ORIGINAL ARTICLE

A new liquid chromatographic method for the simultaneous determination of ketorolac tromethamine and fluorometholone in the presence of hydrochlorothiazide

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Abstract

Introduction: Ketorolac is used to treat eye pain and to relieve the itchiness and burning of seasonal allergies. Fluorometholone is a synthetic glucocorticoid used for treating eye inflammatory diseases. A simultaneous and new RP-HPLC method was developed for the estimation of Ketorolac tromethamine and Fluorometholone in ophthalmic solutions using Hydrochlorothiazide as an internal standard. **Materials and Methods:** Shimadzu Model CBM-20A/20 Alite with phenomenex C8 column (250 mm × 4.6 mm i.d., 5 µm particle size) was used for the chromatographic study. 0.1 % acetic acid and methanol (20: 80, v/v) mixture was used as mobile phase with flow rate 0.7 ml/min and UV detection at 241 nm. Results and **Discussion:** Linearity was observed over the concentration range 1-100 µg/ml with regression equation y = 0.1047x + 0.0006 and correlation coefficient 0.9993 and the method was validated as per ICH guidelines. **Conclusions:** The method is more precise and accurate and suitable for the quality control analysis of combined formulations of Fluorometholone and Ketorolac tromethamine.

Key words: Fluorometholone, hydrochlorothiazide, ketorolac tromethamine, reversed-phase high-performance liquid chromatography, validation

INTRODUCTION

Fluorometholone (FLM)[1] [Figure 1a] is also known as 6α-methyl-9α-fluoro-11β,17α-dihydroxypregna-1,4-diene-3,20-dione, and is a synthetic glucocorticoid which is used in the treatment of inflammatory eye diseases. FLM (CAS No. 426-13-1), it has a molecular formula, $C_{22}H_{29}FO_4$ and molecular 376.462 g/mol (pKa 12.65). It is used in the treatment of steroid-responsive inflammatory conditions of the palpebral and bulbar conjunctiva, cornea, and anterior segment of the eye. FLM is available with brand names Efflumidex, Flucon, FML Forte, etc.

Ketorolac tromethamine (KT)[2] [Figure 1b] is chemically known as (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid with molecular formula, $C_{15}H_{13}NO_3$ and molecular weight 255.27 g/mol. KT (CAS No. 74103-06-3) is freely soluble in methanol with pKa 3.5. Ketorolac acts by inhibiting the bodily synthesis of prostaglandins. An ophthalmic solution of ketorolac is available and is used to treat eye pain and to relieve the itchiness and burning of seasonal allergies. The primary mechanism of action responsible for Ketorolac's anti-inflammatory, antipyretic, and analgesic effects is the inhibition of prostaglandin synthesis by competitive blocking

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of the enzyme cyclooxygenase. KT is available with brand names Toradol, Acular, and Sprix.

Jonvel and Andermann developed a very high-performance liquid chromatographic method[3] as a major advance technique for the determination of FLM in 1983 and only three reversed-phase high-performance liquid chromatography (RP-HPLC) methods were established for the determination of KT.[4-7] Till now only one liquid chromatographic method is available for the simultaneous estimation of KT and FLM in ophthalmic dosage form[8] where the authors have used the mobile phase phosphate buffer:methanol (70:30) with pH adjustment to 3.0 and very low linearity range (2.5–7.5 µg/ml for FLM and 12.5–37.5 µg/ml for KT) has been observed in comparison to the present method. Two spectrophotometric methods were also reported for the simultaneous estimation of KT and FLM.[9,10] In the present study, the authors have made an attempt to develop a simple, validated and robust liquid chromatographic method for the ophthalmic preparations, i.e., ear drops containing both KT and FLM using hydrochlorothiazide (HCTZ) as an internal standard (IS) and the method was validated as per the ICH guidelines.[11]

MATERIALS AND METHODS

Chemicals and Reagents

The combination of FLM and KT is available with brand name Eyetrust (Neiss Labs Ltd, Mumbai) as eye drops containing FLM 0.1% and KT 0.5%. Methanol and acetic acid (AR) were purchased from Merck (India), and all chemicals are of HPLC grade.

Instrumentation

Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with phenomenex C8 column (250 mm × 4.6 mm i.d., 5 µm particle size) was employed for the entire chromatographic study. Isocratic elution was performed using 0.1% acetic acid and methanol (20:80, v/v) as mobile phase (flow rate 0.7 ml/min and UV detection at 241 nm) using HCTZ as IS. The overall run time was 10 min and the chromatographic study was performed at room temperature (25°C±2°C).

Preparation of Stock Solution

The stock solutions of FLM and KT were prepared by transferring each of accurately 25 mg in to 25 ml volumetric flasks separately in methanol (1000 µg/ml), sonicated for 20 min, and dilutions were made with mobile phase as per the requirement. All the solutions were filtered through 0.45 µm membrane filter before injection.

The stock solution of HCTZ (IS) was prepared by transferring accurately 10 mg of HCTZ into a 10 ml volumetric flask in methanol (1000 µg/ml) and 10 µg/ml HCTZ solution was used as an IS during the entire study.

Method Validation

Linearity

During the optimization of the method different mobile phase compositions, flow rates, columns were tried. The chromatogram of the blank run with the optimized conditions was shown in Figure 2a. A series of solutions (1–100 µg/ml) were prepared from the stock solution containing FLM and KT with mobile phase along with the IS, and 20 µL of each of these solutions were injected into the HPLC system. The peak area observed by FLM, KT, and HCTZ was noted from the chromatogram, and then the peak area ratio of FLM and KT to that of the IS (peak area of FLM/peak area of HCTZ) and (peak area of KT/peak area of HCTZ) was calculated. Calibration curves were drawn by taking the concentration of FLM and KT solutions on the X-axis and the corresponding peak area ratio values on the Y-axis. The limit of quantification and limit of detection measured as described in ICH guidelines.[11]

Precision

The intraday precision of the assay method was evaluated at three concentration levels (10, 50 and 100 µg/ml) and the percentage RSD was calculated. The interday precision study was performed on three different days, i.e., day 1, day 2, and day 3 at three different concentration levels (10, 50, and 100 µg/ml) and the percentage RSD was calculated.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100, and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of FLM and KT in the drug product and the percentage recovery was calculated.

Robustness

The robustness of the assay method was established by introducing small changes in the optimized chromatographic conditions such as percentage of organic phase (78 and 82%), flow rate (±0.05 ml/min), and detection wavelength (239 and 243 nm). Robustness of the method was studied using 10 µg/ml of FLM and KT.

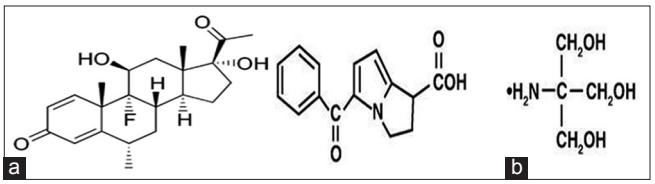

Figure 1: Chemical structures of fluorometholone (a) ketorolac tromethamine (b)

Assay of ophthalmic solutions

The brands available in the local pharmacy store were procured, and FLM and KT were extracted with mobile phase in a volumetric flask. The contents were sonicated for 30 min, filtered and diluted with mobile phase as per the requirement. IS was added to this extracted solution just before injecting into the HPLC system, and the peak area ratio was calculated from the respective chromatograms as described earlier.

RESULTS AND DISCUSSION

The authors have established a validated RP-HPLC method (isocratic mode) for the determination of FLM and KT in ophthalmic preparations using HCTZ as an IS. The optimized method involves the mobile phase composition 0.1% acetic acid:methanol (20:80, v/v) and flow rate 0.7 mL/min where sharp peaks were eluted at 6.493 ± 0.03 min for KT, 5.878 ± 0.02 min for FLM , and 4.051 ± 0.01 min for HCTZ during the chromatographic study [Figure 2b]. The proposed method was validated as per the ICH guidelines. Both FLM and KT have shown linearity over the concentration range 1–100 µg/mL [Table 1] with linear regression equation y = 0.5246x + 0.5759 (R^2 = 0.9993) and y = 0.1304x + 0.1047 (R^2 = 0.9995) for FLM and KT [Figure 3], respectively.

The LOD and LOQ were found to be 0.2944 and 0.892 µg/mL, respectively. The percentage RSD in intraday and interday precision studies was found to be <2% indicating that the method is precise [Table 2]. Good percentage recovery was observed in accuracy studies and the percentage RSD is <2.0 [Table 3] indicating that the method is accurate. The robustness of the method was assessed by exposing the drug solution to deliberate conditions purposely changing from the original established optimized conditions, and the percentage RSD

Table 1: Linearity of fluorometholone and ketorolac tromethamine

Conc. (µg/mL)			*Mean peak area			Peak area ratio	
FLM	KT	HCTZ (IS)	FLM	KT	HCTZ (IS)	FLM/IS	KT/IS
1	1	10	93789	23113	131241	0.7146	0.1761
5	5	10	414127	103516	140241	2.9529	0.7381
10	10	10	768427	186857	123420	6.2261	1.5139
20	20	10	1532735	374694	129697	11.8178	2.8889
50	50	10	4117029	1028710	140720	27.2568	6.5103
100	100	10	7461378	1864546	141770	52.6301	13.1519

*Mean of three replicates. FLM: Fluorometholone, HCTZ: Hydrochlorothiazide, IS: Internal standard, KT: Ketorolac tromethamine

Table 2: Precision studies of FLM and KT

Drugs	Conc. (µg/mL)	Intraday precision		Interday precision	
		*Conc. obtained (µg/mL)±SD	%RSD	*Conc. obtained (µg/mL)±SD	%RSD
FLM	10w	9.99±0.006	0.06	9.98±0.005	0.05
	50	49.8±0.1	0.20	49.9±0.11	0.23
	100	99.8±0.057	0.05	99.9±0.067	0.06
KT	10	9.98±0.005	0.05	9.99±0.006	0.06
	50	49.7±0.09	0.09	49.8±0.1	0.20
	100	99.9±0.067	0.06	99.7±0.004	0.04

*Mean of three replicates. FLM: Fluorometholone, KT: Ketorolac tromethamine

Table 3: Accuracy studies of FLM and KT

Drugs	Spiked conc. (µg/mL)	Total conc. (µg/mL)	*Conc. found (µg/mL)±SD	%RSD	%Recovery
FLM	0.8 (80)	1.8	1.78±0.0058	0.32	98.88
	1 (100)	2	1.99±0.0058	0.29	99.5
	1.2 (120)	2.2	2.18±0.0058	0.26	99.09
KT	4 (80)	9	8.89±0.0031	0.35	98.83
	5 (100)	10	9.92±0.0477	0.48	99.17
	6 (120)	11	10.93±0.0347	0.32	99.38

*Mean of three replicates. FLM: Fluorometholone, KT: Ketorolac tromethamine, SD: Standard deviation, RSD: Relative standard deviation

Table 4: Assay of FLM and KT in ophthalmic solutions

Formulation brand	Drug	Label claim (%)	*Amount found (%)	*% Recovery
Brand I	KT	0.5	0.49	98.0
	FLM	0.1	0.099	99.0

*Mean of three replicates. FLM: Fluorometholone, KT: Ketorolac tromethamine

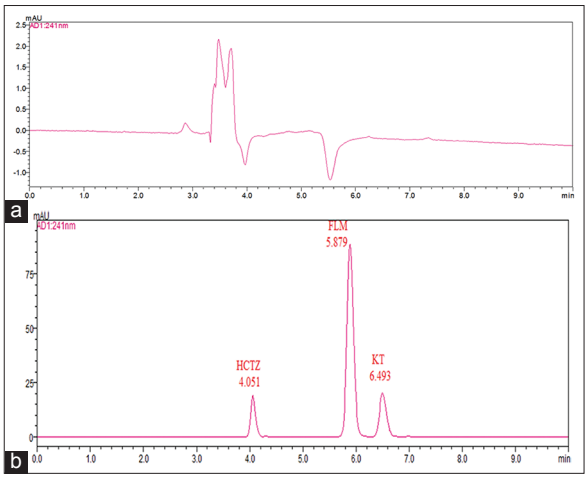

Figure 2: (a) Typical chromatogram of blank (b) Typical chromatogram of fluorometholone and ketorolac tromethamine with internal standard hydrochlorothiazide

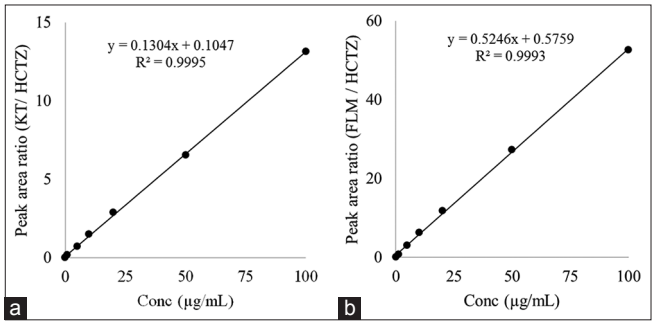

Figure 3: Calibration curves of (a) ketorolac tromethamine and (b) fluorometholone

was found to be <2.0% (0.81–1.01) specifying that the proposed method was robust.

The marketed formulations available were assayed, and the percentage recovery was found to be 98.0–99.0% [Table 4]. The theoretical plates were found to be 7318.927 for HCTZ, 10172.962 for KT, and 9471.254 for FLM which is >2000 and the tailing factor is <1.5 (1.268 for HCTZ, 1.190 for KT, and 1.163 for FLM). The resolution is >2 (2.458 for KT and 8.482 for FLM).

CONCLUSION

The proposed RP-HPLC method was simple, precise, accurate, and robust. This validated method has shown very wide linearity in comparison to the previously published method, and at the same time, the resolution of the peaks was very good. As the work was performed in the presence of an IS, it is more accurate and suitable for the quality control analysis of pharmaceuticals, i.e., at present for the determination of FLM and KT in ophthalmic solutions.

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