



RP-HPLC Method for Simultaneous Estimation of Ciprofloxacin and Dexamethasone in Eye/Ear Drops

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ABSTRACT

High performance liquid chromatography method was applied to the simultaneous determination of ciprofloxacin and dexamethasone. The chromatographic separation was achieved on reversed-phase C18 column (25 cm × 4.6 mm id, 5 μm) in the isocratic mode using methanol-water-triethylamine (55:45:0.6, v/v/v), pH adjusted to 3.0 ± 0.05 with orthophosphoric acid as the mobile phase at a flow rate 0.8 ml/min. Quantitation was achieved with UV detection at 254 nm. In the HPLC method, quantification was achieved over the concentration range of 3-18 and 1-6 μg/ml, with mean recoveries of 99.94 ± 1.51 and 100.28 ± 1.25% for ciprofloxacin and dexamethasone respectively. The proposed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of ciprofloxacin and dexamethasone without any interference from common excipients.

Keywords: Ciprofloxacin, Dexamethasone, Liquid Chromatography, Validation.

INTRODUCTION

Ciprofloxacin (1 cyclopropyl- 6-fluoro- 1, 4-dihydro- 4-oxo- 7[1-piperazinyl] - 3-quinolinecarboxylic acid) is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones.^[1-2] Ciprofloxacin is active against a wide variety of gram-positive and gram-negative organism, use in the treatment of urinary tract infection, conjunctivitis, gonorrhoea and respiratory tract infection.^[3] Dexamethasone (9α-fluoro-11β, 17, 21-trihydroxy- 16α-methylpregna- 1, 4-diene-3, 20-dione) is a synthetic glucocorticoid with varied pharmacological activities including anti-inflammatory, anti-rheumatic and immunosuppressant effect.^[1-3]

Ciprofloxacin (CIP) was determined individually by non-aqueous titration^[4], UV-spectrophotometry^[5], colorimetry^[6-8], high performance liquid chromatography^[9-15], HPLC with mass spectrometry^[16], thin-layer chromatography^[17-18], gas chromatography^[19] and capillary electrophoresis.^[20-21] On the other hand dexamethasone (DEX) was determined individually by UV spectroscopy^[22], HPLC^[23-26], TLC^[27], HPLC with mass spectrometry^[28-30], gas chromatography with mass spectrometry.^[31] Analytical method was reported for Pharmacokinetics of CIPRODEX[®] otic suspension in pediatric and adolescent patients by LC/MS/MS.^[32] Ciprofloxacin and Dexamethasone are formulated together in

the form of eye/ear drops (solution) or otic suspension which is used in the treatment of conjunctivitis, keratitis, acute otitis externa and acute otitis media in pediatric patients with tympanostomy tubes.^[33-35] Ciprofloxacin and Dexamethasone eye/ear drops (solution) are not official in any pharmacopoeia while Ciprofloxacin and Dexamethasone otic suspension is official in United States Pharmacopoeia.^[26] The main problem of spectrophotometric binary mixtures analysis is the simultaneous determination of two drugs without prior separation. No Isocratic HPLC method and spectrophotometric methods are available for simultaneous determination of ciprofloxacin and dexamethasone, so there was a need to develop accurate method for their determination in combination. The aim of this work is to determine both drugs concurrently by simple, rapid, and selective HPLC method which could be used for quality control and routine analysis.

MATERIAL AND METHODS

Instrumentation

HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 AS pump, Rheodyne 7725i injector with a 100 μl loop and SPD-10A UV-Visible detector. A Grace smart reversed-phase C₁₈ column (Grace Discovery and Division; 5 μm, 25 cm × 4.6 mm id) was used as the stationary phase. Class CR10 software was used for data acquisition.

Chemicals and Reagents

CIP powder was procured from Astron Research (Ahmedabad, India). DEX powder was procured from

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Lincoln Pharmaceutical Ltd. (Ahmedabad, India). Purity of CIP and DEX were 99.69 and 99.28 respectively. All experiments were performed with analytical-reagent grade chemicals. For HPLC work, methanol, phosphoric acid and triethylamine were of HPLC grade. Triple distilled water was used. Ciplox-D Eye/Ear Drops (Batch No. MG-8051) was manufactured by Cipla. Zoxan-D Eye/Ear Drops (Batch No. ZXC-9065) was manufactured by FDC. Each eye/ear drop claimed to contain 0.3 % w/v CIP (as free base) and 0.1% w/v DEX. All were purchased from Indian market.

Chromatographic Conditions

A 250 mm × 4.6 mm i.d Grace smart (5µm particle size) reversed-phase C₁₈ column was used for separation and quantitation. The mobile phase consisted of methanol-water-triethylamine (55:45:0.6, v/v/v) pH adjusted to 3.0 ± 0.05 with orthophosphoric acid. The mobile phase was filtered through Millipore filter paper type HV (0.45µm) and degassed by sonication. A flow rate of 0.8 ml/min was maintained. The injection volume was 20µl. The detector was set at 254 nm.

Standard Solutions

Stock standard solutions

Stock standard solutions CIP (as free base, 1 mg/ml) and DEX (1 mg/ml) were prepared separately in methanol.

Working standard solutions

The stock solutions were diluted with mobile phase to prepare final concentrations of 300 and 100µg/ml for CIP and DEX respectively.

Linearity

Aliquot equivalent to 0.1-0.6 ml of CIP working standard solution and 0.1-0.6 mL of DEX working standard solution were transferred separately into 10-mL volumetric flasks from their respective working standard solution and completed to volume with mobile phase. Calibration graphs for both CIP and DEX were obtained by plotting the peak area against concentration, and the corresponding regression equations were calculated.

Preparation of Pharmaceutical Samples

A volume of eye/ear drops (Ciplox-D/Zoxan-D) equivalent to 3 mg and 1 mg of CIP and DEX respectively was diluted up to 10 ml with methanol. Further dilutions were made with mobile phase to reach the calibration range of each drug and the solutions were treated according to the procedure for linearity of HPLC method.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The developed HPLC method has been applied for simultaneous determination of CIP and DEX. To optimize the chromatographic conditions for the separation of CIP and DEX, mobile phase composition, the effect of pH and wavelength of detection were investigated. During the method development work, Grace smart RP-C₁₈ column (250 mm × 4.6 mm i.d.) with particle size of 5µm was used and gave the most suitable resolution. A satisfactory separation was obtained with mobile phase consisting of methanol-water-triethylamine (55:45:0.6, v/v/v); adjustment of pH was performed using drops of orthophosphoric acid to achieve a pH 3.0 ± 0.05 with flow rate of 0.8 ml/min. At lower fractions of organic solvent (<30%), separation occurred but with excessive delay for the DEX chromatographic peak. A very strong mobile phase (organic solvent fraction of >65%) shows low capacity factor. Quantitation based on peak area

was achieved with UV detection at 254 nm. The specificity of HPLC method is illustrated in Fig. 1, which show complete separation of compounds in mixtures. The average retention time ± standard deviation (SD) for CIP and DEX were found to be 4.125 ± 0.014 and 9.688 ± 0.049 respectively, for seven replicates.

External standard calibration method was applied for analysis of CIP and DEX. [36] A linear relation was obtained between peak area and the concentration of the drug in the range of 3-18µg/ml and 1-6µg/ml for CIP and DEX respectively. The regression equations were computed to be:

$$Y = 396299.16X + 11465.47 \quad r = 0.9998 \text{ (for ciprofloxacin)}$$

$$Y = 489368.18X - 53134.28 \quad r = 0.9995 \text{ (for dexamethasone)}$$

Where Y is the peak area, X is the concentration in µg/ml and r is the correlation coefficient.

The method was successfully applied for simultaneous determination of CIP and DEX in different laboratory-prepared mixtures, with mean recoveries of 99.87 ± 0.70 and 100.39 ± 0.91, respectively (Table 2). The proposed method has been also applied for the determination of the two drugs in Ciplox-D and Zoxan-D Eye/Ear Drops (Table 3) and validity was further assessed by applying the standard addition technique (Table 4) and HPLC chromatogram of Ciplox-D and Zoxan-D Eye/Ear Drops are shown in Fig. 2 and Fig. 3.

System Suitability

System Suitability parameters such as number of theoretical plates, HETP, Capacity factor, Tailing factor and Resolution were determined. Results of System Suitability parameters are shown in Table 1.

Table 1: System Suitability Parameters

Parameters	Compound	
	CIP	DEX
Retention time (min)	4.123 ± 0.014	9.688 ± 0.049
Capacity factor (K')	0.52	2.56
Selectivity (α)		4.95
Resolution (R _s)		14.27
Tailing factor (T)	1.05	0.96
RSD of Retention time (%)	0.34	0.51
Injection Repeatability (RSD %)	0.67	0.96
Number of theoretical plates (N)	6155.09	4650.86
Height equivalent to theoretical plates (HETP)	4.09 × 10 ⁻³ cm	5.38 × 10 ⁻³ cm

Validation of the Methods

Linearity and range

The linearity of the HPLC method for the determination of CIP and DEX was evaluated by analyzing a series of different concentrations of each drug. In this study six concentrations were chosen for each drug. Each concentration was repeated six times. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($P = 0.05$) different from zero. The calibration range was established through consideration of the practical range necessary, according to each drug concentration present in the pharmaceutical products to give accurate, precise and linear results. The calibration range of the proposed method is given in Table 5.

Precision

Evaluation of the precision estimates repeatability and intermediate precision were performed at three concentration levels for each drug on three different days. The low values of relative standard deviation (RSD) of the intraday and

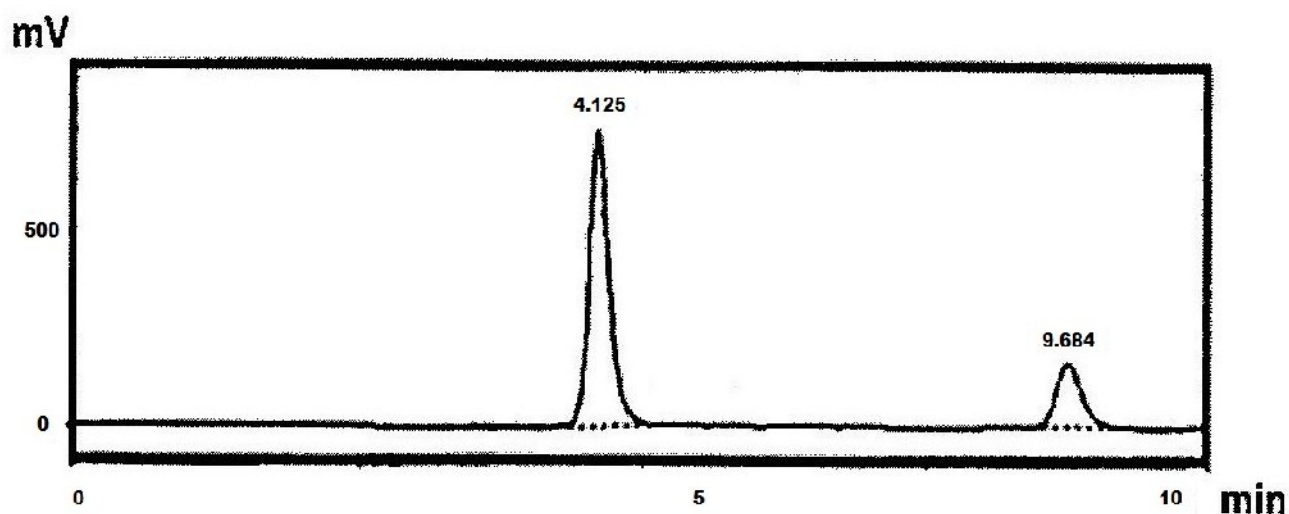


Fig. 1: HPLC Chromatogram of 15µg/ml CIP (RT = 4.125 min) and 5µg/ml DEX (RT = 9.684 min)

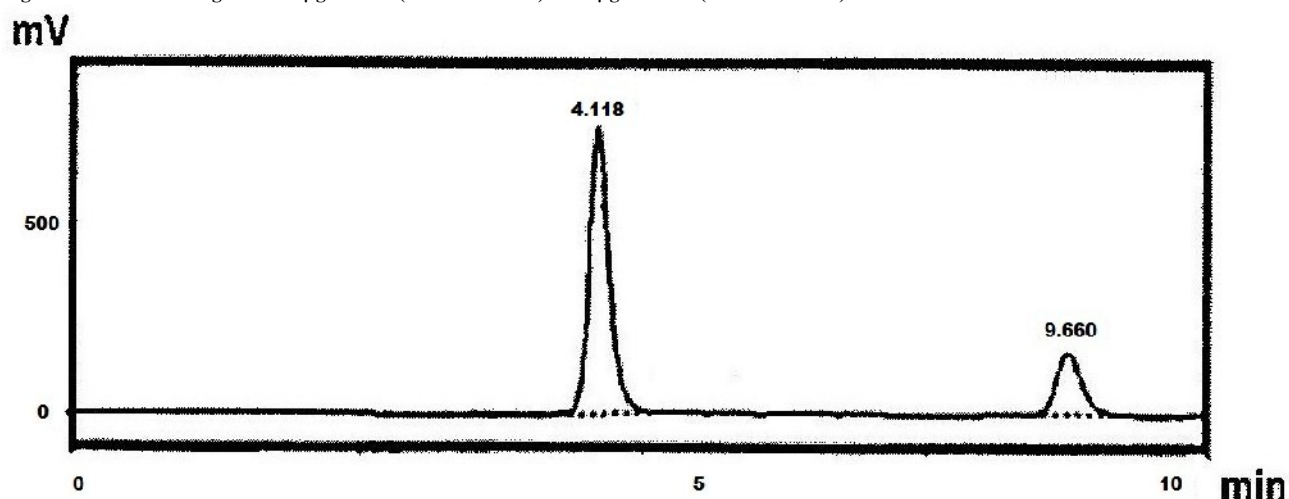


Fig. 2: HPLC Chromatogram of CIP (RT = 4.118 min) and DEX (RT = 9.660 min) in Ciplox-D Eye/Ear Drops

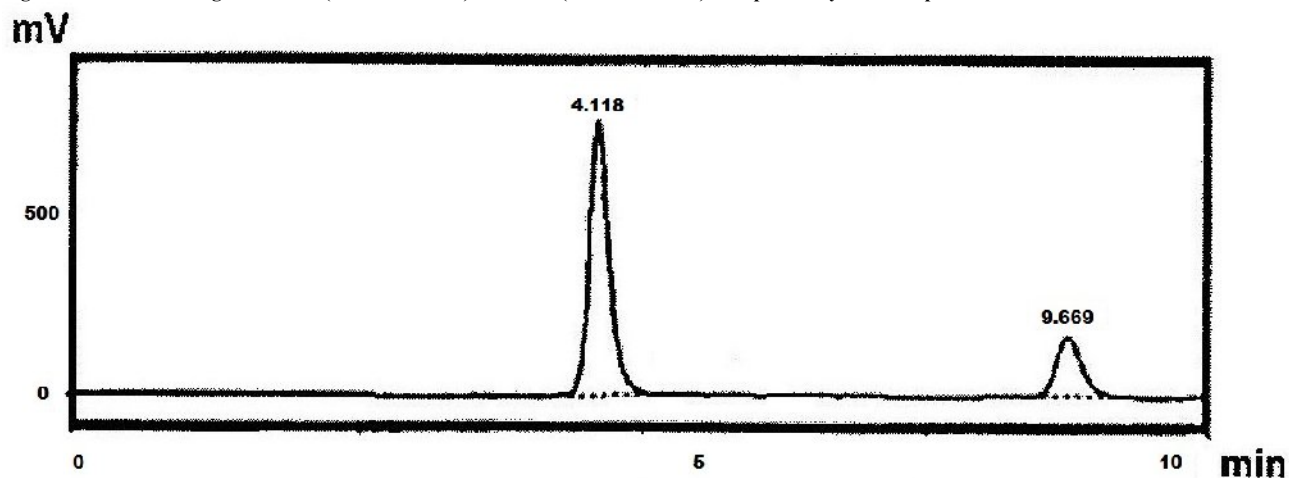


Fig. 3: HPLC Chromatogram of CIP (RT = 4.118 min) and DEX (RT = 9.669 min) in Zoxan-D Eye/Ear Drops

Table 2: Determination of CIP and DEX in laboratory-prepared mixtures by HPLC method

Samples	CIP			DEX		
	Taken (µg/ml)	Found ^a (µg/ml)	Recovery (%)	Taken (µg/ml)	Found ^a (µg/ml)	Recovery (%)
1 (1:1)	3	2.98	99.33	3	3.03	101.00
2 (2:1)	12	12.08	100.67	6	6.05	100.83
3 (3:1)	18	17.93	99.61	6	5.96	99.33
Mean ± S.D.			99.87 ± 0.70			100.39 ± 0.91

^a Average of three experiments.

Table 3: Determination of CIP and DEX in pharmaceutical formulations by HPLC method

Samples	CIP		DEX	
	Label claim (% w/v)	% Recovery Mean $\pm$ S.D. ^a	Label claim (% w/v)	% Recovery Mean $\pm$ S.D. ^a
Ciplox-D	0.3	100.43 $\pm$ 0.78	0.1	100.07 $\pm$ 0.93
Zoxan-D	0.3	97.40 $\pm$ 0.98	0.1	96.94 $\pm$ 1.67

^a Average of three experiments.**Table 4: Application of the standard addition technique to analysis of CIP and DEX in Ciplox-D Eye-Ear drops by HPLC method**

Product	Found ^a %	Pure added (µg/ml)	Pure Found ^a (µg/ml)	Recovery (%)
Ciprofloxacin in Ciplox-D	100.43 $\pm$ 0.78	3	3.04	101.33
Eye-Ear Drop		6	6.01	100.17
Mean $\pm$ S.D.		9	8.85	98.33
				99.94 $\pm$ 1.51
Dexamethasone in Ciplox-D	100.07 $\pm$ 0.93	1	0.99	99.00
Eye-Ear Drop		2	2.03	101.50
Mean $\pm$ S.D.		3	3.01	100.33
				100.28 $\pm$ 1.25

^a Average of three experiments.**Table 5: Validation results obtained by applying proposed method for the determination of Ciprofloxacin and Dexamethasone**

Parameters	HPLC method	
	CIP	DEX
Linearity Range (µg/ml)	3-18	1-6
Slope	396299.16	489368.18
Intercept	11465.47	53134.28
Correlation coefficient (r)	0.9998	0.9995
Repeatability RSD (%)	0.67	0.96
Intraday RSD (%)	0.87-1.38	1.19-1.92
Interday RSD (%)	1.12-2.05	1.51-2.13
Accuracy (% Recovery)	99.94 $\pm$ 1.51	100.28 $\pm$ 1.25
LOD (µg/ml)	0.29	0.25
LOQ (µg/ml)	0.89	0.77

interday determinations (Table 5) show that there was no statistically significant difference between the mean results obtained from one day to another.

Detection and quantitation limits

According to ICH recommendation [37], the approach based on the standard deviation of the response and slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). The theoretical values for HPLC method are given in Table 5.

Selectivity

Method selectivity was achieved by different laboratory-prepared mixtures of the studied drugs at various concentrations within the linearity range. The laboratory-prepared mixtures were analyzed according to the procedure described under the proposed methods. Satisfactory results were obtained (Table 2) indicating the high selectivity of the proposed methods for simultaneous determination of CIP and DEX.

Accuracy

The interference of excipients in the pharmaceutical formulations was studied in detail by proposed methods. For this reason, standard addition method was applied to the pharmaceutical formulation containing these compounds. This study was performed by addition of known amounts of studied drugs to a known concentration of the commercial pharmaceutical product. The excellent recoveries of standard addition method (Table 4) prove the good precision and accuracy of the proposed methods. Consequently, the

excipients in the studied pharmaceutical formulations do not interfere in the analysis of these compounds.

Analysis of Laboratory Mixtures and Pharmaceutical formulations

The proposed HPLC method was applied to the simultaneous determination of CIP and DEX in Laboratory Mixtures (Table 2) and in Ciplox-D and Zoxan-D eye/ear drops (Table 3). Three replicated determinations were made. Satisfactory results were obtained for each compound in good agreement with label claims.

For routine analytical purposes, it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with due accuracy and precision. The proposed HPLC method gives a good resolution between Ciprofloxacin and Dexamethasone within a short time. These methods are rapid, sensitive, selective, accurate and precise and can be used in routine and quality control analysis.

REFERENCES

1. The Merck Index, An Encyclopaedia of Chemical, Drugs and Biologicals, 13th Ed., Merck Research Laboratories, Merck and Co. Inc.: NJ, 2001.
2. Sweetman SC, Martindale, The complete Drug Reference, 36th Ed., Pharmaceutical Press: London, 2009.
3. Hardman JG, Limbird LE, Goodman and Gilman, The Pharmacologic Basis of Therapeutics, 11th Ed., McGraw-Hill: New York, 2006.
4. Kilic E, Koseoglu F, Akay MA. Non-aqueous titrimetric assay of selected antibiotics using tetra-N-butylammonium hydroxide as titrant. J. Pharm. Biomed. Anal. 1994; 12:347.
5. Patel SA, Patel NM, Patel MM. Simultaneous spectrophotometric estimation of ciprofloxacin and ornidazole in tablets. Indian J. Pharm. Sci. 2006; 68:665-667.
6. Amin AS. Spectrophotometric microdetermination of some important aminoquinoline antimalarials, as ion-pair complex. Mikrochim. Acta. 2000; 134:89-94.
7. Abdel-Gawad FM, Issa YM, Fahmy HM, Hussein HM. Spectrophotometric determination of ciprofloxacin in pure form and in tablets through charge-transfer complexation reactions. Mikrochim. Acta. 1998; 130:35.
8. Rizk M, Balal F, Ibrahim F, Ahmed S, Sheribah Z.A. Derivative Spectrophotometric Analysis of 4-Quinolone Antibacterials in Formulations and Spiked Biological Fluids by Their Cu (II) Complexes. J. AOAC Int. 2001; 84: 368.
9. Witte BD, Dewulf J, Demeestere K, Ruyck MD, Langenhove HV. Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography. J. Chromatogr. A. 2007; 1140:126-130.
10. Belal F, Al-Majed, AA, Al-Obaid AM. Methods of analysis of 4-quinolone antibacterials. Talanta, 1999; 50:765-786.
11. Joshi S. HPLC separation of antibiotics present in formulated and unformulated samples. J. Pharm. Biomed. Anal. 2002; 28:795-809.
12. Shervington LA, Abba M, Hussain B, Donnelly J. Simultaneous separation and determination of five quinolone antibiotics using isocratic reversed-phase HPLC: Application to stability studies on an ofloxacin tablet formulation. J. Pharm. Biomed. Anal. 2005; 39: 769-775.
13. Kassab NJ, Singh AK, Hackmam ERMK, Santoro MIRM. Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography. Brazilian J. Pharm. Sci. 2005; 41:507-513.
14. Thoppil SO, Amin PD. Stability indicating reversed-phase liquid chromatographic determination of ciprofloxacin as bulk drug and in pharmaceutical formulations. J. Pharm. Biomed. Anal. 2000; 22:699-703.
15. Gomez DG, Anunciacion Espinosa-Mansilla, Arsenio Munoz de la Pena, Canada FC. HPLC determination of ciprofloxacin, cloxacillin and ibuprofen drugs in human urine samples. J. Pharm. Sci. 2006; 29:1969-1976.
16. Torniainen K, Mattinen J, Askolin CP, Tammilehto S. Structure elucidation of a photodegradation product of ciprofloxacin. J. Pharm. Biomed. Anal. 1997; 15:887-894.

17. Wang PL, Feng YL, Chen LA. Simultaneous Determination of Trace norfloxacin, pefloxacin, and ciprofloxacin by TLC-Fluorescence Spectrodensitometry. *Microchem. J.* 1997; 56:229-235.
18. Novakovic J, Nesmerak K, Nova H, Filka K. An HPTLC method for the determination and the purity control of ciprofloxacin HCl in coated tablets. *J. Pharm. Biomed. Anal.* 2001; 25:957-964.
19. Michalska K, Pajchel G, Tyski S. Determination of ciprofloxacin and related impurities by capillary zone electrophoresis. *J. Chromatogr. A.* 2004; 1051:267-272.
20. Altria KD, Chanter YL. Validation of a capillary electrophoresis method for the determination of a quinolone antibiotic and its related impurities. *J. Chromatogr. A.* 1993; 652:459-463.
21. Michalska K, Pajchel G, Tyski S. Determination of ciprofloxacin and related impurities by capillary zone electrophoresis. *J. Chromatogr. A.* 2004; 1051:267-272.
22. Graham RE, Williams PA, Kenner CT. Detection of decomposition and analytical interferences in pharmaceutical preparations containing corticosteroids. *J. Pharm. Sci.* 1970; 59:1152-1156.
23. Pendela M, Adams E, Hoogmartens J. Development of a liquid chromatographic method for ear drops containing neomycin sulphate, polymyxin B sulphate and dexamethasone sodium phosphate. *J. Pharm. Biomed. Anal.* 2004; 36:751-757.
24. Xiong Y, Xia KP, Rustum AM. Development and validation of a stability-indicating RP-HPLC method to separate low levels of dexamethasone and other related compounds from betamethasone. *J. Pharm. Biomed. Anal.* 2009; 49:646-654.
25. Volin P. HPLC analysis of corticosteroids. *J. Chromatogr. B.* 1995; 671:319-340.
26. United States Pharmacopoeia (30) National Formulary 25, The United States Pharmacopoeial Convention: Rockville, 2007.
27. Gagliardi L, Orsi DD, Giudice MRD, Gatta F, Porrà R, Chimenti P, Tonelli D. Development of tandem thin-layer chromatography-high-performance liquid chromatography method for the identification and determination of corticosteroids in cosmetic products. *Anal. Chim. Acta.* 2002; 457:187-198.
28. Wasch KD, Brabender HFD, Wiele MVD, Vercammen J, Courtheyn D, Impens S. Differentiation between dexamethasone and betamethasone in a mixture using multiple mass spectrometry. *J. Chromatogr. A.* 2001; 926:79-86.
29. Francesco RD, Frerichs V, Donnelly J, Hagler C, Hochreiter J, Tomatore KM. Simultaneous determination of cortisol, dexamethasone, methylprednisolone, prednisone, prednisolone, mycophenolic acid and mycophenolic acid glucuronide in human plasma utilizing liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B.* 2007; 859:42-51.
30. Yang Y, Li H, Gao K, Liu M, Sun Y, Yan T, Fawcett JP, Cui Y, Gu J. Simultaneous quantitation of dexamethasone palmitate and dexamethasone in human plasma by liquid chromatography/tandem mass spectrometry. *J. Chromatogr. B.* 2008; 862:119-124.
31. Hidalgo OH, Lopez MJ, Carazo EA, Larrea MSA, Reuvers TBA. Determination of dexamethasone in urine by gas chromatography with negative chemical ionization mass spectrometry. *J. Chromatogr. B.* 2003; 788:137-146.
32. Spektor Z, Jasek MC, Jasheway D, Dahlin DC, Kay DJ, Mitchell R, Faulkner R, Wall GM. Pharmacokinetics of CIPRODEX[®] otic in pediatric and adolescent patients. *Int. J. of Pediatr. Otorhinolaryngol.* 2008; 72:97-102.
33. Conroy PJ. New Quinolone/Steroid combination for topical treatment of acute otitis: Early- and late-phase study results. *ENT J.* 2003; 82: 1-4.
34. Moe BR, Dupre S, Wall M, Stroman D, Celeste McLean Joseph Dohar, Giles W, Roland P, Bikhazi N, Carroll S, Crenshaw K. Topical Ciprofloxacin/Dexamethasone Superior to Oral Amoxicillin/Clavulanic Acid in Acute Otitis Media with Otorrhea through Tympanostomy Tubes. *Pediatrics.* 2006; 118:1-9.
35. Ermis SS, Aktepe OC, Inan UU, Ozturk F, Altindis M. Effect of topical dexamethasone and ciprofloxacin on bacterial flora of healthy conjunctiva. *Eye.* 2004; 18:249-252.
36. Synder LR, Kirkland JJ, Glajek JL. Practical HPLC Method Development, John Wiley & Sons, Inc.: New York, 1997.
37. ICH Topic Q2B, Note for Guidance on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, The European Agency for the Evaluation of Medical Products: Geneva, 1996.