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Research Article

DEVELOPMENT AND VALIDATION OF VIERDOT'S AND Q-RATIO METHOD FOR THE ESTIMATION OF PARACETAMOL, DOMPERIDONE AND FLUNARIZINE IN SOLID ORAL DOSAGE FORM

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ABSTRACT

Objective: To develop simple, precise and economical UV spectrophotometric method for the estimation of Paracetamol, Domperidone and Flunarizine in the combined dosage form available in the market for the treatment of migraine. Method: The three drugs Paracetamol, Domperidone, and Flunarizine are present in the ratio of 100:2:1 which poses a problem in their simultaneous estimation. Hence, an effective and reproducible extraction method to extract out Paracetamol from the combination was developed and applied successfully to the formulation. The estimation of Paracetamol was done at 256nm. The two drugs Domperidone and Flunarizine were simultaneously estimated by two different methods. The first developed method was Vierdot's method (method A) were in 251nm and 286nm were selected for measuring absorbance of Flunarizine and Domperidone respectively. The second method was Q-ratio method (method-B), wavelength selected were 251nm (λ_{max} of Flunarizine) and 269.83nm (iso-absorptive point). Results:The methods were validated as per the ICH guidelines and the results were statistically validated. Linearity for the two methods was 4-12µg/ml for Paracetamol and 10-40 µg/ml for both Domperidone and Flunarizine. Good recovery results were obtained between 97% to 100% with relative standard deviation below 2%. Conclusion: Two simple, accurate, precise and economical UV-spectrophotometric methods for the estimation of Paracetamol, Domperidone, Flunarizine. The developed method was successfully applied to the formulation and can be used in routine analysis.

Keywords: Vierdot's method, Q-Ratio method, Paracetamol, Domperidone, Flunarizine.

INTRODUCTION

Paracetamol (PARA), (N-(4-hydroxyphenyl)ethanamide), is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer) [1,2].Domperidone (DOM), (5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1yl)propyl]piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one), is an antidopaminergic drug used to suppress nausea and vomiting [1,3]. Flunarizine (FLU) (1-[bis(4-fluorophenyl)methyl-4-[(2E)-3-phenyl-2-en-1-yl]piperazine) is a calcium channel blocker with H_1 blocking activity, effective in the prophylaxis of migraine, occlusive peripheral vascular disease, vertigo of central and peripheral origin, and used as an adjuvant in the therapy of epilepsy [4,5,6].The combination of three drugs is used in the prophylaxis of migraine. FLU (Ca^{++} channel blocker) cures the disease while PARA (analgesic, antipyretic) and DOM (spasmolytic) relieve the symptoms like pain and vomiting associated with the disease. The three drugs alone and in combination with other drugs are reported to be estimated by UV[7-11], HPTLC[12,13], HPLC and RP-HPLC[14-19].The present combination is not official in any pharmacopoeia hence no official method is available. Literature survey does not reveal any UV spectrophotometric method for the estimation of these three drugs from the combined dosage form. In the present work, a first order derivative spectrophotometric method has been developed for the estimation of PARA, DOM and FLU in combined solid oral dosage form. The method was validated as per the ICH guidelines[20].

MATERIALS AND METHODS

Instrument

A double beam shimadzu UV-Visible spectrophotometer model 1800 shimadzu, loaded with UV probe 2.33 software, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1cm matched quartz cells was used for spectroscopy .

Material

Pure PARA and DOM (purity 99.75 & 99.10% respectively) were obtained as gift samples from West Coast Pharmaceutical Pvt.Ltd., Ahmedabad. The standard sample of FLU (99.42% purity) was received as gift sample from RoseLab Biosciences Pvt. Ltd, Ahmedabad. All the other chemicals, reagents and solvents used were of AR grade. The combined dose tablet formulation (Migrest) was purchased from local pharmacy.

Preparation of stock solution

Accurately weighed quantity of PARA (1.25g), DOM (50mg) and FLU (25mg) were transferred to three separate 50ml volumetric flask, sonicated (2 min) and dissolved in methanol and diluted to mark with same solvent. This resulted in stock solution of PARA (25000µg/ml), DOM (1000 µg/ml) and FLU (500 µg/ml).

Method

From the stock solution of PARA, DOM and FLU appropriate volumes were pipette out into a single volumetric flask of 10ml to give a ratio of 100:2:1 for PARA, DOM and FLU. Such six solutions were prepared. The final volume of each was adjusted with ether. The solution was transferred to a separating funnel and extracted with sodium hydroxide (0.1N, 5ml×2). The organic layer (containing FLU) was dried over anhydrous sodium sulphate, collected in a dry boiling tube and evaporated on water bath to dryness. The residue was quantitatively transferred using methanol to a 10ml volumetric flask and solution was scanned from 200-400nm. The λ_{max} for FLU was determined to be 251nm.

The aqueous layer was extracted with chloroform (5ml×2) to extract out DOM. The organic layer was dried over anhydrous sodium sulphate, collected in a dry boiling tube and evaporated on water bath to dryness. The residue was quantitatively transferred using methanol to a 10ml volumetric flask. The solution was scanned from 200-400nm and λ_{max} for DOM was determined to be 286nm.

The aqueous layer (sodium hydroxide, 0.1N) containing PARA was subjected to heat (50°C on waterbath for 15 min) to remove traces of organic solvents, cooled and absorbance was measured at 256nm.

The above procedure was repeated for each solution containing the three drugs. The solutions were scanned in the range of 200-400nm. The overlain spectra (Fig:1) of FLU and DOM exhibited the Iso-absorptive point at 269.83nm.

Method:A

The absorbance of solutions containing each drug were measured at 256, 251, and 286nm for PARA, FLU and DOM respectively. The concentration of individual component (FLU& DOM) in combination calculated using the following simultaneous equation:

$Cx = A_2a_{y1} - A_1a_{y2} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$ .....(1)

$Cy = A_1a_{x2} - A_2a_{x1} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$ .....(2)

Where Cx= Conc of FLU & Cy= Conc of DOM.

A_1 and A_2 are absorbance of sample solution at λ_{max} of FLU (λ_1 251nm) and λ_{max} of DOM (λ_2 286nm) respectively. a_{x1} and a_{x2} are the absorptivities of FLU at 251 and 286 nm respectively and a_{y1} and a_{y2} are the absorptivities of DOM at 251 and 286 nm respectively.

The plot of absobance vs concentration at 256nm gave the calibration curve for PARA (FIG:2)

Method-B

The Concentration of individual component determined by employing following equation:

$C_x = A_1/a_{x1}\ (Q_M - Q_Y/Q_X - Q_Y)$..... (3)

$C_y = A_1/a_{y1}\ (Q_x - Q_M/Q_X - Q_Y)$..... (4)

$Q_M = A_2/A_1$ ..... (5)

$Q_X = a_{x2}/a_{x1}$ and $Q_Y = a_{y2}/a_{y1}$ ..... (6)

Where A_1 and A_2 are absorbance of sample solution at Isoabsorptive point ($\lambda_1$269.83nm) and λ_{max} of flunarizine (λ_2 – 251nm) respectively. a_{x1} and a_{x2} are the absorptivities of FLU at 269.83 and 251 nm respectively and a_{y1} and a_{y2} are the absorptivities of DOM at 269.83 and 251 nm respectively and PARA was estimated at 256 nm.

Method Validation

The proposed method was validated as per the ICH guidelines.

Linearity and Range

Linearity was evaluated for PARA, DOM and FLU, expressed in terms of correlation co-efficient and regression line equation as shown in Tables 1.

Precision

Precision was determined by analyzing PARA, DOM, and FLU three times on the same day for intraday precision and on three consecutive days for interday precision. %RSD was calculated as shown in Tables 2-4.

Accuracy

The recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%) of PARA, DOM and FLU in triplicate. The solution were analyzed, percent recoveries were calculated, shown in Table 5 & 6.

Assay of tablet formulation

Twenty tablets (Migrest) were weighed and powder equivalent to 5mg of FLU(10mg DOM and 500mg of PARA) was transferred into 50ml volumetric flask, sonicated (2min) to dissolve in methanol, volume made up to 50ml with methanol and filtered. 1ml of filtrate was transferred in 10ml volumetric flask and volume made up with ether. Content of flask were taken in separating funnel and extracted with sodium hydroxide (0.1N, 5ml×2). The ether layer (containing FLU) dried over anhydrous sodium sulphate, collected in the dry boiling tube. The sodium hydroxide aqueous layer was extracted with chloroform (5ml×2). The chloroform layer (containing DOM) dried over anhydrous sodium sulphate, collected in the dry boiling tube containing ether extract and evaporated on water bath to dryness. The residue was quantitatively transferred using methanol to a 10ml volumetric flask and spectum was scanned from 200-400nm and absorbance was measured at specified wavelength of each method. The absorbance of aqueous layer (containing PARA) was measured at 256nm in normal spectral mode. Results are reported in Table 7& 8.

RESULTS AND DISCUSSION

The overlain spectra of DOM and FLU reveled the possibility of simultaneous and Q-ratio method for the simultaneous quatification of DOM and FLU in the mixture (Fig.1).

Lineraity

Linearity was observed in the range of 10-30 µg/ml for DOM while 5-25 µg/ml for FLU as shown in **Table: 1**. Regression line computed and regression coefficient found to be near 1 showing good correlation between absorbance and concentration. Calibration curve for PARA shown in Fig:2

Precision

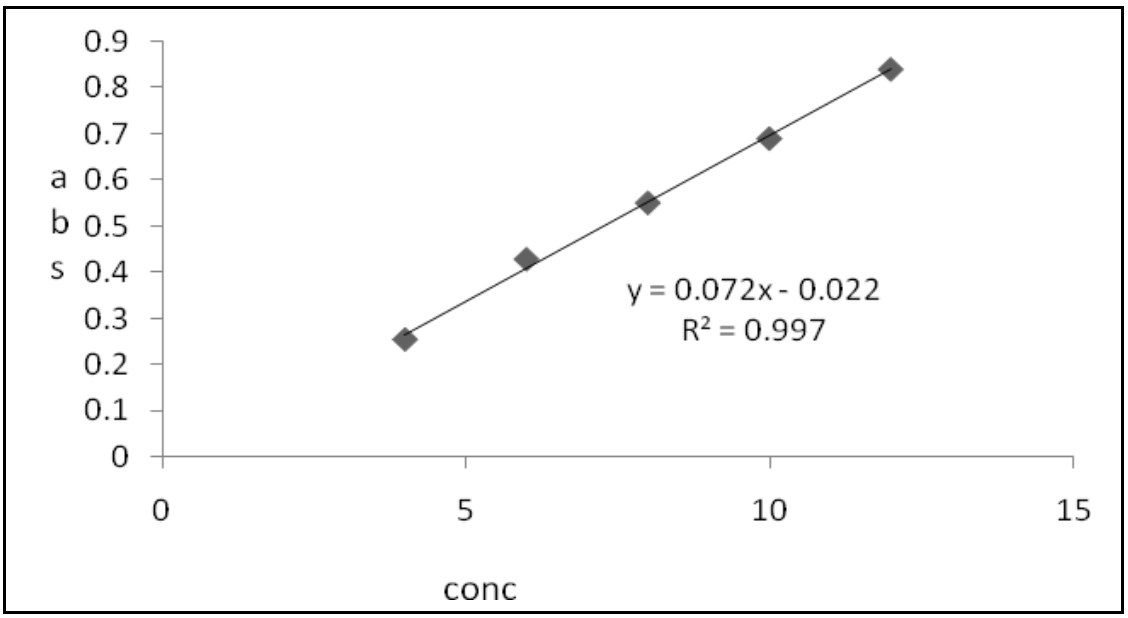
The %RSD for intraday and interday precision was less than 2 indicating the reproducibility of method. (Table:2-4)

Accuracy

Good accuracy results were obtained between 97-101% as shown in Table:5-6

Assay

The % assay results were reproducible and in good agreement with the label claim. (Table:7-8)



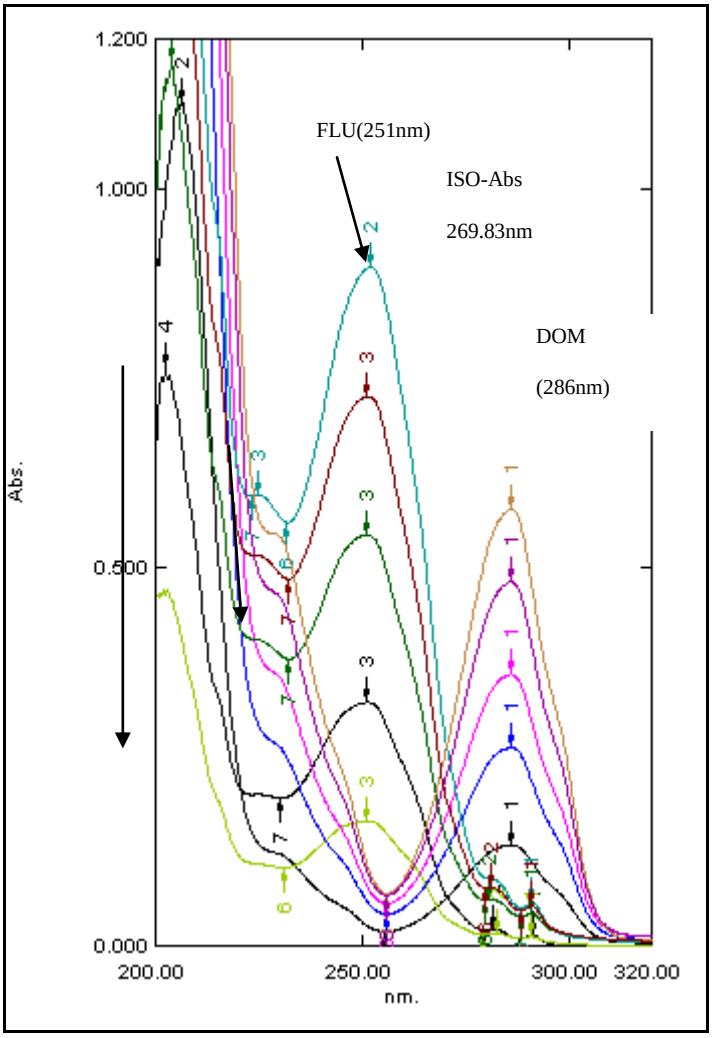


Fig. 1: Overlain spectra of five concentration of DOM and FLU

Table: 1 Quantitative parameters of the method A and B

Parameter	**DOM**		**FLU**		**ISO-ABS**	**PARA**
	251	**286**	**251**	**286**	**269.83**	**256**
Linearity(µg/ml)	10-30	10-30	5-25	5-25	5-25	4-12
Regression equation	Y=0.006x-0.003	Y=0.025x +0.024	Y=0.033x +0.07	Y=0.002x-0.001	Y=0.009x+ 0.029	y=0.072x-0.022
Slope	0.006	0.025	0.003	0.002	0.009	0.072
Intercept	-0.003	0.024	0.070	-0.001	0.029	-0.022
R^2 value	0.992	0.993	0.991	0.992	0.988	0.997
LOD(µg/ml)	1.09	0.84	0.83	1.17	1.37	0.28
LOQ(µg/ml)	3.3	2.57	2.51	3.56	4.17	0.86

Table: 2 Precision (Intraday) for Method A and B

	DOM						**FLU**				
	286		**251**		**269.83**			**251**		**286**	
Conc#	Abs* ±SD	%RSD	Abs* ±SD	%RSD	Abs* ±SD	%RSD	Conc#	Abs* ±SD	%RSD	Abs* ±SD	%RSD
15	0.421± 0.0033	0.78	0.087± 0.0016	1.87	0.175± 0.0020	1.16	**5**	0.21± 0.0008	0.38	0.011± 0.00012	1.09
20	0.545± 0.0050	0.93	0.115± 0.0012	1.08	0.226± 0.0017	0.74	**10**	0.41± 0.0029	0.70	0.027± 0.000471	1.72
25	0.681± 0.0082	1.21	0.145± 0.0030	2.12	0.258± 0.0057	2.21	**15**	0.58± 0.0035	0.60	0.045± 0.00081	1.81

*n=3, SD- Standard Deviation , %RSD- Relative Standard Deviation, #= µg/ml

Table 3: Precision(Interday) for Method A and B

	DOM						FLU				
	286		251		269.83			251		286	
Conc#	Abs* ±SD	%RSD	Abs* ±SD	%RSD	Abs* ±SD	%RSD	Conc#	Abs* ±SD	%RSD	Abs* ±SD	%RSD
15	0.424± 0.0057	1.34	0.083± 0.0012	1.49	0.176± 0.0021	1.22	**5**	0.220±0.0012	0.56	0.012± 0.00017	1.33
20	0.529± 0.0070	1.32	0.114± 0.0008	0.71	0.228± 0.0012	0.54	**10**	0.424±0.0082	1.93	0.028± 0.00047	1.64
25	0.672± 0.0070	1.04	0.145± 0.0012	0.85	0.243± 0.0016	0.67	**15**	0.567±0.0024	0.43	0.047± 0.00047	0.99

* n=3, SD- Standard Deviation , %RSD- Relative Standard Deviation , #= µg/ml

Table: 4 Intraday and interday Precision for PARA at 256nm

	Intraday		Interday	
Conc (µg/ml)	**Abs* ± SD**	**%RSD**	**Abs* ± SD**	**%RSD**
8	0.562 ± 0.0040	0.72	0.552 ± 0.0045	0.82
10	0.690 ± 0.0033	0.48	0.677 ± 0.0024	0.36
12	0.835 ± 0.0045	0.54	0.836 ± 0.0036	0.44

*= Mean of three determinations, SD- Standard Deviation , %RSD- Relative Standard Deviation

Table: 5 Accuracy (Recovery Study for both the methods)

Drug	Level	Amt taken(µg/ml)	Std added (µg/ml)	Total conc. (µg/ml)	Conc.found* (µg/ml) Mean ±SD Methods		% Recovery* ± SD Methods	
					A	**B**	**A**	**B**
DOM	50%	10	5	15	14.96± 0.32	14.76± 0.071	99.66± 2.05	98.39± 0.47
	100%	10	10	20	20.02± 0.39	20.16± 0.15	99.83± 1.58	100.71± 0.84
	150%	10	15	25	24.98± 0.33	24.92± 0.33	99.94± 1.35	99.68± 1.35
FLU	50%	5	3	8	7.9± 0.10	7.99± 0.10	99.30± 1.34	99.94± 1.2
	100%	5	6	11	10.96± 0.11	10.69± 0.10	99.68± 1.09	97.24± 0.93
	150%	5	9	14	13.98± 0.13	13.69± 0.16	99.86± 0.97	99.59± 1.03

*= Mean of three determinations, SD- Standard Deviation .

Table: 6 Accuracy (Recovery study for PARA)

Level	Amt taken(µg/ml)	Std added (µg/ml)	Total conc. (µg/ml)	Conc.found* (µg/ml) Mean ±SD	% Recovery* ± SD
50%	5	2	7	6.93 ± 0.132	99.04 ± 1.89
100%	5	4	9	8.94 ± 0.998	99.37 ± 1.11
150%	5	6	11	10.99 ± 0.205	99.97 ± 1.86

*= Mean of three determinations, SD- Standard Deviation .

Table 7: Results of commercial formulation analysis (Formulation: Migrest)

Label claim (mg/tablet)	Label claim found * (mg/tablet) Mean ± SD		% Assay * ± SD	
	Method-A	**Method-B**	**Method-A**	**Method-B**
DOM (10mg)	9.88± 0.023	9.77± 0.083	98.83± 0.235	97.76 ± 0.834
FLU (5mg)	4.88± 0.062	5.04± 0.081	97.66± 1.24	100.93± 1.63

*= Mean of three determinations, SD- Standard Deviation

Table: 8 Assay results for PARA

Label claim (mg/tablet)	Label claim found * (mg/tablet) Mean ± SD	% Assay * ± SD
PARA (500mg)	493 ± 0.053	98.60 ± 0.53

*= Mean of three determinations, SD- Standard Deviation

CONCLUSIONS

Two spectrophotometric methods viz. viredot's and Q-Ratio were developed for the estimation of PARA, DOM and FLU in the combined dosage form. Methods were found to be simple, rapid, economic, accurate and precise. The results of validation tests were satisfactory and therefore, the developed methods can be applied successfully for routine quality control analysis of the formulation without interference from the excipients.

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