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Research Article

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Simultaneous determination of Flunarizine Dihydrochloride, Domperidone and Paracetamol by RP-HPLC in pharmaceutical dosage form

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ABSTRACT

A simple, rapid and precise reversed-phase liquid chromatographic method is developed for simultaneous determination of Flunarizine dihydrochloride, Domperidone and Paracetamol in combined pharmaceutical dosage form. This method uses an Eclipse XDBC-8 column, length- 4.6 x 150mm, 5µm column. Mobile phase is Acetonitrile and Water (90:10, v/v). The instrumental settings are at a flow rate of 0.8mL/min; the column temperature is 25°C and detector wavelength is 210nm. The sample concentrations are measured on weight basis to avoid the internal standard. The method is validated and shown to be linear. The correlation coefficients for Flunarizine dihydrochloride, Domperidone and Paracetamol are 0.996, 0.999 and 0.995, respectively. The recovery values for Flunarizine dihydrochloride, Domperidone and Paracetamol ranged from 99.48–101.33%, 100.96–102.05% and 99.08–102.58%, respectively. The relative standard deviation for six replicates is always less than 2%. This HPLC method is successfully applied to the simultaneous quantitative analysis of the title drugs in tablets.

Keywords: High Performance liquid chromatography, Validation, Flunarizine dihydrochloride, Domperidone, Paracetamol

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INTRODUCTION

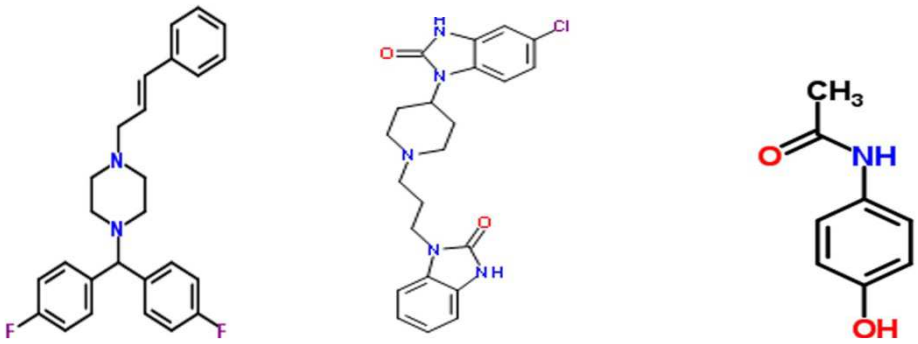
Flunarizine dihydrochloride (FLUN), Domperidone (DOM) and Paracetamol (PCM) are Anti-migraine drugs. FLUN is chemically (E)-1-[Bis (4-fluorophenyl) methyl]-4-(3-phenyl-2-propenyl) piperazine dihydrochloride (Figure 1A), DOM is chemically 5-chloro-1-{1-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1- l) propyl] piperidin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one (Figure 1B) and PCM is chemically acetamide, N-(4-hyroxyphenyl)-4'-hydroxyacetanilide (Figure 1C). The tablets commercially marketed as "Migrest" by Tidal Laboratories Pvt. Ltd. (Mumbai, India). FLUN is official in British Pharmacopoeia[2]. DOM is official in Indian Pharmacopoeia[3] and British Pharmacopoeia[2]. PCM is official in Indian Pharmacopoeia[3], British Pharmacopoeia[2] and United States Pharmacopoeia[1]. Literature survey revealed that various analytical methods like UV[4,5], HPLC [6,7] and HPTLC[8] for determination of DOM and PCM in combined dosage form have been reported. The first order derivative spectroscopy[9] method has been reported for estimation of FLUN, DOM and PCM in tablet formulation. However no chromatographic method for simultaneous analysis of FLUN, DOM and PCM. In the present research work, a reverse-phase HPLC method has been developed for simultaneous determination of FLUN, DOM and PCM.

EXPERIMENTAL SECTION

Chemicals and reagents

FLUN, DOM and PCM used as working standard, were obtained from Cadila Pharmaceuticals Ltd, Ahmedabad, Gujarat, India. Acetonitrile and Methanol were obtained from E. Merck Chemicals Ltd., India and Finar Chemicals Ltd., India respectively. Double distilled water were used throughout the experiment; other chemicals were HPLC

grade. Tablets were purchased from the Indian market, containing: Flunarizine dihydrochloride, 5mg; Domperidone, 10mg; and Paracetamol, 500 mg.



(A) **(B)** **(C)**

Fig 1. Chemical structures of (A) FLUN, (B) DOM and (C) PCM

Apparatus

The instrumentation comprises of a Shimadzu's HPLC (LC-2010, Quaternary Gradient with UV detector, Class VP Software), Agilent Eclipse XDB C-8 column, length- 4.6 X 150 mm, 5µm, Hamilton 20 µL glass syringe, CP 124S analytical balance, ultra sonic sonicator (Ultrasonic cleaner, Mumbai, India) were used for method development.

Chromatographic conditions

A Shimadzu HPLC(LC-2010), which consist of quaternary gradient with UV detector. An Eclipse XDB C-8column, length- 4.6 x 150 mm, 5µm column (Spinco Pvt. Ltd.) was used for the experiment. The instrumental settings are: flow rate, 0.8 mL/min; column oven temperature, 25°C; detector wavelength, 210 nm; and injection volume, 20µL. Data acquisition was made with the Class VP Software. Mobile phase consisted of degassed HPLC grade acetonitrile and water(90:10, v/v).

Preparations of standard solutions

Standard Stock Solution of mixture of FLUN, DOM and PCM was prepared by dissolving 5mg, 5 mg and 50mg of respective drug in methanol in a 50mL volumetric flask and the volume was made up to the mark with methanol to get the final concentration of 100µg/mL of FLUN, 100µg/mL of DOM and 1000µg/mL of PCM.

From above stock solutions 0.5mL FLUN, 1mL DOM and 5mL PCM were taken in to a 10mL volumetric flask and diluted up to the mark with the acetonitrile to get the concentration of 5µg/mL of FLUN, 10µg/mL of DOM and 500µg/mL of PCM.

Preparations of sample solutions

20 tablets were weighed properly, their average weight was calculated then powdered. A quantity of tablet powder equivalent to about 5 mg of FLUN, 10mg of DOM and 500mg PCM was weighed and transferred in to 100mL volumetric flask and dissolved in 50 mL of methanol. The solution was sonicated for 10 minutes for effective solubilization of the drugs. The volume was made up to mark with the mobile phase and the solution was filtered through whatman filter paper no.41. The filtrate was collected in a 100mL volumetric flask to get concentration of 50µg/mL of FLUN, 100µg/mL of DOM and 5000µg/mL of PCM.

From above Stock Solution, 1mL was taken in to 10mL volumetric flask and diluted up to the mark with mobile phase. The concentration of resultant solution was 5µg/mL of FLUN, 10 µg/mL of DOM and 500µg/mL of PCM

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

The chromatographic conditions were optimized by changing various organic and aqueous mobile phases were tried in different compositions at various flow rates to achieve best resolution, peak, and Retention time. As the mobile phase used throughout the experiment, containing acetonitrile and water (90:10, v/v). The sequence of peak elution was observed at the retention times for PCM at 1.90min; DOM, 3.55min; and FLUN, 4.90min (Figure 2), using the described mobile phase with flow rate of 0.8ml/min gave the better result and peak shape.

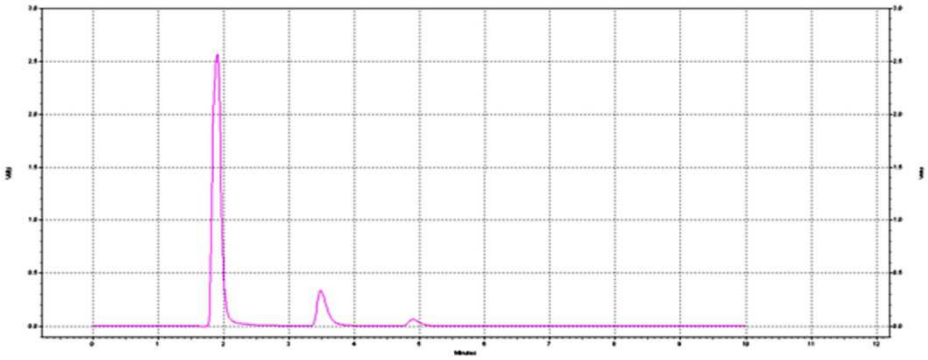


Fig 2. A typical HPLC chromatogram of the tablet (PCM at 1.90 min, DOM at 3.55 min and FLUN at 4.90 min)

Validation of the method[10]

System suitability

For system suitability studies, five replicate injections of mixed standard solutions were injected, and the suitability parameters like relative standard deviation of peak area, theoretical plate, retention time, and tailing factor of the peaks were calculated. Results are shown in Table 1.

Table 1: System Suitability Parameters for the RP-HPLC

Parameters*	FLUN	DOM	PCM
Retention time (min)	4.90	3.11	1.90
Tailing factor	1.54	1.63	1.12
Theoretical plates (N)	3606	2001	2224
Resolution	2.15	3.22	

Calibration and linearity

The mixed standard solutions containing 4–6µg/mL of FLUN, 8–12µg/mL of DOM and 400–600µg/mL PCM in each linearity level. Linearity solutions were injected in triplicate. In the simultaneous determination, the calibration graphs were found to be linear for all the analytes in the mentioned concentrations; the correlation coefficients are shown in Table 2.

Table 2: Regression analysis data

Regression parameters	FLUN	DOM	PCM
Concentration range (µg/ml)	4-6	8-12	400-600
Correlation coefficient (r2)	0.996	0.999	0.995

Precision (Reproducibility)

The precision of the method was studied by determining the concentrations of each ingredient in the tablets for six times. The results of the precision study (Table 3) indicate that the method is reliable and reproducible, with a relative standard deviation less than 2.0%.

Table 3: Validation parameters of evaluated methods

Parameters	FLUN	DOM	PCM
Concentration range(µg/ml)	4-6	8-12	400-600
% Recovery	101.03	100.96	102.05
LOD (µg/ml)	1.113353	0.560816	0.021988
LOQ (µg/ml)	3.373798	1.699443	0.066632
Repeatability (%RSD, n=6)	1.344845	1.459295	0.667386
Intraday Precision (%RSD, n=3)	0.284336	0.091269	0.6008812
Interday Precision (%RSD, n=3)	0.400256	0.319287	0.5652475
Specificity	Specific	Specific	Specific

Accuracy (Recovery test)

Accuracy of the method was studied by recovery experiments by adding known amounts of the drugs in the placebo. The recoveries of the method were performed for three levels, at 80%, 100% and 120% of the label claim per tablet: FLUN, 5mg; DOM, 10mg; and PCM, 500mg. Three samples were prepared for each recovery level. The recovery values for FLUN, DOM and PCM ranged from 99.48–101.33%, 100.96–102.05% and 99.08–102.58%, respectively,

as shown in Table 4. The average recovery of three levels (nine determinations) for FLUN, DOM and PCM were 100.25%, 101.34% and 101.19%, respectively.

Table 4: Recovery studies

Level of addition (%)	Ingredient	Amount added (mg)	% Recovery*
80	**FLUN**	4	99.94
	DOM	8	99.48
	PCM	400	101.33
100	**FLUN**	5	101.03
	DOM	10	100.96
	PCM	500	102.05
120	**FLUN**	6	100.19
	DOM	12	102.58
	PCM	600	99.08

**n=3*

Determination of the limit of detection and quantitation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope [Table 3]. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of LOD and LOQ. The LOD for FLUN, DOM and PCM were 1.113µg/mL, 0.560µg/mL and 0.021µg/mL and LOQ were 3.373µg/mL, 1.699µg/mL and 0.06µg/mL, respectively.

Robustness

To verify the robustness of the method, the analysis was done under variable flow rates, wavelength and mobile phase composition. Sample solutions were injected as 20µL injection and run under set chromatographic conditions. Results are shown in Table 5.

Table 5: Robustness Study

Parameter	FLUN		DOM		PCM	
	Mean peak area	**RSD (%)**	**Mean peak area**	**RSD (%)**	**Mean peak area**	**RSD (%)**
Flow rate (±0.2 mL/min)	772033.7	1.862898	3600794	0.31309	23903760	1.691255
Mobile phase Ratio (ACN: Water)	766171.3	0.84416	3590884	0.040517	24440207	0.429662
Wavelength (±2 nm)	757558	1.037579	362496.3	0.411399	25243025	0.559498

CONCLUSION

This method can be used for the simultaneous determination of FLUN, DOM and PCM in pharmaceutical dosage form. The method was validated and shown to be accurate, precise and robust.

Acknowledgments

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