation coefficient 0,999 (Figure 3.).

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The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the equations LOD = 3SD/b and LOQ = 10SD/B, where „SD" is the standard deviation of the blank and „b" is the slope of the calibration curve.

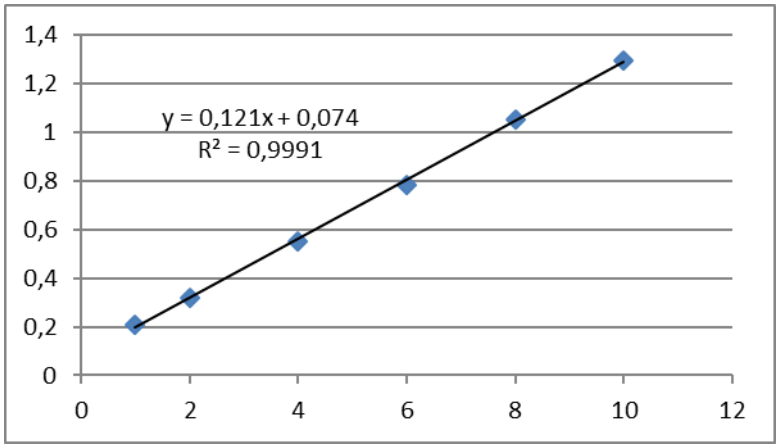

Fig 4: Calibration curve for ethacridin lactate

Accuracy:

The accuracy of the proposed methods was checked by recovery studies. Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration by addition of standard drug and taken absorbance of each solution in triplicate. The recovery results showed that the proposed method has an acceptable level of accuracy for ethacridine lactate which for 80% - 120% of test concentration was 99.29% - 100.23%.

Precision:

Precision was determined by studying the repeatability and intermediate precision. Repeatability was determined using three concentrations (2, 4, 6 µg/mL) and analyzed (n=6) without changing the parameter of the proposed method. Precision of the method was demonstrated by intra-day and inter-day variation studies, for the three concentrations, three times during the day and in several days. The percentage relative standard deviation (RSD) values were <0,20 % (intra-day) and <2% (inter-day), indicating the high precision of the methods. All validation parameters are given in Table 1.

Table 1: Summary of the validation parameters

Linearity	Concentration 1-10 µg/mL Correlation coefficient 0,999 Standard deviation 0 - 0,022 RSD (%) 0 – 0,71
Accuracy	Concentration 3,8 – 4,2 µg/mL; (80-120 %) Recovery99.29% -100.23%. Standard deviation 0,001 – 0,002 RSD (%) 0,18 – 0,29
LOD	0,0454(µg/mL)
LOQ	0,1512(µg/mL)
Precision (n= 6)	Inter day 0,16 % – 0,18 % Intra Day 1,75 % – 1,99 %
Repetability	Concentration 2, 4, 6 µg/mL Standard deviation0,000231- 0,00153 RSD (%) 0,04 – 0,18

Sample analysis

The proposed method was applied for determination of the ethacridine lactate content in the tested samples. For the analysis were used four *ex tempore* prepared solutions, and the same solution after one, two and three months. The results of analysis are presented in Table 2.

It can be seen that the content of ethacridine lactate is in correlation with our preliminar microbiological investigation which confirmed the efficacy of *ex tempore* prepared sample and reduced but acceptable sample efficacy 30 days after preparation [14].

Table 2: Ethacridine lactate content in analysed samples

	ex tempore prepared			30 days after preparation			60 days after preparation			90days after preparation		
Sample	*X	RSD (%)	content (%)	*X	RSD (%)	content (%)	*X	RSD (%)	content (%)	*X	RSD (%)	content (%)
1	0,609	0,00	107,8	0,529	0,10	94,12	0,525	0,10	93,41	0,523	0,08	93,06
2	0,611	0,10	108,7	0,518	0,10	92,17	0,510	0,35	90,70	0,447	0,12	79,54
3	0,607	0,07%	108,0	0,597	0,00	106,22	0,590	0,00	104,98	0,524	0,10	93,23
4	0,608	0,00%	108,1	0,537	0,00	96,55	0,530	0,23	94,31	0,469	0,09	82,21

*X – absorbance average (n=6)

CONCLUSION

The developed and validated analytical method is simple, sensitive, rapid and specific and it can be used for the routine analysis and the quality control of ethacridine lactate solution during the period of use. The method has good linearity with correlation coefficient 0,999 , and it was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.29-100.23%) were obtained for each tested concentrations, indicating that the method was accurate. The LOD and LOQ were found 0,0454 µg/mL and 0,1512 µg/mL indicating the sensitivity of the method. The content of the ethacridine lactate in the tested samples decreases with the duration of use and according to this results it is recommended to use the extemporaneous prepared ethacridine lactate solution within 30 days after preparation and opening.

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