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Original Article

A validated analytical method for the estimation of Oxetacaine from its pharmaceutical formulation by RP-HPLC

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OBJECTIVE: The aim of the current study was to develop an analytical procedure for the determination and quantification of Oxetacaine, from its marketed formulation which is simple, precise, accurate and a validated, reverse phase high performance liquid chromatography (RP-HPLC) method.
METHOD: The optimized chromatographic condition was achieved on a kromasil C_{18} (150 X 4.6 mm i.d., 5µ) as stationary phase and Acetonitrile**:** 10mM Potassium dihydrogen-ortho-phosphate (pH 3.5) in the ratio 30:70 % v/v as mobile phase, with a flow rate of 1.0 ml/min. The detector response for the method was determined, and the quantification was carried out at 214 nm.
RESULT: Oxetacaine was eluted at 6.0 min. The quantification was performed using calibration curve method, and the linearity was achieved from 10-50 µg/ml. The percentage recovery was found to be 98.0 ± 1.20 respectively. The correlation coefficient was found to be 0.9954 respectively. The limit of detection (LOD) and limit of quantification (LOQ) for the current method was achieved at 1µg/mL and 5µg/mL respectively.
CONCLUSION: The current study was performed at ambient temperature, and the method is simple, selective, linear, precise, accurate and sensitive which can be used for the routine analysis of Oxetacaine tablets. The developed method was validated as per ICH guidelines.

ABSTRACT:

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INTRODUCTION

Most manufacturing industries rely upon quantitative chemical analysis to ensure that the raw materials used and the final products obtained meet certain specifications in the final product. Chromatography is the method by which a mixture is separated into its individual components as a result of the relative ability of each component to be flushed along or through a stationary phase and mobile phase [1-7].

Alumina gel and magnesium hydroxide react chemically to neutralize or buffer existing quantities of acid. It has no direct effect on the production of gastric acid. Gastroscopic observations have shown that alumina gel, if taken undiluted, forms a coating over the inflamed mucosa for a variable period. Oxetacaine is a topical potent anesthetic. Combining Oxetacaine with alumina gel exerts a prolonged topical anesthetic action when applied to mucous membranes

Oxetacaine chemically known as (2,2'-(2-hydroxyethylimino)bis[*N*-(1,1-dimethyl-2-phenylethyl)-*N*-methylacetamide]) is a potent safe topical anesthetic drug substance having a molecular formula of $C_{28}H_{41}N_3O_3$ and a molecular weight 467.64 g/mol(fig.1).

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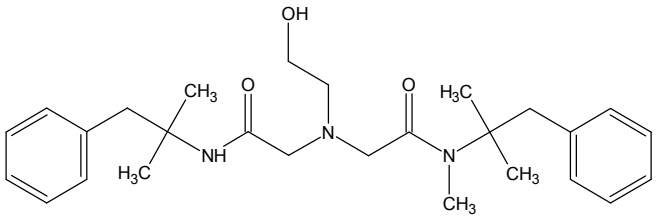

Fig. 1: Chemical structure of Oxetacaine

Extensive literature survey has revealed that several methods are reported for the combination with other drugs [8-9].However, there is no method reported for the individual estimation. Overall the proposed method was to develop specific, precise, accurate, optimized and validated RP-HPLC method which can be used for the quantification of Oxetacaine.

MATERIALS AND METHODS

Chemicals and Reagents

Working Standard of Oxetacaine was obtained from the manufacturers as gift sample. Acetonitrile of HPLC grade, Ortho-Phosphoric acid AR grade and potassium dihydrogen ortho-phosphate AR grade were supplied by Rankem Chemicals. Water HPLC grade was obtained from Milli-Q RO system (Millipore, Bedford, USA) was used.

Equipment &Chromatographic condition

High Performance Liquid Chromatography **(**Shimadzu gradient HPLC system**)** equipped with a solvent delivery system **(**Model-LC-10 AT-VP**)**, Rheodyne injector **(**Model-7725i with 20 l loop**)**, UV detector **(**Model-SPD M-10A VP).

The data were recorded using Class VP data station software. Kromasil C_{18} **(**150 x 4.6 mm i.d.,5µ**)** was used for method development and validation at ambient temperature **(**25°C**).** In this current method, the separation was achieved using 1ml/minflow rate, detection at 214nm with an injection volume of 20µl.

Preparation of mobile phase:

0.136 gm. of potassium di hydrogen ortho-phosphate buffer was taken and dissolved diluted to 1000 ml with Millipore water. Further, the pH of the buffer solution was adjusted to pH 3.5 with ortho-phosphoric acid.

Preparation of Standard solution

0.01 gm. of bulk drug Oxetacaine was diluted to 10 ml with Acetonitrile. The further working concentrations of the standard drug was carried out using diluent Acetonitrile: water (50:50, v/v).

Standard solution for CC

10ml each of 10.0, 20.0, 30.0, 40.0, and 50.0 µg/ml of Oxetacaine standard solutions were prepared from the Oxetacaine standard solution using diluent Acetonitrile: water (50:50, v/v).

Preparation of sample solution

The weight equivalent to 5ml of Oxetacaine suspension was weighed and transferred to a 100ml volumetric flask, dissolved the content with little Acetonitrile and make up the volume with diluent to obtain a concentration of 100 µg/ml of Oxetacaine. The above solution was further diluted with the diluent (50: 50, v/v) to produce the concentration of 10, 30 and 50 µg/ml (LQC, MQC and HQC)**.**

Selection of wavelength

An UV spectrum of 10µg/ml Oxetacaine in acetonitrile was recorded by scanning the solution in the range of 200 nm to 400 nm from the UV spectrum wavelength of 214 nm was selected (fig.2). At this wavelength Oxetacaine showed maximum absorbance.

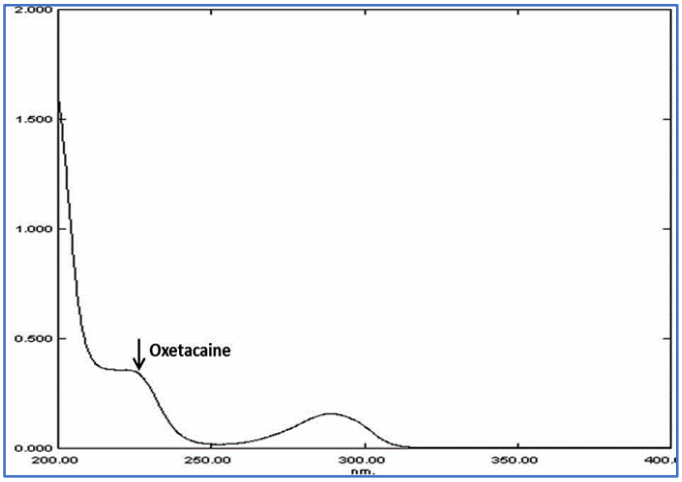

Fig. 2: UV spectrum of Oxetacaine

Method validation

The method was validated for System suitability, selectivity, linearity, accuracy, precision, recovery, stability, detection limit, and quantification limit according to the principles of the ICH guidelines [10-12].

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of other drugs, excipients and their potential impurities.

Linearity

Linearity was checked on a concentration range between 10-50 µg/ml by average of six determinations of the nominal standard concentration. The linearity of the proposed method was evaluated by using calibration curve to calculate the coefficient correlation, slope and intercept values.

Accuracy

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The accuracy of the method was determined by recovery studies by standard addition method according to ICH guidelines

Precision

The precision of the method was evaluated by inter-day and intra-day precision studies samples of three concentration levels at six replicates were prepared as low (LQC), medium (MQC) and high (MQC) quality controls, corresponding to 10, 30 and 50 ng/ml, respectively. Percent relative standard deviation (%RSD) of the regressed concentration was used to report precision.

Limit of detection and limit of quantification

Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by injecting low concentration of the standard solution using developed HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio 3).

The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio 10). LOD and LOQ value can be measure by the following formula:

$$\mathbf{LOD = 3.3\sigma/S} \text{ and } \mathbf{LOQ = 10\sigma/S};$$

Where σ = Standard deviation of the response; S = Slope of the deviation curve.

Robustness and Ruggedness

The ruggedness and robustnessof the methods were studied by changing the experimental conditions (operators, source of reagents and column of similar type) and optimized conditions (pH, mobile phase ratio and flow rate).

System suitability

System suitability test is an integral part of method development and was used to ensure adequate performance of the chromatographic System. Retention time **(**RT**)**, number of theoretical plates **(**N**)** and Tailing factor **(**T**)** were evaluated for three replicates injections of the sample solution. To determine precision, Oxetacaine standard solution was prepared and injected for six times into HPLC system. The SD and % RSD for peak areas of Oxetacaine was calculated.

Linearity

Linearity was established by least square linear regression analysis of the calibration curve. The constructed calibration curve was linear over the concentration range of 10-50 µg/ml, peak area was plotted against the respective concentration in the mobile phase, and linear regression analysis performed on the resultant curves was confirmed by the value of correlation coefficient 0.9954 respectively (Fig. 4).

Precision

Precision of the method was calculated by the intra-day and inter-day precision studies at three different concentrations (LQC, MQC and HQC) and they were found to be within the limits (Table 1).

Accuracy

The accuracy of the method was determined by recovery studies **(Table 2)** by standard addition method according to ICH guidelines. The pre-analyzed samples were spiked with standard drug Oxetacaine. The mixtures were analyzed by the proposed method and found to be within the limit.

RESULTS AND DISCUSSION

Specificity

The specificity test demonstrates that the used excipients did not interfere with the peak of the main compound. No peaks were eluted along with the retention time of Oxetacaine (Fig.3). Hence, the results showed that the developed method was selective for determination of Oxetacaine in the formulation.

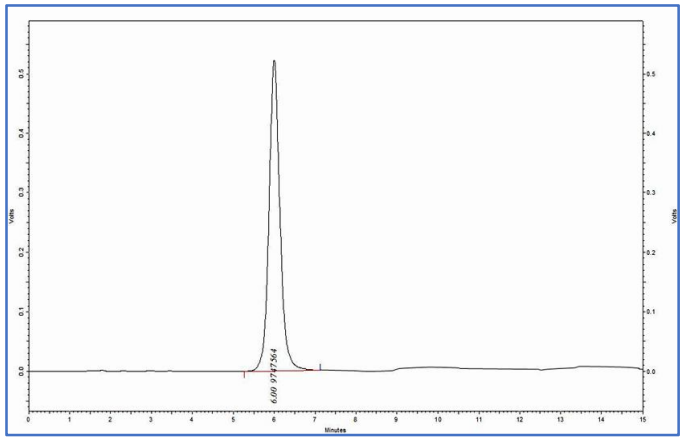

Fig. 3: Typical chromatogram of standard

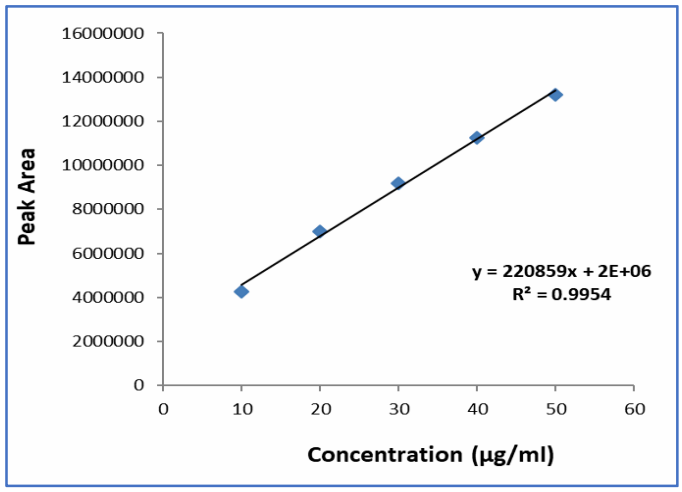

Fig. 4: Linearity of Oxetacaine

Limit of detection and quantification

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The limits of detection (LOD) and limit of quantification (LOQ) represents the sensitivity of the proposed method. The LOD and LOQ value obtained was 1 g/ml and 5 g/ml respectively (table 3). It indicates the high sensitivity of the proposed method.

System suitability

The results of system suitability were within acceptable limits (Table 3).A system suitability test can be defined as a test to ensure that the method can generate results of acceptable, accuracy and precision. The requirement for system suitability usually designed after method development. And, the method has been validated as per ICH guidelines.

Table 1: Precision Studies

S.No	Concentration Added (µg/ml)	Intra-day(µg/ml) ±SD (n=6)	Inter-day(µg/ml) ±SD (n=6)
1	10	9.87 ± 0.14	9.79 ± 0.04
2	30	29.6 ± 0.28	29.0 ± 0.18
3	50	49.8 ± 0.37	49.2 ± 0.17

Table 2: (a) Recovery Studies

S.No	Concentration Added (µg/ml)	Concentration Recovered (µg/ml) ±SD (n=6)	Percentage Recovery
1	10	9.85 ± 0.04	98.5
2	30	29.5 ± 0.18	98.34
3	50	49.3 ± 0.27	98.6

Table 2: (b) Recovery Studies in Formulation

Drug	Labeled amount	Amount taken for assay (µg/ml)	Amount obtained(µg/ml) ±SD (n=6)	Percentage Recovery
Oxetacaine	**10mg**	10	0.8 ± 0.01	96.5 ± 2.20
		30	29.1 ± 0.13	99.0 ± 0.10
		50	49.0 ± 0.23	99.6 ± 0.09

Table 3: System suitability studies

S.No.	Parameters	Oxetacaine
1	Retention Time (min)	6.083
2	Theoretical Plates (N)	20785
3	Tailing Factor	1.8
2	Linearity Range (µg/ml)	10-50
3	Slope	220859
4	Correlation Coefficient	0.9954
5	LOD (µg/ml)	1
6	LOQ (µg/ml)	5

Robustness and Ruggedness

No significant changes in the chromatographic parameters were observed when the experimental conditions were changed, proving that the developed method was found to be robust.

CONCLUSION

A rapid,simple, sensitive, precise, accurate reverse phase high-performance liquid chromatography method has been developed and validated with high recovery. Sample preparation and extraction procedure requires less time and the run time was less than 10min. The developed method can be successfully applied in drug testing laboratory and in quality control departments.

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