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Research Article

RP-HPLC-PDA Method for the Determination of Paracetamol, Famotidine, Diclofenac Potassium and Chlorzoxazone in Bulk and Marketed Formulation

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ABSTRACT
A simple, specific and accurate RP-HPLC-PDA method was developed for the simultaneous determination of paracetamol, famotidine, diclofenac potassium and chlorzoxazone in bulk and marketed formulation. For present study, a reversed-phase Altima C-18 column (150 mm * 4.5 mm i.d., particle size 5 µ) with mobile phase consisting of acetonitrile and 20 mM phosphate buffer (pH of buffer 6.6 adjusted with ortho phosphoric acid) taken in a gradient program was used. The flow rate was maintained at 1.0 ml/min and the analytes were monitored at 270 nm. The mean retention times of paracetamol, famotidine, diclofenac potassium and chlorzoxazone were found to be 4.8, 6.6, 7.7 and 8.8 min, respectively. The method was validated following ICH guidelines including parameters like linearity, range, specificity, system suitability, accuracy, precision and robustness. The proposed method was successfully applied for the estimation of paracetamol, famotidine, diclofenac potassium and chlorzoxazone in combined tablet dosage form.

Keywords: RP-HPLC, Paracetamol, Famotidine, Diclofenac potassium, Chlorzoxazone, Simultaneous estimation.

INTRODUCTION

Paracetamol (PARA) is an analgesic and antipyretic agent and chemically it is N-(4-hydroxyphenyl)acetamide. Famotidine (FTD) chemically 3-([2-(diaminomethyleneamino) thiazol-4-yl] methylthio)- *N'*-sulfamoylpropanimidamide is a H_2 receptor antagonist used in the treatment of peptic ulcer. Diclofenac potassium (DLF) chemically 2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid is a non steroidal anti-inflammatory drug used to treat chronic pain associated with inflammation. Chlorzoxazone (CLZ) chemically 5-chloro-3*H*-benzooxazol-2-one. It acts centrally as a muscle relaxant inoder to treat muscle spasm. Structures of PARA, FTD, DLF and CLZ are given in Fig.1. These drug substances like PARA, DLF and CLZ are frequently used in pharmaceutical formulations as anti-inflammatory and antipyretic, FTD is a H2-receptor antagonist used to treat ulcer caused by taking the other three drugs. All the four drugs are present in formulations with an important imbalance between the quantities of different active ingredients in the dosage forms. Polartity wise all the four drugs differ and therefore their chromatographic behaviour is not similar. The mentioned combination is available in tablet dosage form as a single unit dose with concentrations of these active ingredients in varying concentrations which are 325mg of PARA, 10mg of FTD, 50mg of DLF and 250 mg of CLZ. Thus the process of analysing the four drugs by using a single method becomes difficult. The literature reveals number of analytical methods published for PARA, FTD, DLF and CLZ with some other drug combinations. Many HPLC & UV methods for the estimation of paracetamol and also its combinations in pharmaceuticals or in biological fluids have already been reported[1-6]. UPLC, HPLC and UV methods for Famotidine and in combination with Ibuprofen have been reported[7-13]. Diclofenac potassium has been reported to be quantified in combination with other active ingredients by spectrophotometry[14] HPLC[15]. Chlorzoxazone has been quantified in combination with some other active ingredients by HPLC[16-17]. Till the date no single HPLC method is reported to determine all these four ingredients quantitatively in this combination. Hence, we planned to develop a new method for simultaneous estimation and validation of paracetamol, famotidine, diclofenac potassium and chlorzoxazone in bulk and combined tablet dosage form.

EXPERIMENTAL

Instrumentation

Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. Online degasser was equipped within the HPLC system which

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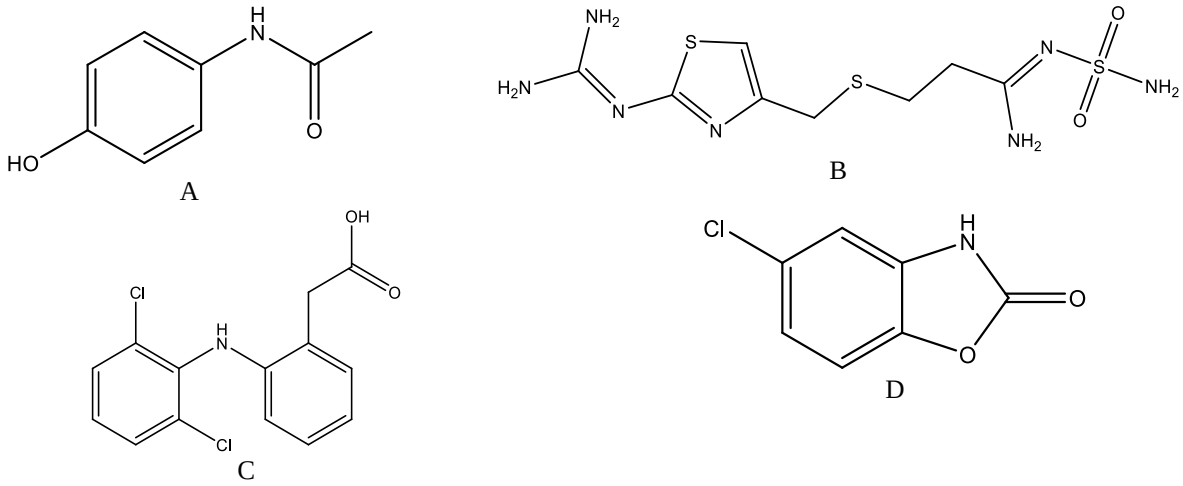

Figure 1: The structures of A) Paracetamol (PARA), B) Famotidine (FTD), C) Diclofenac (DLF) and D) Chlorzoxazone (CLZ).

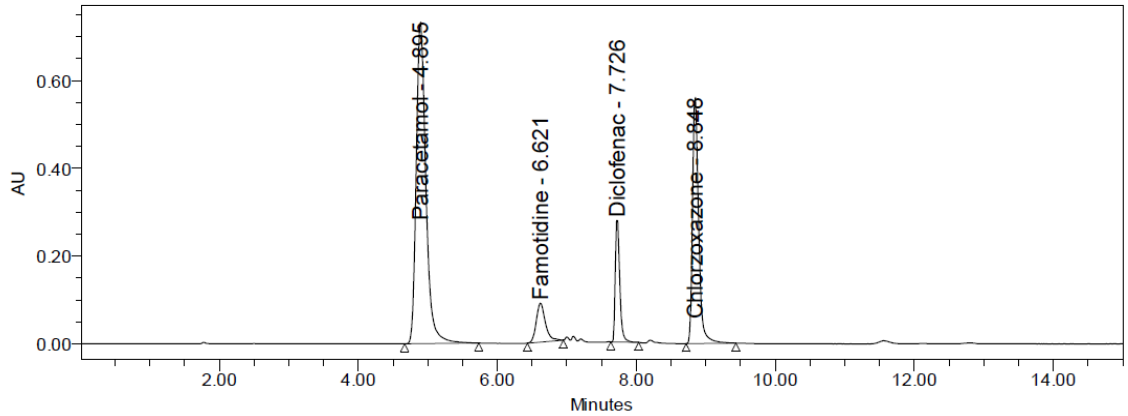

Figure 2: HPLC chromatogram obtained during simultaneous determination of Paracetamol (PARA), Famotidine (FTD), Diclofenac potassium (DLF) and Chlorzoxazone (CLZ).

Table 1: Gradient Programme.

Time	Flow	%Buffer	%Acetonitrile
0	1	90	10
3.5	1	90	10
5	1	50	50
10	1	50	50
11	1	90	10
14	1	90	10

Table 2: System suitability of PARA, FTD, DLF and CLZ.

STD. Sol.	Parameters (n=6) R_t	Resolution	Tailing	Theoretical Plates
PARA	4.8	-	1.18	6533
FTD	6.6	8.99	1.04	17686
DLF	7.7	7.70	1.08	76940
CLZ	8.8	8.88	1.09	58627

degasses the mobile phase and prevents the pressure fluctuations; along with this a column compartment was present in order to control the temperature. The HPLC system operates with Empower2 software and it is used for analysis, data acquisition followed by reporting the data.

Reagents and chemicals

Pharmaceutically pure sample of paracetamol, famotidine, diclofenac potassium and chlorzoxazone were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. The chemicals required for the preparation of mobile phase i.e HPLC grade acetonitrile, methanol were obtained from Merck, Mumbai. Millipore water (HPLC grade) used for the preparation of buffer and solutions was obtained from Milli-Q water purification system. Commercial tablets of Andic-MR tablets (Label Claim: 325mg of paracetamol, 20mg of famotidine, 50mg of diclofenac potassium and 250 mg of chlorzoxazone) were procured from Decisive Pharma Pvt. Ltd.

Preparation of standard stock solution

These solutions were prepared by dissolving 325 mg of paracetamol, 20 mg of famotidine drug, 50 mg of diclofenac potassium and 250 mg of chlorzoxazone into a clean and dry 50 ml volumetric flask, 35ml of diluent was added, sonicated for 5 minutes and volume was made up to 50 ml with diluent to get Stock Solution.

Preparation of working standard solutions

Aliquot of 0.125ml, 0.25ml, 0.375ml, 0.5ml and 0.625ml

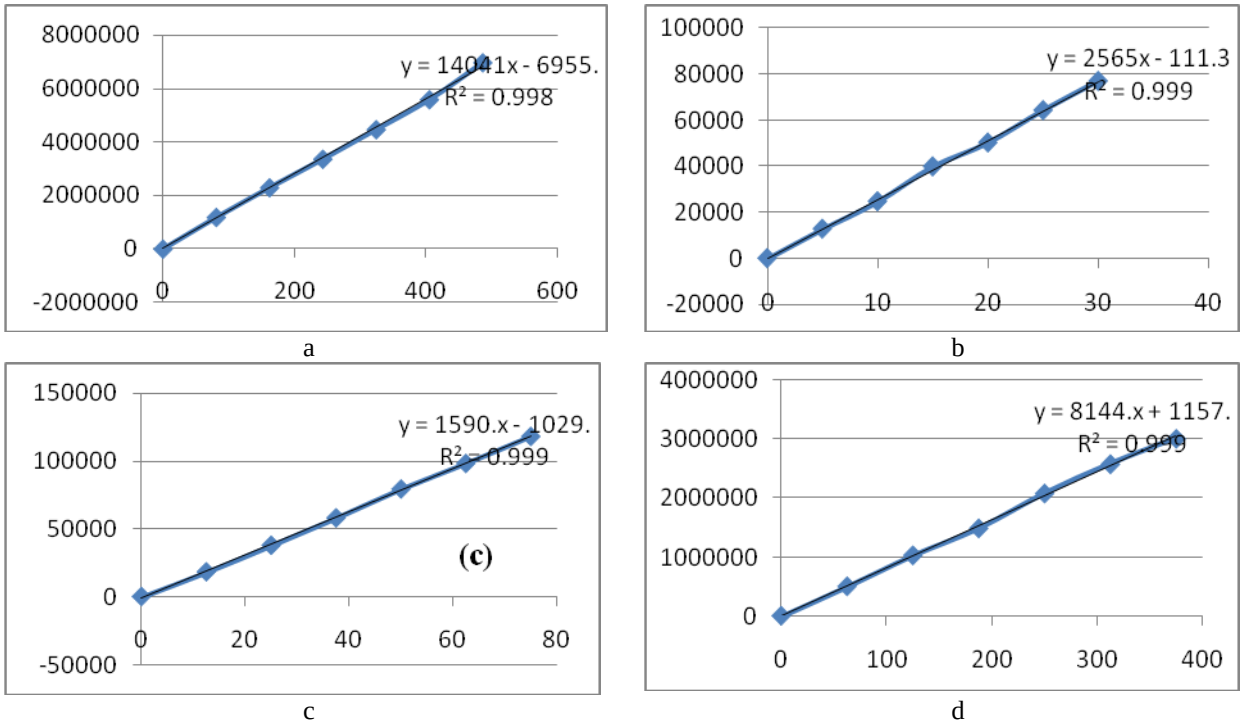

Figure 3: Calibration curves of (a) Paracetamol, (b) Famotidine, (c) Diclofenac potassium and (d) Chlorzoxazone.

Table 3: Accuracy studies of PARA, FTD, DLF and CLZ.

Recoverylevel (%)	Standard added	Amount added (mg)	Mean recovery (mg) (n=3)	Mean % Recovery
50	PARA	162.5	160.95	99.05
	FTD	10	10.17	101.73
	DLF	25	25.45	101.83
	CLZ	125	125.17	100.14
100	PARA	325	325.97	100.30
	FTD	20	20.03	100.16
	DLF	50	50.53	101.07
	CLZ	250	252.92	101.17
150	PARA	487.5	491.59	100.84
	FTD	30	30.21	100.70
	DLF	75	75.12	100.17
	CLZ	375	376.12	100.30

and 0.75ml were pipette out from stock solution into 10 ml volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 81.25µg/ml, 162.5µg/ml, 243.75µg/ml, 325µg/ml,406.25µg/ml and 487.5µg/ml respectively for paracetamol, 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml and 30µg/ml respectively for famotidine,12.5µg/ml, 25µg/ml, 37.5µg/ml, 50µg/ml, 62.5µg/ml and 75µg/ml respectively for diclofenac potassium and 62.5 µg/ml,125 µg/ml,187.5 µg/ml,250 µg/ml, 312.5 µg/ml and 325 µg/ml respectively for chlorzoxazone.

Sample preparation

Twenty samples were selected from the lot which were pre weighed and powdered. Powder equivalent to weight of five samples was transferred into a conical flask and dissolved in 250 ml diluent, sonicated for 20 min and filtered through PVDF 0.45µ filter. From the filtrate, 0.5 ml was pipetted and transferred into a 10ml volumetric flask and the solution was made up to the volume with diluents

Method validation

Validation parameters like system suitability, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and solution stability were performed as per ICH guidelines.

RESULT AND DISCUSSION

Method development

In order to optimize the method for drug analysis in pharmaceutical formulations, primary tests were conducted to select the best and optimal conditions. Parameters such as an ideal mobile phase and their ratios at optimum pH were studied in detail so as to achieve a reasonable degree of separation of analytes many eluents were tested using varied proportions of solvents such as acetonitrile, methanol, water and buffer at different pH conditions. But, the best results were obtained by phosphate buffer (20Mm) pH 6.6 adjusted with diluted

Table 4: Precision studies.

Concentration µg/ml	Mean measured concentration	
	Repeatability (n=6)	Intermediate precision(n=6)
PARA		
162.5	161.80	163.65
243.75	244.77	244.89
325	327.17	328.02
FTD		
10	10.09	10.09
15	15.14	15.14
20	20.19	20.18
DLF		
25	25.34	25.27
37.5	36.88	37.77
50	49.06	49.63
CLZ		
125	126	123.37
187.5	188.71	189.24
250	251.65	246.75

orthophosphoric acid and Acetonitrile taken in Gradient Programme shown in Table 1 at flow rate of 1ml/min followed by detection at 270nm. Fig.2 shows the chromatogram obtained from standard mixture by using the above optimized method.

Method Validation

System suitability

This test was performed to ensure the validity of the analytical procedure. Data from six injections of 10µL of the working standard solutions of PARA, FTD, DLF and CLZ were used for the evaluation of the system suitability. From the result obtained the %RSD of all the six injections was within the limit. Theoretical plates were found to be more than 5000 and the peak tailing was less than 1.2 which shows that it is within the range. Purity angle for the drug peaks was less than the purity threshold which implies that no interference was there at the retention time of main peak. Results of all these parameters were shown in the following Table 2.

Linearity

By appropriate aliquots of the standard PARA, FTD, DLF and CLZ solutions with the mobile phase, six working solutions ranging between 81.25-487.5µg/mL, 5-30 µg/mL,12.5-755µg/mL and 62.5-375 µg/mL were prepared and injected (n=3). The peak areas were plotted against the concentration of PARA, FTD, DLF and CLZ to obtain the calibration curve and the results were shown Fig 3. The linearity were represented by a linear regression equation as follows: y (PARA)= 14041.x – 6955.4 (r^2=0.998), y (FTD)= 2565.x -111.36 (r^2=0.999), y (DLF)= 1590.9.x -1029.5 (r^2=0.999) and y(CLZ)= 8144.5.x + 1157.9 (r^2=0.999). Fig 3 shows the calibration curves of PARA, FTD, DLF and CLZ.

Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as %recovery. In the present study standard addition method was followed to determine the % recovery. And it is determined by spiking the active ingredients at different concentrations 50%, 100% and 150% each of the labelled claim and injected in developed chromatographic conditions in triplicate. The recovery was found to be between 99.05 –101.84 for all the four drugs and it is shown in Table 3.

Precision

Repeatability and intermediate precision were determined in accordance with ICH guidelines. The samples (n=6) were assayed on the day of analysis and also the consequent day. Six replicate injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for PARA, FTD, DLF and CLZ were found to be within acceptable limit of ≤2. Both the repeatability and intermediate precision were carried out with three different concentrations. No significant difference was observed in the precision results carried out on two consecutive days and the results were shown in Table 4.

Robustness

The robustness of the method was performed by changing the chromatographic conditions. The change in the % organic strength (±5%), column temperature (±5^{0}c) and the flow rate (±0.1mL) did not bring any significant changes in the chromatography pattern. and the %RSD were also within the acceptance limits, showing that the method is robust and results were shown in Table 5.

Stability of sample solution

The sample solution injected after 24 hr did not show any appreciable change (Table 6).The standard deviation

Table 5: Robustness studies of PARA, FTD, DLF and CLZ.

Parameters		%RSD of peak area response				Mean tailing factor				Mean retention time in min			
		PARA	FTD	DLF	CLZ	PARA	FTD	DLF	CLZ	PARA	FTD	DLF	CLZ
flow	+0.2	1.3	1.4	1.8	0.6	1.83	1.21	1.55	1.07	4.1	6.2	7.1	8.0
rate	std	1.1	0.9	0.8	1.0	1.18	1.04	1.08	1.09	4.8	6.6	7.7	8.8
	-0.2	0.8	1.3	0.2	0.1	1.33	1.25	1.40	1.31	5.1	6.9	7.9	9.0
%	+5	0.8	0.1	0.6	0.5	1.28	1.18	1.39	1.32	4.3	6.4	7.6	8.7
Organic	std	1.1	0.9	0.8	1.0	1.18	1.04	1.08	1.09	4.8	6.6	7.7	8.8
phase	-5	0.6	0.1	1.4	0.1	1.29	1.34	1.31	1.33	4.8	6.7	7.8	8.9
Column	+5	0.3	0.1	0.9	1.8	1.28	1.22	1.29	1.36	4.4	6.0	7.6	8.6
Tempera	std	1.1	0.9	0.8	1.0	1.18	1.04	1.08	1.09	4.8	6.6	7.7	8.8
ture	-5	0.1	1.0	0.5	0.8	1.29	1.14	1.31	1.34	4.8	6.7	7.7	8.8

Table 6: Stability studies of PARA, FTD, DLF and CLZ.

Drug	Amount of drug found at 0 hr*(mg)	Amount of drug found at 24hr*(mg)	Amount of Deviation(mg)
PARA	327.17	329.16	1.99
FTD	20.19	19.85	0.34
DLF	49.06	50.86	1.8
CLZ	251.65	245.42	6.22

* n=6 for each parameter

Table 7: LOD and LOQ values of PARA, FTD, DLF and CLZ.

Drug	LOD	LOQ
PARA	0.4321	1.2959
FTD	0.6213	1.8849
DLF	0.3126	0.9368
CLZ	0.5762	1.7212

Table 8: Analysis of marketed formulation by proposed method.

Marketed formulation	Ingredients	Labeled amount (mg)	Amount found (mg)	Found %
Andic – MR tablets	PARA	325	322.62	99.27
	FTD	20	20.04	100.23
	DLF	50	49.35	98.7
	CLZ	250	252.77	101.11

and relative standard deviation were less <2 which shows that the sample solution is stable.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOD) of PARA, FTD, DLF and CLZ were determined by calibration curve method. Solutions of PARA, FTD, DLF and CLZ were prepared in linearity range and injected (n=3). The graph was plotted against average peak areas and concentration. The LOD and LOQ were calculated by using the equations, LOD = (3.3 ×Syx)/b and LOQ= (10.0×Syx)/b, Where Syx is residual variance due to regression; b is slope. LOD and LOQ of PARA, FTD, DLF and CLZ were determined by calibration curve method and the results were shown in the Table 7.

Tablet Analysis

Content of PARA, FTD, DLF and CLZ was found in the tablets by the proposed method and results were shown in Table 8.

CONCLUSION

A novel RP-HPLC-PDA method has been developed for the simultaneous estimation of PARA, FTD, DLF and CLZ in bulk and tablet in which the active agents are present in variable concentrations. Because of the wide variability among the drugs, their polarities and also there concentrations in the dosage form it became a tough task to optimise the method which gave good resolution for all the four drugs with a short run time (15 min). The developed method was validated according to ICH guidelines. The developed method was simple, specific as the excipients have no interference in the determination of main components, precise, accurate, and sensitive. The proposed method can be used for routine analysis of PARA, FTD, DLF and CLZ in combined dosage form which are present in variable concentrations. It can be also applied in the quality control of bulk manufacturing of presented API's.

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