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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BROMFENAC SODIUM AND MOXIFLOXACIN IN THEIR COMBINED DOSAGE FORM

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ABSTRACT
A simple, rapid, accurate and precise method was developed for Bromfenac sodium and Moxifloxacin. BDS Hypersil C-18 column (250 mm × 4.6 mm id) 5 µm particle size was used as stationary phase. The mobile phase used was Buffer KH_2PO_4: Acetonitrile : Triethylamine at pH 4.0 adjusted with Ortho phosphoric acid. The mobile phase was delivered at flow rate 1.0 ml/min. UV detection was set at 275 nm. The retention time for Bromfenac sodium and Moxifloxacin was found 4.637 minutes and 7.630 minutes respectively. The linearity was observed over the range of 2.25-6.75 mcg/ml and 12.5-37.5 mcg/ml for Bromfenac sodium and Moxifloxacin respectively. The LOD was found 0.46 mcg/ml and 0.64 mcg/ml for Bromfenac sodium and Moxifloxacin respectively; whereas LOQ was found to be 1.41 mcg/ml for Bromfenac sodium and 1.94 mcg/ml for Moxifloxacin. Moreover, the % RSD for repeatability, Inter and intra-day precision was found to be less than 2 % which reveals method is precise. The correlation co-efficient found to be 0.997 and 0.999 for Bromfenac sodium and Moxifloxacin respectively. The % recovery was found to be 99.79-100.09 % for Bromfenac sodium and 99.90-100.03 % for Moxifloxacin. The assay percentage was found to be 98.65 % and 97.50 % for Bromfenac and Moxifloxacin respectively. All the validation parameters were checked according to ICH guidelines finally it is concluded that the developed method is precise and accurate.
Keywords: Bromfenac sodium, Moxifloxacin, Method validation, HPLC.

INTRODUCTION

The aim of present work is to develop simple, rapid, precise and economical method and to validate the method according to ICH guidelies. Various articles from different journals show at the time I have started my work that there was no RP-HPLC method developed. Bromfenac sodium is non-steroidal anti inflammatory drug (NSAID) for ophthalmic use. Chemically it is known as 2-[2-amino-3-(4-bromobenzoyl)phenyl]acetic acid sodium salt. Ophthalmic NSAIDs are becoming a counterstone for management of ocular pain and inflammation. Their well-characterized anti-inflammatory activity, analgesic property and established safety record have also made NSAIDs an important tool to optimize surgical outcomes. The high degree of penetration and potency of bromfenac sodium can be attributed to halogenations of molecule: by adding bromine moiety the NSAIDs becomes highly lipophilic which allows for rapid, sustained drug levels in the ocular tissues. Moxifloxacin is an antibiotic in a group of drugs called fluoroquinolones (flor-o-KWIN-o-lones). Moxifloxacin fights bacteria in the body. Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibacterial agent. It is marketed worldwide (as the hydrochloride) under the brand names Avelox, Avalox and Avelon for oral treatment. Moxifloxacin is used to treat different types of bacterial infections, Respiratory tract infections, Cellulitis, Intra-abdominal infections, Endocarditis, Meningitis and Tuberculosis. Moxifloxacin may also be used for purposes not listed in this medication guide. Chemically it is known as 1-cyclopropyl-7-[(1s,6s)-2,8-diaz[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-quinoline carboxylic acid[1,2,4,5].

MATERIALS AND EQUIPMENTS

API samples were gifted by sun pharma ltd. The combination pharmaceutical dosage form used for experiment was manufactured by opticarma pharma ltd, HPLC grade methanol (Samir Tech-Chem Pvt Ltd.), Orthophosphoric acid, Phosphate buffer, Sonicator (EIE instruments Pvt ltd.), pH meter (Welltronix PM100), Micro analytical balance (Shimadzu BL-220H), HPLC instrument (Shimadzu LC 20AT) etc were used.

Optimized Chromatographic Condition

The chromatographic column BDS hypersil C-18 (250 mm × 4.6 mm id × 5 µm particle size) was used as stationary phase and Buffer (0.1 M Potassium Dihydrogen Phosphate with Orthophosphoric acid, pH 4.0), Acetonitrile and Triethylamine in ratio of 55:45:0.1 %v/v/v was used. The flow rate was set at 1.0 ml/min. The column temperature maintained at 25°C. The elluent was detected at 275 nm with 20 µl of injection volume.

Selection of analytical wavelength

9 mcg/ml and 50 mcg/ml solutions of Bromfenac sodium and Moxifloxacin were being made. The solutions were scanned in range of 400-200 nm. The overlay graph shows that both the drugs can significantly detected at 275 nm.

Preparation of standard stock solution of Bromfenac sodium

Weigh accurately 4.5 mg of Bromfenac sodium into 100 ml volumetric flask and dissolve in 10 ml of diluents. Then the flask was sonicated for 10 minutes. Volume was made up to the mark to get 45 mcg/ml solutions. From this take 1 ml to the 10 ml volumetric flask and volume was adjusted with diluents to get the final concentration of 4.5 mcg/ml.

Preparation standard stock solution of Moxifloxacin

Weigh accurately 25 mg of Moxifloxacin into 100 ml volumetric flask and dissolve in 10 ml of diluents. Then the flask was sonicated for 10 minutes. Volume was made up to the mark to get 250 mcg/ml solutions. From this take 1 ml to

the 10 ml volumetric flask and volume was adjusted with diluents to get the final concentration of 25 mcg/ml.

Preparation of mobile phase

A mixture of 55 ml phosphate buffer, 45 ml acetonitrile and 0.1 ml of triethylamine was filtered through 0.45 µm filter paper then sonicated for 10 minutes. It was then used as mobie phase.

Preparation of 0.1 M Phosphate Buffer

Potassium dihydrogen phosphate 1360 mg was accurately weighed and dissolved in 100 ml of HPLC grade water pH was adjusted to 4.0 with the help of orthophosphoric acid.

Assay

Prepare standard stock solutions of both the drugs as given above. Prepare sample solutions from marketed formulation; from the formulation take 0.5 ml in volumetric flask make up volume up to 10 ml from this take 1 ml make up to 10 ml which gives solution of 4.5 mcg/ml of bromfenac sodium and 25 mcg/ml of moxifloxacin. Both were measured at 275 nm and % assay is calculated.

Method

Validation

The developed method is validated with respect to validation parameters like accuracy, precision, linearity and range, LOD and LOQ, Robustness, ruggedness, system suitability etc.

Table 1: Linearity Data of Bromfenac Sodium and Moxifloxacin

	BROM		MOXI	
	Conc.(mcg/ml)	Area	Conc.(mcg/ml)	Area
	2.25	950.515	12.5	2187.756
	3.375	1308.052	18.75	3142.431
	4.5	1852.875	25	4272.08
	5.625	2288.957	31.25	5277.285
	6.75	2731.848	37.5	6292.076
SLOPE	315.9		218.5	
SD	44.53		42.50	
LOD	0.46		0.64	
LOQ	1.40		1.94	

Table 2: Intraday and Interday Precision of Brom

Concentration (µg/ml)	Intraday (Area* ± SD)	% RSD	Interday (Area* ± SD)	% RSD
2.25	929.37 ± 1.85	0.199	930.3 ± 1.84	0.198
4.5	1879.20 ± 3.74	0.199	1881.08 ± 3.73	0.198
6.75	2811.79 ± 5.598	0.199	2814.59 ± 5.61	0.199

Table 3: Intraday and Interday Precision of Moxi

Concentration (µg/ml)	Intraday (Area* ± SD)	% RSD	Interday (Area* ± SD)	% RSD
12.5	2130.32±11.51	0.54	2137.66±3.07	0.143
25	4319.54±3.899	0.09	4330.81±5.43	0.125
37.5	6467.69±7.296	0.11	6475.03±6.74	0.104

Table 4: Repeatability Data of Bromfenac Sodium and Moxifloxacin

	BROM		MOXI	
	Conc.(mcg/ml)	Area	Conc.(mcg/ml)	Area
	4.5	1884.886	25	4345.734
	4.5	1888.685	25	4354.458
	4.5	1892.401	25	4357.211
	4.5	1886.754	25	4350.086
	4.5	1890.504	25	4358.816
	4.5	1894.331	25	4354.511
AVERAGE	1889.593		4353.469	
SD	3.527		4.815	
% RSD	0.186		0.110	

Table 5: Robustness Data of Brom and Moxi

Conditioned varied	Changed condition	Area		%RSD	
		BROM (4.5 µg/ml) ± SD	MOXI (25 µg/ml) ± SD	BROM	MOXI
Change in flow rate of mobile phase (ml/min)					
Change in flow rate	0.8 ml/min	1962.063 ± 11.43	4517.89 ± 16.91	0.582	0.374
	1.2 ml/min	1848.867 ± 11.33	4258.18 ± 19.78	0.613	0.464
Change in contents of mobile phase (Buffer: Acetonitrile :Triethylamine55:45:0.1%v/v/v)					
Change in mobile phase	57:47	1847.01 ± 11.33	4253.05 ± 18.68	0.613	0.439
	53:43	1939.24 ± 11.39	4465.86 ± 17.58	0.587	0.393
Change in pH of the mobile phase					
Change in pH mobile phase	4.2	1809.61 ± 11.275	4164.57 ± 17.17	0.623	0.412
	3.8	1940.54 ± 12.38	4471.89 ± 23.87	0.638	0.533

Table 6: Recovery Data of Bromfenac Sodium and Moxifloxacin

	Conc. Level (%)	Amount of sample present (µg)	Amount of std added (µg)	Amount found (µg)	% Recovery mean ± % RSD
BROM	80	2.25	1.8	1.80	100.09 ± 1.12
	100	2.25	2.25	2.23	99.79 ± 0.65
	120	2.25	2.7	2.69	99.79 ± 0.49
MOXI	80	12.5	10	9.99	100.02 ± 1.1
	100	12.5	12.5	12.49	100.01 ± 0.71
	120	12.5	15	14.98	99.91 ± 0.61

Table 7: System Suitability Data of Brom and Moxi

Drugs	Retention time (Rt) (minute)	Tailing Factor	Theoretical plates	Resolution
Bromfenac Sodium (4.5 µg/ml)	4.637	1.448	7423	
Moxifloxacin (25 µg/ml)	7.630	1.451	6277	9.970

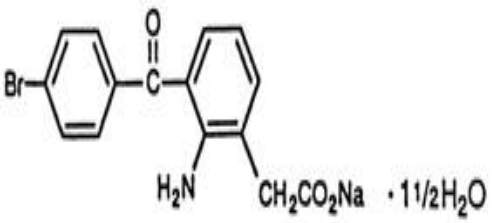

Figure 1: Structure of bromfenac sodium[4]

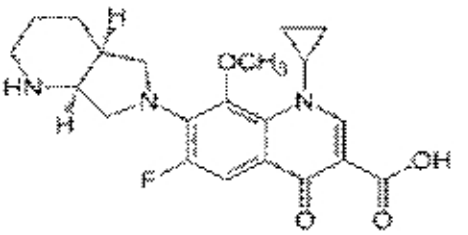

Figure 2: Structure of moxifloxacin[5]

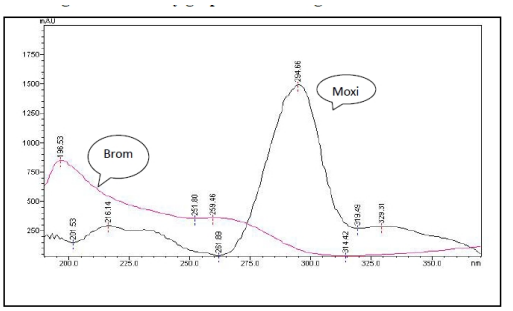

Figure 3: Overlain Spectra of Both Drugs

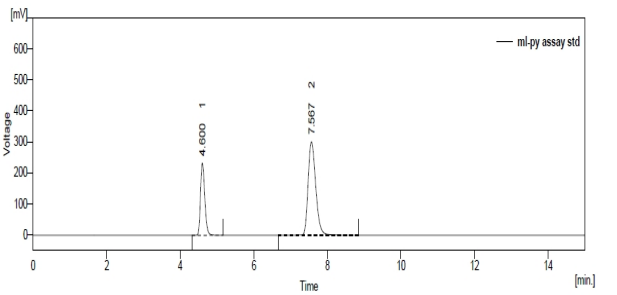

Figure 4: Mix Standard Chromatogram of Moxi and Brom

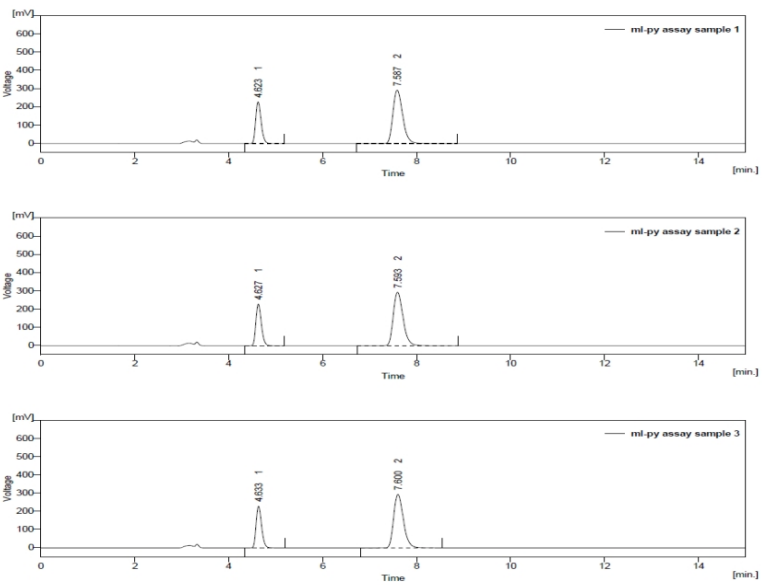

Figure 5: Chromatogram of Test Samples

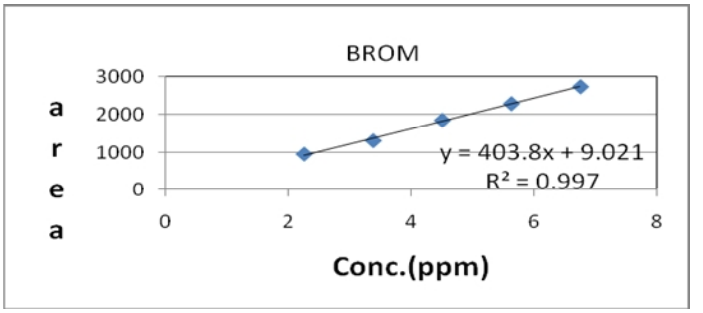

Figure 6: Linearity Chart of Bromfenac Sodium

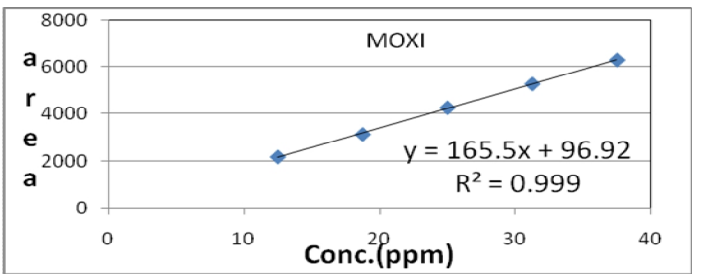

Figure 7: Linearity Chart of Moxifloxacin

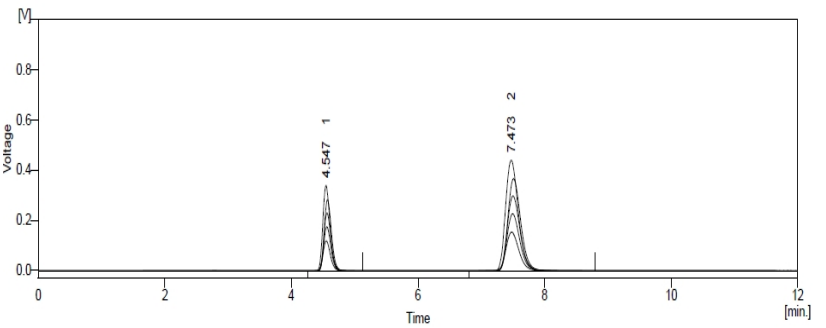

Figure 8: Overlain Chromatogram of Linearity of Both Drugs

RESULTS

Initially solubility test were conducted in various solvents and finally mobile phase was selected from it. To optimized chromatographic condition several trials were taken for system suitability. The graphs given below give idea about it. The assay was performed under optimized condition which gives chromatographs. The mobile phase used was Buffer KH_2PO_4: Acetonitrile: Triethylamine at pH 4.0 adjusted with Ortho phosphoric acid. The mobile phase was delivered at flow rate 1.0 ml/min. UV detection was set at 275 nm. The retention time for Bromfenac sodium and Moxifloxacin was found 4.637 minutes and 7.630 minutes respectively. The linearity was observed over the range of 2.25-6.75 mcg/ml and 12.5-37.5 mcg/ml for Bromfenac sodium and Moxifloxacin respectively. The LOD was found 0.46 mcg/ml and 0.64 mcg/ml for Bromfenac sodium and Moxifloxacin respectively; whereas LOQ was found to be 1.41 mcg/ml for Bromfenac sodium and 1.94 mcg/ml for Moxifloxacin. Moreover, the % RSD for repeatability, Inter and intra-day precision was found to be less than 2 % which reveals method is precise. The correlation co-efficient found to be 0.997 and 0.999 for Bromfenac sodium and Moxifloxacin respectively. The % recovery was found to be 99.79-100.09 % for Bromfenac sodium and 99.90-100.03 % for Moxifloxacin. The assay percentage was found to be 98.65 % and 97.50 % for Bromfenac and Moxifloxacin respectively. All the validation parameters were checked according to ICH guidelines. The tables contain data of Assay, Precision, repeatability, Robustness, % Recovery and system suitability.

DISCUSSION

The assay was performed on the eye drops and % drug contents found to be 98.65 % and 97.50 % for Bromfenac sodium and Moxifloxacin respectively. Limit for precision is % RSD should not be more than 2 %. Limit for repeatability % RSD should not be more than 2 %. Limit for robustness % RSD should not more than 2 %. Limit for % Recovery % RSD should not more than 2 %. N = 3 determinations of 3 concentrations.

CONCLUSION

It is concluded that the developed method is simple, rapid, accurate and precise. All the validation parameters checked according to ICH guidelines and they were in the limits so it is concluded that the developed method is simple, precise and rapid. It is also concluded that this method can be used to

analyze the drugs in bulk as well as in combination and also can be used in industry.

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REFERENCES

1. Ahuja S and Rasmussen HT. HPLC Method Development for Pharmaceuticals. Academic Press; 2007.
2. Ahuja S and Dong MW. Handbook of Pharmaceutical Analysis by HPLC. Elsevier/Academic Press; 2005.
3. Kasture AV, Vadodkar SG, Manik KR and More HN. Pharmaceuticals analysis. 17th ed. Niraliprakashan; 2007.
4. www.cdsco.nic.in/listofdrudarovedmain.html
5. www.drugs.com/monograph/bromfenac
6. www.drugs.com/monograph/moxifloxacin
7. Patel M, Patel V and Koradia S. Development and validation of colorimetric method for the estimation of bromfenac sodium in bulk and pharmaceutical formulation. Intern Journal of universal Pharmacy and bio sciences 2013; 217-228.
8. Nambiar H, Subrahmanyam EVS and Shabaraya AR. Development of new analytical method for the determination of bromfenac in bulk and marketed formulation and its validation. Intern Journal of Pharmtech research 2013; 5 suppl 2: 486-491.
9. Patel M, Gohil M and Koradia S. Development and validation of stability indicating rp-hplc method for estimation of bromfenac sodium in the Pharmaceutical Formulation. Intern Journal of universal Pharmacy and bio sciences 2013; 438-453.
10. Boddeda B, Ramaprasad LA, Vijayaratna J and Biswal A. Rapid UPLC method for estimation of bromfenac and alication to eye drops. Intern Journal of Pharmacy and Pharmaceutical Sciences 2013; 5 suppl 2: 456-461.
11. Rama subbaiah P, Kumudhavalli MV, Saravanan C, Kumar M and Margret chandira R. Method development and validation for estimation of moxifloxacinhcl in tablet dosage form by RP-HPLC method. Pharm Anal Acta 2010; 2 suppl 1: 1-2.
12. Sultana N, Saeed AM, Akhtar M, Shamim S, Gul S and Mehboob KM. High-performance liquid chromatography assay for moxifloxacin in bulk, pharmaceutical formulations and serum: alication to *in-vitro* metal interactions. J. Chin. Chem. Soc. 2010; 57 suppl 4A: 1-10.
13. Abdellaziz LM and Hosny MM. Development and validation of spectrophotometric, atomic absorption and kinetic methods for determination of moxifloxacin. Analytical Chemistry Insight; 2011. p. 67-78.
14. Patel S and Patel K. Spectrophotometric estimation of loteprednoletabonate and moxifloxacin Hcl in eye drops by Q-absorbance ratio method. Int. Res. J. Pharm 2013; 4 suppl 1: 186-189.
15. Patel M, Kakadiya J and Shah N. Development and validation of first order derivative spectrophotometric method for simultaneous estimation of cefiximetrihydrate and moxifloxacin hydrochloride in combined tablet dosage form. Asian Journal of Pharm Science and Technology 2013; 3 suppl 1: 19-24.
16. Razzaq S, Khan I, Mariam I and Razzaq S. Stability indicating HPLC method for the simultaneous determination of moxifloxacin and prednisolone in pharmaceutical formulations. Chemistry Central Journal; 2012. http://dx.doi.org/10.1186/1752-153X-6-94
17. Dabhi MJ, Patwari AH, Desai UH, Doshi DB, Rathod IS and Suhagia BN. Simultaneous determination of moxifloxacin hydrochloride and dexamethasone sodium phosphate in eye drops by HPLC and absorbance correction method. Journal of Chemical and Pharmaceutical Research 2012; 4 suppl 10: 4462-4467.
18. Parmar A, Parmar R, Patel V and Shah D. The simultaneous estimation of moxifloxacin hydrochloride and bromfenac sodium in eye drops by UV. Journal of pharm sci and bioscientific research 2012; 2 suppl 1: 36-39.
19. Vyas PJ, Dave JB, Patel CN. Simultaneous estimation of Moxifloxacin hydrochloride and bromfenac Sodium in eye drops by spectrophotometric methods. International Journal of Pharmaceutical Sciences and Research 2012; 3 suppl 7: 2137-2142.
20. Reddy N, Samidha T, Sushma E, Sagar P, Sudheerkumar D, Sreekanth G. Simple RP-HPLC method development and validation for simultaneous estimation of Moxifloxacin hydrochloride and Bromofenac sodium on eye drops. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5suppl 4: 689-698.

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