

Simultaneous quantitation of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium in affixed dose combinative formulation by HPLC-UV method

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Abstract: A high-pressure liquid chromatography (HPLC-UV) based simple and specific method for simultaneous quantitative determination of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium has been developed and validated according to ICH guidelines. Chromatographic separation of the three drugs was carried out on 4.6 x 250mm x 5 μ Licospher RP Select B Column, using mobile phase constituted of methanol and phosphate buffer pH 3.5 (650: 350), pH adjusted to 3.5 \pm 0.05 with dilute ortho-phosphoric acid and delivered at a flow rate of 1ml/min. The eluents were detected at UV wavelength of 220nm and the retention times for Ofloxacin, Fexofenadine HCl and Diclofenac Potassium were 2.5 minutes, 4 minutes and 11.5 minutes, respectively. This method is suitable and specific for the three drugs and was found to be linear ($R^2 > 0.996$), accurate, specific, reproducible and robust over a concentration range of 0.05 to 0.15mg/ml for Ofloxacin, 0.015 to 0.045mg/ml for Fexofenadine HCl and 0.0125 to 0.0375mg/ml for Diclofenac Potassium. The proposed method is simple and convenient, hence easily utilized for the characterization and quantitation of the three drugs in a single formulation for combination therapy of rheumatoid arthritis, sepsis, infection with fever and flu.

Keywords: Ofloxacin, Fexofenadine HCl and Diclofenac Potassium, HPLC-UV, analysis, validation.

INTRODUCTION

Major health problems in countries like Pakistan arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. (Gal, 1965) Diseases like sepsis, rheumatoid arthritis, chest infection, uncomplicated cystitis, bronchospasm etc. are threat to human life, and doctors often prescribe antibiotic, anti-inflammatory & anti allergic in individual dosage form for the treatment. Multiple unit dosage or drugs at a same time cause several discomfort to patients. They may forget to take different medicine on time. This therapy is also expensive for patients of developing countries. Therefore, combination pharmacotherapy has been implemented which provide greater efficiency than traditional care. Combining several drugs at low dose is likely to be more effective and have fewer side effects than high dose therapy with a single dose. One more advantage of fixed dose combination is to improve medication compliance by reducing the pill burden of patients.

Ofloxacin is a synthetic broad-spectrum antimicrobial agent. Ofloxacin, a fluorinated carboxyquinolone, is racemate (\pm)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-

6-carboxylic acid. It is slightly soluble in water, alcohol, dichloromethane, and methyl alcohol; sparingly soluble in chloroform. The solubility of ofloxacin varies depending upon pH. (Arayne, Sultana, Shehnaz, & Haider, 2011) It is a broad-spectrum antibiotic, which belongs to the third generation of fluorinated quinolone. Ofloxacin is indicated for the treatment of adults with mild to moderate infections (Ferraro, 1997) (Standards, 1997). The antibacterial action, the good tolerance and the easy administration of Ofloxacin make it a useful antibiotic in the treatment of osteoarticular infections. (Lopitiaux, Hermet, Sirot, Terver, & Levai, 1988) and is one of the most frequently used fluorinated quinolone antibiotics. (Gal, 1965) Fexofenadine hydrochloride, is a histamine H₁-receptor antagonist with the chemical name (\pm)-4-[1 hydroxy-4-[4- (hydroxydiphenylmethyl)-1-piperidiny]-butyl]- α , α -dimethyl benzene acetic acid hydrochloride. Its molecular mass is 501.68gm/mol. It is freely soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane. (Aventis Pharmaceuticals, 2000). Fexofenadine hydrochloride is a medicine, which is used in seasonal allergic rhinitis. (Lopitiaux *et al.*, 1988) It has greater antihistamine activity than loratadine. (Boyle, Ridout, Meadows, Johnsen, & Hindmarch, 2005). Diclofenac potassium is a non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities. Its formula is C₁₄H₁₁Cl₂NO₂ Its molecular mass is 296.148

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gm/mol. It is freely soluble in methanol, soluble in ethanol and practically insoluble in chloroform and in dilute acid. Diclofenac potassium is soluble in water. The n-octane/water partition coefficient is 13.4 at pH 7.4 and 1545 at pH 5.2 (Aventis Pharmaceuticals, 2000). It is used to treat pain, inflammatory disorders, and dysmenorrhea. (Lopitiaux *et al.*, 1988) It is often used to treat chronic pain associated with cancer, (World Health Organization scheme for treatment of chronic pain). (Massart, Vandeginste, Buydens, Lewi, & Smeyers-Verbeke, 1997) Therefore, it may have the capacity to treat uncomplicated urinary tract infections caused by *E. coli*. (Mazumdar, Dutta, Dastidar, Motohashi and Shirataki, 2006) Use of a COX-2 inhibitor with an anti-allergic produces significantly more relief for most symptoms. The significance of this is as yet unclear, but it is hoped that these results will further our understanding of chronic inflammation. (Takechi, 2004)

Although various analytical methods based on HPLC-UV have been developed for the quantitation of Ofloxacin, Fexofenadine HCl & Diclofenac potassium individually & in combination with other drugs. (Groeneveld & Brouwers, 1986) (Arayne *et al.*, 2011) (Pandey, 2011) (D. Venkatanarayana Rao & Ravindranath, 2012) (Nag *et al.*, 2012). But there has been no report in the literature on the simultaneous determination of these drugs. Therefore, in the present study a HPLC-UV based simple, accurate, precise and robust analytical method has been developed and validated for a combination formulation. To the best of our knowledge, this is the first study, which reports an analytical method for simultaneous determination of these aforementioned drugs.

MATERIALS AND METHODS

Chemicals and reagents

Ofloxacin was obtained from Jiangxi Dadi Pharmaceutical Limited China. Fexofenadine HCl was supplied from Ami Life Sciences (Pvt.) Ltd, India. Diclofenac potassium was received from Amoli organics (Pvt.) Ltd, India. HPLC grade methanol and sodium dihydrogen phosphate monohydrate were purchased from Merck KGA, Germany and Ortho-phosphoric acid was provided from Sigma Aldrich, Germany.

Instrumentation and chromatographic conditions

Separation of drugs and their subsequent analysis (eluent) were performed on single chromatographic system composed of LC-10AT chromatographs (SSI, Kyoto, Japan) and SPD-10AVPUV-detector (SSI, Kyoto, Japan). The parameters were recorded by Chromatography Station for Windows (CSW) (Water, USA). For the separation of drugs Licrospher RP select B (Waters Corporation, MA, USA) reverse phase column (4.6x250mm; 5 μ m) was used as a stationary phase.

Preparation of mobile phase

Phosphate buffer pH 3.5 was prepared by dissolving 2.72g sodium dihydrogen phosphate monohydrate in 800ml purified water, pH adjusted to 3.5 \pm 0.05 with dilute phosphoric acid and volume was made up with purified water up to 1000ml. Mobile Phase was prepared by mixing methanol and phosphate buffer pH 3.5 in a ratio of 650:350 and pH was then adjusted to 3.5 \pm 0.05 with dilute ortho-phosphoric acid. The prepared mobile phase was passed through 0.2 μ m membrane filter (Millipore, USA) and degassed by using ultrasonic bath.

Preparation of standard and sample solutions

Standard solution of Ofloxacin 100mg, Fexofenadine HCl 60mg and Diclofenac Potassium 50mg were prepared by using mobile phase as diluent at final concentration of 0.1mg/ml, 0.03mg/ml and 0.025mg/ml respectively. Sample solution was prepared by crushing 20 tablets and mean weight of each formulation was taken, diluted with mobile phase to a concentration equivalent to standard. Placebo solution was prepared by weighing all the excipients separately, mixed in the diluent and then filtered the solution through 0.2 μ m membrane filter.

Method validation

The newly developed HPLC-UV based analytical procedure for simultaneous determination of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium in the formulation was validated according to guidelines of International Conference on Harmonization (ICH). The parameters included were system suitability and specificity, linearity, accuracy, precision, reproducibility, robustness and range (Guideline, 2005).

System suitability and specificity

Suitability of the system for performing the analysis was determined by running 5 replicates each of blank (mobile phase) and standard solutions. Area under the curves of individual drugs (replicates) in the standard solution were calculated for percentage relative standard deviation (%RSD; $\leq 2\%$), theoretical plates (≥ 1000) and tailing factor (≤ 2.0) as per USP.

System specificity for the objective analytes were evaluated by injecting placebo (formulation constituents without active drug ingredients), blank (mobile phase only) and standard solutions separately and respective chromatograms were observed for any interference between the active drug ingredients and excipients present in the finished formulation.

Linearity and accuracy

The linear range of most analytical instruments is known to be limited. Therefore, during method validation the linearity of the calibration curve should be assessed and the working range of the calibration curve should be determined. (European Economic Community, 1990) (Massart *et al.*, 1997). To estimate the linear

proportionality of the yields with the concentration of analytes, system linearity was calculated from 50% to 150% of standard concentrations. Five concentrations (50 %, 80%, 100 %, 120%, and 150 %) of standard solution were prepared by diluting the stock standard solution and run on HPLC. The accuracy of the system was determined by evaluating the percent recovery of three concentrations (50 %, 100 % and 150 % of standard solution) in triplicate.

Precision and intermediate precision

Precision of the system was carried out by comparing the percentage assay of six independently prepared samples with standard (100%) solution.

Intermediate precision of the systems was carried out by performing assay tests each on two separate but similar instruments by two individual analyst son two different days. All the other procedures and factors were kept same as mentioned above for precision testing.

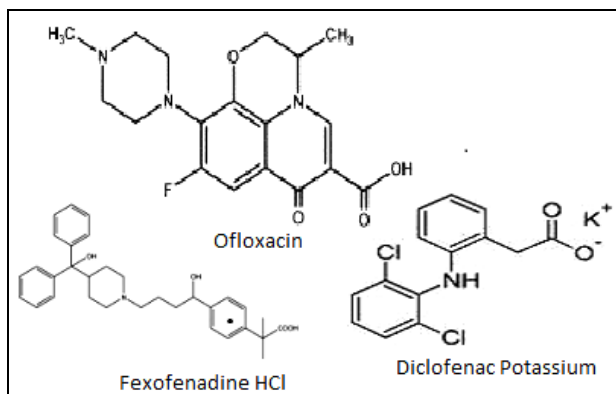


Fig. 1: Structural formula of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium.

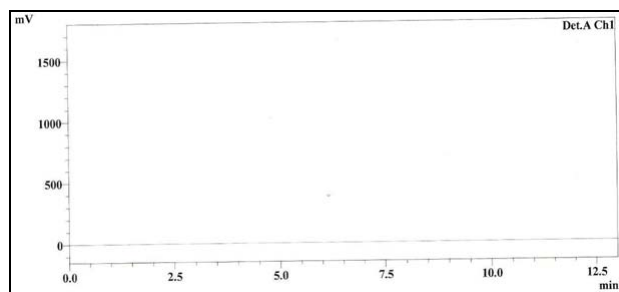


Fig. 2: HPLC-UV chromatogram of placebo solution was showing no chromatographic Interference in the developed method due to any additive material found in the formulation

Robustness

To measure the capacity of this newly developed analytical procedure to withstand small changes in the method, samples of standard solution were run in mobile phases with different pH values (pH 3 & 4). More overestimation of assay was also carried out at two different wavelengths (215 nm and 225nm).

Range

To determine the range of this analytical procedure, various concentrations prepared for linearity testing were calculated to determine highest and lowest possible concentrations with acceptable accuracy and precision.

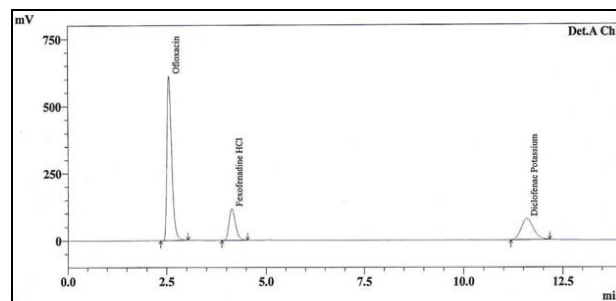


Fig. 3: HPLC-UV chromatogram of standard solution showing resolution of peaks Ofloxacin, Fexofenadine HCl and Diclofenac Potassium.

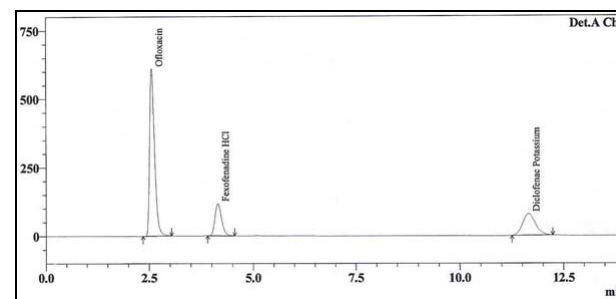


Fig. 4: HPLC-UV chromatogram of sample solution showing resolution of peaks of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium.

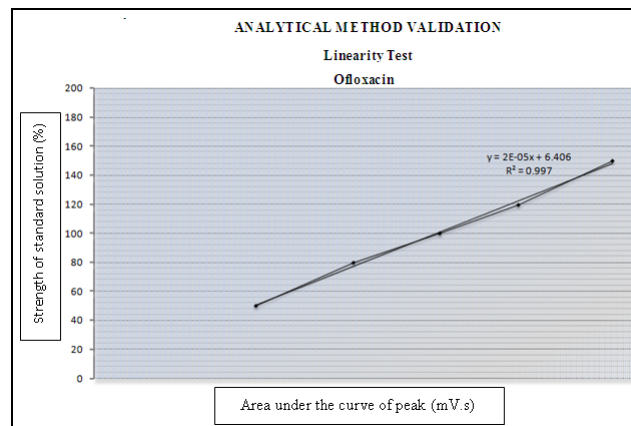


Fig. 5: Graph showing linearity of Ofloxacin.

RESULTS

Preliminary investigations for robust HPLC based analytical method for simultaneous determination of Ofloxacin, Fexofenadine HCl & Diclofenac Potassium were made in the light of reported literature. The eluents were determined at different wavelengths in the range of 210 to 230nm and the final detection was carried out at 220nm with good selectivity and sensitivity. After

different runs, 65% (v/v) methanol solution in deionized (DI) water was selected as the best possible composition for mobile phase. The pH of the mobile phase also affected the quality of eluent's peaks, therefore, after multiple runs at different pH values; pH 3.5 (adjusted with dilute orthophosphoric acid) was finally selected. The retention times for the three eluents were found to be 2.5 minutes for Ofloxacin, 4.0 minutes for Fexofenadine HCl and 11.5 minutes for Diclofenac potassium in the standard and sample solutions as shown in figs. 2 and 3.

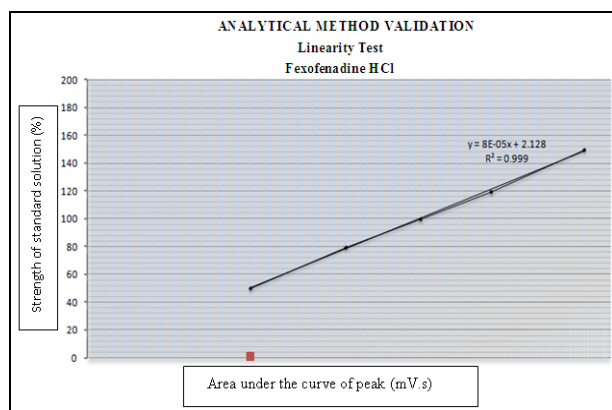


Fig. 6: Graph showing linearity of Fexofenadine HCl

DISCUSSION

Method validation

The validation of this newly developed analytical procedure for simultaneous quantitative determination of Ofloxacin, Fexofenadine HCl & Diclofenac Potassium was successfully demonstrated following the criteria set as per ICH (European Economic Community, 1990). The validation of this procedure included all the parameters ascribed in USP for validation of compendia methods under category-I18. Method development was right from optimization of the condition and parameters i.e. selection of system, column, mobile phase. A new chromatographic method has been developed and subsequently validated as per ICH guidelines and also followed the compendia requirements (USP).

System suitability and specificity

Suitability of this analytical method with the system was evaluated by calculating % RSD for peak areas, theoretical plates and tailing factor of 5 replicate runs of the standard solution. All the parameters were satisfactory as per USP requirements for % RSD ($\leq 2\%$), theoretical plates (≥ 1000) and tailing factor (≤ 2.0). Parameters calculated for system suitability of Ofloxacin, Fexofenadine HCl & Diclofenac Potassium are given in table 1.

Placebo (excipients without active ingredients), standard solution and blank were run on HPLC in order to investigate interference of any excipient with the elution

of objective analytes. No chromatographic interference was observed in the developed method due to any additive material found in the formulation as illustrated in figs. 2, 3 and 4.

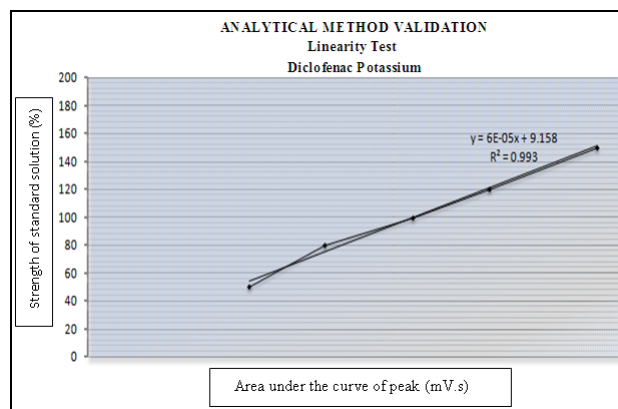


Fig. 7: Graph showing linearity of Diclofenac potassium

Linearity and accuracy

The estimation of degree of proportionality between the concentration of analytes and their respective amounts recovered after analyses was made by performing linearity testing. Five working concentrations (50 %, 80%, 100 %, 120 %, and 150 %) of standard solution were run and their respective amounts recovered were found to be linearly correlated (Residual sum of square ≥ 0.992) for all the three drugs which were all above the USP limit of ≥ 0.99 as shown in figs. 5, 6, 7 (table 2).

The accuracy of the method was determined by 3 replicate runs of three different concentrations (50%, 100% and 150%) of standard solution. The system was found to be very accurate as the mean recovery of analytes obtained was found well within the range of 98% to 102% with % RSD calculated ≤ 2.0 as per USP requirements given in table 3.

Precision and intermediate precision

The newly established method was highly precise as the % RSD of mean recovery of 6 independently prepared standard solutions were 0.523%, 0.715% and 1.16% for Ofloxacin, Fexofenadine HCl and Diclofenac Potassium respectively (table 4). These values were within the USP range of ≤ 2.0 %.

Intermediate precision of the method was also observed to be satisfactory as the overall mean % RSD of 6 assays of standard solution on two separate instruments by two individual analysts on two different days were 0.161, 0.091 and 0.519 for Ofloxacin, Fexofenadine HCl and Diclofenac Potassium respectively which were again well within the USP range of $\leq 2.0\%$ (table 4).

Robustness

No significant difference in the recovery of eluents was

Table 1: System suitability parameters

Analyte (n=5)	Area of Replicate 1	Area of Replicate 2	Area of Replicate 3	Area of Replicate 4	Area of Replicate 5	Mean Area	% RSD	USP Theoretical plates	USP Tailing Factor
Ofloxacin	4739518	4760220	4794119	4719603	4800898	4762871.6	0.73086584	2361.654	1.417
Fexofenadine HCl	1167737	1165187	1168887	1168722	1168706	1167847.8	0.13315575	2707.808	1.0786
Diclofenac Potassium	1575872	1619984	1617202	1572108	1629493	1602931.8	1.67463823	2508.875	0.986

Table 2: Calculated parameters for method linearity peak areas of various concentrations.

Analyte (n=3)	50 %	80 %	100 %	120 %	150 %	Slope	Intercept value	Correlation coefficient	Residual sum of square
Ofloxacin	2317306	3708561	4948394	6069836	7417628	0.000	6.406	0.998	0.997
Fexofenadine HCl	582551	921761	1189722	1440676	1774266	0.000	2.129	0.999	0.999
Diclofenac Potassium	805718	1182760	1622621	2007377	2545418	0.000	9.158	0.996	0.993

Table 3: Calculated parameters for method accuracy Percent mean recovery of standard solution with various concentrations.

Analyte	50 %	100 %	150 %	Over all Mean % Recovery	Over all Standard Deviation	Over All % RSD
Ofloxacin	99.593	100.032	100.236	99.950	0.480	0.480
Fexofenadine HCl	100.459	99.783	99.832	100.024	0.575	0.575
Diclofenac Potassium	100.102	100.088	99.645	99.945	0.448	0.448

Table 4: Calculated parameters for method precision reproducibility and precision intermediate. Mean drug recovered (%)

Analyte (n=6)	Analyst 1		Analyst 2		Over all Mean % Recovery	Over all %RSD
	Mean	% RSD	Mean	% RSD		
Ofloxacin	99.438	0.523	99.210	1.800	99.320	0.161
Fexofenadine HCl	99.640	0.715	99.510	1.070	99.570	0.091
Diclofenac Potassium	100.325	1.160	99.590	0.414	99.950	0.519

Table 5: Method robustness

Analytes (n=3)	Mean percent recovery of analyte detected at two different wavelengths		Mean recovery of analyte at two different pH of Mobile Phase	
	215nm	225nm	pH 3.0	pH 4.0
Ofloxacin	99.627	99.098	98.91	99.10
Fexofenadine HCl	99.654	99.338	99.351	99.618
Diclofenac Potassium	99.63	99.23	99.40	99.99

observed by introducing slight deliberate changes in pH of mobile phase and detection wavelength as given in table 5. The % RSD of mean recovery of the three drugs was found to be within the acceptable range of $\leq 2\%$.

Range

The range for this analytical procedure was established after estimating the accuracy, precision and linearity for the highest and lowest possible concentrations. These

parameters were found to be within the satisfactory limits as per pharmacopoeia standards. The concentration ranges for the three drugs were calculated as 0.050 to 0.150 mcg/ml for Ofloxacin, 0.015 to 0.045 mcg/ml for Fexofenadine and 0.0125 to 0.0375 mcg/ml for Diclofenac potassium as presented in table 2. This implies that the newly developed analytical procedure is valid over a wide range of concentrations for the objective drugs.

CONCLUSION

Present study describes a new facile HPLC based analytical method for the simultaneous determination of Ofloxacin (200mg), Fexofenadine HCl (60 mg) and Diclofenac Potassium (50mg) in a single formulation. This method offers various advantages including easy to constitute with shorter run time and high resolution of the analyte's peaks. The method has been validated according to ICH guidelines and is found to be simple and convenient to perform, sensitive and specific for the objective drugs, and accurate, precise and robust over a wide range of analytes concentration. The advantages of this method for analytical purposes lie in the rapid determination, its cost effectiveness, easy preparation of the sample and good reproducibility. It is more simple, economic, accurate and practical. Therefore, the proposed method can be used for routine analysis of combined formulation of Ofloxacin, Fexofenadine HCl and Diclofenac Potassium in any analytical setting of either pharmaceutical industry or research organization or any academic institution.

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