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Validated spectrophotometric methods for simultaneous estimation of Paracetamol, Domperidone and Tramadol HCl in pure and tablet dosage form

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Abstract: Two methods are developed for simultaneous estimation of paracetamol, domperidone and tramadol HCl in pure and tablet dosage form by using 0.1N NaOH as a solvent. Paracetamol, domperidone and tramadol HCl show absorbance maximums at 256 nm, 289.6 nm and 218.4 nm respectively. Shimadzu UV 1700, capable of multicomponent analysis, was used for quantitation. In method I, absorbance of the sample solution was measured at 256 nm and 289.6 nm for the estimation of paracetamol, domperidone respectively as tramadol HCl does not absorb at these wavelengths. Estimation of tramadol HCl was carried out at 218.4 nm. Method II is based on a multiwavelength spectroscopic method. Validation study reveals that the methods are specific, accurate, precise, and reproducible. All three drugs obey Beer's law in the concentration ranges used for the methods. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% COV< 2.0 and S.D. < 2.0) for both accuracy and precision study. High recovery and low % COV reveals the reliability of the method for quantitative study of three drugs in tablet formulation. The methods are simple, rapid accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of paracetamol, domperidone and tramadol HCl in pure and tablet dosage form.

Keywords: multi-wavelength spectroscopy; paracetamol; domperidone; tramadol HCl.

1. Introduction

Chemically, paracetamol is 4-hydroxy acetanilide, used as an analgesic and antipyretic drug. Domperidone (DMP) is chemically 5-chloro-1-[1-[3-(2-oxo-2,3dihydro-1H-benzimidazol-1-yl)propyl]-piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one used as antiemetic drug and tramadol HCl is (+-/)-cis-2-[(dimethylamino)methyl]-1-(3-methoxy-phenyl)cyclohexanol-hydrochloride, administrated orally non-steroidal anti-inflammatory drug which possesses good analgesic properties and good tolerability profile in variety of painful conditions. Paracetamol is official in Indian Pharmacopoeia [1] and British Pharmacopoeia [2]. These two pharmacopoeias suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations. Domperidone is official in BP [2] where assay is described by titrimetric method. Tramadol is official in BP [2]. These two pharmacopoeias suggest titrimetric (potentiometric) assay method for tramadol in bulk. However

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there are many methods reported for the determination of paracetamol by spectroscopy [3,4,5], chemometric-assisted spectrophotometric [6], RP-HPLC [7,] for domperidone by spectroscopy [8] and RP-HPLC [9] and for tramadol hydrochloride by GC [10] in plasma and brain tissue of mice and rats, using HPLC [11,12], in plasma and urine, and spectrohotometry [13] in combination with other drugs in pharmaceutical formulation. A combination of paracetamol, domperidone and tramadol HCl is commercially available in tablet dosage form. Literature reveals that no spectrophotometric method is available for simultaneous determination of these three drugs in combination. We communicate here rapid and cost-effective quality-control tool for routine quantitative analysis of three drugs in pure and their combined dosage forms by spectrophotometry.

2. Materials and Methods

2.1 Materials

UV-visible double beam spectrophotometer, Shimadzu model 1700 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 tablet, Ramcet-D (Label claim: paracetamol I.P.-325 mg, domperidone B.P.-10 mg, tramadol HCl B.P.-37.5 mg) was procured from local market.

2.2 Selection of common solvent

After assessing the solubility of drugs in different solvents 0.1N NaOH was used as common solvent for developing spectral characteristics.

2.3 Preparation of standard stock solution

The standard stock solutions of paracetamol, domperidone and tramadol HCl were prepared by dissolving 25 mg of each drug in 10 mL of 0.1N NaOH and final volume adjusted with 0.1NaOH in 100 mL 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 obtain the absorbance spectra and overlain spectra.

2.4 Method I: Three wavelength spectrophotometry

Overlain spectra (Fig. 1) suggest that, paracetamol, domperidone and tramadol HCl show absorbance maximums at, 256 nm, 289.6 nm and 218.4 nm and tramadol hydrochloride does not show absorbance at 256 nm (λmax of paracetamol) and 289.6 nm (λmax of domperidone), paracetamol and domperidone was estimated by following simultaneous equations.

$$C_{Paracetamol} = \frac{A_2 \times 0.0137 - A_1 \times 0.0298}{-0.0017} \quad \text{……..…Eqn.1}$$

$$C_{Domperidone} = \frac{A_1 \times 0.0312 - A_2 \times 0.0726}{-0.0017} \quad \text{……Eqn.2}$$

Where $C_{Paracetamol}$ and $C_{Domperidone}$ are concentrations of paracetamol and domperidone and A_1 and A_2 are the absorbance of the sample at 256 nm and 289.6 nm respectively.

Tramadol HCl is estimated at 218.4 nm. For this purpose the standard calibration curve of tramadol HCl was prepared at 218.4 nm in the concentration range of 0-25 mg/mL. A reference solution containing exactly the same concentration of, paracetamol and domperidone as determined

from the absorbance measurement at 256 nm and 289.6 nm was prepared. The absorbance of the sample solution containing paracetamol, domperidone and tramadol HCl was measured at 218.4 nm against the reference solution containing paracetamol and domperidone. The concentration of tramadol HCl is obtained from the calibration curve plotted at 218.4 nm.

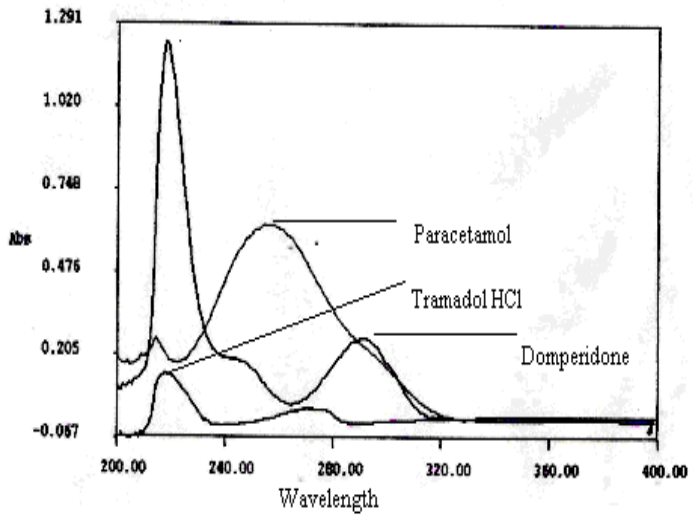

Figure 1. Overlain spectra of paracetamol, domperidone and tramadol HCl

2.5 Estimation of paracetamol, domperidone and tramadol HCl in tablet dosage form:

2.5.1 Estimation of paracetamol and domperidone in tablet dosage form:

Twenty tablets (RAMCET-D) were taken, their average weight was determined, and were crushed to fine powder. Then powder equivalent to 50 mg of paracetamol was put into a 100 mL volumetric flask and dissolved in 30 mL of NaOH with vigorous shaking for 5–10 minutes. Finally volume is adjusted with same solvent upto the 100 mL. This solution was transferred to a 100 mL of volumetric flask through a Whatman #41 filter paper. The residue was washed twice with 0.1N NaOH, and the combined filtrate was made up to the 100 mL mark with 0.1N NaOH. The above solution was further diluted with 0.1N NaOH to get a solution containing 10 mg/mL of paracetamol and corresponding concentration of domperidone. This was analyzed at 256 nm and 289.6 nm wavelengths, and the values of the absorptions were substituted in Eqns. (1) and (2) to obtain the content of paracetamol and domperidone. The results of this analysis are shown in Table 1.

2.5.2 Estimation of tramadol HCl:

Having determined the concentration of paracetamol and domperidone reference solution containing exactly the same concentration of paracetamol and domperidone (mg /mL) as contained in stock solution was prepared. The absorbance of the stock solution was measured at 218.4 nm against reference solution in the spectrum mode of the instrument and the concentration of tramadol HCl was obtained from the calibration curve of tramadol HCl. Standard addition of domperidone and tramadol HCl were done to improve its absorbance.

2.6 Method II: Multi-wavelength Spectroscopy

The overlain spectrum of paracetamol, domperidone and tramadol HCl is shown in Fig. 2. The use of five mixed standards and four sampling wavelength 218.4 nm, 256 nm, 289.6 nm, and 295 nm were found to serve the purpose of the experiment. Five mixed standard solutions containing paracetamol, domperidone and tramadol HCl in the concentration ratio of 5:0.575:0.15, 10:1.15:0.30, 15:1.725:0.46, 20:2.3:0.610, 25:2.875:0.770, (mg/mL) were prepared in 0.1N NaOH. All the mixed standard solutions were scanned over the range of 400 to 200 nm in the multicomponent mode using the four sampling wavelengths previously mentioned. Recording the absorbance of the mixed standard solutions was processed by the instrument by means of matrix equations and was then corrected to determine the concentrations of all the drugs in the tablet sample solutions. A tablet sample solution was prepared as described under method I. The spectrophotometric analysis of the resulting solution was carried out using the multicomponent mode of the instrument.

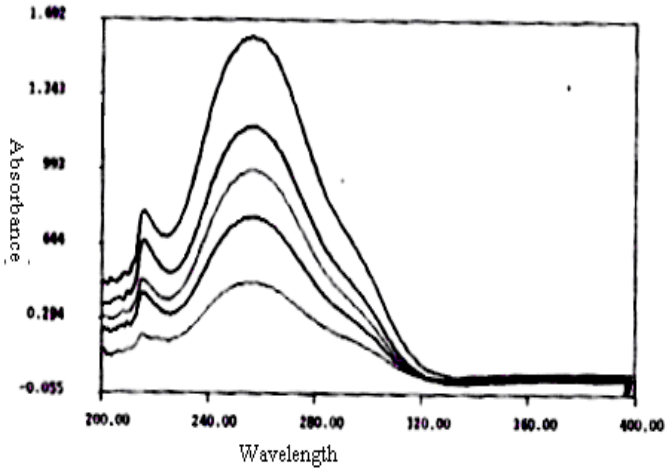

Figure 2. Overlain spectra of mixed standard of paracetamol, domperidone and tramadol HCl

2.7 Validation

2.7.1 Accuracy

Accuracy was confirmed by recovery study as per ICH norms [14] at three different concentration levels 80%, 100%, 120% by replicate analysis (n = 3). Here to a preanalysed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The result of accuracy study was reported in Table 2. From the recovery study it is clear that the method is accurate for quantitative estimation of paracetamol, domperidone and tramadol HCl in tablet dosage form as the statistical parameters are within the acceptance range (S.D. < 2.0).

2.7.2 Precision

Precision was determined as a repeatability and intermediate precision.

2.7.3 Repeatability

Repeatability result indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated. Repeatability was performed for six times with tablets formulation. The results of statistical evaluation are given in Table 1.

2.7.4 Intermediate Precision (Inter-day and Intra-day precision)

An intermediate precision was carried out by intra and inter day precision study. In intra day study concentration drugs were calculated on the same day at an interval of one hour. In inter day study the drug contents were calculated on three different days. Study expresses within laboratory variation in different days. In both intra and inter-day precision study for the methods % COV are not more than 1.0 indicating good intermediate precision (Table 3).

2.7.5 Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range is 0-40 µg/mL for paracetamol and domperidone and 0-25 µg/mL for tramadol HCl. The linearity data for both methods are presented in Table 3.

2.7.6 Limit of Detection (LOD) and Limit of Quantization (LOQ)

The LOD and LOQ of paracetamol, domperidone and tramadol HCl by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ 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.

3. Results and Discussion

The Beer- Lambert's concentration range is 0-25 µg/mL for paracetamol, domperidone and tramadol HCl at 256 nm, 289.6 nm and 218.4 nm wavelengths with coefficient of correlation 0.9793, 0.9904, and 0.9740 respectively. Drugs show good regression values at their respective wavelengths and the recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

Percentage estimation in tablet dosage form is 98.98, 99.95, 100.79 by method I where as 101.40, 98.04, 100.88 by method II for paracetamol, domperidone and tramadol HCl respectively with standard deviation <2.

In method II five mixed standard and three sampling wavelengths are selected through rational experimentation keeping in view the amount of drugs in the formulation and molar absorptivity coefficients (Fig. 2). The method requires no manual calculations, produces comparable results to the first method and is more suitable as compared to method I.

The validity and reliability of proposed methods are assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggests non-interference of formulation additives in estimation (Table 2).

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error are calculated for paracetamol, domperidone and tramadol HCl (Table 1). 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 1.0 indicating good intermediate precision (Table 3).

The LOD values are 0.8642, 0.0457 and 0.1487 µg/mL and LOQ values are 1.4571, 0.4875 and 0.7812 µg/mL for paracetamol, domperidone and tramadol HCl respectively. Low LOD and LOQ indicates good sensitivity of proposed methods.

Table 1. Analysis data of tablet formulation

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV
I	PAR	325	321.685	98.98	0.7574	0.7652
	DOM	10	9.995	99.95	0.4522	0.4524
	TRA	37.5	37.796	100.79	0.8654	0.8586
II	PAR	325	329.55	101.40	0.6785	0.6691
	DOM	10	9.804	98.04	0.9087	0.9268
	TRA	37.5	37.83	100.88	1.4532	1.4405

PAR: Paracetamol, DOM: Domperidone, TRA: Tramadol HCl, S.D.: Standard deviation**,** COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation.

Table 2. Result of recovery studies

Method	Recovery level (Added amount)	Percent recovery ± SD #		
		PAR	DOM	TRA
I	80%	98.99 ±0.0456	100.20±0.1045	101.20±0.3558
	100%	99.85 ± 0.0345	101.20±0.7567	98.90+0.4567
	120%	99.50±0.2321	100.10±0.6578	99.90±0.3452
II	80%	100.40±0.2341	99.60±0.567	100.5±0.876
	100%	101.10±0.1679	99.60±0.6784	99.30±0.7850
	120%	99.50±0.2451	100.3±0.3452	98.90±0.8704

PAR: Paracetamol, DOM: Domperidone, TRA: Tramadol, S.D.: Standard deviation, # Average of three estimation at each level of recovery.

Table 3. Optical characteristics data and validation parameters

Parameters	Values		
	PAR	DOM	TRA
Working λmax	256 nm	289.6 nm	218.4 nm
Beer's law limit (µg/mL)	0-25	0-25	0-25
Absorptive*	0.0726	0.0298	0.0179
Correlation coefficient*	0.9793	0.9904	0.9740
Intercept*	0.0312	0.0673	-0.038
Slope*	0.351	0.117	0.108
LOD* (µg/mL)	0.8642	0.0457	0.1487
LOQ*(µg/mL)	1.4571	0.4875	0.7812
Intra-Day* (Precision) (% COV)	0.6754	0.2317	0.3410
Inter-Day (Precision) (% COV) n=3	0.9834	0.9865	0.6709

PAR: Paracetamol, DOM: Domperidone, TRA: Tramadol HCl, COV: Coefficient of variation, * Average of six determination.

4. Conclusion

The proposed methods are simple, rapid accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of paracetamol, domperidone and tramadol HCl in pure and tablet dosage form.

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