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Simultaneous analysis of Paracetamol and Tramadol - Analytical method development & validation

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Abstract

In the present study the development and validation of analytical method was carried out for simultaneous analysis of paracetamol and tramadol using first derivative method. The λ max was determined for paracetamol and tramadol and standard curves were plotted. The stability of the stock solutions with due course of time was also determined and compared using t-Test. Further the simultaneous analysis at 1:1, 8:1 and 1:8 ratios revealed the need of a higher derivative method for the simultaneous estimation of paracetamol and tramadol in various dosage forms.

Keywords: Paracetamol, tramadol, simultaneous estimation, method development, validation.

Introduction

UV spectroscopy is concerned with ranges from 190 to 370 nm. Compound which are colourless absorb radiation in UV range. In both UV as well as visible spectroscopy only the valence electrons absorb the energy there by molecule undergoes transition from ground state to exited state. This absorbance is characteristic and depends on the nature of the electron present. The intensity of the electron depends on the concentration and path length as given by Lambert beer's law. According to Beer's law: The intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically. According to Lambert's law: The rate of decrease of intensity (monochromatic light) with the thickness pf medium is directly proportional to the intensity of incident light

$$A = \varepsilon . c . t$$

where A is absorbance, **ε** is the molar absorbtivity with units of L mol^{-1} cm^{-1}, t is the path length of the sample - that is, the path length of the cuvette in which the sample is contained. We will express this measurement in centimeters, c is the concentration of the compound in solution, expressed in mol L^{-1} [1].

Method development is the setting up of an analytical procedure that will be appropriate for the analysis of a particular sample and makes the analysis simpler, reliable and easier. It starts with the choice of the technique according to the work. Efficient UV method development requires expert knowledge of Spectroscopic science and extensive practical experience. Methods validation is the process of demonstrating that analytical procedures are suitable for their intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support the analytical procedures. For pharmaceutical methods, guidelines from the United States Pharmacopeia (USP), International Conference on Harmonisation (ICH), and the Food and Drug Administration (FDA) provide a framework for performing such validations [2, 3]

Tramadol is a synthetic, centrally acting opioid analgesic that has a weak action on μ-opioid receptors (6000 times weaker than morphine)1 and additionally inhibits reuptake of noradrenaline and enhances serotonin release within central pain pathways.2 Paracetamol is a non-opioid, non-salicylate analgesic with an unclear mechanism of action. It appears to have some central actions including inhibition of N-methyl-D-aspartate, substance P mediated nitric oxide synthesis and release of prostaglandin E_2 [4]. Paracetamol (325mg), a non-opioid and non salicylate analgesic. It is indicated for the treatment of moderate to severe pain [5].

Tramadol is a potent drug which is used in the treatment of moderate to severe pain in combination with Paracetamol. Most of analytical work has been done using H.P.L.C. method which is complex and very costly. So, based on our literature survey and need of today, I have tried to develop a new method which is easier, reliable, cheaper, accurate and which can be easily performed in laboratory using simple instrument like UV Spectrophotometer.

Results and Discussion

UV Spectroscopy is a very good method for the analysis of Drugs. It can be use for simultaneous analysis of several drugs. In this project the drugs selected viz. Paracetamol and Tramadol are available in combination in Tablet form. In the tablet composition the amount of Tramadol is 37.5 mg and Paracetamol is 325 mg. Therefore the ratio is around 1:10.

λmax determination:

The λmax of Paracetamol and Tramadol were observed at 248 nm and 273 nm as shown in Figure 1(a) and Figure 1(b), respectively.

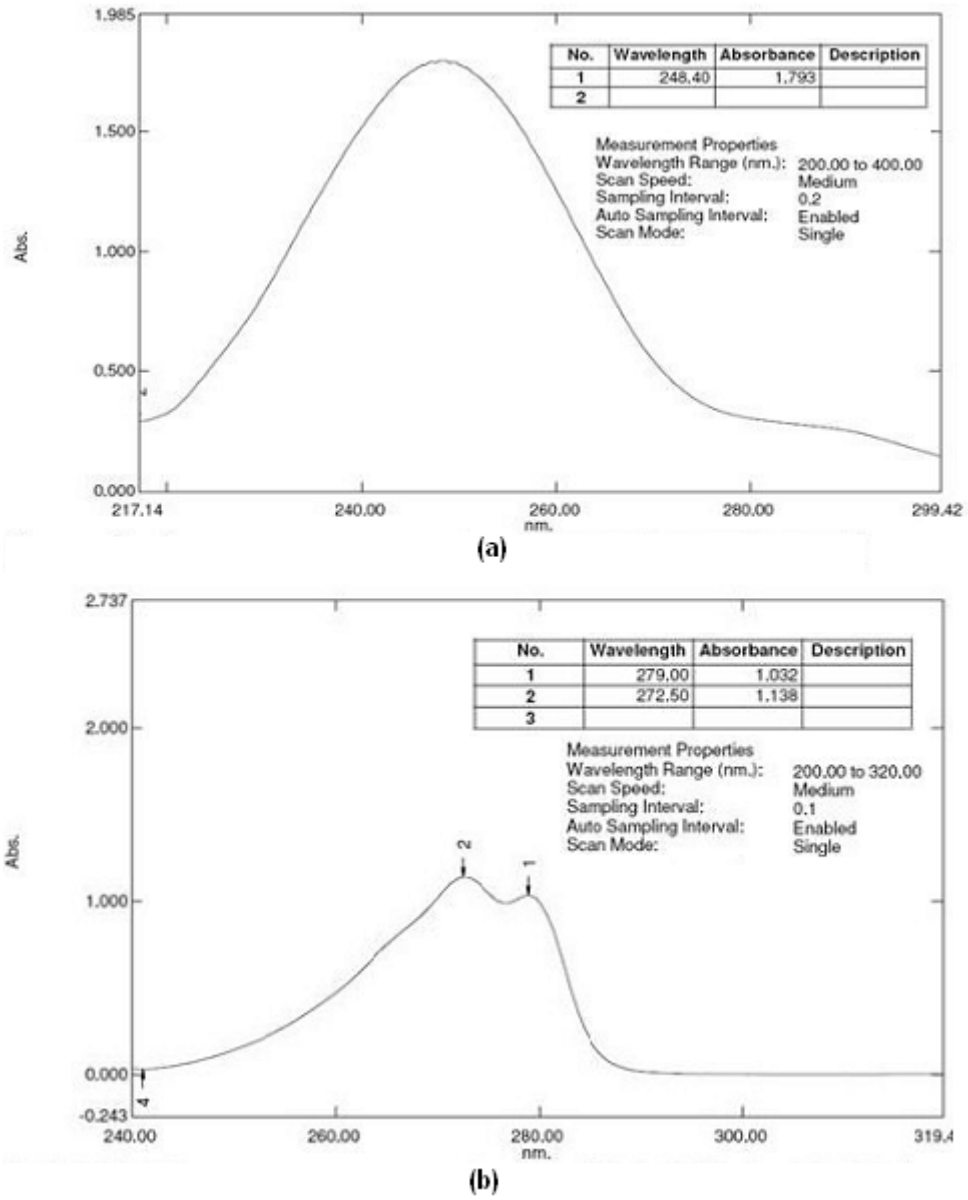


Figure 1: Spectrum point pick report for (a) Paracetamol and (b) Tramadol

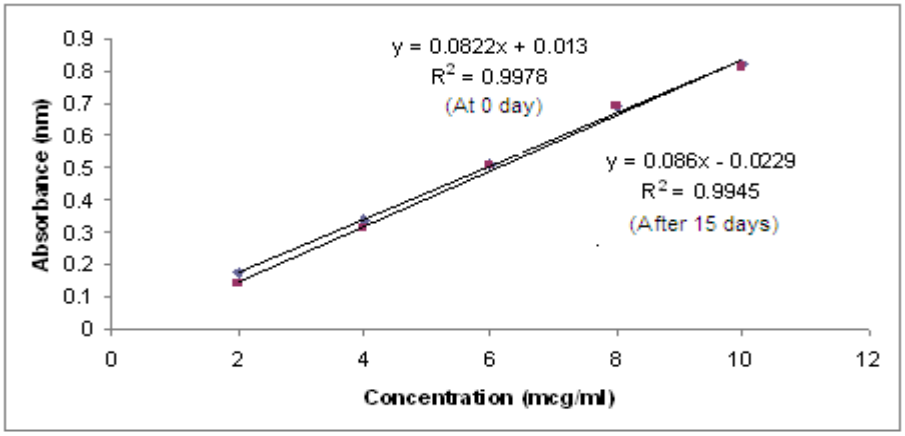
Stability of the Drugs:

From the experiment it was observed that there is no significant change in the stability of the solutions of Paracetamol after 15 days, as shown in Figure 2(a). From the t-test it was proved that there is no significant difference between the stability of sample on day 1 and day 15 (Table 1).

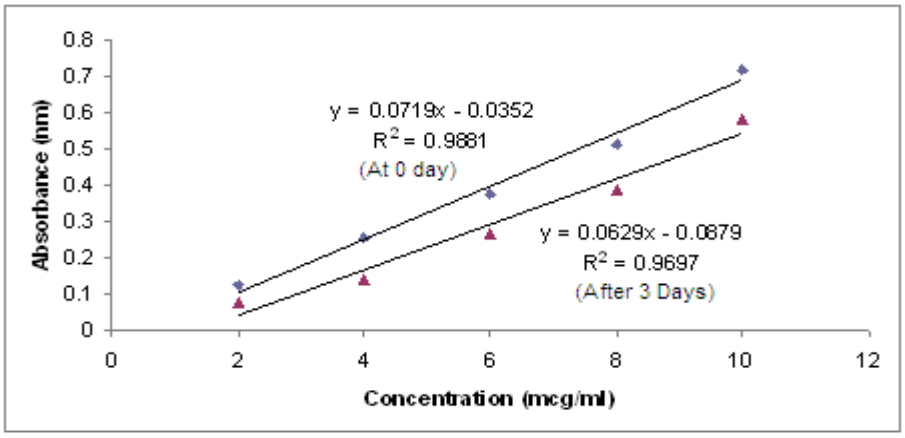
In case of Tramadol analysis it was observed that our hypothesis for the t-test was false (Table 1) and there was a very significant difference between the stability of sample on day 1 and day 3 (Figure 2 b). This suggests that Tramadol is a very unstable drug compared to Paracetamol.

Table 1: t-Test :- Paired Two Sample for Means

	Paracetamol		Tramadol	
	Variable 1	***Variable 2***	***Variable 1***	***Variable 2***
Mean	0.5064	0.4924	0.3962	0.2892
Variance	0.067633	0.074632	0.052321	0.040734
Observations	5	5	5	5
Pearson Correlation	0.999566		0.99586	
Hypothesized Mean Difference	0		0	
Df	4		4	
t Stat	**2.046469**		**7.192719**	
P(T<=t) one-tail	0.055066		0.00099	
t Critical one-tail	**2.131846**		**2.131846**	
P(T<=t) two-tail	0.110131		0.00198	
t Critical two-tail	2.776451		2.776451	



(a)



(b)

Figure 2: Comparison of two Standard curves of (a) Paracetamol and (b) Tramadol

Simultaneous Analysis of Paracetamol and Tramadol:

Figure 3 shows the overlapped view of Paracetamol & Tramadol separately and here the sharp peak of both Paracetamol and Tramadol is clearly seen (Figure 3 a). In case of Paracetamol and Tramadol (1:1 ratio), no sharp peak of Paracetamol or Tramadol is obtained (Figure 3 b). With an increase in concentration of paracetamol viz. Paracetamol & Tramadol (8:1 ratio), a sharp peak of Paracetamol is obtained but there is no peak of Tramadol (Figure 3 c). Similarly in case of Paracetamol & Tramadol (1:8 ratio), sharp peak of Tramadol is obtained but peak of Paracetamol is not clearly visible (Figure 3 d).

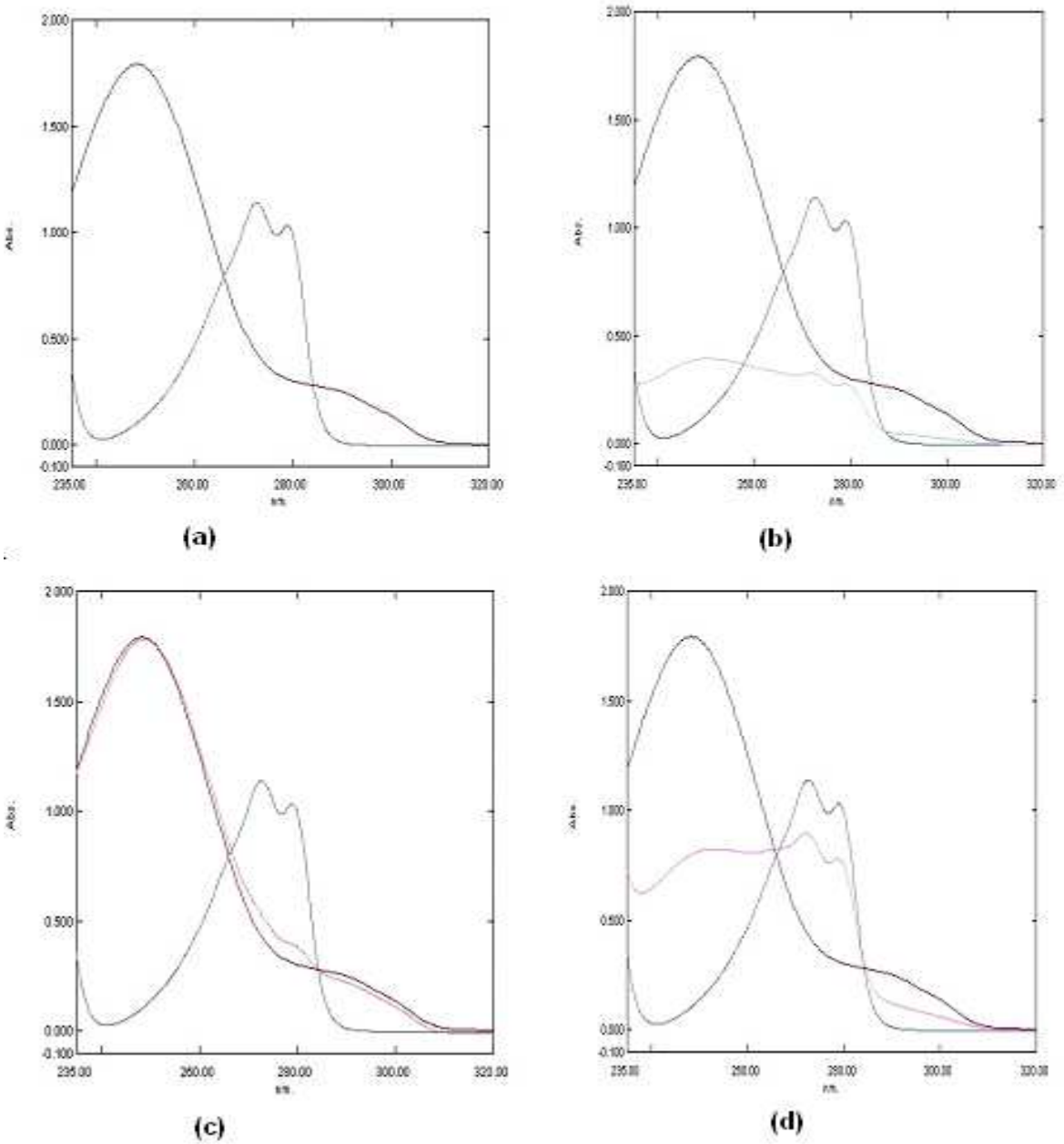


Figure 3: Overlapped view of Paracetamol & Tramadol (a) separately; (b) 1:1 ratio; (c) 8:1 ratio and (d) 1:8 ratio.

From the analysis and the graphs at different concentration ratios of Paracetamol and Tramadol it was observed that both drugs suppress the absorbance peak of one another when they are present in their higher concentration (Figure 3 c and 3 d).

From the experiment done it was obtained that it might be due to interference of both peaks, since, the λmax of both of the drug is very close to each other. So, it is not possible to go in for the simultaneous analysis of the drugs in the Tablet. Therefore is not possible to analyze Paracetamol and Tramadol simultaneously using first derivative method. For the simultaneous analysis we have to go in for higher derivative method by U.V Spectroscopy.

Materials and Methods

Tramadol and Paracetamol were obtained ex-gratia from Ind-Swift Laboratories Ltd., Baddi, India. Methanol was procured from S. D. Fine Chem Ltd., Bombay, India. UV spectrophotometer - Single Beam : UV-117 Series (SYSTRONICS)

λmax Determination of Paracetamol:
Paracetamol was dissolved in Methanol and scanning was done in the wavelength region of 200nm to 400nm against Methanol as blank using UV spectrophotometer (UV-117 Series, SYSTRONICS India Ltd.).

Preparation of stock solution for Paracetamol:
Standard solution of the drug Paracetamol was prepared separately dissolving 100mg of drug in 100 ml of Methanol solution. Shake it properly to dissolve the drug and then adjusted the volume with the Methanol to get 1000 mcg/ml [6].

Plotting of Standard Curve for Paracetamol:
The prepared stock solution was further diluted with Methanol to get working standard solution of 2 mcg/ml, 4 mcg/ml, 6 mcg/ml, 8 mcg/ml & 10 mcg/ml, to construct standard plot for Paracetamol. The absorbance of each solution was measured at 248 nm for Paracetamol against Methanol as blank. The standard graph for Paracetamol was plotted by taking Concentration of drug on X-axis and Absorbance on Y-axis [6]. The standard curve was plotted on 0 day and after a period of 1 day each until a significant difference was observed in the two standard curves.

t-Test Hypothesis: There is no significant change in the stability of the solutions of Paracetamol after 15 days. To prove the hypothesis the two samples were paired for means.

λmax Determination of Tramadol:
Tramadol was dissolved in Methanol and scanning was done in the wavelength region of 200nm to 400nm against Methanol as blank using UV spectrophotometer.

Preparation of stock solution for Tramadol:
Standard solution of the drug Tramadol was prepared separately dissolving 100mg of drug in 100ml of Methanol solution. Shake it properly to dissolve the drug and then adjusted the volume with the Methanol to get 1000 mcg/ml [7].

Plotting of Standard Curve for Tramadol:
The prepared stock solution was further diluted with Methanol to get working standard solution of 20 mcg/ml, 40 mcg/ml, 60 mcg/ml, 80 mcg/ml & 100 mcg/ml, to construct standard plot for Tramadol. The absorbance of each solution was measured at 273 nm against Methanol as blank. The standard graph for Tramadol was plotted by taking Concentration of drug on X-axis and Absorbance on Y-axis [7]. The standard curve was plotted on 0 day and after a period of 1 day each until a significant difference was observed in the two standard curves.

t-Test Hypothesis: There is no significant change in the stability of the solutions of Tramadol after 3 days. To prove the hypothesis the two samples were paired for means.

Conclusion

The developed method is suitable for the determination and quantification of the simultaneous determination of paracetamol and tramadol in dosage forms having low ratios. But the method is not suitable enough for those having high ratios. A higher derivative method is further required to quantify the higher percentages.

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