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Development and validation of spectrophotometric methods for simultaneous estimation of Paracetamol and Meloxicam in pure and tablet dosage form

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Abstract

Two simple, accurate, economical and reproducible spectrophotometric methods for simultaneous estimation of paracetamol and meloxicam in pure and tablet dosage form have been developed. Method I is based on solving simultaneous equation. Paracetamol and meloxicam show absorbance maximums at 256 and 268.8 nm so absorbance was measured at the same wave lengths for the estimation of paracetamol and meloxicam. Method II is based on determination of Q-value. Absorbance was measured at 308 nm (Isobestic point) and 256 nm (λmax of paracetamol). Both drugs obey the Beer Lambert's law in the concentration range of 5-30 µg /mL. Methods are validated according to ICH guidelines and can be adopted for the routine analysis of paracetamol and meloxicam in pure and tablet dosage form.

Keywords: paracetamol, meloxicam, simultaneous equation method, absorbance ratio method, validation

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INTRODUCTION

Chemically, paracetamol is 4-hydroxy acetanilide, used as an analgesic and antipyretic drug. Meloxicam is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazoly)-2H-1,2-benzo-thiazine-3-carboxamide-1,1dioxide commonly prescribed as non-steroidal anti-inflammatory drug. Paracetamol is official in Indian and British Pharmacopoeia. Both the pharmacopoeias suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations. Meloxicam is official in British Pharmacopoeia which suggests gradient RP-HPLC method. However many methods are reported for the determination of paracetamol in combination with other drugs by spectroscopy [1-3], chemometric-assisted spectrophotometric [4], and HPLC [5]. Also numbers of methods are reported in the literature to determine meloxicam by spectrophotometry [6-12], electrophoretic [13], chromatography [14] and

polarography [15-19]. HPLC is the technique that most commonly used for the determination of meloxicam in plasma [20-22].

Method validation [23] is an important issue in pharmaceutical analysis. It confirms that the analytical procedure employed for the analysis is suitable and reliable for its intended use. In present study, all validation parameters for quantitative analysis of paracetamol and meloxicam in tablets were tested and data were evaluated according to their acceptance criteria.

As combination of paracetamol and meloxicam is available in market and no spectrophotometric method is reported for their simultaneous estimation, in the present work, a successful attempt has been made to develop simple and validated UV spectrophotometric methods for simultaneous estimation of paracetamol and meloxicam.

MATERIALS AND METHODS

UV-visible double beam spectrophotometer, Systronics model 2201 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. The commercially available tablets, Melodol (Label claim: paracetamol- 325 mg, meloxicam-7.5 mg) was procured from local market.

Preparation of standard stock solution and calibration curve

The standard stock solutions of paracetamol and meloxicam were prepared by dissolving 0.025 gm of each drug in 0.1N NaOH and final volume was adjusted with same solvent in 100 mL of volumetric flask to get a solution containing 250 µg/mL of each drug.

Working standard solutions of 10 µg/mL were scanned in the entire UV range of 400-200 nm to determine the λmax. The λmax of paracetamol and meloxicam is 256 nm and 268.8 nm respectively and from overlain spectra (Fig. 1) it is evident that isobestic point is at 308 nm. Six working standard solutions with concentration 5, 10, 15, 20, 25 and 30 µg/mL were prepared in 0.1N NaOH from stock solution. The absorbances of resulting solutions were measured at their respective λmax and isobestic point and plotted a calibration curve to get the linearity and regression equation.

Method I (Simultaneous equation method)

Simultaneous equation method of analysis is based on the absorption of drugs (paracetamol and meloxicam) at their wavelength maximum. Two wavelengths selected for the development of the simultaneous equations are 256 nm and 268.8 nm. The absorptivity values determined for paracetamol are 0.0667 (ax_1), 0.0561 (ax_2) and for meloxicam are 0.0231 (ay_1), 0.0300 (ay_2) at 256 nm and 268.8 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of drugs.

$$C_{paracetamol} = \frac{(A_2 x 0.0231) - (A_1 x 0.0300)}{-0.0007} \quad \text{.....Eqn.1}$$

$$C_{meloxicam} = \frac{(A_1 x 0.0561) - (A_2 X 0.0667)}{-0.0007} \quad \text{……..Eqn.2}$$

Where $C_{paracetamol}$ and $C_{meloxicam}$ are concentration of paracetamol and meloxicam respectively in mcg/mL. A_1 and A_2 are the absorbance of the mixture at 256 nm and 268.8 nm respectively.

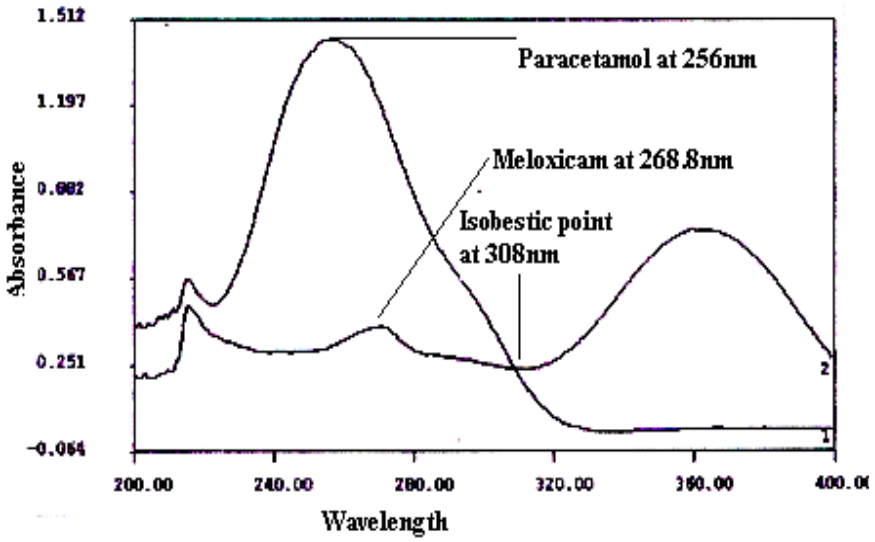

Fig 1: Overlain spectra of paracetamol and meloxicam

Method II (Absorbance ratio method)

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isobestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig. 1) 308 nm (isobestic point) and 256 nm (λmax of paracetamol) are selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorptivity values determined for paracetamol are 0.0140 (ax_1), 0.0667 (ax_2) and for meloxicam are 0.0192 (ay_1), 0.0231 (ay_2) at 308 nm and 256 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of drugs.

$$C_{paracetamol} = \frac{Q_M - 1.203}{3.5612} x \frac{A_1}{0.0140} \quad \text{…..Eqn.3}$$

$$C_{meloxicam} = \frac{Q_M - 4.7642}{-3.5612} x \frac{A_1}{0.0192} \quad \text{……Eqn.4}$$

Q_M, Q_X, and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1},\ Q_X = \frac{ax_2}{ax_1} = 4.7642,\ Q_Y = \frac{ay_2}{ay_1} = 1.203$$

Where $C_{paracetamol}$ and $C_{meloxicam}$ are concentration of paracetamol and meloxicam respectively in mcg/mL. A_1 and A_2 were the absorbance of the sample at 308 nm and 256 nm respectively.

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of paracetamol was transferred to 100 mL volumetric flask and dissolved in 0.1N NaOH and final volume was made up with 0.1N NaOH. The sample solution was then filtered through Whatman filter paper No.41. From the above solution 10 mL of solution was taken and diluted to 50 mL with 0.1N NaOH to get a solution containing 100 µg/mL of paracetamol and corresponding concentration of meloxicam. From above 2 mL of solution was transferred in 10 mL volumetric flask, to this added 5 µg/mL of pure meloxicam and diluted with 0.1N NaOH. Addition of 5 µg/mL of pure meloxicam to final solution is to bring the concentration in linearity range. With this addition, the concentration of paracetamol and meloxicam in the samples was brought in the ratio of 20:5.46. Analysis procedure was repeated six times with tablet formulation. The results of tablet analysis are reported in Table 2.

Validation of the developed methods

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method I and II, the Beer- Lambert's concentration range was found to be 5-30 µg/mL for paracetamol and 5-30 µg/mL for meloxicam. The linearity data for both methods are presented in Table 1.

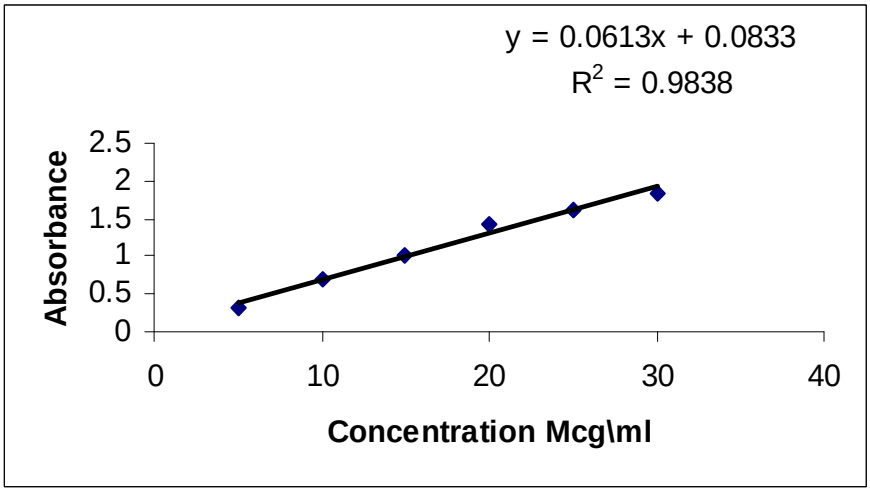

Fig 2: Linearity of paracetamol

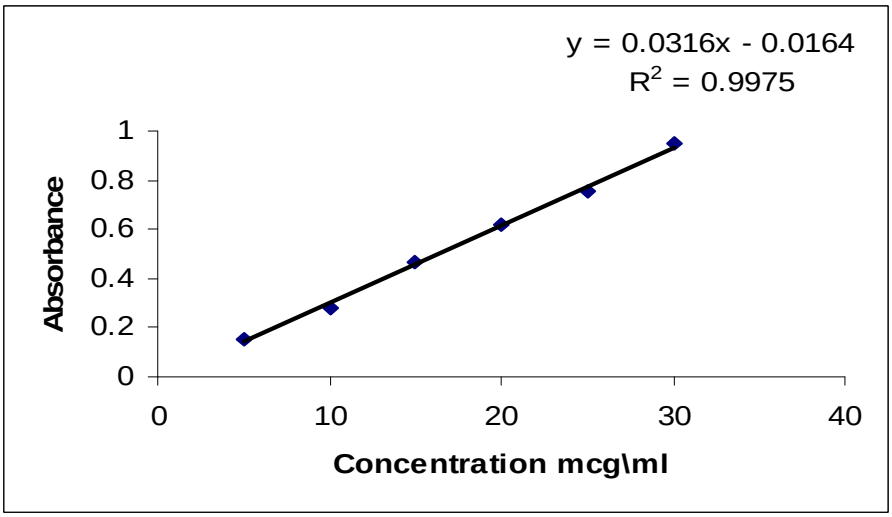

Fig 3: Linearity of meloxicam

Table 1: Optical Characteristics Data of Paracetamol and Meloxicam

Parameters	Values			
	PAR	MEL	PAR at isobestic point	MEL at isobestic point
Working λ	256 nm	268.8 nm	308 nm	308 nm
Beer's law limit (µg/ml)	5-30	5-30	5-30	5-30
Absorptive value*	0.0667	0.0300	0.0140	0.0192
Correlation coefficient*	0.9838	0.9975	0.9790	0.8720
Intercept*	0.0833	-0.0164	0.0190	0.0270
Slope*	0.0613	0.0316	0.0120	0.0160

*PAR: paracetamol, MEL: meloxicam, *Average of six estimation*

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and 120 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are quoted in Table 2.

Precision

Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The results of statistical evaluation are given in Table 2.

Intermediate Precision (Interday and Intraday precision)
The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table 3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ of paracetamol and meloxicam by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISCUSSION

Linearity range for paracetamol and meloxicam is 5-30 µg/mL and 5-30 µg/mL at respective selected wavelengths. The coefficient of correlation for paracetamol at 256 nm and for meloxicam at 268.8 nm is 0.9838 and 0.9975 respectively. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

Percentage estimation of paracetamol and meloxicam from tablet dosage form by method I is 100.81 and 99.95 and by method II is 99.42 and 100.56 respectively with standard deviation <2 (Table 2).

The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods is in good agreement with their respective label claims, which suggest non interference of formulation additives in estimation (Table 3).

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval of time and interassay precision. The standard deviation, coefficient of variance and standard error were calculated for paracetamol and meloxicam. The results were mentioned in Table 2. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter day precision study for both the methods % COV are not more than 2.0% indicates good repeatability and intermediate precision (Table 2).

The LOD values are 0.1576, 0.1255 µg/mL while LOQ values are 0.4778, 0.3805 µg/mL in method I and the LOD values are 0.1576, 0.0808 µg/mL while LOQ values are 0.4778, 0.2449 µg/mL in method II for paracetamol and meloxicam respectively. Low values of LOD and LOQ indicates good sensitivity of proposed methods.

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E*.	Amount Added		% Recovery #
								(%)	mg\mL	
I	PAR	325	327.63	100.81	0.8952	0.8880	0.3635	80	260	99.89
								100	325	99.00
								120	390	100.02
	MEL	7.5	7.49	99.95	1.0204	1.0209	0.4166	80	6	98.98
								100	7.5	98.50
								120	9	99.50
II	PAR	325	323.11	99.42	0.9538	0.9593	0.4083	80	260	99.50
								100	325	98.95
								120	390	100.04
	MEL	7.5	7.54	100.56	0.8802	0.8753	0.3594	80	6	99.60
								100	7.5	98.60
								120	9	99.00

*PAR: paracetamol, MEL: meloxicam, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery.*

Table 3: Validation Parameters

Method	Drug	LOD* µg/ml	LOQ* µg/ml	Precision (% COV)			
				Intraday n=3	Interday*		
					First day	Second day	Third day
I	PAR	0.1576	0.4778	0.6495	0.9058	0.5918	0.6884
	MEL	0.1255	0.3805	0.9424	0.7321	0.9320	0.7773
II	PAR	0.1576	0.4778	0.7807	0.9850	0.5031	0.5097
	MEL	0.0808	0.2449	0.7135	0.5918	0.6629	0.5929

*PAR: paracetamol, MEL: meloxicam, COV: Coefficient of variation, * Average of six determination.*

CONCLUSION

The proposed methods are simple, rapid and validated in terms of linearity, accuracy, precision, specificity and reproducibility. These two methods can be successfully used for simultaneous estimation of paracetamol and meloxicam in pure and tablet dosage form.

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