

Development and Validation of Antipyretic and Non Steroidal Anti-Inflammatory Drug by Spectroscopic Methods and its Validation

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ABSTRACT

Two methods for simultaneous estimation of Paracetamol and Zaltoprofen in combined tablet dosage form have been developed. The first UV spectrophotometric method was a determination using the simultaneous equation method at 245 nm and 227 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 237.5 nm (isobestic point) and at 227 nm the maximum absorption of Zaltoprofen. The linearity ranges for Paracetamol and Zaltoprofen were 2–10 µg/ml and 4–12 µg/ml respectively. The accuracy of the methods was assessed by recovery studies was found to be 100.02 ± 0.467 and 99.87 ± 0.532 for simultaneous equation method and 99.82 ± 0.483 and 99.84 ± 0.512 for Q analysis (absorption ratio) method for Paracetamol and Zaltoprofen respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

INTRODUCTION:

Paracetamol and Zaltoprofen are available in tablet dosage form. Chemically, Paracetamol (PARA) is N acetyl-p-aminophenol and its structure is shown in Fig. 1. It has antipyretic and analgesic activity¹. Zaltoprofen (ZAL) (\pm)-2-(10, 11-dihydro-10-oxodibenzo [b, f] thiepin-2-yl) propionic acid and its structure is shown in Fig. 2. Zaltoprofen (ZLT) is a non-steroidal anti-inflammatory drug, and has excellent effects even on post-surgery or post-trauma chronic inflammation. It is used in the treatment of rheumatoid arthritis, osteoarthritis, and other chronic inflammatory Pain conditions⁶. Literature survey reveals many analytical methods for determination of paracetamol such as UV Spectrophotometry, HPLC, and Capillary electrophoresis methods from pharmaceutical preparations¹⁻⁴. Few analytical methods for determination of Zaltoprofen using UV Spectroscopy and HPLC in pharmaceutical formulation have been reported⁵⁻⁹. However, there are no reported methods for simultaneous estimation of both drugs in combination. This paper presents two simple, rapid, reproducible and economical methods for the simultaneous analysis estimation of both the drugs from pharmaceutical dosage form.

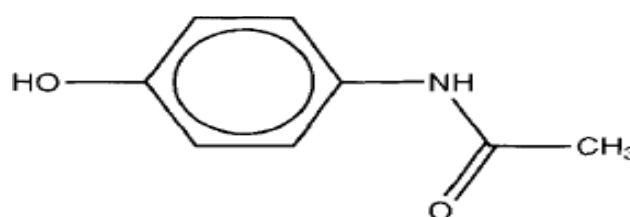


Fig. 1: Structure of Paracetamol

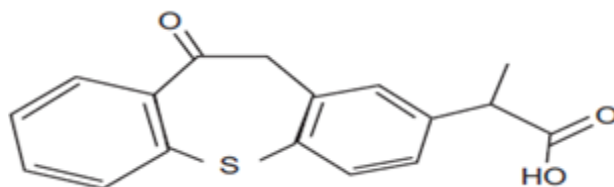


Fig. 2: Structure of Zaltoprofen

Experimental Work Instruments & Chemicals:

Pharmaceutically pure samples of Zaltoprofen were obtained as gifts from IPCA Lab Ltd. Mumbai & Paracetamol of AR grade was purchased from Research Lab. Methanol AR grade (Research Lab) and distilled water (1:4) was used as solvent in the study. Double beam UV spectrophotometer Shimadzu 1800 with a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solution.

Preparation of standard stock solution:

Accurately 10 mg each of Paracetamol and Zaltoprofen was weighed separately and transferred to two different 100 ml volumetric flask. Each drug was dissolved by 10 min sonication in 20 ml methanol and then volume was made up to the mark with distilled water. The standard stock solutions (100 µg/ml) were further diluted separately to obtain working standard of concentration 10 µg/ml of Paracetamol and Zaltoprofen each.

Study of spectra and selection of wavelengths:

Each working standard solution was scanned between the range 200-400 nm in 1 cm cell against blank. Maximum absorbing wavelength of Paracetamol and Zaltoprofen were selected from spectral data and isobestic wavelength selected from overlain spectra of zero order. The λ_{max} for Paracetamol, Zaltoprofen and isobestic point was 245 nm, 227 nm and 237.5 nm respectively.

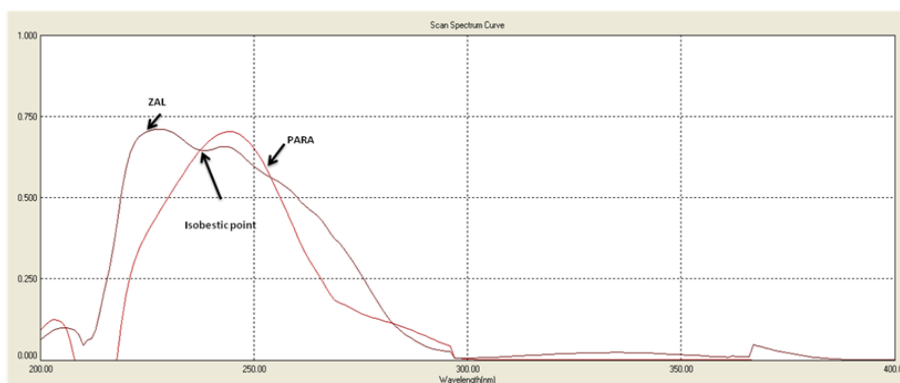


Fig. 3: Overlain Spectra of Paracetamol and Zaltoprofen

Method I:

In quantitative estimation of two components by simultaneous equation method, absorbances were measured at the maximum absorption wavelengths of two drugs. From the spectra of Paracetamol and Zaltoprofen absorbances were measured at selected wavelengths i.e., 245 nm (λ_1) and 227 nm (λ_2) the maximum absorption of Paracetamol and Zaltoprofen respectively. The absorptivity coefficients of each drug at both wavelengths were determined. The concentration of each drug in laboratory mixture and tablet formulation was determined by substituting the absorbance and absorptivity coefficient. Where, A_1 and A_2 are absorbances of mixture at 245 nm and 227 nm respectively, a_{x1} and a_{x2} are absorptivities of Paracetamol at λ_1 and λ_2 respectively and a_{y1} and a_{y2} are absorptivities of Zaltoprofen at λ_1 and λ_2 respectively. C_x and C_y are concentrations of Paracetamol and Zaltoprofen respectively.

Method II:

In Q analysis method the absorbances were measured at the isobestic point and maximum absorption wavelength of Zaltoprofen. From overlain spectra of Paracetamol and Zaltoprofen (Fig.3) absorbances were measured at the selected wavelengths i.e., 237.5 nm (isobestic point) and at 227 nm, the maximum absorption of Zaltoprofen. The absorptivity coefficients of each drug at both wavelengths were determined. The

concentration of each drug in laboratory mixture and tablet formulation was determined by substituting the absorbance and absorptivity coefficients.

Procedure for analysis of tablet formulation:

Twenty tablets were accurately weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 5 mg Zaltoprofen was dissolved in 20 ml methanol and sonicated for 50min and volume was made up to 250ml by distilled water. The solution was filtered through Whatmanfilter paper No 41 and aliquot portion of filtrate was diluted to produce solution having concentration of 4 µg/ml of Zaltoprofen and 8.125µ/ml of Paracetamol. The absorbance of sample solution was measured at selected wavelengths and the concentrations of the two drugs were estimated using simultaneous equation method and absorbance ratio method. The analysis procedure was repeated six times and the results are depicted in Table 1.

Validation:

The methods were validated with respect to linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and ruggedness. To study accuracy of the developed methods, recovery studies were carried out using standard addition method at three different levels. Percent recovery and low relative standard deviation for six replicates of sample solution was less than 2%, which met the acceptance criteria established for spectrophotometric methods.

Ruggedness of the proposed method was determined by analysis of sample solution prepared by proposed methods between different days. The percent relative standard deviation was found to be less than 2% showed ruggedness of the spectrophotometric methods. The results obtained are summarized in Tables.

Table 1: Linear Regression Analysis of Calibration Curves with their Respective Absorptivity values

Parameter	Method I		Method II	
	PARA	ZAL	PARA	ZAL
Beer's Law limit (µg/ml)	2-10	4-12	2-10	4-12
Correlation Coefficient (r)	0.9998	0.9997	0.9995	0.9998
Molar Absorptivity (Lit/mole/cm)	8492.67	13796.2	9355.48	18401.4
Slope	0.048	0.045	0.065	0.059
Intercept	0.046	0.021	0.02	0.013
LOD (µg/ml)	0.7	0.5	0.9	0.8
LOQ (µg/ml)	2.5	1	2	1

Table 2: Results of Recovery Studies

Level of Recovery %	Amount of pure drug added (mg)		Simultaneous equation method % recovery		Absorbance ratio method % recovery	
	PARA	ZAL	PARA	ZAL	PARA	ZAL
80	8	4	100.09	100.61	100.74	99.52
100	10	5	99.47	98.82	100.22	100.37
120	12	6	100.51	100.23	98.51	99.63
Mean % Recovery			100.02	99.87	99.82	99.84
SD*			0.467	0.532	0.483	0.512
CV**			0.422	0.398	0.532	0.213

*Mean of six readings

Table 3: Results of Analysis of Tablet Formulation

Drug	Simultaneous equation method % ± SD (n=6) *	Absorbance ratio method % ± SD (n=6) *
PARA	100.37 ± 0.231	100.51 ± 0.123
ZAL	100.12 ± 0.216	99.86 ± 0.214

*Mean of six readings

Table 4: Results of Intermediate Precisions

Day	Method I		Method II	
	% Label claim estimated (Mean±%RSD)*		% Label claim estimated (Mean±%RSD)*	
	PARA	ZAL	PARA	ZAL
Intraday	98.34 ±0.546	99.28 ±0.874	100.46 ±0.453	98.98 ±0.931
Interday	100.21 ±0.245	99.54 ±0.563	99.51 ±0.653	98.64 ±0.328

*Mean of six readings

RESULTS AND DISCUSSIONS:

The overlain spectra of Paracetamol and Zaltoprofen exhibit λ max of 245 nm and 227 nm for Paracetamol and Zaltoprofen respectively which are quite se Paracetamol ted from each other. Additionally, one iso absorptive point was observed at 237.5 nm, this wavelength was selected for simultaneous estimation of Paracetamol and Zaltoprofen for Q value analysis and it is assumed to be sensitive wavelength. Standard calibration curves for Paracetamol and Zaltoprofen were linear with correlation coefficients (r) values in the range of 0.997 – 0.999 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.2465 – 0.7126. The methods were repeated three times in a day and the average % RSD was found to be 0.546 for Paracetamol and 0.874 for Zaltoprofen for method I and 0.453 for Paracetamol and 0.931 for Zaltoprofen for method II. Similarly the method was repeated for three different days and average % RSD was found to be 0.245 for Paracetamol and 0.563 for Zaltoprofen for method I and 0.653 for Paracetamol and 0.328for Zaltoprofen for method II. The accuracy of the methods was confirmed by recovery studies from tablet at three different levels of standard additions; recovery in the range of 98.51 – 100.74% justifies the accuracy of method.

CONCLUSIONS:

The proposed UV spectrophotometric methods are a simple, accurate, precise, rapid and economical for the simultaneous estimation of Paracetamol and Zaltoprofen in tablet dosage form. The proposed methods use inexpensive reagents, solvents and instruments that are available in laboratories. Hence, these methods can be conveniently adopted for the routine analysis in quality control laboratories.

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