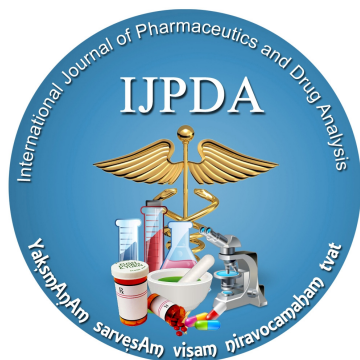


VALIDATED HPLC METHOD FOR SIMULTANEOUS QUANTITATION OF PARACETAMOL, DICLOFENAC POTASSIUM AND FAMOTIDINE IN BULK DRUG AND FORMULATION

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Abstract:

A simple, sensitive, precise and accurate HPLC method of analysis for paracetamol, diclofenac potassium, and famotidine both as a bulk drug and in tablet formulation was developed and validated. This method is based on HPLC separation of the three drugs on the PerfectSil-100 ODS-3-C₁₈ (250 mm × 4.6 mm, 5.0 μ) column from Germany with isocratic mobile phase containing acetonitrile: water (30: 70, v/v) pH 9.0 adjusted with triethylamine at flow rate 1 mL/min. UV detection was performed at 274 nm with R_t of 3.80, 5.40 and 6.33 min for Paracetamol, Famotidine and Diclofenac Potassium respectively. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 81.25-406.25 μg/mL for Paracetamol, 5-25 μg/mL for Famotidine and 12.5-62.5 μg/mL for Diclofenac Potassium. The method was validated for linearity, precision, robustness and recovery according to International Conference on Harmonization guidelines. Chromatographic interference from the tablet excipients was not found. Statistical analysis showed that the method was repeatable and selective for the simultaneous quantitation of the three drugs in tablet formulation and for routine quality control of raw materials of the drugs.

Keywords:

Paracetamol; Diclofenac Potassium; Famotidine; HPLC; Validation

Introduction

A combination of paracetamol (PAR), diclofenac potassium (DCL) and famotidine (FAM) is used as an analgesic, antipyretic and antacid for the treatment of fever, pain and acidity. PAR [N-(4-hydroxyphenyl) acetamide] (**Fig. 1**) has analgesic and antipyretic effects. The mechanism of action of PAR is by inhibition of the cyclooxygenase enzyme and prostaglandin synthesis in the central nervous system and its direct activity on the centre for body temperature regulation in the hypothalamus [1]. DCL as the potassium salt, is a benzeneacetic acid

derivative, designated chemically as 2-[(2, 6-dichlorophenyl) amino] benzeneacetic acid, monopotassium salt (**Fig. 2**). The mechanism of action of DCL, like that of other nonsteroidal anti-inflammatory drugs, is not completely understood but may be related to prostaglandin synthetase inhibition [2, 3]. FAM is chemically 3-[(2-(diaminomethyleneamino) thiazol-4-yl] methylthio)-N'sulfamoylpropanimidamide (**Fig. 3**). FAM binds to H₂-receptors located on the basolateral membrane of the parietal cell, blocking histamine effects [4].

A literature review revealed that methods have been reported for analysis of PAR, DCL and FAM in pharmaceuticals, including HPLC and HPLC/MS methods for quantitation in human plasma [5–14], HPLC and HPTLC methods for quantitation in formulations [15–33], either alone or in combination with other drugs. This paper reports for the first time the simultaneous quantitation of PAR, DCL and FAM by HPLC in bulk drug and pharmaceutical dosage form. The proposed method was validated according to International Conference on Harmonization (ICH) guidelines (34).

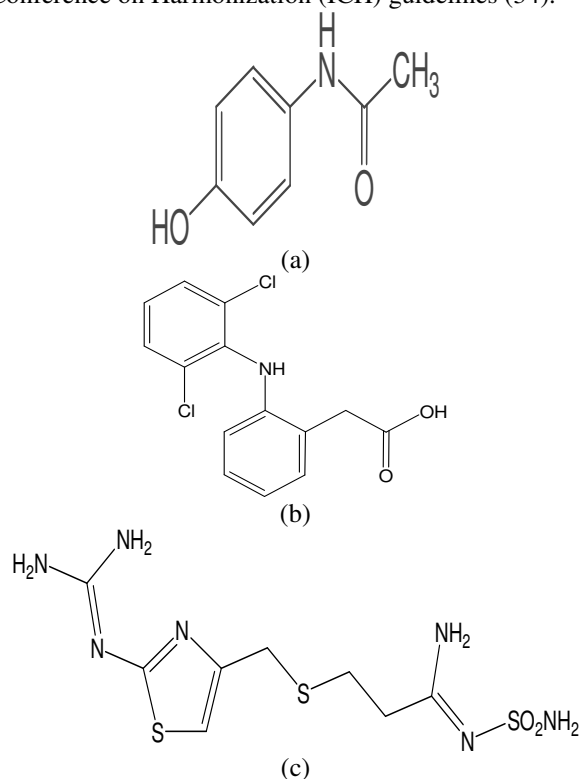


Fig.1 Structure of a) Paracetamol, b) Diclofenac, c) Famotidine

Experimental

Materials

Working standards of pharmaceutical grade PAR (Batch No. 260738), DCL (Batch No. 160-2008), and FAM (Batch No. 0410708) were obtained as generous gift samples from Bal Pharmaceuticals Ltd., Pune; Brihans Laboratories, Pune; and Vaasava Pharmaceuticals Pvt. Ltd., Solapur (Maharashtra, India) respectively. Fixed-dose combination tablets (MAHAGESIC PLUS, Batch No. MHP-24) containing 325 mg PAR, 50 mg DCL, and 20 mg FAM were procured from WINDLABS Biotech Ltd., Deharadun (Uttar Pradesh, India). All chemicals and reagents were of analytical grade and were purchased from Merck Chemicals, Mumbai (Maharashtra, India).

Instrumentation

The HPLC system consisted of Intelligent HPLC Pump (model, Jasco PU 2080) with sampler

programmed at 20 μ L capacity per injection was used. The detector consisted of UV/ VIS (Jasco UV 2075) model operated at a wavelength of 274 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was PerfectSil-100 ODS-3-C₁₈ (250 mm $\times$ 4.6 mm, 5.0 μ) from Germany.

Preparation of Standard Stock Solutions

Standard stock solutions of concentration 32.5 mg/mL PAR, 5 mg/mL DCL and 2 mg/mL FAM were prepared separately using methanol. From the Standard stock solutions, the mixed standard solution was prepared using the methanol to contain 0.406 mg/mL PAR, 0.062 mg/mL DCL and 0.025 mg/mL FAM. The stock solutions were stored at 2-8 $^{\circ}$ C protected from light.

Optimization of HPLC Method

The HPLC procedure was optimized to develop a simultaneous assay method for PAR, DCL and FAM. The mixed standard stock solution (0.406 mg/mL PAR, 0.062 mg/mL DCL and 0.025 mg/mL FAM) was injected into HPLC. Different ratios of acetonitrile and water were tried and it was found that peak shape for DCL was not good. Hence triethylamine was used to adjust the pH. From the mobile phases tried, the optimal composition was determined to be acetonitrile: water (30: 70, v/v) pH 9.0 adjusted with triethylamine at flow rate 1 mL/min which gives acceptable retention time, plates and good resolution for PAR, DCL and FAM (Fig. 4).

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and range

The mixed standard stock solution was further diluted to obtain concentrations of 81.25-406.25 μ g/mL, 12.5-62.5 μ g/mL and 5-25 μ g/mL for PAR, DCL and FAM, respectively. Linearity of the method was studied by injecting five concentrations of the drug prepared in the mobile phase in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (81.25, 243.75, 406.25 μ g/mL for PAR; 12.5, 37.5, 62.5 μ g/mL for DCL and 5, 15, 25 μ g/mL for FAM) six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Robustness of the method

To evaluate robustness of a HPLC method, few parameters were deliberately varied at three levels (-1, 0 and 1). The parameters included variation of flow rate and pH of the mobile phase. Robustness of the method was done at three different concentration levels (81.25, 243.75, 406.25 µg/mL for PAR; 12.5, 37.5, 62.5 µg/mL for DCL and 5, 15, 25 µg/mL for FAM respectively.)

Specificity

Complete separation of PAR, DCL and FAM in presence of excipients and other parameters like retention time (t_r), capacity factor (k), tailing or asymmetrical factor (T) etc. was checked.

Recovery

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i.e. multiple level recovery studies. Apply the method to drug sample (PAR, DCL and FAM combination tablet) to which known amount of PAR, DCL and FAM standard powder corresponding to 80, 100 and 120% of label claim had been added (Standard addition method), mixed. The powder was extracted & analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of PAR, DCL and FAM in conventional tablet (Brand name: MAHAGESIC PLUS, Label claim: 325 mg PAR, 50 mg DCL and 20 mg FAM per tablet), 20 tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 325 mg PAR, 50 mg DCL and 20 mg FAM was transferred into a 50 mL volumetric flask containing 20 mL methanol, sonicated for 30 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (6.5, 1.0 and 0.4 mg/mL for PAR, DCL and FAM, respectively). Then 250 µL of the above supernatant was diluted to 10 mL to produce a concentration of 162.5, 25 and 10 µg/mL for PAR, DCL and FAM, respectively, and 20 µL of the same solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 274 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

Results and discussion

The results of validation studies on simultaneous estimation method developed for PAR, DCL and FAM using acetonitrile: water (30: 70 v/v) pH 9.0 adjusted with triethylamine as mobile phase are given below.

Linearity

The drug response was linear over the concentration range between 81.25-406.25 µg/mL for PAR, 12.5-62.5 µg/mL for DCL, and 5-25 µg/mL for FAM (**Table 1**).

Precision

The results of the repeatability and intermediate precision experiments are shown in **Table 2**. The developed method was found to be precise as the RSD values for both repeatability and intermediate precision studies were < 2 %, as recommended by ICH guidelines.

Robustness of the method

Insignificant differences in peak areas and less variability in retention time were observed. The low values of the RSD, as shown in Table 3, indicated robustness of the method

Specificity

The method was found to be specific as complete separation of PAR, DCL and FAM in presence of excipients was observed and excipients from formulation were not interfering with the assay. The average retention time $\pm$ standard deviation for PAR, DCL and FAM were found to be 3.80 ± 0.15 , 6.33 ± 0.06 and 5.40 ± 0.08 min respectively for six replicates.

Recovery Studies

As shown from the data in **Table 4** good recoveries of the PAR, DCL and FAM in the range from 95 to 99 % were obtained at various added concentrations

Analysis of a formulation

Experimental results of the amount of PAR, DCL and FAM in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 96.5 % for PAR, 97.4 % for DCL and 98.1% for FAM. Two different lots of PAR, DCL and FAM combination tablets were analyzed using the proposed procedures as shown in **Table 5**.

Conclusion

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds. The drug was analyzed by HPLC method using PerfectSil-100 ODS-3- C_{18} (250 mm $\times$ 4.6 mm, 5.0 µ) column from Germany with isocratic conditions and simple mobile phase containing acetonitrile: Water (30:70, v/v) pH: 9 adjusted with triethylamine at flow rate of 1 mL/min using UV detection at 274 nm. The procedure has been evaluated for the linearity, accuracy, precision, recovery and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 81.25-406.25 µg/mL for PAR, 12.5-62.5 µg/mL for DCL and

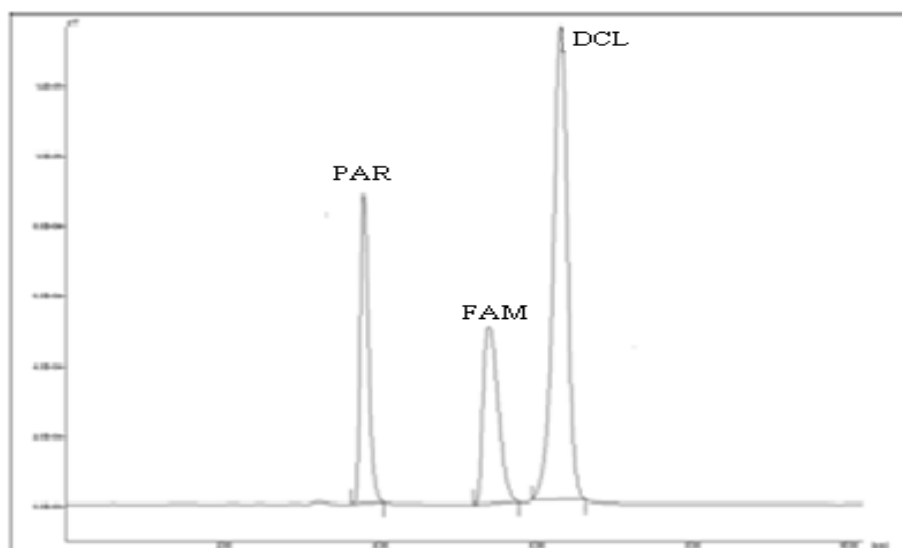


Figure 4: Chromatogram of Paracetamol R_t (3.80 min), Famotidine R_t (5.40 min) and Diclofenac R_t (6.33 min)

Table 1 : Linear regression data for the calibration plots^a (^a n=3)

Parameters	Paracetamol	Diclofenac	Famotidine
Linearity range	81.25-406.25 $\mu\text{g/mL}$	12.5-62.5 $\mu\text{g/mL}$	5-25 $\mu\text{g/mL}$
r^2	0.9996	0.9993	0.9996
Slope	24487	70268	35870
Intercept	79814	41545	42479

Table 2 : Precision studies

Conc. $\mu\text{g/mL}$	Repeatability (n=6)		Intermediate precision (n=6)	
	Measured conc. $\pm$ SD	% RSD	Measured conc. $\pm$ SD	% RSD
Paracetamol				
81.25	78.99 $\pm$ 0.15	0.196	77.64 $\pm$ 0.30	0.391
243.75	238.43 $\pm$ 1.77	0.744	238.01 $\pm$ 0.14	0.06
406.25	390.22 $\pm$ 1.37	0.351	392.5 $\pm$ 0.45	0.155
Diclofenac				
12.5	12.03 $\pm$ 0.09	0.739	12.04 $\pm$ 0.12	0.967
37.5	36.3 $\pm$ 0.25	0.699	36.49 $\pm$ 0.50	1.375
62.5	60.77 $\pm$ 0.78	1.290	60.55 $\pm$ 0.51	0.852
Famotidine				
5	4.87 $\pm$ 0.02	0.574	4.88 $\pm$ 0.01	0.286
15	14.34 $\pm$ 0.11	0.775	14.15 $\pm$ 0.13	0.927
25	24.03 $\pm$ 0.09	0.407	24.10 $\pm$ 0.16	0.701

Table 3 : Robustness testing

Chromatographic Factors	Level	Chromatographic changes in R _t		
		Paracetamol	Famotidine	Diclofenac
Flow rate (mL/min)				
0.90	-0.1	3.91	5.48	6.42
1.00	0	3.80	5.40	6.33
1.10	+0.1	3.7	5.35	6.28
Mean ± S.D. (n=6)		3.80±0.10	5.41 ± 0.06	6.34±0.07
pH of Buffer				
8.9	-0.1	3.72	5.46	6.25
9	0	3.80	5.40	6.33
9.1	+0.1	3.84	5.32	6.44
Mean ± S.D. (n=6)		3.78.±0.06	5.39±0.07	6.34±0.09

Table 4 : Recovery study (^a n=3)

Label Claim (mg/ tablet)	Amount added %	Total amount	Amount recovered (mg ± % RSD)	Recovery %
Paracetamol 325	80	585	564 ± 1.27	96.41
	100	650	638 ± 0.614	98.15
	120	715	690 ± 0.452	96.50
Diclofenac 50	80	90	87.6 ± 0.527	97.33
	100	100	96.3 ± 1.14	96.30
	120	110	105 ± 0.748	95.45
Famotidine 20	80	36	34.5 ± 1.02	95.83
	100	40	38.3 ± 1.32	95.75
	120	44	42.1 ± 0.851	95.68

Table 5 : Analysis of commercial formulation

Paracetamol 325 mg	Paracetamol found (mg/tablet)	
	Mean $\pm$ SD (n= 6)	Recovery (%)
1 st Lot	325.1 $\pm$ 0.92	100.03
2 nd Lot	324.9 $\pm$ 1.01	99.96
Diclofenac 50 mg	Diclofenac found (mg/tablet)	
	Mean $\pm$ SD (n= 6)	Recovery (%)
1 st Lot	49.87 $\pm$ 0.23	99.74
2 nd Lot	50.01 $\pm$ 0.32	100.02
Famotidine 20 mg	Famotidine found (mg/tablet)	
	Mean $\pm$ SD (n= 6)	Recovery (%)
1 st Lot	19.97 $\pm$ 0.09	99.85
2 nd Lot	20.01 $\pm$ 0.34	100.05

5-25 μ g/mL for FAM. Statistical analysis proves that the method is suitable for the analysis of PAR, DCL and FAM as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of PAR, DCL and FAM and also for its estimation in plasma and other biological fluids.

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