

RESEARCH ARTICLE

DEVELOPMENT OF A UV-SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF TRAMADOL HYDROCHLORIDE AND PARACETAMOL IN BULK AND MARKETED PRODUCT

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A specific, rapid and simple UV spectrophotometric method with good sensitivity was developed and validated for the simultaneous quantification of tramadol HCl and paracetamol in bulk and marketed product by simultaneous equation method. From the optical characteristics of the proposed methods, it was found that the λ_{max} of tramadol-HCl and paracetamol was found to 271 nm and 248 nm respectively. Tramadol HCl and paracetamol obey linearity within the concentration range of 2.5-15 $\mu\text{g/ml}$ and 3-15 $\mu\text{g/ml}$. The %RSD is less than 2%. The percentage recovery values of pure drug from the pre-analyzed formulations were in between 99-103%. The analysis of the formulation showed good result in concentration in range of 98-101%. This analytical method is also applicable in ordinary laboratories and can be adopted for quality control tests for these drugs in marketed formulation.

Key words: UV spectrophotometric method, Tramadol-HCl, Paracetamol, Simultaneous determination.

INTRODUCTION

Tramadol-HCl (\pm cis-2-[(dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride) and paracetamol (N-(4-hydroxyphenyl)acetamide) have been extensively used as antipyretic and analgesic drugs (**Figure 1a, 1b**).

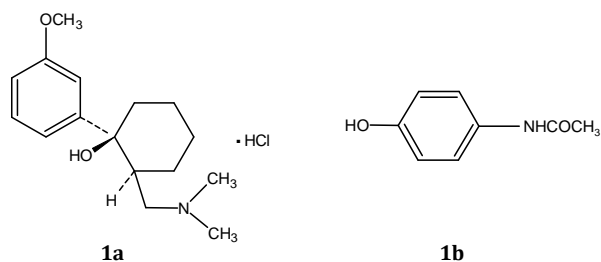


Figure 1a, 1b. Structures of tramadol-HCl and paracetamol

They are frequently prescribed in admixture with each other or in combination with other drugs. Literature revealed that several methods have been reported for the quantification of tramadol-HCl and paracetamol individually but yet no analytical method using UV spectrophotometer for their simultaneous quantification is reported. However, literature is enriched with reports showing application of spectrophotometric methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in tablet (Puranik *et al* 2006), application of liquid chromatographic assay/GC-MS for simultaneous determination of tramadol and its active metabolite in human plasma

(Campanero *et al* 1998; Li and Wang, 2006; Chan and Ho, 1998; Lintz and Uragg, 1985), HPLC determination and validation of tramadol hydrochloride in capsules (Kartinasari *et al* 2004), determination of tramadol in capsules by high performance thin layer chromatography - densitometry (Venkateshwarlu *et al* 2008), simultaneous determination of acetaminophen and tramadol by second derivative spectrophotometry (Toral *et al* 2008), simultaneous densitometric analysis of tramadol hydrochloride and chlorzoxazone by high-performance thin-layer chromatography (Gandhimathi and Ravi, 2008), conducting reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form (Godse *et al* 2009), application of high performance thin-layer chromatographic method for simultaneous estimation of paracetamol and valdecoxib in tablet dosage form (Gandhimathi *et al* 2007).

A successful attempt has been proposed in present investigation to quantify the tramadol-HCl and paracetamol simultaneously by spectrophotometer. The UV spectrophotometric analyses are often preferred in quality control testing and ordinary laboratories due to its broad availability and suitability. The objective of this study was to develop and validate a simple and specific UV spectrophotometric method for the simultaneous determination of tramadol-HCl and paracetamol in pure drug and marketed formulation. This method exhibits precise, accurate and cost effective assay for pure drug and marketed formulation.

MATERIALS AND METHODS

Materials

Tramadol HCl (purity 99.91%) and paracetamol (purity 99.99%) samples were provided by Zydus Cadila healthcare, Ahmedabad. All chemicals utilized were of analytical grade and were used without further purification. Tablets manufactured by Johnson and Johnson Ltd were utilized in present work. Double distilled water was used in the present study. UV-Vis spectrophotometer (ELICO SL 164 instrument) with spectral bandwidth of 0.1 nm and wavelength accuracy of ± 0.5 nm with automatic wavelength correction was used in current work.

Methods

Preparation of stock solutions

For the preparation of stock solution, 100 mg of paracetamol and tramadol hydrochloride were weighed and dissolved individually in a 100 ml

standard flask. From this, appropriate dilution of the solution was made to contain 2-20 $\mu\text{g/ml}$ and 1.5-20 $\mu\text{g/ml}$ of paracetamol and tramadol hydrochloride respectively. The mixing of both the drugs was done in final dilution.

Method development

Selection of an appropriate solvent system:

Based on the solubility, the physical and chemical characters of both the drugs, ethanol was considered to be suitable for the current work and the selected solvent showed a null absorbance in UV range.

Selection of wavelength:

Both the drugs were diluted to a common concentration of 10 μg in the selected solvent and scanned in the range of 200-365 nm against blank.

Determination of absorptive value and concentration in pure drug:

Method - simultaneous equation was adopted for determination of absorptive value and concentration in pure drug. The absorbance at 249 nm for paracetamol and at 271 nm tramadol hydrochloride was measured and calibration curves were plotted. The absorptive values were determined with both the wavelengths in mixture. The absorbance and absorptive values at particular wavelength were calculated and substituted in the equation to obtain the concentration.

$$C_{PM} = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \quad \text{Eq 1}$$

$$C_{TRD} = A_2ax_1 - A_1ax_2 / ax_2ay_1 - ax_1ay_2 \quad \text{Eq 2}$$

The concentration of tramadol HCl and paracetamol in mixture was determined by solving equations 1 and 2.

Method validation

Working solutions of both drugs in their respective concentration range were determined by scanning with a wide dilution and linearity was found to be 3-15 $\mu\text{g/ml}$ and 2.5-15 $\mu\text{g/ml}$ for paracetamol and tramadol hydrochloride respectively (**Table 1**). Standard calibration curve for paracetamol and tramadol HCl are represented by **Figure 1, 2** respectively. **Figure 3** represents the overlaying spectra of tramadol-HCl and paracetamol. To check precision (percentage RSD) of method, six replicate samples of 6 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ concentrations of paracetamol and tramadol-HCl were analyzed simultaneously. To study the accuracy and reproducibility of the proposed

Table 1. Validation parameter

Parameter	Paracetamol		Tramadol HCl	
	249 nm		271 nm	
Linearity range ($\mu\text{g/ml}$)	3-15 $\mu\text{g/ml}$		2.5-15 $\mu\text{g/ml}$	
	Concentration	Absorbance	Concentration	Absorbance
	3	0.223	2.5	0.142
	6	0.423	5	0.296
	9	0.621	7.5	0.443
	12	0.832	10	0.596
	15	1.01	12.5	0.749
			15	0.926
Coefficient of correlation (r^2)	0.9992		0.9991	
Slope	0.0685		0.0617	
Y-intercept	0.0102		0.0016	
Repeatability (RSD)*	0.7002		0.518	

*RSD for six determinations

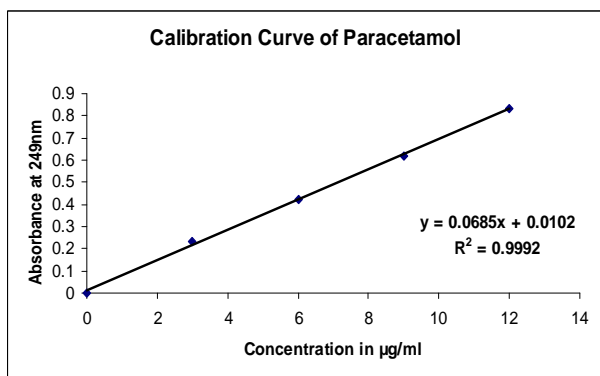


Figure 1. Standard curve of paracetamol at 249 nm

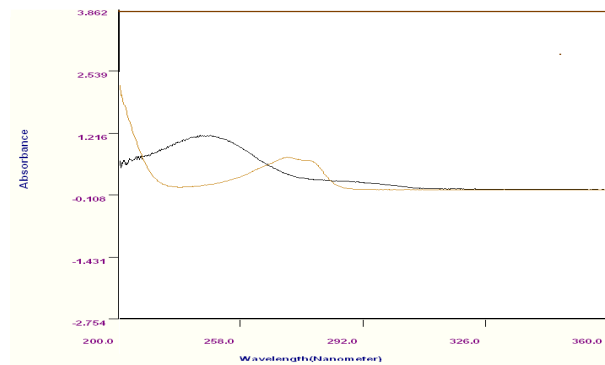


Figure 3. Overlaying spectra for paracetamol and tramadol·HCl

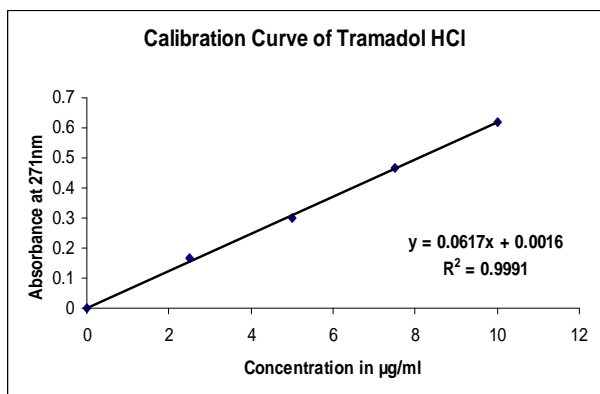


Figure 2. Standard curve of tramadol·HCl at 271 nm

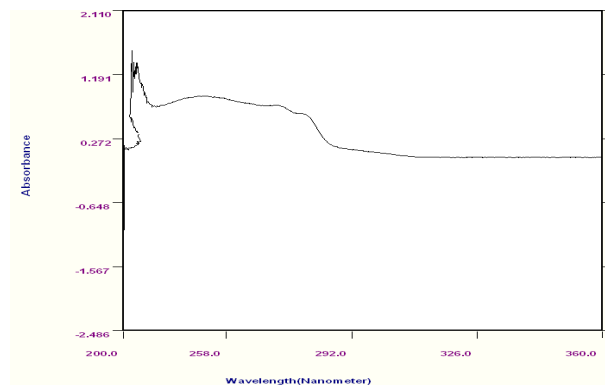


Figure 4. Formulation containing paracetamol and tramadol·HCl

Table 2. Recovery study

S. No.	Drug	% Label Claim (\pm %RSD*)	Recovery % (\pm %RSD*)		
			80%	100%	120%
1	Paracetamol	98.35 \pm 0.65	101.50 \pm 0.20	99.81 \pm 0.11	100.71 \pm 0.78
2	Tramadol·HCl	98.51 \pm 0.69	100.47 \pm 0.55	100.89 \pm 0.89	100.06 \pm 0.01

*RSD for three determinations

Table 3. Analysis of formulation

Formulation	Label Claim (g/tab)	Amount found gm/tab \pm RSD*	% Assay \pm RSD*
Paracetamol	0.325	0.319 \pm 0.65	98.35 \pm 0.65
Tramadol·HCl	0.0375	0.0369 \pm 0.69	98.51 \pm 0.69

*RSD for three determinations

method, recovery studies were carried out by adding known amount of drugs to pre-analyzed sample at 3 levels and the % recovery was calculated.

Analysis of formulations

Twenty tablets (brand name - Ultracet, manufactured by Johnson and Johnson Ltd.) were taken and their average weight was determined. Tablets were crushed to fine powder and powder equivalent to 11.54 mg of tramadol·HCl and 100 mg of paracetamol was transferred to 100 ml volumetric flask. The tramadol·HCl present in tablet powder could not be found accurately due to low absorbance. Hence, to increase the accuracy, accurately weighed 40 mg pure tramadol·HCl was added to the crushed tablet, which increased the amount of tramadol·HCl to 51.54 mg. It was then, dissolved in 50 ml of ethanol by intermittent shaking for 4-5 min. The volume was made up to 100 ml by same solvent, and the solution was filtered through Whatman filter paper no. 41. Appropriate dilutions were prepared from the above stock solution to get the final concentration of paracetamol 10 μ g/ml and

tramadol 5.15 μ g/ml and the amount of drug was determined (**Figure 4, Table 3**).

RESULTS AND DISCUSSION

The regression coefficient for paracetamol and tramadol hydrochloride was found to be 0.9992 at 249 nm and 0.9991 at 271 nm respectively which indicated good correlation between concentration and absorbance within the concentration range tested. The results suggested good precision for paracetamol (0.7002%) and tramadol·HCl (0.518%) (**Table 1**). The intra- and inter-day precision was found to be less than 2% whereas the recoveries were found to be 100.70 \pm 0.36% and 100.47 \pm 0.48% for paracetamol and tramadol·HCl (**Table 2**).

CONCLUSION

It can be concluded from the results that this method for the simultaneous determination of tramadol·HCl and paracetamol in bulk drug and marketed product is specific, rapid and simple with sufficient sensitivity which is applicable in ordinary laboratories and can also be adopted for quality control tests for raw material and combined dosage form in tablets.

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