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# Development and validation of an analytical method for the simultaneous quantification of dexamethasone and ketorolac tromethamine in ophthalmic solution using reverse-phase high-performance liquid chromatography

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## ABSTRACT

A reverse-phase high-performance liquid chromatography method was developed to simultaneously determine dexamethasone and Ketorolac Tromethamine in Pharmaceutical marketed preparation. The kromasil C18 (250 mm x 4.6mm) column achieved the reverse phase high-performance liquid chromatographic separation. Methanol and 0.1% orthophosphoric acid in the ratio of 65:35 v/v, were selected as mobile phases with a flow rate of 1mL $min^{-1}$. The detection wavelength was 283 nm. Dexamethasone and ketorolac tromethamine had retention times of 4.87 and 5.96, respectively. According to the international conference on harmonization's guidelines for linearity, accuracy, precision, detection limit, quantification limit, and robustness, the method was validated. Linearity was seen for dexamethasone and ketorolac tromethamine at 2-30 µg mL-1 and 10-150 µg $mL^{-1}$, respectively. The established approach was proven to be accurate, precise, and sensitive for the simultaneous quantification of dexamethasone and ketorolac tromethamine in pharmaceutically marketed preparations.

**KEY WORDS:** Dexamethasone; Ketorolac tromethamine; Orthophosphoric acid; LOD; LOQ

## INTRODUCTION

Chemically, ketorolac tromethamine (KTM), also known as 2-amino-2-(hydroxymethyl) propane 1,3-diol (1RS)-5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylate, is a non-steroidal anti-inflammatory medication[1]. It contains anti-inflammatory, antipyretic, and analgesic properties and inhibits the cyclooxygenase (COX) enzyme, which prevents the formation of prostaglandins[2]. Ketorolac tromethamine is available in single and mixed dosage forms in a variety of method development and validation methods, such as the HPLC method development in the formulation of the drug's eye drops[3],tablet dosage form[2], and more simultaneous technique development and validation by RP-HPLC, such as a mixture of ketorolac tromethamine with phenylephrine hydrochloride and febuxostat[4], ketorolac tromethamine with moxifloxacin hydrochloride[5],ketorolac tromethamine and tramadol hydrochloride[6].

Dexamethasone (DMS) is a steroid drug with anti-inflammatory and immune suppressor effects used in treating different pathologies. A literature survey revealed RP-HPLC method development of dexamethasone[7] in the single formulation and also available in combination dosage form with granisetron[8], vildagliptin, metformin hydrochloride, and ciprofloxacin

hydrochloride[9], but no HPLC method development and validation are available in a combination of ketorolac tromethamine with Dexamethasone. The goal of this work was to create a sensitive, accurate, and exact method for measuring dexamethasone and ketorolac tromethamine in combination form by reverse phase high-performance liquid chromatography.

## MATERIAL AND METHODS

### Materials

Dexamethasone (SGS Pharmaceutical Pvt. Ltd., Uttarakhand, India) and ketorolac tromethamine (Innova Captab Ltd, Baddi, India) were procured as a gift sample and a marketed formulation of KDX eye drop was purchased from a local pharmacy store.

Methanol (HPLC grade), and orthophosphoric acid were procured from Qualigens (Thermo fisher scientific) and HPLC grade water was purchased from Moreshwar Enterprises, India.

### Instruments

Analytical balance (Aczet CY224), HPLC (Agilent 1260 Infinity II autosampler with open lab Ezchrome workstation software), ultrasonicator (Biotechnics India 12L300H), pH meter (Labman-LMPH-10)

### Methods

*Preparation of standard stock solution*

With 20 mg of ketorolac tromethamine, a stock solution of the drug was prepared and transferred into a 20 mL volumetric flask. After adding 15 mL of distilled water and thoroughly dissolving it using a sonicator, the volume was finally adjusted. (1000 µg mL-1). A 20 mL volumetric flask was used to make a stock solution of dexamethasone by mixing 20 mg of dexamethasone with 15 mL of distilled water. It was then sonicated for proper dissolution before being diluted with distilled water (1000 $\mu g\ mL^{-1}$).

*Preparation of standard solution*

Precise Dexamethasone stock solution (1 mL) and ketorolac tromethamine stock solution (5 mL) were pipetted into a 50 mL volumetric flask, and the volume was adjusted with diluent (20$\mu g\ mL^{-1}$ of dexamethasone and 100 $\mu g\ mL^{-1}$ of ketorolac tromethamine).

*Preparation of sample solution*

Weighed accurately 1010 mg of sample (equivalent to 1mg of dexamethasone and 5 mg of ketorolac tromethamine). Transfer it to a clean and dry 20 mL of a volumetric flask, and add 15 mL of distilled water to sonicate it for 15 minutes with intermittent shaking every 5 minutes. volume was properly adjusted up to the mark with distilled water. The further solution was filtered through a suitable 0.45 µm syringe filter and 4 mL of the filtrate was diluted to make 10 mL with diluent (20$\mu g\ mL^{-1}$ of dexamethasone and 100 $\mu g\ mL^{-1}$ of ketorolac tromethamine).

*Detection Method*

The complete analysis was carried out under isocratic conditions at a column oven temperature $40^{0C}$ through the column, Kromasil, C18, 250mm x 4.6mm, 5µm. The injection volume was 20 µL for all samples and standard solutions and the flow rate of the mobile phase was run at $1mLmin^{-1}$ for 9 min. The analysis was carried out at 283nm with UV detection (Figure.1).

**Method Development**

Different tests were conducted by altering the mobile phase, its ratio, and wavelength to optimize suitable chromatographic conditions.

**Method Validation**[10]

*System suitability*

System suitability tests were conducted to establish a sensitive precise and accurate method.

*Specificity*

The capacity of the analytical method to distinguish between the analyte(s) and the other elements in the sample matrix is known as specificity.

*Linearity*

Linearity was performed at 5 levels by injecting the working solution range from 10% to 150% of the solution in triplicates.

*Precision*

The precision of the assay procedure was analyzed by injecting six samples in triplicates of the same batch prepared six times and analyzed.

*Accuracy*

To assess the accuracy of the proposed method, recovery studies using the usual addition technique were conducted. To do this, dexamethasone and ketorolac tromethamine in known concentrations were added to a pre-quantified sample solution. Then, the experimental and real values were compared.

*Robustness*

To evaluate the method's robustness, deliberate changes were made to the experimental setup of the suggested approach. Minor modifications to flow rate, wavelength, and temperature were performed for this purpose. It was then determined how these modifications affected chromatographic metrics such as retention duration, tailing factor, and the number of theoretical plates.

*Limit of detection (LOD) and Limit of quantification (LOQ)*

The following formula was used to calculate LOD and LOQ values to use a residual linearity plot to check the detection and quantification limits.

$LOD = 3.3 * \sigma/S$

$LOQ = 10 * \sigma/S$

Where ‘σ' stands for standard deviation and 'S' for the slope of the calibration curve.

## RESULT AND DISCUSSION

### System Suitability

The standard solution was injected five times and the percentage relative standard deviation, asymmetry, and theoretical plate number were calculated. (Table-1) The % RSD of the area under the curve was found less than 2 and average asymmetry was shown > 2, and the number of theoretical plates was calculated < 2000.

### Specificity

The placebo solution without containing dexamethasone and ketorolac tromethamine, blank, and sample was injected and evaluated specificity. The blank and placebo not shown noisiness at the retention time of both drugs, and peak purity was found within the acceptable range for standard and test samples (Figure.2).

### Linearity

The linearity graph was plotted by concentration and the mean area of each triplicate by calculating 5 Levels of Concentration created from 10% to 150% of working concentration,

(Figure.3) and calculated the intercept, slope, and regression coefficient (Table-2).

### Limit of detection and limit of quantification

The residual values between a set of observed and expected values were used to calculate the standard deviation, and the calibration curve's slope and residual analysis were used to estimate the LOD and LOQ. (Table-2).

### Precision

Intra-day and Inter-day experiments were conducted to ensure precision. For both intraday and between-day trials, the precision experiments involved injecting a prepared test solution at a particular concentration at six times, and the percentage relative standard deviation was calculated. The method's precision was demonstrated by the low RSD (%) value, which was within the acceptable range of ±2% (Table-3).

### Accuracy

The conventional addition approach was used for accuracy studies. As a percentage of the standard spiked to the previously examined test sample, accuracy is expressed. A sample that had already been examined was spiked with the active components at various concentrations, including 50%, 100%, and 150% of each claim made on the label, and this was done in triplicate under established chromatographic conditions (Table-4).

### Robustness

Small changes to flow rate, wavelength, and temperature were performed respectively ±10%, ±3nm, and $\pm 2^{0C}$ for the determination of the robustness of the proposed method (Table-5).

## CONCLUSION

The developed RP-HPLC method for simultaneous determination of dexamethasone and ketorolac tromethamine is accurate, precise, and sensitive. Hence this method can be used for routine analysis of DMS and KTM in the marketed dosage form.

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## Conflict of interest

The authors did not have any conflict of interest.

## REFERENCES

1. Khairnar, D.A., Chaudhari, C.S. and Anantwar, S.P., Method development and validation of ketorolac tromethamine in tablet formulation by RP-HPLC method. *International Journal of Pharmaceutical Sciences and Research*, 2014; *5*(9), p.3696.
2. Sandoval HP, Fernández de Castro LE, Vroman DT, Solomon KD. A review of the use of ketorolac tromethamine 0.4% in the treatment of post-surgical inflammation following cataract and refractive surgery. Clinical Ophthalmology. 2007;1;1(4):367-71.
3. Sunil G, Jambulingam M, Thangadurai SA, Kamalakannan D, Sundaraganapathy R, Jothimanivannan C. Development and validation of Ketorolac Tromethamine in eye drop formulation by RP-HPLC method. Arabian Journal of Chemistry. 2017; 1;10:S928-35.
4. El Yazbi FA, Hassan EM, Khamis EF, Ragab MA, Hamdy MM. Development and validation of a high-performance thin-layer chromatographic method for the simultaneous determination of two binary mixtures containing ketorolac tromethamine with phenylephrine hydrochloride and with febuxostat. Journal of chromatographic science. 2016;1;54(5):819-28.
5. Raghavi, R., Saravanan, D. and Maheswaran, A., Analytical method development and validation for simultaneous estimation of moxifloxacin hydrochloride and ketorolac tromethamine by using RP-HPLC. *International Journal of Research in Pharmaceutical sciences and Technology*,2020; *2*(1), pp.25-31.
6. Srinivas K. A new RP-HPLC method development and validation for the simultaneous estimation of ketorolac tromethamine and tramadol hydrochloride in pharmaceutical dosage forms. Asian Journal of Pharmaceutical and Clinical Research. 2017;1:186-90.
7. Urban, M.C.C., Mainardes, R.M. and Gremião, M.P.D., Development and validation of HPLC method for analysis of dexamethasone acetate in microemulsions. *Brazilian journal of pharmaceutical sciences*, 2009; *45*, pp.87-92.
8. Heda AA, Kathiriya JM, Gadade DD, Puranik PK., Development and validation of RP-HPLC method for simultaneous determination of granisetron and dexamethasone. *Indian Journal of Pharmaceutical Sciences.* 2011;73(6), pp.696.

9. Fouad MM. RP-UPLC method development and validation for simultaneous estimation of vildagliptin with metformin hydrochloride and ciprofloxacin hydrochloride with dexamethasone sodium phosphate. *World Journal of Pharmaceutical Sciences*. 2015; pp.1755-62.
10. Srivastava S, Dhaneshwar S, Kawathekar N., Development of validated stability-indicating HPTLC method for the estimation of ulipristal acetate in bulk and dosage form. Journal of Applied Pharmaceutical Science. 2021;11(11),pp.161-7.

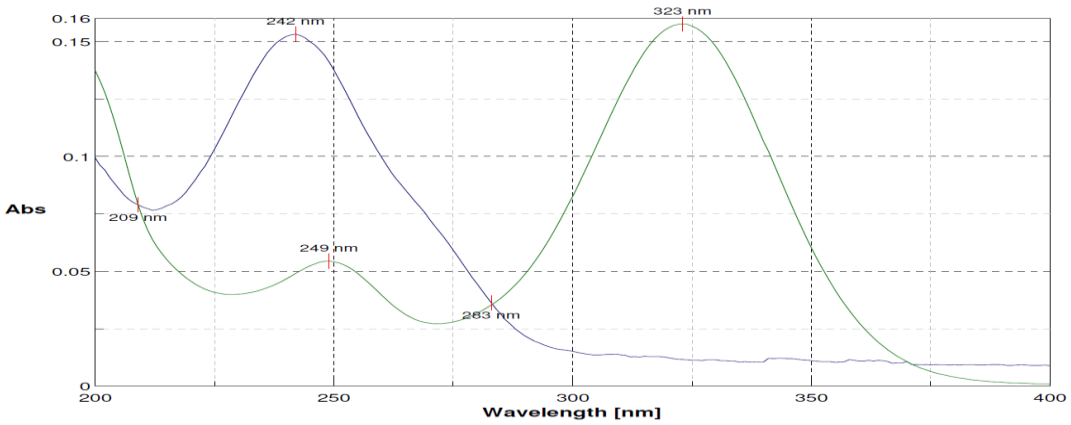


**Figure.1** Selection of wavelength for the proposed study

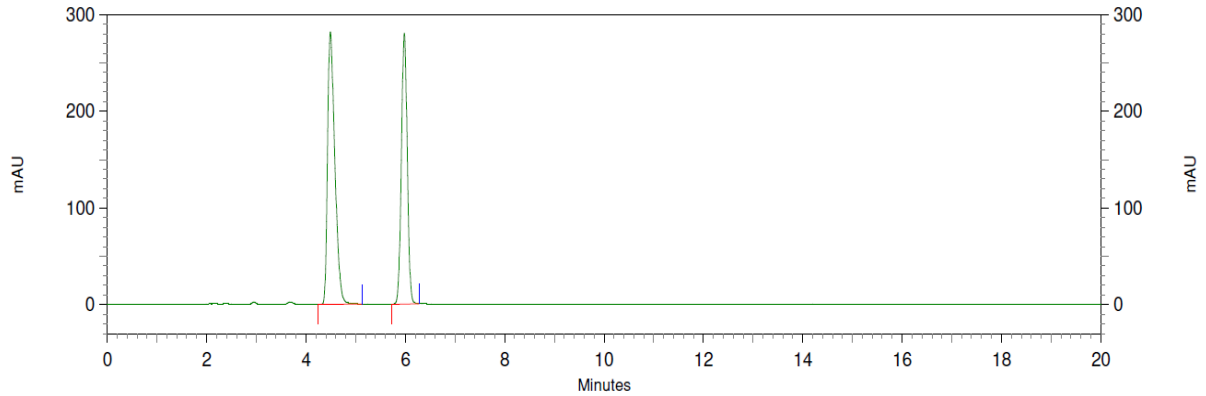


**Figure.2** Determination of retention time of DMS and KTM

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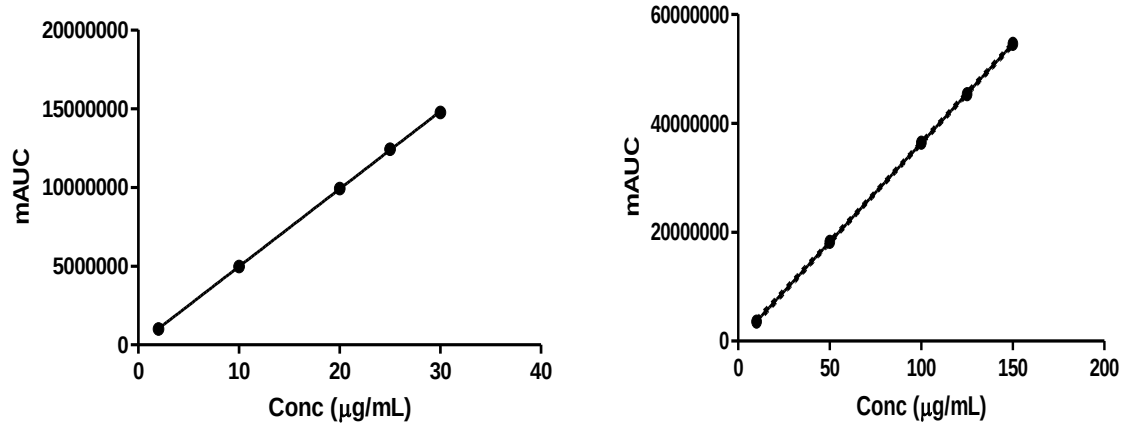


**Figure.3** Calibration curve of dexamethasone(a) and ketorolac tromethamine(b).

Table-1 Result of system suitability

| **Parameters** | **DMS** | **KTM** |
|---|---|---|
| % RSD (n=5) | 0.21 | 0.41 |
| Theoretical plates | 7042 | 11838 |
| Asymmetry | 1.21 | 1.05 |

Table-2 Linearity, LOD, and LOQ

| **Parameters** | **DMS** | **KTM** |
|---|---|---|
| Linearity range (µg/mL) | 2-30 | 10-150 |
| Best fit values | | |
| y-intercept | 41100 | -6158 |
| Slope | 492900 | 363587 |
| Goodness of fit | | |
| Correlation coefficient($r^2$) | 0.9999 | 0.9999 |
| Sensitivity | | |
| LOD(µg/mL) | 0.34 | 0.82 |
| LOQ(µg/mL) | 1.03 | 2.49 |
| Specificity | Specific | Specific |

Table-3 Results of precision

| **Drugs** | **Parameters** | **Intraday precision** | **Inter-day precision** |
|---|---|---|---|
| DMS | %RSD | 0.158 | 0.391 |
| | Mean % assay | 99.89 | 99.71 |
| KTM | %RSD | 99.72 | 99.88 |
| | Mean % assay | 0.364 | 0.223 |

Table-4 Accuracy of the proposed method

| **Drug** | **Level** | **Spiked concentration (µg/mL)** | **Recovered concentration (µg/mL)** | **% Recovery** | **Mean Recovery** | **%RSD** |
|---|---|---|---|---|---|---|
| | 50% | 10.01 | 10.02 | 99.94 | | |
| | | 9.99 | 10.02 | 99.70 | 99.86 | 0.139 |
| | | 10.01 | 10.02 | 99.94 | | |
| DMS | 100% | 20.26 | 20.04 | 101.11 | | |
| | | 20.20 | 20.04 | 100.78 | 100.95 | 0.163 |
| | | 20.23 | 20.04 | 100.95 | | |
| | 150% | 29.97 | 30.06 | 99.69 | | |
| | | 30.24 | 30.06 | 100.61 | 99.79 | 0.772 |
| | | 29.78 | 30.06 | 99.08 | | |
| | 50% | 50.46 | 50.01 | 100.89 | 100.41 | 0.752 |
| | | 49.78 | 50.01 | 99.54 | | |
| | | 50.41 | 50.01 | 100.80 | | |
| KTM | 100% | 99.71 | 100.02 | 99.69 | 100.08 | 0.347 |
| | | 100.37 | 100.02 | 100.35 | | |
| | | 100.23 | 100.02 | 100.21 | | |
| | 150% | 149.84 | 150.03 | 99.87 | 99.93 | 0.159 |
| | | 149.74 | 150.03 | 99.81 | | |
| | | 150.20 | 150.03 | 100.11 | | |

Table-5 Result of Robustness

| **Parameters** | **DMS** | | **KTM** | |
|---|---|---|---|---|
| | **Asymmetry** | **Theoretical Plate** | **Asymmetry** | **Theoretical Plate** |
| Flow rate (±10%) | | | | |
| 1.1mL/min | 1.21 | 6426 | 1.05 | 11034 |
| 0.9 mL/min | 1.24 | 7727 | 1.03 | 12997 |
| Wavelength (±3nm), | | | | |
| 286nm | 1.24 | 7090 | 1.04 | 11872 |
| 280nm | 1.23 | 7065 | 1.05 | 11882 |
| Column-oven temperature(±$2^{0c}$) | | | | |
| $42^{0c}$ | 1.25 | 6975 | 1.07 | 11678 |
| $38^{0c}$ | 1.26 | 6807 | 1.06 | 11609 |