

Stability indicating RP-HPLC method for simultaneous determination of gatifloxacin and dexamethasone in binary combination

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In this study, conditions were optimized for development of a simple RP-HPLC method for simultaneous analysis of gatifloxacin and dexamethasone in different matrices like pharmaceuticals, human serum and urine. Good separation of gatifloxacin and dexamethasone from the induced degradation products was accomplished using C8 as stationary phase; 0.02 M phosphate buffer (pH 3.0) and methanol (42:58 v/v) as mobile phase. The concentration was measured with DAD at 270 nm. Linearity was observed in the range of 0.000040-0.000280 mol/L for gatifloxacin ($r^2 \geq 0.999$) and 0.000013-0.000091 mol/L for dexamethasone ($r^2 \geq 0.999$). Both the analyte peaks were completely separated from the peaks of induced degradation products as indicated by the peak purity index (≥ 0.9999 for both analytes). The optimized method is recommended to be used for concurrent analysis of gatifloxacin and dexamethasone in different matrices.

Uniterms: Gatifloxacin. Dexamethasone. High performance liquid chromatography. Validation. Plasma. Degradation products.

INTRODUCTION

Gatifloxacin (Figure 1A), a broad spectrum antibiotic is primarily used to treat bacterial infections like conjunctivitis, keratitis, pre and post-operative (Martindale, 2009). Dexamethasone (Figure 1B), a corticosteroid, used primarily for ocular inflammation conditions and where the risk of bacterial ocular infection or its risk is found (Martindale, 2009). The determination of gatifloxacin has widely been reported using spectrophotometry (Sonali *et al.*, 2006; Venugopal, Ranendra, 2005), HPLC (Santoro *et al.*, 2006), atomic absorption spectrophotometry, conductometry, colorimetry (Sheikh, 2008) and spectrofluorimetry (Ocaña, Barragán, Callejón, 2005). Similarly, the estimation of dexamethasone has also been reported widely by HPLC (Iqbal *et al.*, 2006; Huetos *et al.*, 1999;

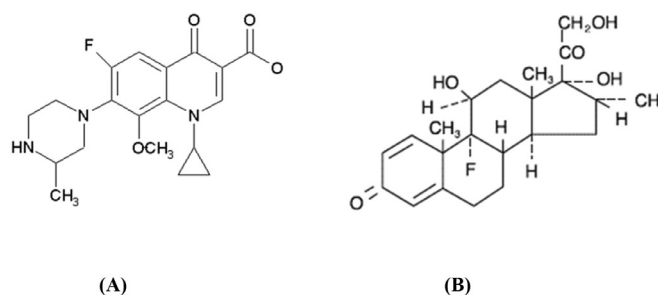


FIGURE 1 - Chemical Structures of gatifloxacin (A) and dexamethasone (B).

Mallinson *et al.*, 1995; Chen *et al.*, 2008; Lemus Gallego, Pérez Arroyo, 2002; Cocenza, Mainardes, Gremião, 2009; Kwak, D'Amico, 1995), GC-MS (Mallinson *et al.*, 1995) and TLC (Huetos *et al.*, 1999).

Although gatifloxacin and dexamethasone fixed dose combination (FDC) is available for sale, however not officially adopted by any pharmacopeia. Scientific literature was also found scarce of any stability indicating method for this FDC. In order to fill this knowledge

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gap, the present study was designed to optimize the chromatographic conditions in order to estimate them in different matrices (pharmaceutical formulations, human urine and serum). In the past few years, different FDC have already been reported by our research group after developing suitable analytical methods (Ashfaq *et al.*, 2013; 2014; Khan *et al.*, 2013; 2014; Razzaq *et al.*, 2013; 2014; John *et al.*, 2015a, b; Saleem *et al.*, 2015).

MATERIAL AND METHODS

Chemicals and Reagents

Gatifloxacin (99.7%) and dexamethasone (99.2%) reference standards were obtained as gift samples from a pharmaceutical laboratory located at Lahore, Pakistan. GATE DX and GATIBLU-D ophthalmic preparations (3 mg/mL gatifloxacin and 1 mg/mL dexamethasone) were analyzed during the applications of the method. All other chemicals and reagents were of highest analytical grades available in the market. For filtration of all solutions including mobile phase, 0.45 μm nylon filters (Millipore, USA) were used.

Equipment and Chromatographic Conditions

Liquid chromatography as well as integration of peak areas and chromatographic parameter were calculated using Shimadzu LC-20A system (Kyoto, Japan) at 270 nm. Hypersil C8 column (0.250 m X 4.6 mm, 5 mm) was used as stationary phase while 0.02 M phosphate buffer pH 3.0 (adjusted by 1 mL triethylamine and phosphoric acid) and methanol (42:58 v/v) were used as mobile phase at 1.5 mL/min.

Preparation of Standard Solution (A) and Working Standard Solution (B)

Stock solution (A) of gatifloxacin (0.0040 mol/L) and dexamethasone (0.00130 mol/L) was prepared by dissolving 150 mg gatifloxacin and 50 mg dexamethasone in few mL of methanol in 100 mL measuring flask, sonicated for few minutes and then diluted up to the mark with mobile phase. Solution was then subsequently diluted to get working solution (B) of gatifloxacin (0.000160 mol/L) and dexamethasone (0.000052 mol/L).

Preparation of Sample Solution

An appropriate volume of commercial ophthalmic solution was diluted with mobile phase to get

0.000160 mol/L gatifloxacin and 0.000052 mol/L dexamethasone.

Preparation of Human Urine Samples

1250 μL of solution A was mixed with equal volume of human urine for 2 minutes, followed by its centrifugation for 10 minutes at 4000 rpm. The supernatant (200 μL) was added in 2300 μL of the mobile phase. The final concentration of solution thus obtained was equal to (0.000160 mol/L of gatifloxacin and 0.000052 mol/L of dexamethasone).

Preparation of Human Serum Samples

Solucion A, 50 μL , was mixed with 250 μL human serum, followed by adding 950 μL mobile phase. The mixture was centrifuged for 10 minutes at 4000 rpm and the supernatant was shifted in polypropylene tubes having concentration equal to 0.000160 mol/L for gatifloxacin and 0.000052 mol/L for dexamethasone.

Linearity

Linearity was established by analyzing each solution in triplicate in the range of 0.000040-0.000280 mol/L (0.000040, 0.000080, 0.000120, 0.000160, 0.000200, 0.000240 and 0.000280 mol/L) of gatifloxacin and 0.000013-0.000091 mol/L of dexamethasone (0.000013, 0.000026, 0.000039, 0.000052, 0.000065, 0.000078 and 0.000091 mol/L).

Accuracy

Synthetic mixtures of both analytes were analyzed in order to evaluate accuracy. The synthetic mixture was prepared by mixing appropriate amounts of gatifloxacin, dexamethasone, benzalkonium chloride and sodium chloride in 1L purified water. The solution was then diluted to get solutions of three different concentrations (50-150%) of working solution concentration (0.000160 mol/L gatifloxacin and 0.000052 mol/L dexamethasone). These solutions were further used to test recovery studies.

Precision

Precision was evaluated by calculating % RSD of the samples analyzed within one day for five times and for sample analyzed for three days. Three different concentration of solutions were used for both within-day and between-day precision.

Specificity (Stress Testing)

Acid Degradation Studies

To four separate measuring flasks, each contained 1 mL solution A, 5 M hydrochloric acid (1 mL) was mixed and kept in chamber at 40 °C/75% RH for 1, 8, 16 and 23 h followed by neutralization and completing to the mark.

Base, Oxidative and Thermal Degradation Studies

Same procedure was used for base, oxidative and thermal stress studies except using 5 M NaOH instead of 5 M HCl in basic stress, 6% H₂O₂ for oxidative stress (1, 8, 23 and 70 h) and 1, 16, 24 and 70 h time for thermal stress.

Photolytic Degradation Studies

Three separate 25 mL measuring flasks containing 1 mL of solution A were exposed to direct sunlight for 2, 4 and 6 h, followed by marking the volume.

RESULTS AND DISCUSSION

Reverse phase (RP) chromatography has been used widely in the last two decades for the separation of many organic molecules particularly pharmaceuticals because of containing major non-polar groups as well as containing unsaturated π electrons. As both the investigating pharmaceuticals have this common feature in their structure, so in this study, an RP-HPLC was developed for simultaneous analysis of gatifloxacin and dexamethasone in different matrices like pharmaceuticals, human serum and urine.

Optimization of Mobile Phase and Stationary Phase

Both dexamethasone and gatifloxacin have been analyzed individually in previous methods (Razzaq *et al.*, 2013; 2014; Khan *et al.*, 2014). In order to develop a method that can quantify both of these simultaneously, we took advantage of the problems faced during the previous studies, where dexamethasone peak showed high tailing on C18, Phenyl-2, and Cyano columns but symmetrical/good peak on C8 (Razzaq *et al.*, 2013; 2014; Khan *et al.*, 2014). So we chose C8 column for this FDC. Some minor changes in the composition of organic and aqueous phase as well as pH were good enough to resolve both these compounds from each other as well as from induced degradation products. The final composition of the mobile phase thus used was, methanol: phosphate buffer 0.02 M, pH 3.0 (58:42, v/v) that resulted in good/symmetrical peaks.

Analytical Method Validation

Validation parameters like linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation as recommended by ICH (International Conference on Harmonization, 1996) were evaluated when validating this method.

Linearity was established by analyzing each solution in triplicate in the range of 0.000040-0.000280 mol/L (0.000040, 0.000080, 0.000120, 0.000160, 0.000200, 0.000240 and 0.000280 mol/L) of gatifloxacin and 0.000013-0.000091 mol/L of dexamethasone (0.000013, 0.000026, 0.000039, 0.000052, 0.000065, 0.000078 and 0.000091 mol/L). The linear regression equation for gatifloxacin was found to be $Y = 46312 X + 4983$ with correlation coefficient ≥ 0.999 whereas for dexamethasone it was $Y = 94196 X + 1566$ with correlation coefficient ≥ 0.999 .

Limit of detection (LOD) was found to be 0.0000008 mol/L and 0.0000003 mol/L (Figure 3) for gatifloxacin and dexamethasone, respectively (Figure 6). LOQ was found to be 0.0000027 mol/L and 0.0000001 mol/L for gatifloxacin and dexamethasone, respectively.

Synthetic mixtures of both analytes were analyzed to evaluate accuracy. Solutions of three different concentrations (50-150%) of working solution concentration (0.000160 mol/L gatifloxacin and 0.000052 mol/L dexamethasone) were used to test recovery. The results are presented in (Table I), indicating good recovery.

Precision was evaluated by calculating % RSD of the samples analyzed within one day for five times and for sample analyzed for three days. Three different concentration of solutions were used for both within-day and between-day precision. The results are presented in (Table II).

Slight variation in the chromatographic conditions were made in order to evaluate robustness. Results presented in (Table III and Table IV) showed little effect on chromatographic parameters.

The chromatograms involving forced stresses are shown in (Figure 2), whereas the results describing percentage degradation and extent of degradation are provided (Table V).

Both the drugs were degraded with ease under most of the stress conditions (except thermal and oxidative). When comparing the vulnerability of both drugs, it was observed that dexamethasone was easy to degrade compared to gatifloxacin (31% and 95 % degradation under acidic and basic conditions). Up to 4 degradation products were observed during the mentioned stresses.

TABLE I - Accuracy of the proposed HPLC method

Nature of Sample	Spiked Concentrations mol/L	Measured Concentration mol/L \pm RSD (%)	Recovery (%)
Pharmaceutical Formulations			
Gatifloxacin	0.000080	0.0000806 \pm 1.19	100.7
	0.000160	0.000160 \pm 0.41	100.0
	0.000240	0.0002431 \pm 0.91	101.3
Dexamethasone	0.000026	0.0000259 \pm 0.49	99.50
	0.000052	0.0000523 \pm 1.24	100.5
	0.000078	0.0000785 \pm 0.61	100.7
Spiked Serum			
Gatifloxacin	0.000080	0.000072 \pm 1.65	90.0
	0.000160	0.000147 \pm 3.24	91.9
	0.000240	0.000219 \pm 1.33	91.4
Dexamethasone	0.000026	0.0000258 \pm 1.39	99.2
	0.000052	0.0000525 \pm 1.93	100.9
	0.000078	0.000079 \pm 1.90	101.3
Spiked Urine			
Gatifloxacin	0.000080	0.0000796 \pm 1.14	99.5
	0.000160	0.000163 \pm 0.68	101.8
	0.000240	0.000244 \pm 1.79	101.5
Dexamethasone	0.000026	0.0000261 \pm 1.94	100.2
	0.000052	0.0000523 \pm 1.61	100.5
	0.000078	0.0000782 \pm 1.31	100.3

TABLE II - Intra-Day and Inter-Day precision of the proposed HPLC method

Drugs	Actual Concentration mol/L	Intra-Day Precision Measured Concentration mol/L \pm RSD (%)	Inter-Day precision Measured Concentration mol/L \pm RSD (%)
Pharmaceutical Formulations			
Gatifloxacin	0.000080	0.0000783 \pm 1.21	0.0000803 \pm 1.87
	0.000160	0.0001614 \pm 1.92	0.0001627 \pm 0.99
	0.000240	0.0002400 \pm 0.81	0.0002406 \pm 0.82
Dexamethasone	0.000026	0.0000262 \pm 1.87	0.0000260 \pm 1.41
	0.000052	0.0000525 \pm 0.51	0.0000526 \pm 1.89
	0.000078	0.0000780 \pm 1.22	0.0000777 \pm 1.20
Spiked Serum			
Gatifloxacin	0.000080	0.0000767 \pm 1.58	0.0000758 \pm 2.26
	0.000160	0.0001471 \pm 2.99	0.0001511 \pm 2.94
	0.000240	0.0002252 \pm 1.10	0.0002205 \pm 2.09
Dexamethasone	0.000026	0.0000260 \pm 1.15	0.0000261 \pm 2.14
	0.000052	0.0000526 \pm 2.17	0.0000520 \pm 2.43
	0.000078	0.0000780 \pm 1.95	0.0000782 \pm 1.18
Spiked Urine			
Gatifloxacin	0.000080	0.0000797 \pm 1.54	0.0000804 \pm 2.16
	0.000160	0.0001623 \pm 1.08	0.0001647 \pm 1.04
	0.000240	0.0002402 \pm 1.54	0.0002406 \pm 1.72
Dexamethasone	0.000026	0.0000262 \pm 1.08	0.0000262 \pm 1.54
	0.000052	0.0000523 \pm 1.53	0.0000525 \pm 1.13
	0.000078	0.0000781 \pm 1.29	0.0000776 \pm 1.34

TABLE III - Robustness study of Gatifloxacin

Chromatographic conditions	Assay (%)	t_R (min)	Plates	Tailing
Methanol: buffer (61:39)	98.7	2.99	4507	1.42
Methanol: buffer (58:42)	100.6	3.38	4588	1.41
Methanol: buffer (55:45)	100.9	3.69	4478	1.44
Flow rate (1.3 mL/min)	100.9	3.75	4601	1.43
Flow rate (1.5 mL/min)	99.8	3.38	4577	1.43
Flow rate (1.7 mL/min)	100.5	2.89	4587	1.41
Buffer (pH 2.8)	102.0	3.34	4691	1.44
Buffer (pH 3.0)	101.5	3.38	4511	1.41
Buffer (pH 3.2)	99.6	3.38	4547	1.40

TABLE IV - Robustness study of Dexamethasone

Chromatographic conditions	Assay (%)	t_R (min)	Plates	Tailing
Methanol: buffer (61:39)	101.7	7.52	6821	1.20
Methanol: buffer (58:42)	100.9	7.75	6799	1.21
Methanol: buffer (55:45)	100.0	7.92	6647	1.21
Flow rate (1.3 mL/min)	99.9	9.19	6788	1.20
Flow rate (1.5 mL/min)	99.9	7.75	6718	1.22
Flow rate (1.7 mL/min)	99.5	6.82	6895	1.23
Buffer (pH 2.8)	100.7	7.75	6805	1.22
Buffer (pH 3.0)	98.7	7.77	6705	1.24
Buffer (pH 3.2)	100.7	7.74	6611	1.22

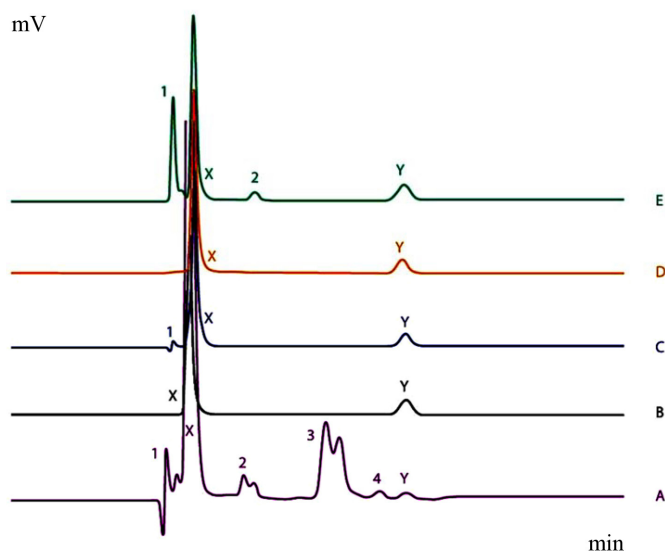


FIGURE 2 - A typical chromatogram of gatifloxacin and dexamethasone under acidic, basic, thermal, photolytic and oxidative stress conditions. Where (X) is gatifloxacin peak, (Y) is dexamethasone peak, (1, 2, 3, and 4) are degradation/impurities peaks, (A) chromatogram of basic stress, (B) chromatogram of thermal stress, (C) chromatogram of acidic stress, (D) chromatogram of photolytic stress and (E) chromatogram of oxidative stress.

The developed method was finally used to determine gatifloxacin and dexamethasone in commercially available ophthalmic solutions (Table VI), spiked human serum and urine (Figure 3). Excellent results were obtained from these experiments.

CONCLUSION

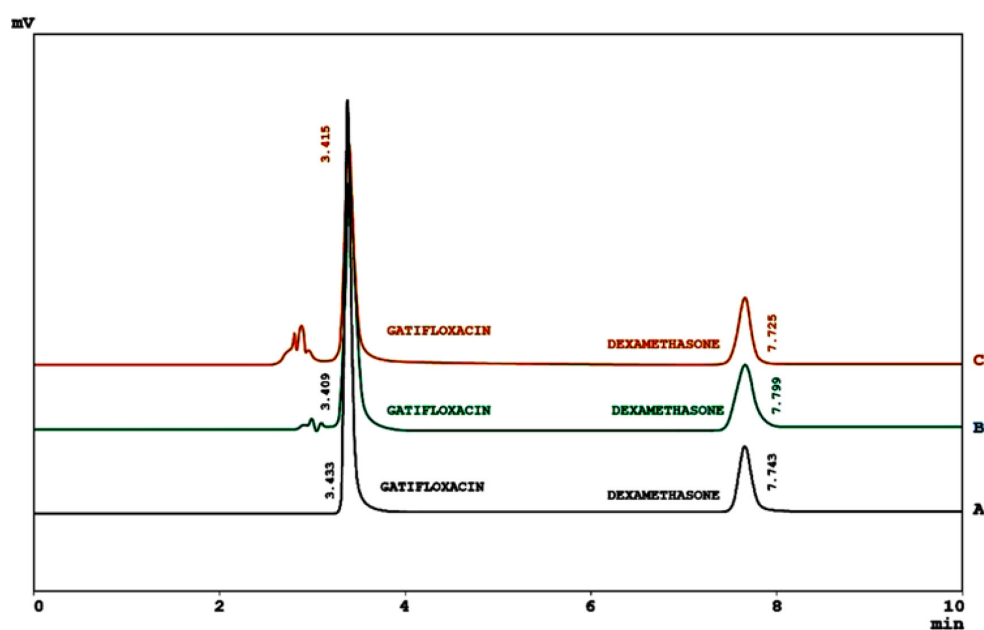
In this study, an RP-HPLC method was developed for simultaneous analysis of gatifloxacin and dexamethasone in different matrices like pharmaceuticals, human serum and urine. Method validation was performed as recommended by ICH. The optimized method is recommended to be used for concurrent analysis of gatifloxacin and dexamethasone in different matrices because of its excellent results with inexpensive reagents.

TABLE V - Stress testing results of gatifloxacin and dexamethasone

Nature of stress	Storage condition (°C/%RH)	Time (h)	Amount of gatifloxacin remaining \pm RSD (%)	Amount of dexamethasone remaining \pm RSD (%)	Extent of decomposition
5 M HCl	(40/75)	1	100.1 \pm 1.2	101.3 \pm 0.30	None
	(40 /75)	8	99.7 \pm 1.0	97.6 \pm 1.1	None
	(40/75)	16	97.2 \pm 0.80	90.0 \pm 1.5	Slight
	(40/75)	23	100.3 \pm 0.90	68.6 \pm 1.2	Substantial
5 M NaOH	(40/75)	16	93.7 \pm 1.7	4.8 \pm 2.3	Substantial
6% H2O2	(40/75)	1	98.6 \pm 1.5	100.8 \pm 1.5	None
	(40/75)	8	101.2 \pm 1.4	100.5 \pm 1.4	None
	(40/75)	23	95.5 \pm 1.4	100.6 \pm 1.5	None
	(40/75)	70	96.8 \pm 1.1	96.7 \pm 1.9	None
Thermal	(40/75)	1	100.3 \pm 0.80	99.5 \pm 1.5	None
	(40/75)	16	99.7 \pm 0.40	100.4 \pm 1.4	None
	(40/75)	24	100.0 \pm 1.3	102.8 \pm 1.5	None
	(40/75)	70	98.5 \pm 1.0	93.2 \pm 1.7	None
Photolytic	Sunlight	2	99.8 \pm 1.9	102.9 \pm 1.2	None
	Sunlight	4	98.6 \pm 1.4	102.3 \pm 1.4	None
	Sunlight	6	91.3 \pm 1.9	78.4 \pm 1.4	Substantial

TABLE VI - Assay results of gatifloxacin and dexamethasone in commercial eye drops

Product	Ingredient	Label value (mg/mL)	Recovery (%) \pm RSD (%)
GATE DX	gatifloxacin	3	100.3 \pm 0.30
	dexamethasone	1	99.00 \pm 0.70
GATIBLU-D	gatifloxacin	3	100.7 \pm 0.10
	dexamethasone	1	98.90 \pm 0.70

**FIGURE 3** - Chromatograms of gatifloxacin and dexamethasone in Pharmaceutical Formulations (A), Human Serum (B) and Urine (C).

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