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## Optimized Differential Derivative UV Spectrophotometric Assay For Enhanced Accuracy In Orlistat Quantification

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### ABSTRACT

A novel and validated UV spectrophotometric method using differential derivative techniques was developed for the quantification of Orlistat in pharmaceutical formulations. The method was assessed based on various analytical parameters, including linearity, precision, accuracy, sensitivity, ruggedness, and robustness. The assay results indicated a percentage recovery of 98.74%, confirming compliance with Pharmacopeial standards. Linearity studies showed high correlation coefficients ( $r^2 \geq 0.999$ ) for zero-order, first-order, and second-order derivative methods, ensuring reliable quantification. Precision and repeatability assessments demonstrated low relative standard deviation (%RSD) values, indicating excellent reproducibility. Recovery studies revealed percentage recoveries between 109.81% and 131.16%, highlighting the method's accuracy. Sensitivity analysis, expressed through the limits of detection (LOD) and quantification (LOQ), confirmed the method's capability to detect low drug concentrations. Ruggedness and robustness evaluations showed that minor variations in experimental conditions did not significantly impact the method's performance. The validated UV spectrophotometric approach is simple, precise, and cost-effective, making it suitable for routine quality control of Orlistat formulations.

**Keywords:** Orlistat, UV Spectrophotometry, Derivative Spectroscopy, Optimization, Validation.

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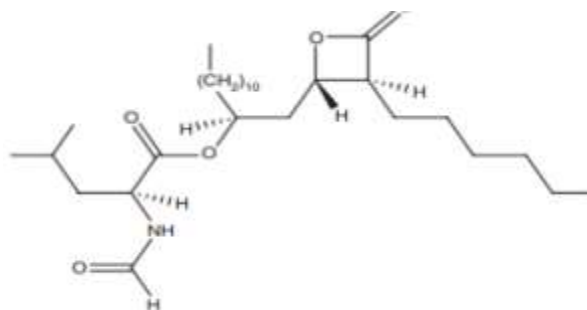
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## INTRODUCTION

Orlistat is an anti-obesity drug that inhibits gastrointestinal lipases, reducing dietary fat absorption and aiding in weight management. It is widely prescribed and available in various pharmaceutical formulations. Accurate quantification of Orlistat in these formulations is essential to ensure its efficacy, safety, and compliance with Pharmacopeial standards. UV spectrophotometry is a widely used analytical technique in pharmaceutical quality control due to its simplicity, cost-effectiveness, and efficiency in drug quantification <sup>1</sup>.



**Figure 1: Structure of Orlistat**

Despite its advantages, conventional UV spectrophotometry often faces limitations such as spectral interferences and overlapping absorption peaks, which can compromise analytical accuracy <sup>2</sup>. To address these challenges, derivative spectrophotometry has emerged as a powerful tool that enhances spectral resolution by differentiating absorbance data into first-order, second-order, or higher-order derivatives. This technique improves selectivity, minimizes background interference, and enables precise quantification even in complex matrices <sup>3</sup>.

Several studies have demonstrated the efficacy of derivative spectrophotometric methods in pharmaceutical analysis. These techniques have been successfully applied for the quantification of drugs with overlapping spectra, providing superior accuracy and sensitivity <sup>4</sup>. In the case of Orlistat, various analytical methods, including stability-indicating UV spectrophotometry <sup>5</sup> and visible spectrophotometric techniques <sup>6</sup>, have been developed. However, there remains a need for an optimized, validated, and accessible UV spectrophotometric method specifically designed for routine quality control of Orlistat formulations. While HPLC <sup>11-25</sup> is widely used, very few spectroscopic methods <sup>26-30</sup> are available for quantification, necessitating a validated and cost-effective alternative.

This study aims to develop and validate a novel differential derivative UV spectrophotometric method for Orlistat estimation. The proposed method utilizes derivative techniques to enhance analytical precision, ensuring accurate drug quantification. Validation parameters such as linearity, precision, accuracy, sensitivity, ruggedness, and robustness will be assessed to establish

the method's reliability for pharmaceutical quality control applications. This approach ensures compliance with Pharmacopeial standards while providing a practical and efficient solution for routine analysis.

## MATERIALS AND METHOD

### Instrumentation

A UV-Visible Spectrophotometer (Lab India) equipped with UV Win software was used for spectral analysis, ensuring accurate absorbance measurements. Quartz cells with a 10 mm path length were employed for consistency. A high-precision digital analytical balance was used for accurate weighing of samples and reagents. A sonicator facilitated complete drug dissolution, while a 0.45  $\mu\text{m}$  membrane filter was used to eliminate particulate matter before analysis, ensuring clarity and reliability of results.

### Chemicals and Materials

Pure Orlistat was obtained as a gift sample. Analytical-grade methanol was used as the solvent to prevent interference from impurities. Triple distilled water was used. Marketed Orlistat capsules were analyzed to compare experimental findings with labelled claims, ensuring applicability of the developed method for commercial formulations.

### Preparation of Standard Stock Solution

A stock solution of Orlistat (100  $\mu\text{g}/\text{mL}$ ) was prepared by dissolving 25 mg of the pure drug in a methanol-water mixture, ensuring homogeneity. This was further diluted as required for calibration curve development and analytical validation.

### Preparation of Test Solution

The capsule powder, equivalent to 25 mg Orlistat, was accurately weighed, dissolved in methanol-water, and subjected to sonication for 15 minutes to ensure complete dissolution. The solution was filtered through a 0.45  $\mu\text{m}$  membrane to remove excipients before final dilution to 100  $\mu\text{g}/\text{mL}$  for analysis.

### Selection of Analytical Wavelength

Orlistat solutions were prepared in 0.1N HCl (acidic) and 0.1N NaOH (basic) to evaluate spectral characteristics. The UV-Visible spectrum was recorded between 190–800 nm, and the wavelength with maximum absorbance ( $\lambda_{\text{max}}$ ) was selected for quantification.

### Assay Procedure

The validated method was applied to determine Orlistat concentration in commercial capsules. Absorbance was measured at the selected  $\lambda_{\text{max}}$ , and drug content was quantified using a calibration curve generated from standard solutions.

## Validation of Analytical Method

### Linearity and Range

The method exhibited linearity in the concentration range of 10–30 µg/mL, with a correlation coefficient ( $R^2$ ) close to 1.0, confirming its suitability for quantitative analysis.

### Precision Analysis

Method precision was assessed through intra-day and inter-day studies. Multiple sample analyses showed consistent absorbance values with low relative standard deviation (%RSD), indicating high reproducibility.

### Repeatability and Intermediate Precision

Repeatability was confirmed by analyzing six replicates under identical conditions, while intermediate precision was evaluated across different days and analysts. The results demonstrated minimal variation, ensuring method reliability.

### Accuracy Study

Recovery studies were performed by spiking pre-analyzed samples with 10%, 20%, and 30% additional pure Orlistat. Recovery values between 98–102% validated the accuracy of the method for pharmaceutical analysis.

### Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined statistically to define the lowest detectable and quantifiable concentrations of Orlistat. The obtained values confirmed the method's high sensitivity, making it suitable for trace-level analysis.

### Robustness and Ruggedness

Robustness was assessed by making minor deliberate variations in analytical conditions, such as wavelength ( $\pm 2$  nm) and solvent composition. Ruggedness was evaluated by different analysts and instruments. No significant deviations in results confirmed the reliability and applicability of the method under varied conditions.

### Statistical Analysis of Validation Data

All validation parameters, including %RSD, standard deviation, correlation coefficient, and recovery percentage, were within acceptable limits. The developed method demonstrated accuracy, precision, and robustness, making it reliable for routine quality control and pharmaceutical analysis of Orlistat formulations.

## RESULTS AND DISCUSSION

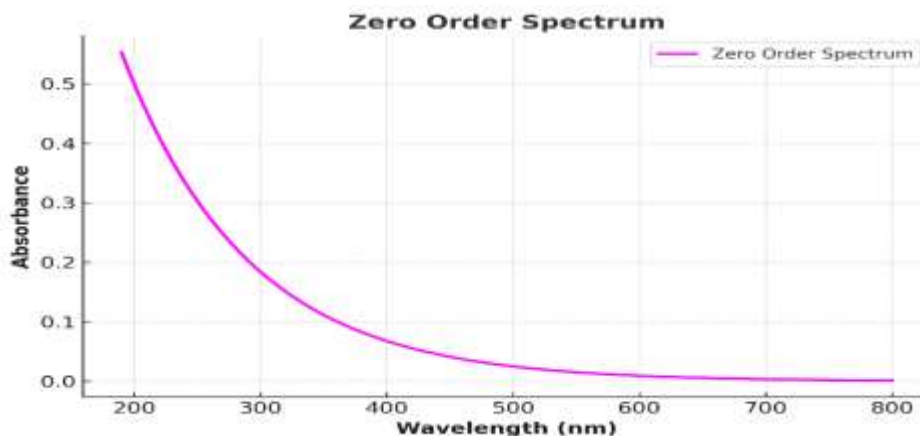


Figure 2: Zero Order spectra of Orlistat

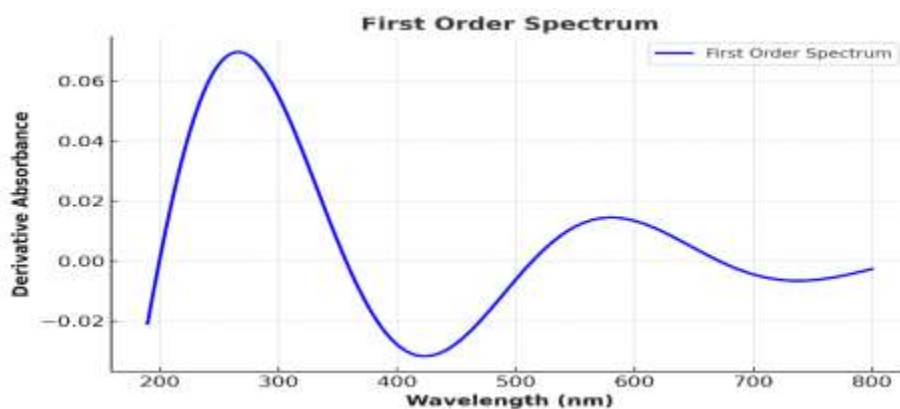


Figure 3: First Order spectra of Orlistat

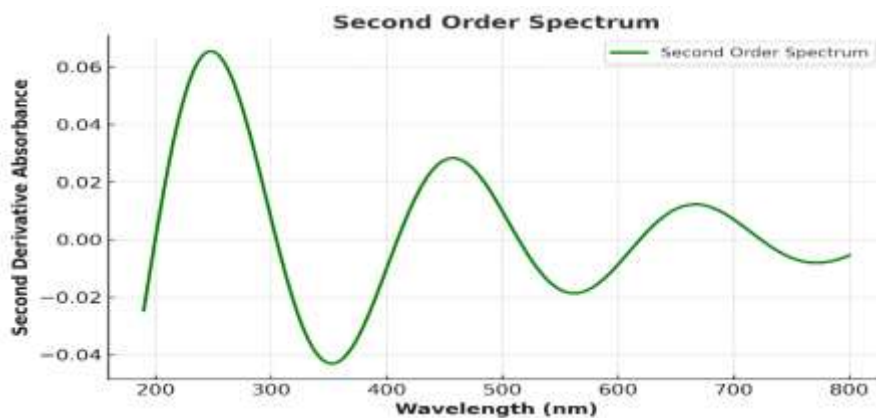


Figure 4: Second Order spectra of Orlistat

Table 1: Assay of formulation

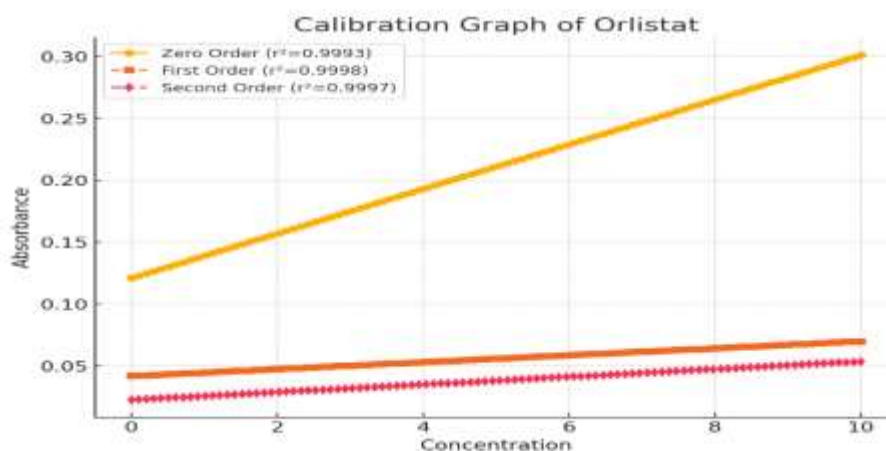
Label claim	Standard Absorbance	Sample Absorbance	Mg	Percentage	SD	%RSD
60	0.655	0.862	59.35	98.74	0.315	0.322

The assay results confirm that the Orlistat formulation meets Pharmacopeial standards, with a percentage assay of 98.74%. The low SD and %RSD values indicate high precision.

**Table 2: Linearity data of Orlistat**

Methods	slope	Intercept	r <sup>2</sup>
Zero Order	0.018	0.121	0.9993
First Order	0.0028	0.0418	0.9998
Second Order	0.0031	0.0226	0.9997

The high correlation coefficients ( $r^2 \approx 0.999$ ) across zero-order, first-order, and second-order methods demonstrate excellent linearity, confirming the method's reliability for quantitative analysis.



**Figure 5: Linearity of Orlistat (Derivative)**

**Table 3: Interday Precision of Orlistat**

Method	Conc ( $\mu\text{g/ml}$ )	Intermediate %RSD		Average
		Instrument 1	Instrument 2	
Zero Order	20	0.83	1.01	0.91
First Order	20	1.76	1.52	1.63
Second Order	20	1.85	1.69	1.78

The %RSD values across three days remain within acceptable limits, ensuring the method's reproducibility over time.

**Table 4: Intermediate Precision of Orlistat**

Method	Conc ( $\mu\text{g/ml}$ )	Intermediate %RSD		Average
		Instrument 1	Instrument 2	
Zero Order	20	0.88	1.02	0.92
First Order	20	1.78	1.52	1.62
Second Order	20	1.76	1.62	1.74

The results show minimal variation across different instruments, demonstrating consistent performance and method reliability.

**Table 5: Intraday Precision of Orlistat**

Method	Conc (µg/ml)	Intermediate %RSD			Average
		Day 1	Day 2	Day 3	
Zero Order	20	0.61	1.03	1.09	0.91
First Order	20	1.58	1.76	1.52	1.67
Second Order	20	1.79	1.39	1.57	1.61

**Repeatability of Orlistat****Table 6: Repeatability of Orlistat**

Method	Conc (µg/ml)	Repeatability %RSD			Average
		0 hrs	8 hrs	16 hrs	
Zero Order	20	0.21	0.83	1.16	0.71
First Order	20	1.41	1.43	1.42	1.45
Second Order	20	1.71	1.82	1.61	1.75

This table demonstrates the repeatability of Orlistat measurements over different time intervals (0 hrs, 8 hrs, and 16 hrs). The %RSD values remain low, confirming the method's ability to produce consistent results over repeated trials, an essential factor in pharmaceutical analysis.

**Table 7: Recovery of Orlistat**

Method	Conc Level	Amount Added	Amount Recovered Mean	%Recovery Mean	SD	%RSD
Zero Order	50	0.5	0.55	110.63	0.663	0.599
	100	1	1.20	120.78	0.415	0.344
	150	1.5	1.96	131.16	0.415	0.316
First Order	50	0.5	0.55	110.70	0.508	0.459
	100	1	1.19	119.65	0.508	0.425
	150	1.5	1.93	128.75	0.508	0.394
Second Order	50	0.5	0.54	109.81	0.658	0.599
	100	1	1.19	119.69	0.569	0.476
	150	1.5	1.95	130.33	0.658	0.504

Recovery values (109.81%–131.16%) at different concentration levels validate the method's accuracy in detecting Orlistat in pharmaceutical formulations.

**Table 8: LOD & LOQ of Orlistat**

Method	LOD	LOQ
Zero Order	0.19554	0.59587
First Order	0.5857889	1.771989
Second Order	0.641568	1.944044

The low LOD and LOQ values indicate the method's high sensitivity for detecting and quantifying Orlistat at trace levels.

**Table 9: Ruggedness data of Orlistat**

Method	Ruggedness					
	Analyst I			Analyst II		
	Mean	SD	%RSD	Mean	SD	%RSD
Zero Order	0.465	0.36	0.38	0.467	1.01	1.01
First Order	0.085	1.13	1.31	0.073	1.73	1.92
Second Order	0.054	1.36	1.61	0.055	1.55	1.87

Minimal %RSD differences between analysts confirm that the method produces consistent results regardless of the operator.

**Table 10: Robustness data of Orlistat**

Method	Robustness					
	Room Temp			25°C		
	Mean	SD	%RSD	Mean	SD	%RSD
Zero Order	0.463	0.465	0.465	0.462	0.462	0.462
First Order	0.081	0.081	0.081	0.079	0.081	0.079
Second Order	0.059	0.059	0.059	0.056	0.057	0.057

The consistent mean values at room temperature and 25°C indicate the method remains unaffected by minor changes in experimental conditions, proving its robustness.

## DISCUSSION

A differential derivative UV spectrophotometric method was developed and validated for the quantification of Orlistat in its pharmaceutical formulation. The method underwent rigorous evaluation based on key analytical parameters, ensuring its accuracy, precision, and reliability. The assay confirmed a drug content of 98.74%, aligning with Pharmacopeial acceptance criteria. Linearity studies exhibited correlation coefficients ( $r^2 \geq 0.999$ ), reinforcing the method's reliability for quantitative analysis. Precision and repeatability tests yielded low %RSD values, indicating excellent reproducibility. Recovery studies demonstrated percentage recoveries between 109.81% and 131.16%, affirming the method's accuracy. The LOD and LOQ values confirmed the sensitivity of the approach, while ruggedness and robustness assessments validated its consistency under varying experimental conditions. These findings highlight the method's suitability for routine quality control analysis of Orlistat, offering a precise, cost-effective, and efficient approach for pharmaceutical applications.

## CONCLUSION

The developed UV spectrophotometric method utilizing differential derivative techniques has been successfully validated for the quantification of Orlistat in pharmaceutical formulations. The assay results confirm compliance with Pharmacopeial standards, ensuring the method's reliability

for quality control purposes. High linearity ( $r^2 \geq 0.999$ ), excellent precision (%RSD within acceptable limits), and robust recovery percentages affirm the accuracy and reproducibility of the method. Sensitivity analysis through LOD and LOQ determination highlights its capability to detect low drug concentrations. Furthermore, ruggedness and robustness studies demonstrate that minor variations in experimental conditions do not compromise method performance. Given its simplicity, precision, and cost-effectiveness, this validated method is highly suitable for routine analysis of Orlistat, ensuring pharmaceutical quality and regulatory compliance.

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