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New validated UV spectrophotometric method for the estimation of rebamipide and tramadol in bulk and dosage forms

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ABSTRACT

New UV spectrophotometric (UV method) for the quantitative estimation of rebamipide, a potent antiulcer agent and tramadol, a centrally acting analgesic in pure form and in solid dosage forms was developed in the present study. The linear regression equations for rebamipide was Abs=0.09944Conc. (in µg/mL)+0.022 and tramadol was Abs=0.0059Conc. (in µg/mL)+0.003 measured at 228 nm and 271 nm respectively. The detection limit for rebamipide and tramadol was found to be 0.27µg/mL and 0.24µg/mL. The results of analysis were treated statistically, as per ICH guidelines for validation of analytical procedures, and by recovery studies.

KEYWORDS: Rebamipide, tramadol, uv-method, validation, Spectrophotometry

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INTRODUCTION

Rebamipide [2-(4-chlorobenzoyl) amino]-3-(2-oxo-1H-quinolin-4-yl) propanoic acid] (Figure. 1A), a novel quinolinone derivative, is a potent antiulcer agent with its main pharmacological actions being mediated by increasing endogenous prostaglandin synthesis and by scavenging the oxygen-derived free radicals, which play an important role in gastric mucosal cell damage [1-2]. It is reported to increase the synthesis of mucus, to increase the mucosal concentration of endogenous prostaglandin, and to promote rapid ulcer healing [3-4].

Tramadol hydrochloride [cis-2-((dimethylamino) methyl)-1-(3-methoxyphenyl) cyclohexanol hydrochloride] (Figure. 1B), is a centrally acting analgesic, used in the treatment of moderate to severe acute and chronic pain [5].

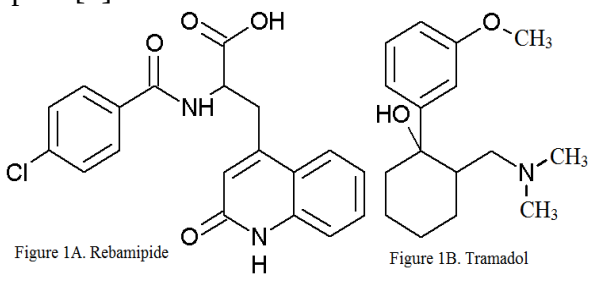


Figure 1A. Rebamipide

Figure 1B. Tramadol

As the use of rebamipide and tramadol is increasing rapidly, it is essential to develop simple and suitable analytical method for its estimation in formulations. Such method should provide better sensitivity and selectivity for routine quality control analysis, dissolution or similar studies. A survey of literature had not revealed any UV-visible spectrophotometric method for rebamipide; however three liquid chromatographic methods for rebamipide estimation in serum [6-7] and [8] have been reported; UV methods for estimation of tramadol was reported [9-10] is only suitable for estimating ampoule dosage forms and requires addition of reagent. In present study, simple, economical, accurate and reproducible analytical method with better detection range for estimation of rebamipide and tramadol in its pure form and its solid dosage forms were developed. This paper describes a UV method (UV spectroscopic method) for estimation of rebamipide and tramadol in pharmaceutical formulations. Rebamipide was taken in 25% v/v methanol in phosphate buffer pH 7.4 [14] and estimated at 228 nm. Tramadol was taken in phosphate buffer pH 6.8 [14] and estimated at 271 nm. The UV method was aimed at developing an easy and rapid assay for rebamipide without any time consuming sample preparation steps for routine analysis to be adopted in quality control and drug testing laboratories, and at the same time ensuring satisfactory recovery during drug estimation from pharmaceutical formulations. In the proposed method there is no need to extract the drug from the formulation excipient matrix there by decrease in the error in quantization. Formulation sample can be directly used after dissolving and filtration. The developed methods were used to estimate the total drug content in commercially available formulation of rebamipide and tramadol.

MATERIALS AND METHODS

1. Experimental

1.1. Chemicals

HPLC grade methanol was purchased from S.D. Fine Chemicals Ltd., Mumbai, India. Sodium lauryl sulfate, Potassium phosphate, Monobasic and Sodium Hydroxide pellets were purchased from S.D. Fine Chemicals, Mumbai, India. High Pure water was prepared using Millipore purification system (Millipore, Molsheim, France, model Elix SA 67120). Tramadol was obtained as a gift sample from IPCA Labs Ltd., Mumbai, India. The commercially available tablet of rebamipide (Rebagen), tramadol (tragesic capsules and tramazac tablets) was taken from Indian market. The tablets normally contain common additives like diluents (lactose, aerosil, etc.), glidants and lubricants (magnesium stearate, etc.).

1.2. Equipment

A UV-visible-NIR spectrophotometer (Jasco V-570, Tokyo, Japan) with automatic wavelength accuracy of 0.1nm, and 10 mm matched quartz cells with spectra manager software was used for all absorbance measurements.

1.3. Method Development

To develop a robust and suitable UV spectrophotometric method for the analysis of rebamipide and tramadol in formulations different solvent systems were used. The criteria employed for assessing the suitability of particular solvent system for the drug was cost, time required for analysis, sensitivity of assay, solvent noise, preparatory steps involved and the use of same solvent system for extraction of drug from formulation excipient matrix for the estimation of drug content.

1.4. Preparation of standard curve for UV method

A 100µg/mL standard stock solution of rebamipide was prepared by dissolving 10 mg of drug in 100mL of 25% v/v methanol- potassium phosphate buffer (pH 7.4) mixture. The λ_{max} of

rebamipide in the media was determined by various dilutions made to obtain solutions of 2, 4, 6, 8 and 10µg/mL. Similarly, tramadol was dissolved in phosphate buffer (pH 6.8) and various dilutions made to obtain solutions of 20, 40, 60, 80 and 100µg/mL, and absorbance were measured for each dilution. The results are listed in the Table 1. The stability of the two drugs in solvent system during the actual analysis (Figure. 1B and 2B) was also investigated.

1.5. Method validation

1.5.1. Accuracy and Precision: as a part of determining accuracy of the proposed methods, different levels of drug concentrations (LQC, MQC and HQC respective media) were prepared from independent stock solution and analyzed (n = 10). Accuracy was assessed as mean percentage recovery shown in Table 2.

1.5.2. Linearity: Five separate series of solutions of rebamipide, 2-10µg/mL and tramadol 20-100µg/mL were prepared from the stock solution and analyzed.

1.5.3. Specificity: Series of five solutions of rebamipide (6µg/mL) and tramadol (60µg/mL) were prepared from the stock solution meant for method validation and analyzed.

1.5.4. Limit of quantization (LOQ) and Limit of detection: (LOD): LOQ and LOD were calculated on the basis of response of blank and slope of the regression equation. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected.

1.5.5. Robustness: Robustness of the method for rebamipide was determined by varying the pH of the phosphate buffer between 7.2 and 7.6 and performing the analysis at 23%, 25% and 27% of methanol and for tramadol was determined by varying the pH of the phosphate buffer between 6.6 and 7.0.

1.6. Estimation of rebamipide and tramadol from commercial tablet formulation:

The commercially available tablets of rebamipide (Rebagen) (at this time only one manufacturer is available) were taken from the Indian market for estimation of the total drug content per tablet by the proposed method. Twenty tablets were weighed, and contents were thoroughly mixed and an accurately weighed aliquot amount (equivalent to 5 mg of rebamipide) was transferred to a series of 50 mL volumetric flasks (five in each case) and volume was made using (25:75) methanol-phosphate buffer pH 7.4. Similar procedure was followed for tramadol and diluted with phosphate buffer pH 6.8. Finally all the above solutions were filtered through Whatman filter paper number 1 and the filtrate was suitably diluted to get final concentration within the limits of linearity as given in Table 2. From the absorbance value the drug content per tablet (on average weight basis) was calculated as described in recovery studies and Table 4.

RESULTS AND DISCUSSION

1.7. Method development

For developing the UV method for these two drugs, various solvent systems investigated were high pure water, 0.5-2% of sodium lauryl sulfate, 0.5-2% of tween 20, 40, 60 and 80, 0.1N hydrochloric acid, 0.1N sodium hydroxide, methanol and phosphate buffers of various pH (6.8-7.8). Methanol (15-30%) with different buffers was employed to improve the sensitivity of rebamipide. In such combinations, pH of the buffer selected was one in which drug gave maximum stability and absorbency. The final decision for using 25% methanol in phosphate buffer pH 7.4 as the solvent for rebamipide and phosphate buffer pH 6.8 for tramadol was selected based on sensitivity, ease of preparation, suitability for drug content estimation, time

and cost in that order. The overlay spectra of rebamipide in (25:75) methanol: phosphate buffer (pH 7.4) and tramadol in phosphate buffer pH 6.8 is shown in Fig.2A and 3A. The λ_{max} was found to be 228 nm for rebamipide and 271 nm for tramadol. The statistical analysis of data obtained for estimation of rebamipide and tramadol in respective solutions indicated a high level of precision for the proposed method as evidenced by low standard deviation values Table 1, further established the precision of the proposed method. The drug solutions were stable for a period of 48 hours and there was no microbial growth in the used media.

Table 1: Calibration curve points of the proposed methods for estimation of rebamipide and tramadol.

UV method-Rebamipide (in 25:75 Methanol-Phosphate buffer, pH 7.4)			
Concentration (µg/mL)	Mean absorbance value [a]	CV(%)	Standard error
2	0.2253± 0.0035	1.56	0.0011
4	0.4147 ±0.0029	0.65	0.0009
6	0.6144 ±0.0027	0.44	0.0008
8	0.8177 ±0.0040	0.49	0.0012
10	1.0207 ±0.0078	0.79	0.0024
UV method-Tramadol (in Phosphate buffer, pH 6.8)			
Concentration (µg/mL)	Mean absorbance value [a]	CV(%)	Standard error
20	0.1243±0.0008	0.68	0.0002
40	0.2387±0.0021	0.87	0.0006
60	0.3600±0.0030	0.83	0.0009
80	0.4798±0.0047	0.99	0.0015
100	0.6000±0.0089	1.48	0.0028

[a] *average of ten determinations with standard deviation.*
[b] *coefficient of variance (Relative Standard Deviation)*

Table 2: Validation Parameters

Rebamipide	**Predicted conc. (µg/ml)** [a]			**Mean % Recovery ± S.D**
Level	**Range**	**Mean ± S.D.**	**% R.S.D.**	
LQC	1.97-2.08	2.002 ± 0.04	1.99	99.89±0.33
MQC	5.4-5.82	5.69 ± 0.08	1.42	100.72±0.14
HQC	8.86-9.05	9.01 ± 0.13	1.21	101.98±0.37
Tramadol				
LQC	24.84-26.09	25.43±0.21	0.82	100.02±0.48
MQC	54.92-56.13	55.84±0.62	1.11	99.98±0.22
HQC	94.89-96.06	95.35±0.64	0.67	99.96±0.32
Linearity	**(µg/mL)**			
Rebamipide	2-10			
Tramadol	20-100			
Specificity				
Rebamipide	A 6µg/mL solution will show an absorbance of 0.61446±0.00274 at 228 nm.			
Tramadol	A 60µg/mL solution will show an absorbance of 0.36004±0.00302 at 271 nm.			
Limit of detection [b]	**(µg/mL)**			
Rebamipide	0.27			
Tramadol	0.24			
Limit of quantization	**(µg/mL)**			
Rebamipide	0.82			
Tramadol	0.75			
Robustness	**(Mean %recovery±S.D.)**			
Rebamipide	100.66±1.0818			
Tramadol	99.98±0.02439			

[a] *Predicted concentrations were calculated by linear regression equation.*
[b] *Based on standard deviation of blank response and slope of regression curve.*

Table 3: Results of optical characteristics and least square regression analysis data for the estimation of Rebamipide and tramadol by the proposed method

Parameters	Rebamipide	Tramadol
Molar absorptivity (1 $mol^{-1}cm^{-1}$)	3.82×10^{4}	1.56×10^{3}
Regression equation[a]	Y=0.0994X+0.022	Y=0.0059X+0.003
Correlation coefficient (r)	0.9999	0.9999
Standard error of slope	3.08×10^{-5}	4.11×10^{-5}
Standard error of intercept on ordinate	2.2×10^{-3}	1.4×10^{-3}
Standard error of the estimate	1.2×10^{-4}	1.6×10^{-3}
Slope without intercept	0.09944	0.005958
Y-intercept when X=0.0	0.02243	0.003432
X-intercept when Y=0.0	-0.2255	-0.5760
95% c.i.[b] of the slope	0.0978 to 0.1011	0.00587 to 0.0060
95% c.i. [b] of the intercept	-0.3296 to -0.1244	-0.0020 to 0.0089
Calculated F-value (critical F-value)[c]	0.81 (2.7587)[d]	1.24 (2.8660)[e]

[a] Based on five calibration values; Y Absorbance ; X Concentration of the drug in µg/mL; [b] Confidence interval
[c] Based on one way ANOVA test at p=0.05 level of significance; [d] F(4, 25),), [e]F(4, 24), Theoretical values of .

Table 4: Assay of rebamipide and tramadol commercial formulations by the proposed methods

Sample	Label claim (mg)	Percentage recovery		
		Amount Found	CV%	Assay (%)
Rebamipide				
Rebagen tablets	100	98.84±1.21	1.249	98.8
Tramadol				
Tragesic capsules	50	49.1±0.59	1.201	98.2
Trazac tablets	50	48.5±0.74	1.525	97.0

The linear regression equation obtained for rebamipide and tramadol was Y = 0.09944X + 0.022(r = 0.9999) and Y = 0.0059X + 0.003(r = 0.9999), where Y is the absorbance and X is the concentration (in µg/mL) of pure drugs in respective solutions. The correlation coefficient values obtained were highly significant for the method given in Table 3.

Figure 2A: Absorbance spectra of Rebamipide at difference concentrations (2-10 µg/mL) of Rebamipide

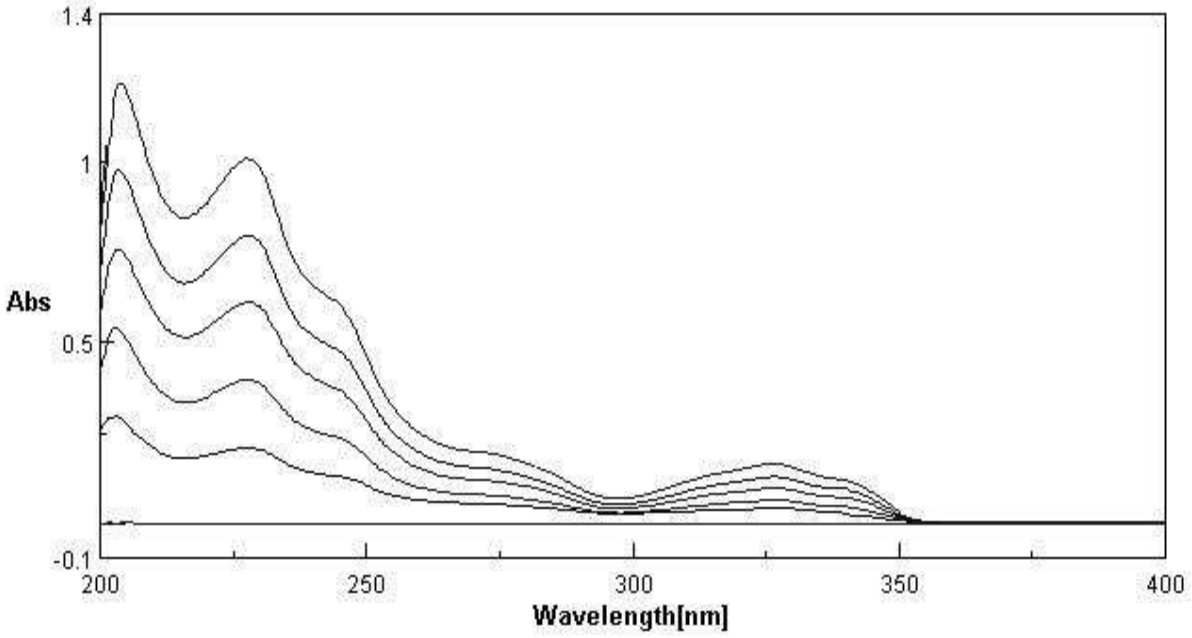


Figure 2B: Absorption spectra of 6 µg/mL concentration of Rebamipide in 25% Methanol-Phosphate buffer pH 7.4 at 0^{th} h and 24^{th} h. (Dark line at 0 h; light broken line- 24^{th} h).

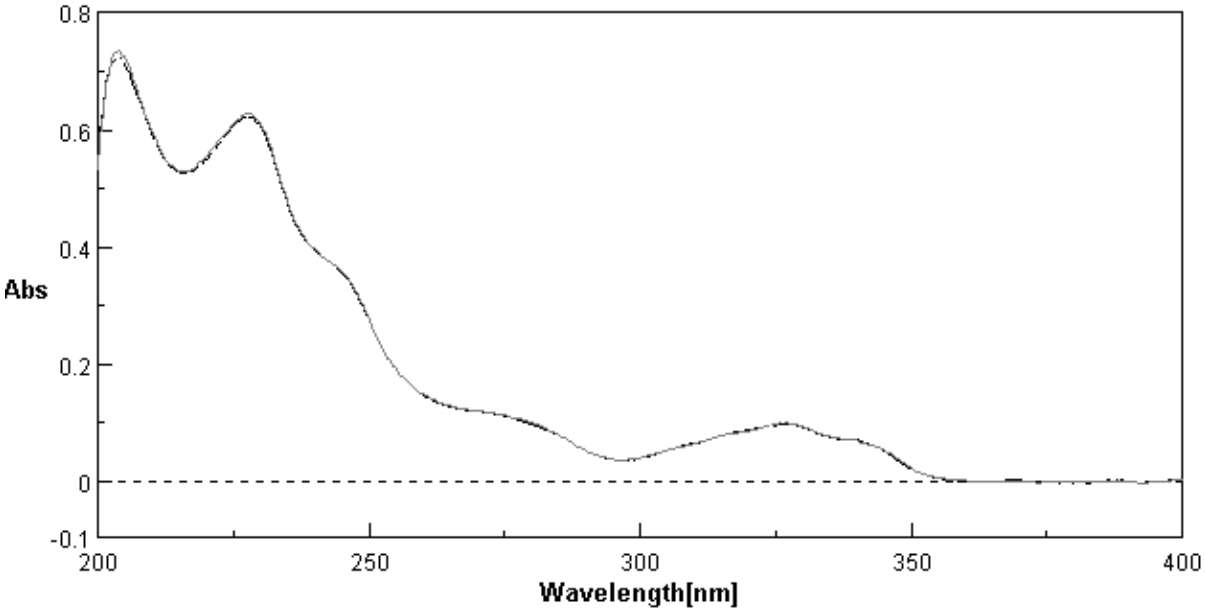


Figure 2C: Absorption spectra of 6 µg/mL concentration of Rebamipide (bulk form and marketed formulation) (25% methanol –Phosphate Buffer pH 7.4) (Dotted line-drug in marketed formulation, dark line-pure drug).

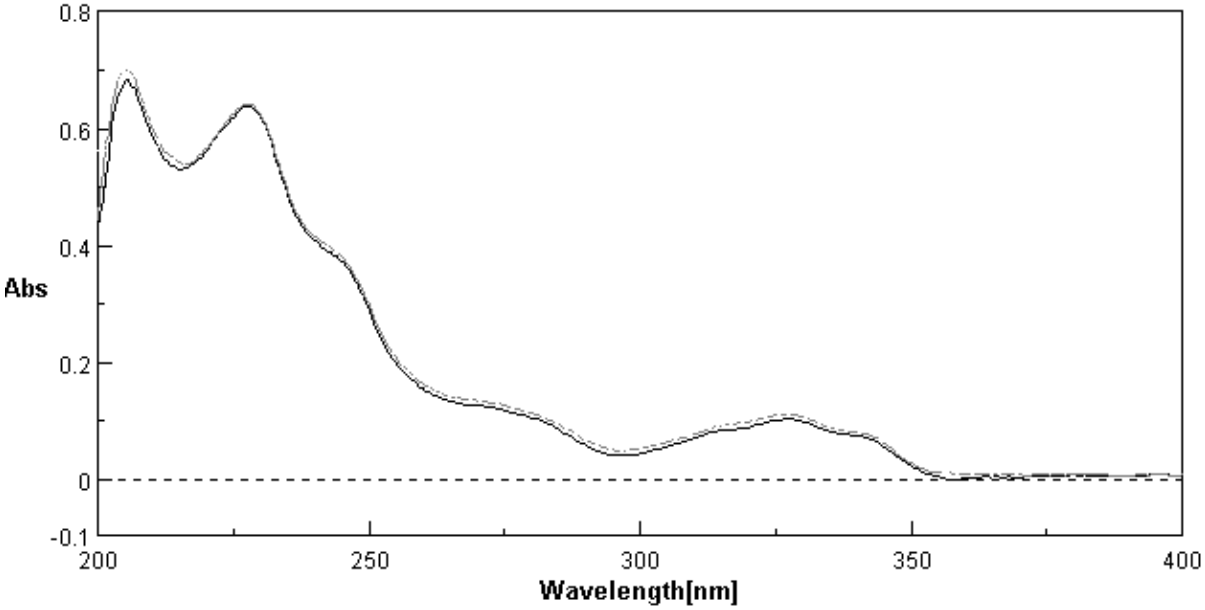


Figure 3A: Absorbance of Tramadol at different concentrations in the range of 20-100µg/ml.

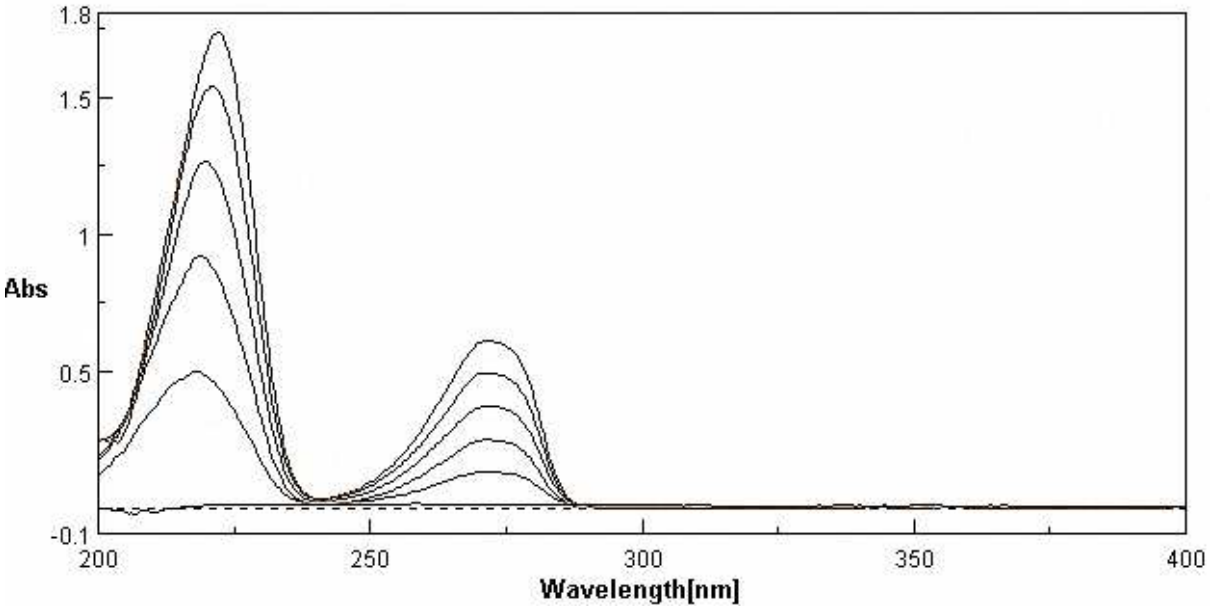


Figure 3B: Absorption spectra of 20 µg/mL concentration of Tramadol in Phosphate Buffer pH 6.8 at 0th h and 24th h. (Light line-drug in at 0 h; light broken line-drug after 24th h)

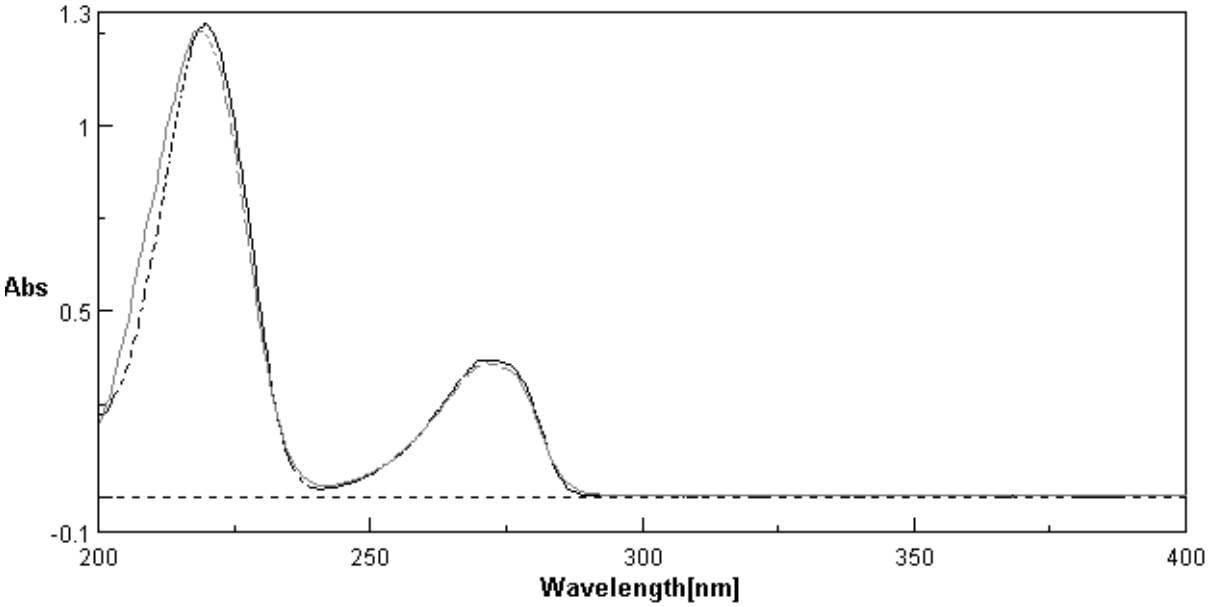
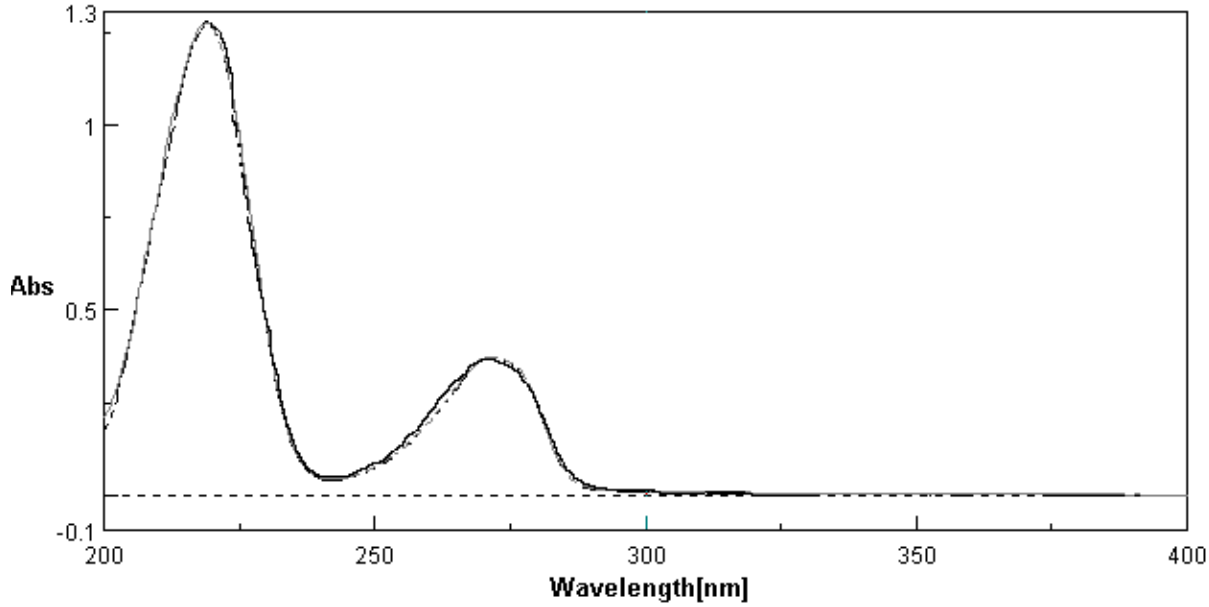


Figure 3C: Absorption spectra of 60 µg/mL concentration of Tramadol (bulk form and marketed formulation) in Phosphate Buffer pH 6.8. (Dotted line-drug in marketed formulations, dark line-pure drug).



A one-way ANOVA test [11], [12] was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions. The calculated F-value was found to be less than the critical F-value at 5% significance levels in this method given in Table 3.

Validation of the developed Methods

The developed methods were validated according to standard procedures [13], [14] and the results obtained are tabulated in Table 2.

The linearity range for rebamipide was 2-10 µg/mL at a λ_{max} of 228 nm and tramadol was 20-100 µg/mL at a λ_{max} of 271 nm. The limit of detection (LOD) and limit of quantization (LOQ) for rebamipide (0.27 µg/mL and 0.82 µg/mL) and tramadol (0.24 µg/mL and 0.75 µg/mL) given in Table 2.

For the developed UV method, varying the pH of the phosphate buffer from 7.4-7.8 for rebamipide, 6.6-7.0 for tramadol did not affect the sensitivity of the method. Although varying the percent of methanol did not affect the rebamipide linearity range of the UV method ±5% change observed in the absorbency at different concentrations of the calibration curve. The validation parameters of the method are presented in Table 2. The intra- and inter- day variations calculated on the basis of percentage relative standard deviation on replicate set of calibration samples (n=5 at each concentration) was less than 3%. In Table 2, the accuracy is reported in terms of % recovery and precision in terms of % RSD. The low values of these parameters reflect excellent measurement of accuracy and precision of the method of estimation of rebamipide and tramadol.

1.8. Recovery studies

The method was evaluated by estimation of rebamipide in pharmaceutical formulation by the proposed methods and analysis of pure drug solution as a reference. The percentage recovery (mean and standard deviation for five triplicate determinations) of drug from this (Rebagen)

formulation by the proposed method was found to be 98.84±1.21% and % assay was found to be 98.8%. The percentage of recovery (mean and standard deviation for five triplicate determinations) of drug from (tragesic capsules and trazac tablets) formulations by the proposed method varied from 97% to 98.2%. The estimated drug content with extremely low value of standard deviation established the precision of the proposed methods. The accuracy of the results of estimation for rebamipide and tramadol was further tested by recovery by adding known amount of pure drug to pre analyzed samples of the formulation. Recovery experiments using the developed assay procedures further indicated absence of interference from commonly encountered pharmaceutical excipients used in the selected formulation.

CONCLUSIONS

The proposed method of estimation of rebamipide and tramadol was found to be accurate, precise, and easy. As the LOQ is very low for rebamipide (0.82µg/mL) and tramadol (0.75µg/mL), the method can be adopted for routine quality testing and dissolution studies.

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