Journal of Pharmaceutical and Biomedical Analysis Letters
ISSN: 2347-4742 | https://doi.org/10.30904/j.jpbmal.2026.4953
J. Pharm, Biomed. A. Lett., 2026; 14(1): 21-24

Simultaneous Estimation of Drugs in their Combined Dosage forms by UV-Visible Spectroscopy

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Keywords
UV–VIS spectrophotometry, Beer–Lambert's law, Pharmaceutical analysis, Method validation, Drug combinations, Quality control

Received 28-01-2026
Revised 16-02-2026
Accepted 25-02-2026
Published 15-03-2026

ABSTRACT
The present study aims to develop and validate simple, accurate, precise, rapid, and economical UV–Visible spectrophotometric methods for the simultaneous estimation of selected multicomponent pharmaceutical formulations. With the increasing use of combination drug therapy for enhanced therapeutic efficacy and patient compliance, the need for reliable analytical methods has become essential. In this work, simultaneous equation and absorbance ratio methods were developed for the estimation of drug combinations such as Ofloxacin–Tinidazole, Alprazolam–Propranolol, and Amlodipine–Metoprolol. The developed methods are based on the measurement of absorbance at selected wavelengths, including λmax and isoabsorptive points, and obey Beer–Lambert's law within the specified concentration ranges. Various analytical parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were evaluated in accordance with standard validation guidelines. The results demonstrated good correlation coefficients, high recovery values close to 100%, and low %RSD values, indicating excellent reproducibility and reliability. The proposed methods were successfully applied for the analysis of pharmaceutical formulations without interference from excipients. Due to their simplicity, sensitivity, and cost-effectiveness, these methods can be effectively used for routine quality control analysis of multicomponent drug formulations in pharmaceutical industries and laboratories.

INTRODUCTION

The increasing prevalence of diseases has led to a growing demand for both herbal and synthetic pharmaceutical products. While herbal medicines are preferred for long-term treatment due to their safety and cost-effectiveness, allopathic medicines are widely used for rapid and targeted therapeutic action [Kala et al., 2006, Jawla et al., 2009, Nagori et al., 2011]. The rapid advancement in pharmaceutical technology has significantly enhanced drug development and manufacturing processes, making the pharmaceutical industry one of the fastest-growing sectors globally.

The introduction of new drug molecules and combination therapies necessitates the development of reliable analytical methods to ensure drug quality, safety, and efficacy. Since no drug is completely free from adverse effects at higher doses, qualitative and quantitative analysis of pharmaceutical substances is essential to maintain consistency in composition and therapeutic performance [Rahman et al., 2009, Gadkari, 2012]. Analytical methods play a vital role in evaluating drug purity, determining dosage accuracy, and supporting pharmacokinetic studies.

Traditionally, classical analytical methods such as gravimetric and titrimetric techniques were widely used for drug estimation. These methods are simple and economical but are often time-consuming and less sensitive. With the advancement of analytical instrumentation, modern techniques such as spectroscopic, chromatographic, and electroanalytical methods have gained prominence due to their high sensitivity, precision, accuracy, and rapid analysis [Berry and Nash, 1993, Skoog et al., 2004].

Among these techniques, UV–Visible spectrophotometry is one of the most widely used methods in pharmaceutical analysis. It is based on the measurement of absorbance of electromagnetic radiation in the UV and visible regions and follows Beer–Lambert's law, which correlates absorbance with concentration. Various spectrophotometric methods such as simultaneous equation method, absorbance ratio (isoabsorptive point) method, and derivative spectrophotometry are commonly employed for the analysis of multicomponent formulations.

Multicomponent pharmaceutical formulations are increasingly used in clinical practice due to their improved therapeutic efficacy, reduced side effects, and enhanced patient compliance. However, simultaneous estimation of multiple drugs in such formulations presents analytical challenges, necessitating the development of selective and accurate methods [Saraf et al., 2006]. Several spectrophotometric methods have been reported

for simultaneous estimation of drug combinations, demonstrating good linearity, accuracy, and precision within Beer's law limits [Malathi et al., 2014, Nayak et al.,2012, Darak et al., 2011].

The drugs selected in the present study include Ofloxacin, Tinidazole, Alprazolam, Propranolol, Amlodipine, and Metoprolol, which are widely used in the treatment of bacterial infections, protozoal infections, anxiety disorders, hypertension, and cardiovascular diseases. These drugs exhibit different physicochemical properties and mechanisms of action, making their simultaneous estimation important for quality control and regulatory compliance.

Therefore, the present study focuses on the development and validation of simple, rapid, precise, and economical UV–Visible spectrophotometric methods for the simultaneous estimation of selected drug combinations. The proposed methods aim to provide reliable alternatives for routine quality control analysis in pharmaceutical industries and academic laboratories.

MATERIALS AND METHODS

Instrumentation

- All apparatus was calibrated before use.
- A double beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) having two matched cell of 1cm.
- An electronic analytical balance (Shimadzu AUX220) having capacity of 220 g and readability of 0.1mg was used for weighing.
- An ultrasonic cleaner Power Sonica 410 (Hwashin Technology Seoul, Korea) was used for sonication.

Reagents and Pharmaceutical Preparations

Pure sample of drugs used were kindly gifted by industries and used without further purification. All chemicals used were either of analytical grade (UV method). Commercial pharmaceutical preparations with claimed amount of each drug were used in analysis.

Table 1: Pure Drugs used in Analysis and their Supplier

Drug	Supplied by
Amlodipine	Symbiosis Pvt. Ltd.
Metoprolol	Symbiosis Pvt. Ltd.
Propranolol	Biochemix Pvt. Ltd.
Alprazolam	Biochemix Pvt. Ltd.
Ofloxacin	Zee Laboratories Pvt. Ltd.
Tinidazole	Zee Laboratories Pvt. Ltd.
Paracetamol	Brother Pharma Ltd.
Promethazine	Brother Pharma Ltd.

Preparation of Standard (Calibration) Curve

The standard curve was determined for each drug independently. The working standards were prepared by suitable dilution of stock solution with appropriate solvent. Absorbance of standard solution was taken at their respective λ_{max} and also on the λ_{max} of another component and at the isoabsorptive point for further calculations (Table 5 & 6 for Combination I, 18 & 19 for Combination II and 25 for Combination III). The concentration on X-axis is plotted against absorbance at Y-axis. Linearity graphs, Beer's limit and other optical characteristics calculated for Combination I, II and III are shown in Fig. 3, 5 and 7 and Table 13, 20 and 26 respectively.

Determination of Molar Absorptivities at Selected Analytical Wavelengths: The molar absorptivities for drugs were calculated using equation:

$A = \varepsilon bc$ (Beckett and Stanlake, 2002).

Where, A = Absorbance of the sample solution.

ε = molar absorptivity.

b = Path length of sample cell (b = 1cm).

c = Concentration of sample.

Analysis of Tablet

Twenty tablets were accurately weighed and crushed to fine powder separately. A powder equivalent to desired amount of pure drug was weighed (a known amount of pure drug was also added in cases where contribution of drug is very less) and the stock solution was made as described for the pure drug. It was sonicated, filtered and made upto the desired volume. The working solution (10µg/mL) was made after further dilution of stock solution and absorbance was taken at selected wavelengths. The values observed were then equated in suitable equations for further calculations. The standard solution of pure drug was also prepared (10µg/mL).

Method Validation

Validation of method as per ICH guidelines was performed (ICH, 2000 and ICH, 2002) with respect to accuracy, linearity, precision, LOD and LOQ.

Accuracy (Recovery studies)

Recovery studies were carried out by accurately weighing desired amount of drug from tablet dosage form to which known quantity of standard drug corresponding to 80%, 100% and 120% of drug sample taken was added (standard addition method, ICH, 2000). The contents were dissolved in small amount of specified solvent and then sonicated for 10 min. in ultrasonicator after which volume was made with selected solvent up to mark. The solution was diluted further to appropriate working concentration (10µg/mL) and before use, was filtered through 41 no. Whatmann filter paper. Absorbance was then observed at selected wavelengths and drug concentration was calculated using equations. As per ICH guidelines, three determinations were performed at each level of recovery studies for which % RSD should be less than or equal to 2 for method to be accurate (ICH, 2000). The recovery results for Combination I, II and III are shown in Table 16, 23 and 29 respectively.

Precision (Repeatability)

Intraday Precision

Precision was studied by measuring absorbance of three replicate solutions of known concentration in triplicate on same day (Intraday precision) and on three consecutive days (Inter-day precision). Variation of results within same day (Intraday precision) and on consecutive days (Interday precision) was analyzed and statistically validated. The % RSD should be less than or equal to 2.

Linearity

The linearity range was estimated by measuring absorbance of the prepared dilutions from the standard stock solution and then calibration curve was plotted between absorbance and concentration of drug. Results are then evaluated by linear regression analysis and shown in Fig. 3, 5 and 7 for

Combination I, II and III respectively.

Limit of Detection (LOD) and Quantitation (LOQ)

It was calculated by using equations

DL =3.3 σ/S

QL =10 σ /S

RESULTS AND DISCUSSION

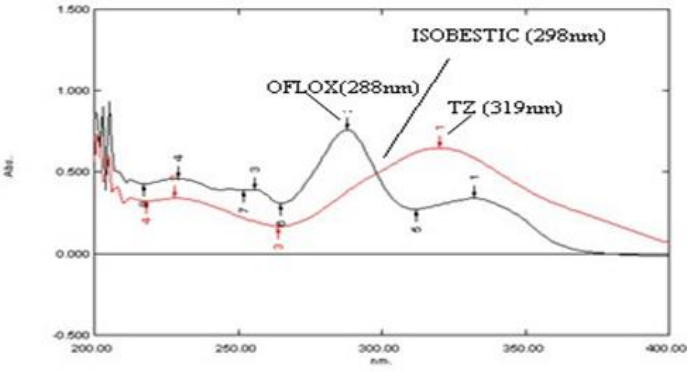

Fig.1: Fig.1: Overlain spectrum of OFLOX and TZ showing λmax at 288 nm and 319 nm respectively and isobestic point at 298 nm in 0.1N NaOH

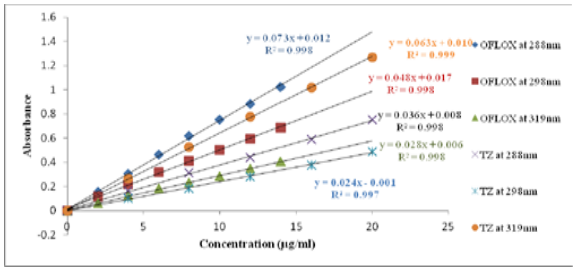

Fig.2: Linearity graphs of OFLOX and TZ plotted between concentration (µg/mL) on X-axis and absorbance on Y-axis

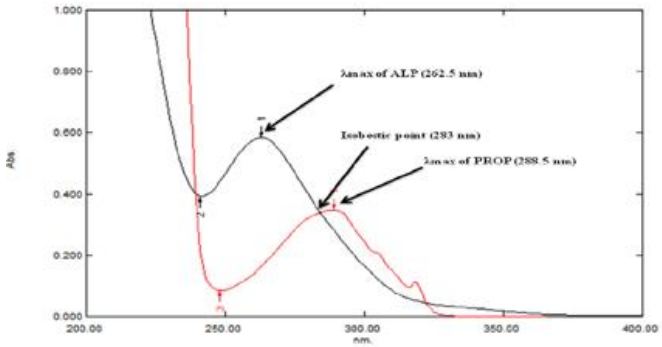

Fig.3: Overlain spectrum of ALP and PROP showing λmax at 262.5nm and 288.5 nm respectively & isobestic point at 283 nm in 0.1N HCl

Table 2: Absorbance for Ofloxacin at 288 nm, 319nm and 298nm in 0.1N NaOH

Conc (µg/mL)	288nm		319nm		298nm	
	Absorbance ± SD	% RSD	Absorbance ± SD	% RSD	Absorbance ± SD	% RSD
0	0 ± 0	0	0 ± 0	0	0 ± 0	0
2	0.156 ± 0.001	0.641	0.064 ± 0.002	3.125	0.118 ± 0.002	1.695
4	0.304 ± 0.002	0.657	0.127 ± 0.002	1.574	0.219 ± 0.006	2.739
6	0.465 ± 0.001	0.215	0.184 ± 0.005	2.717	0.318 ± 0.001	0.314
8	0.619 ± 0.001	0.162	0.234 ± 0.000	0	0.412 ± 0.007	1.699
10	0.752 ± 0.002	0.266	0.285 ± 0.001	0.350	0.502 ± 0.011	2.191
12	0.882 ± 0.000	0	0.348 ± 0.007	2.011	0.595 ± 0.002	0.336
14	1.025 ± 0.001	0 .098	0.407 ± 0.006	1.474	0.687 ± 0.001	0.145

Table 3: Absorbance for Alprazolam at 262.5 nm, 288.5nm and 283nm in 0.1N HCl

Concentration (µg/mL)	262.5nm		288.5nm		283nm	
	Absorbance ± SD	% RSD	Absorbance ± SD	% RSD	Absorbance ± SD	% RSD
0	0 ± 0	0	0 ± 0	0	0 ± 0	0
5	0.200 ± 0.001	0.5	0.101 ± 0.001	0.99	0.115 ± 0.002	1.739
10	0.391 ± 0.000	0	0.197 ± 0.001	0.508	0.222 ± 0.001	0.450
15	0.594 ± 0.001	0.168	0.283 ± 0.000	0	0.339 ± 0.002	0.589
20	0.81 ± 0.001	0.123	0.372 ± 0.001	0.269	0.463 ± 0.001	0.216
25	1.02 ± 0.001	0.098	0.466 ± 0.000	0	0.584 ± 0.001	0.171

DISCUSSION

The present study was undertaken to develop and validate simple, precise, accurate, rapid, and economical UV–Visible spectroscopic methods for the simultaneous estimation of selected multicomponent pharmaceutical formulations. With the increasing use of combination drug therapy to enhance therapeutic efficacy and patient compliance, the need for reliable analytical methods for routine quality control has become essential. UV–Visible spectroscopy was selected due to its simplicity, cost-effectiveness, sensitivity, and suitability for routine laboratory analysis. Simultaneous equation and absorbance ratio methods were successfully developed for Ofloxacin–Tinidazole, Alprazolam–Propranolol combinations using appropriate solvents and optimized wavelength selections. A simultaneous equation method was developed for Amlodipine–Metoprolol combination using methanol as solvent. All methods showed good linearity within the specified concentration ranges and complied with Beer's law. The developed methods demonstrated low LOD and LOQ values, indicating good sensitivity. Accuracy studies showed mean percent recovery values close to 100%, confirming the reliability of the methods. Precision studies revealed percentage relative standard deviation (%RSD) values less than 2%, indicating excellent repeatability and reproducibility. The methods were validated according to ICH guidelines and were found to be specific, robust, and free from interference by common excipients.

CONCLUSION

The developed UV–Visible spectroscopic methods for the simultaneous estimation of multicomponent pharmaceutical dosage forms were found to be simple, rapid, precise, accurate, sensitive, and economical. The validation results confirmed that the methods meet ICH acceptance criteria for linearity, accuracy, precision, and sensitivity. These methods do not require prior separation steps and can be effectively employed for routine quality control analysis of combined tablet formulations in pharmaceutical industries and academic laboratories. Therefore, the proposed spectrophotometric methods provide a reliable alternative for routine estimation of drug combinations while ensuring quality, safety, and regulatory compliance.

ACKNOWLEDGMENT

The authors thank the, Saastra College of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India, for technical assistance and support.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not for profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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