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*Research Article*

# Simultaneous Determination of Prasugrel and Aspirin by Second Order and Ratio First Order Derivative Ultraviolet Spectrophotometry

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Two simple, accurate, and precise UV derivative spectrophotometric methods for the simultaneous determination of Prasugrel and Aspirin in synthetic mixture form have been developed. The first method involves measurement of second order derivative spectra of Prasugrel and Aspirin. The zero crossing wavelengths 267.62 nm and 252.40 nm were selected for estimation of Prasugrel and Aspirin, respectively. In the second method, the first order derivatives of ratio spectra were calculated and used for the determination of Prasugrel and Aspirin by measuring the peak intensity at 268 nm and 290 nm, respectively. The methods were validated as per the ICH guideline Q2 (R1). Beer's law is followed in the range of 5–45 $\mu$g/mL for Prasugrel and 25–150 $\mu$g/mL for Aspirin by second order derivative method and 6–22 $\mu$g/mL for Prasugrel and 45–165 $\mu$g/mL for Aspirin by ratio first order derivative method. The recovery studies confirmed the accuracy of the methods. Relative standard deviations for repeatability and inter- and intraday assays were less than 2%. Hence, the described derivative spectrophotometric methods are simple, accurate, precise, and excellent alternatives to sophisticated chromatographic techniques and can be potentially used for the simultaneous determination of Prasugrel and Aspirin in combined dosage form.

## 1. Introduction

Aspirin (ASP), 2-(acetyloxy)benzoic acid, (Figure 1) has anti-inflammatory and antipyretic properties and acts as an inhibitor of cyclooxygenase which results in the inhibition of the biosynthesis of prostaglandins. It also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis [1]. Aspirin is official in IP [2], BP [3], and USP [4].

Prasugrel (PRA), 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4H,5H,6H,7H-thieno [3,2-c]pyridin-2-yl acetate, a thienopyridine derivative (Figure 2), is a platelet activation and aggregation inhibitor structurally and pharmacologically related to clopidogrel and ticlopidine. It is a prodrug that requires enzymatic transformation in the liver to its active metabolite R-138727. R-138727 irreversibly binds to P2Y12 type ADP receptors on platelets thereby inhibiting ADP-mediated platelet activation and aggregation [5]. It is not official in any pharmacopoeia. Aspirin in combination with Prasugrel is used to prevent thrombotic complications of Acute Coronary Syndrome (ACS) and percutaneous coronary intervention (PCI) [6].

The literature survey reveals that some analytical methods have been reported for determination of PRA [7–11] and ASP [12–14] individually and Aspirin in combination with other drugs [15–17]. HPLC and UV simultaneous methods are available for estimation of PRA and ASP in their combined dosage form [18, 19]. The objective of the current study was to develop simple, accurate, and reproducible second order derivative and ratio first order derivative analytical methods for the simultaneous estimation of Prasugrel and Aspirin in presence of excipients. The methods have advantage over other methods that they completely eliminate the spectral interference from one of the two drugs while estimating the other drug at selected wavelength. The second order

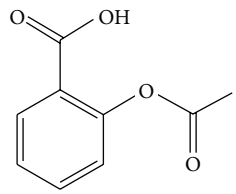


Figure 1: Chemical structure of Aspirin.

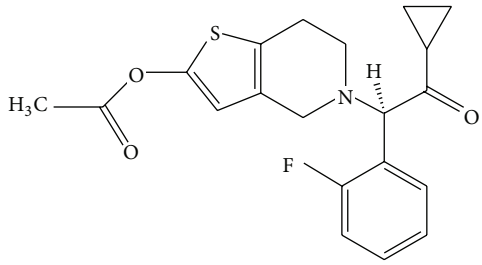


Figure 2: Chemical structure of Prasugrel.

derivative method eliminates interference by selection of zero crossing point (ZCP), while the ratio first order derivative method eliminates it by dividing the zero order spectra of mixture with one of the two drugs and transforming the resulting spectra into their respective first order derivatives to estimate other drug. The methods were developed and validated as per ICH guideline Q2 (R1) [20].

## 2. Materials and Methods

*2.1. Instruments and Chemicals.* A double beam UV-visible spectrophotometer (Shimadzu, UV1800) having two matched quartz cells with 1 cm pathlength, UVProbe v2.35 software, Ultrasonic Cleaner (Electroquip), and electronic analytical balance (Denver SI 234) were used. Calibrated volumetric flasks, pipettes of borosilicate glasses, and Whatman grade no. 3 filter paper were used throughout the experiment. Methanol AR grade was procured from Sisco Research Laboratories Pvt. Ltd. Lactose, Corn Starch, and Magnesium Stearate were procured from SD Fine Chem Ltd.

*2.2. Preparation of Standard Stock Solution.* Accurately weighed quantities of PRA (25 mg) and ASP (62.5 mg) were transferred separately to 25 mL volumetric flasks, dissolved, and diluted upto the mark with methanol to get 1000 $\mu$g/mL PRA and 2500 $\mu$g/mL ASP. The working standard solutions of the respective drugs were prepared by serial dilution in methanol.

*2.3. Sample Preparation.* Synthetic mixture of PRA and ASP was prepared by using common excipients like Corn Starch, Lactose, and Magnesium Stearate by calculating formula for 20 tablets having label claim for PRA and ASP 10 mg and 75 mg, respectively. From this mixture, powder equivalent to 10 mg Prasugrel and 75 mg Aspirin was dissolved in 100 mL methanol, then sonicated for 15 min, and filtered through Whatman filter paper. From this solution, 1 mL aliquot was taken in 10 mL volumetric flask and diluted upto the mark with methanol to make final concentration of PRA and ASP 10 $\mu$g/mL and 75 $\mu$g/mL, respectively, which was used for assay.

## 3. Method Development

*3.1. Method D2: Second Order UV Spectrophotometric Method (Determination of Zero Crossing Point (ZCP)).* In the second order derivative method, aliquots of PRA and ASP standard stock solutions were accurately transferred into 10 mL volumetric flasks, separately, and diluted upto the mark with methanol. Each of the working standard solutions was scanned between 400 and 200 nm at a medium scanning speed. It showed wavelength maxima at 254 nm for PRA and 276 nm for ASP. All the zero order spectra were then transformed to their respective second order derivative spectra (D2) using the UVProbe v2.35 software with $\Delta\lambda = 10$ nm and ZCPs of PRA and ASP were found to be at 252.40 nm and 267.62 nm respectively. Responses of each of the above solutions were measured for PRA and ASP at 267.62 nm, and 252.40 nm, respectively. The calibration curves were constructed and the concentration of individual drug present in the mixture was determined against the calibration curve.

*3.2. Method RD1: First Order Derivative of the Ratio Spectra.* Previously scanned absorption spectrum of PRA solutions prepared at different concentrations (6–22 $\mu$g/mL) in its binary mixture with ASP was divided by the spectrum of the standard solution of ASP (75 $\mu$g/mL in methanol) to get the ratio spectra of PRA. The first derivatives of the ratio spectra were than calculated. The amount of PRA was determined by measuring the first derivative signal at 268 nm (minima). A similar procedure was followed for different concentrations of ASP (45–165 $\mu$g/mL) with PRA and was used for division spectrum of the standard solution of PRA (10 $\mu$g/mL in methanol). Similarly, content of ASP was determined by measuring the first derivative signal at 290 nm (minima).

*3.3. Assay of Synthetic Mixture*

*3.3.1. Method D2.* In the second order derivative method, the absorption spectrum was recorded against methanol as a blank for the solution prepared in methanol from the synthetic mixture of PRA and ASP. The resulting zero order spectra were transformed to their second order derivative spectra. The intensity of the second derivative spectra of the mixture were measured at 267.62 nm and 252.40 nm for estimation of PRA and ASP, respectively. The procedure was repeated 2 more times. The concentrations of PRA and ASP were calculated from their corresponding regression equations and % amount was determined.

*3.3.2. Method RD1.* In the ratio first order derivative method, the previously scanned zero order absorption spectra for the mixture were divided by the spectrum of ASP (75 $\mu$g/mL) and

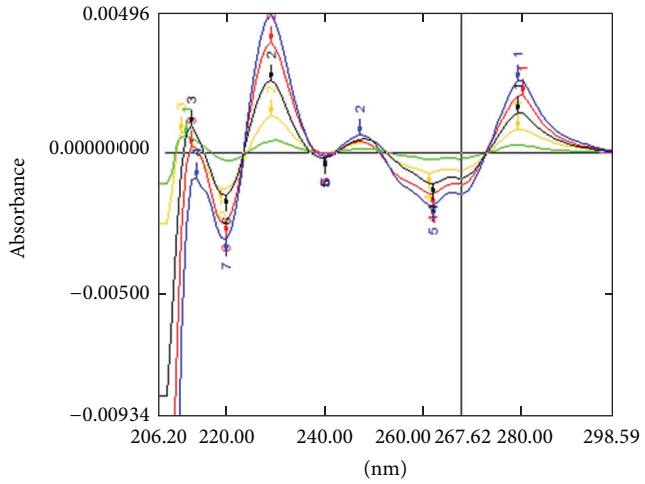


Figure 3: Second order derivative UV absorption overlain spectra of PRA (5–45 $\mu$g/mL).

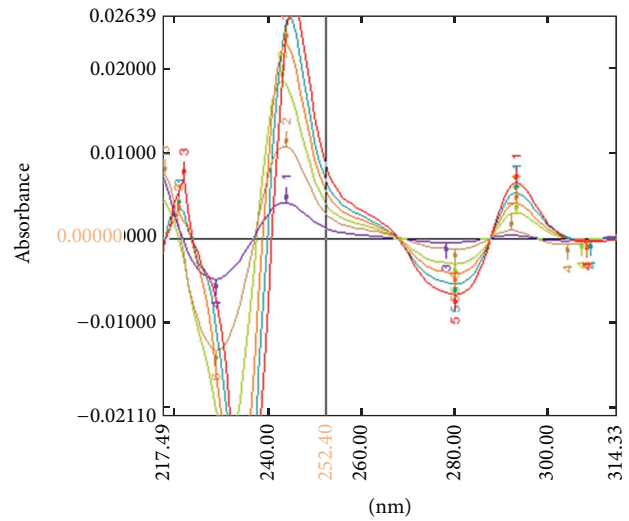


Figure 4: Second order derivative UV absorption overlain spectra of ASP (25–150 $\mu$g/mL).

by spectrum of PRA (10 $\mu$g/mL) separately for the determination of PRA and ASP, respectively. The concentrations of PRA and ASP were calculated from their corresponding regression equations measuring the intensity of signals at 268 nm and 290 nm, respectively, and % amount was determined.

## 4. Validation of the Methods

Newly developed methods were validated for specificity, linearity, accuracy, precision, limits of quantitation, and limits of detection according to the ICH guideline Q2 (R1).

*4.1. Linearity and Range ($n = 5$).* For the second order derivative method, the linearity response was determined by analyzing 5 independent calibration curves in the range of 5–45 $\mu$g/mL (5, 15, 25, 35, and 45 $\mu$g/mL) for PRA and 25–150 $\mu$g/mL (25, 50, 75, 100, 125, and 150 $\mu$g/mL) for ASP. In the ratio first order derivative method, the linearity response was determined in the range of 6–22 $\mu$g/mL (6, 10, 14, 18, and 22 $\mu$g/mL) for PRA and 45–165 $\mu$g/mL (45, 75, 105, 135, and 165 $\mu$g/mL) for ASP. Correlation coefficient and regression line equations for PRA and ASP were calculated for both the methods.

*4.2. Accuracy ($n = 3$).* It was carried out to determine the suitability and reliability of the proposed methods. Accuracy was determined by calculating the % recovery of PRA and ASP from the synthetic mixture by the standard addition method in which known amounts of standards samples of PRA and ASP at 80%, 100%, and 120% levels were added to the preanalysed samples. The procedure was repeated 2 more times and the recovered amounts of PRA and ASP were calculated at each level and % recovery was reported as % recovery = $((C_{\text{total}} - C_{\text{assay}})/C_{\text{added}}) * 100$, where $C_{\text{total}}$ is the total drug concentration found after standard addition, $C_{\text{assay}}$ is the drug concentration in the formulation mixture, and $C_{\text{added}}$ is the concentration of standard added.

*4.3. Precision*

*4.3.1. Repeatability ($n = 6$).* The repeatability was checked by scanning and measurement of the responses of solutions of PRA (10 $\mu$g/mL) and ASP (75 $\mu$g/mL) without changing the parameters of the proposed methods. The procedure was repeated six times and % RSD was calculated.

*4.3.2. Intermediate Precision ($n = 3$).* The intraday and interday precisions of the second order derivative method were determined by analyzing corresponding responses in triplicate on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of PRA (5, 10, and 15 $\mu$g/mL) and ASP (50, 75, and 100 $\mu$g/mL). Similarly, the intraday and interday precisions of ratio first order derivative method were determined by taking mixtures having concentration of PRA (10, 14, and 18 $\mu$g/mL) and that of ASP (75, 105, and 135 $\mu$g/mL).

*4.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ).* ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio, and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\sigma/S$ equations, respectively, where $\sigma$ is the standard deviation of $y$-intercepts of regression lines and $S$ is the slope of the calibration curve.

## 5. Results and Discussion

Prasugrel and Aspirin both possess good solubility and considerable UV absorption in methanol. Thus methanol was selected as solvent for the present work. From the zero order overlain UV spectra of PRA (10 $\mu$g/mL) and ASP

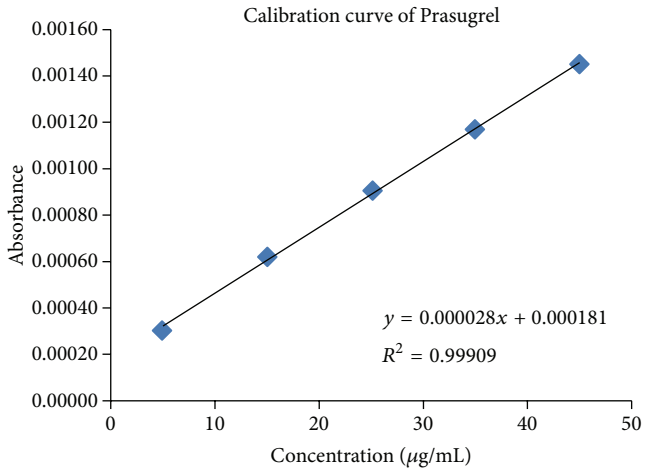


Figure 5: Calibration curve of Prasugrel (5–45 μg/mL) by second order derivative method.

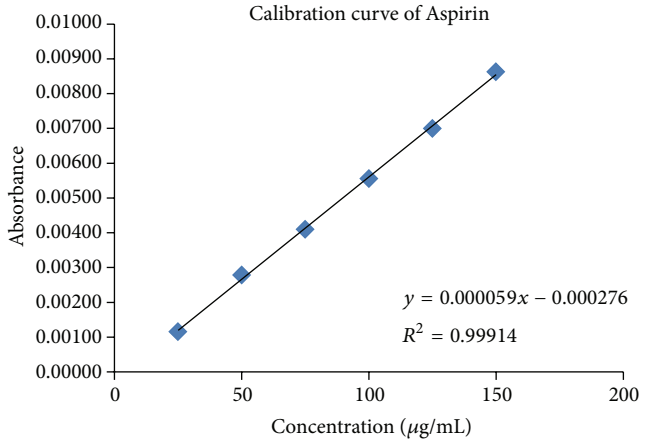


Figure 6: Calibration curve of Aspirin (25–150 μg/mL) by second order derivative method.

(75 μg/mL) and their mixture, it was observed that the spectra are overlapping each other, demonstrating the complexity in measuring these drugs by direct UV absorption measurement in a binary mixture.

*5.1. Second Order Derivative Method.* The UV derivative method has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point in the derivative spectra of each drug at selected wavelength. The zero order spectra of PRA and ASP were transformed to first and second order derivative spectra with help of UVProbe software ($\Delta\lambda$ = 10 nm). The first order spectra of PRA did not show any zero crossing points (ZCP), while the second order spectra of both the drugs showed ZCPs with considerable sensitivity for the estimation of respective drugs on each other's ZCPs. Hence second order derivative method was used in the present work. The ZCPs for PRA and ASP were found to be 252.40 nm and 267.62 nm, respectively. Hence, the estimation of PRA was done at 267.62 nm (Figure 3) while that of ASP was done at 252.40 nm (Figure 4). Calibration graphs were established for PRA and ASP in the concentration range of 5 to 45 μg/mL (Figure 5) and 25 to 150 μg/mL (Figure 6), respectively, with good correlation.

*5.2. Ratio First Order Derivative Method.* The ratio first order derivative method (RD1) permits the determination of each component in their mixture at the wavelengths corresponding to a maximum or minimum. The main advantage of this method is the chance of easy measurements in correspondence with peaks so it permits the use of the wavelength of the highest value of analytical signals (maximum or minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in presence of other active compounds or excipients which possibly interfere with the analysis. Determination of PRA was done by using spectrum of 75 μg/mL of ASP as a divisor

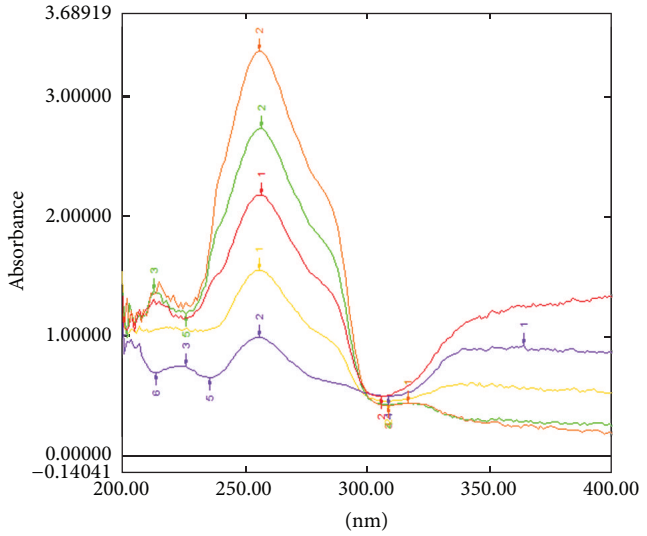


Figure 7: Ratio spectra of PRA (6–22 μg/mL) using 75 μg/mL solution of ASP as divisor.

(Figure 7). The corresponding ratio spectra of PRA were then transformed to first order derivative spectra and PRA was estimated at wavelength minima 268 nm (Figure 8). Similarly, determination of ASP was done by using spectrum of 10 μg/mL of PRA as a divisor (Figure 9) and converting these spectra into their respective first order derivative (Figure 10). ASP was estimated at wavelength 290 nm (minima). The calibration curves were constructed in the range of 6 to 22 μg/mL for PRA (Figure 11) and 45 to 165 μg/mL for ASP (Figure 12) with good correlation.

*5.3. Validity of Methods.* The proposed methods were validated as per ICH guideline. The results of validation are summarized in Table 1. The linearity within the specified range was established by high degree of correlation shown in Table 1. The LOD and LOQ for both the methods and both the drugs were calculated by using standard deviation of response. The %RSD values (Table 1) for repeatability,

Table 1: Validation parameters for second order and ratio first order derivative spectrophotometric methods.

| Validation parameters | Method D2* | | Method RD1** | |
|---|---|---|---|---|
| | PRA | ASP | PRA | ASP |
| Linearity and range ($\mu$g/mL) | 5–45 | 25–150 | 6–22 | 45–165 |
| Wavelength (nm) | 267.62 | 252.40 | 268 | 290 |
| Correlation coefficient ($r^2$) | 0.99909 | 0.99914 | 0.99975 | 0.99972 |
| Slope | 0.000028 | 0.000059 | 0.00228 | 0.00714 |
| Intercept | 0.000181 | −0.000276 | 0.00476 | −0.10455 |
| Precision (%RSD) | | | | |
| Repeatability | 1.13% | 0.91% | 0.88% | 0.72% |
| Intraday precision | 0.94%–1.88% | 1.09%–1.31% | 0.56%–1.78% | 1.14%–1.52% |
| Interday precision | 0.92%–1.90% | 0.54%–1.96% | 1.05%–1.69% | 1.27%–1.50% |
| Accuracy (%recovery) | 98.81%–100.19% | 99.53%–100.04% | 100.07%–100.75% | 99.48%–101.6% |
| LOD ($\mu$g/mL) | 0.73 | 2.35 | 0.44 | 1.31 |
| LOQ ($\mu$g/mL) | 2.22 | 7.12 | 1.35 | 3.95 |

*Second order derivative method. **Ratio first order derivative method.
PRA: Prasugrel; ASP: Aspirin. %RSD: percent relative standard deviation.

Table 2: Analysis of synthetic mixture.

| Drug | Amount of drug (mg) | Method D2 ($n$ = 3) % amount found (mean% ± SD) | Method RD1 ($n$ = 3) % amount found (mean% ± SD) |
|---|---|---|---|
| Prasugrel | 10 | 100.83% ± 2.06% | 100.39% ± 0.58% |
| Aspirin | 75 | 101.38% ± 0.67% | 101.48% ± 0.77% |

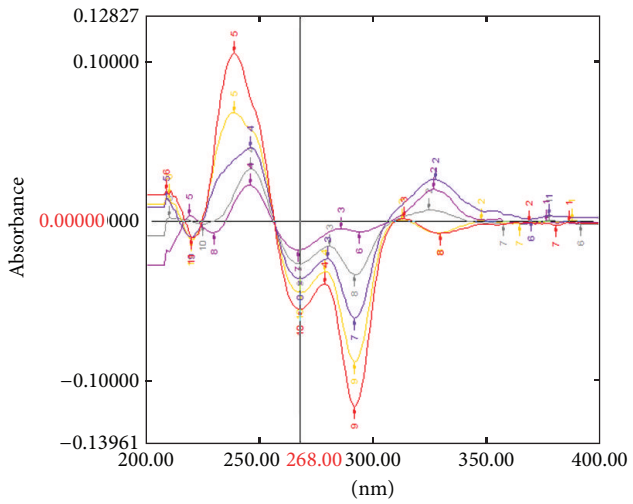


Figure 8: First derivative ratio spectra of PRA (6–22 $\mu$g/mL).

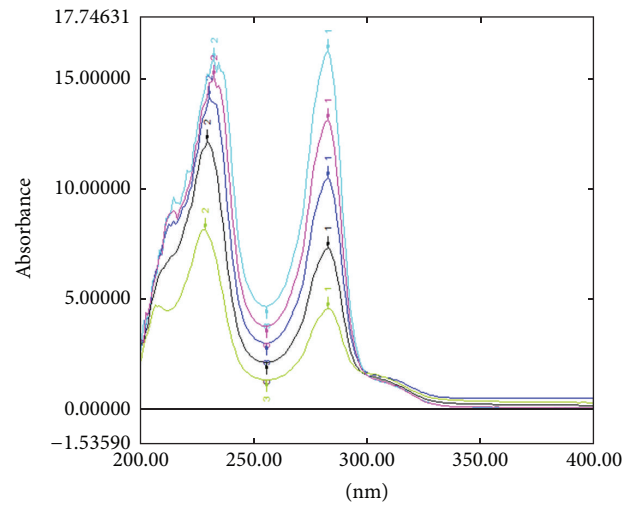


Figure 9: Ratio spectra of ASP (45–165 $\mu$g/mL) using 10 $\mu$g/mL solution of PRA as divisor.

intraday and interday variations for both drugs in both the methods were within the acceptable range (<2%) indicating that these methods have excellent repeatability and reproducibility in the current experimental condition. To demonstrate the specificity and applicability of the proposed methods, the quantitative analysis of PRA and ASP was carried out in the presence of excipients by using laboratory prepared synthetic mixture (Table 2). Furthermore, the validity and reliability of the proposed methods were assessed by determining the mean percentage recovery at 80%, 100%, and 120% levels. The results showed that the % recovery values are within the acceptable limits with low standard deviation indicating high accuracy of the proposed analytical methods. The average % recoveries by both the methods are presented in Tables 3(a) and 3(b).

Table 3: (a) Recovery studies for Prasugrel. (b) Recovery studies for Aspirin.

(a)

| % level | Amount of PRA (mg) | Amount of ASP (mg) | Amount of standard PRA added (mg) | Amount of standard ASP added (mg) | Method D2 ($n = 3$) % recovery of PRA (mean% ± SD) | Method RD1 ($n = 3$) % recovery of PRA (mean% ± SD) |
|---|---|---|---|---|---|---|
| 80% | 10 | 75 | 8 | 0 | 99.70% ± 2.57% | 100.75% ± 1.01% |
| 100% | 10 | 75 | 10 | 0 | 98.81% ± 2.06% | 100.07% ± 1.28% |
| 120% | 10 | 75 | 12 | 0 | 100.19% ± 1.71% | 100.46% ± 0.68% |

(b)

| % level | Amount of PRA (mg) | Amount of ASP (mg) | Amount of standard PRA added (mg) | Amount of standard ASP added (mg) | Method D2 ($n = 3$) % recovery of ASP (mean% ± SD) | Method RD1 ($n = 3$) % recovery of ASP (mean% ± SD) |
|---|---|---|---|---|---|---|
| 80% | 10 | 75 | 0 | 60 | 99.53% ± 1.14% | 101.60% ± 0.27% |
| 100% | 10 | 75 | 0 | 75 | 100.04% ± 0.69% | 100.92% ± 0.78% |
| 120% | 10 | 75 | 0 | 90 | 99.87% ± 1.89% | 99.48% ± 0.58% |

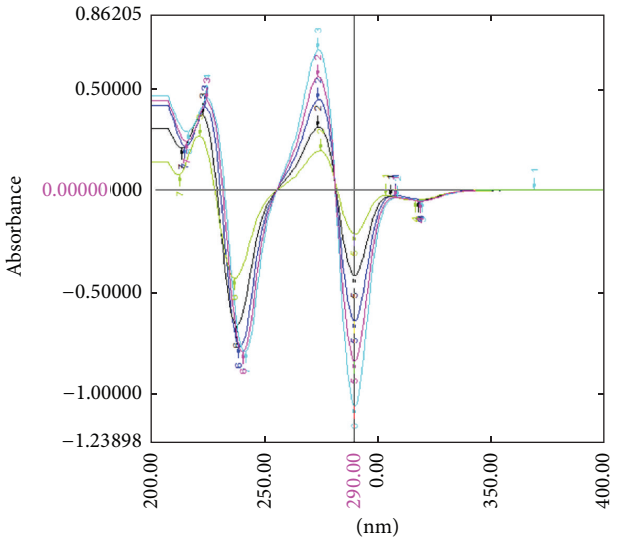


Figure 10: First derivative ratio spectra of ASP (45–165 $\mu$g/mL).

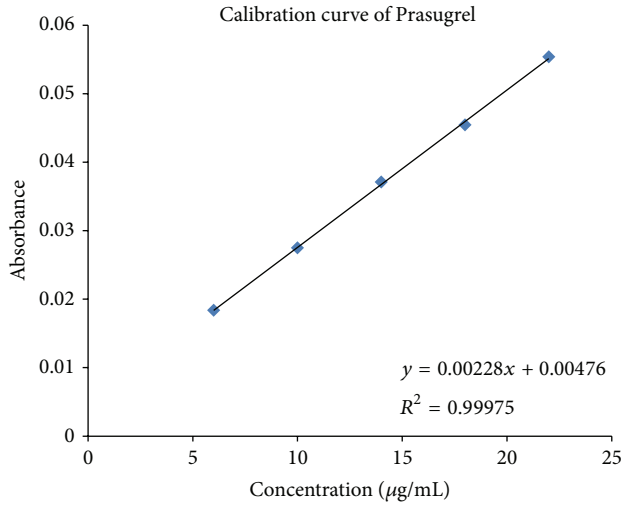


Figure 11: Calibration curve of Prasugrel (6–22 $\mu$g/mL) by ratio first order derivative method.

## 6. Conclusion

Two new, simple, accurate, and precise derivative UV spectroscopic methods were developed for the simultaneous estimation of PRA and ASP in bulk drugs and in the presence of tablet excipients. The recovery studies suggested noninterference of formulations excipients in the estimation. Moreover, the methods have advantage over other methods that they completely eliminate the spectral interference from one of the two drugs while estimating the other drug at selected wavelength; hence the proposed methods can be used for the quality control of the cited drugs and can be extended for routine analysis of the drugs in their pharmaceutical preparations.

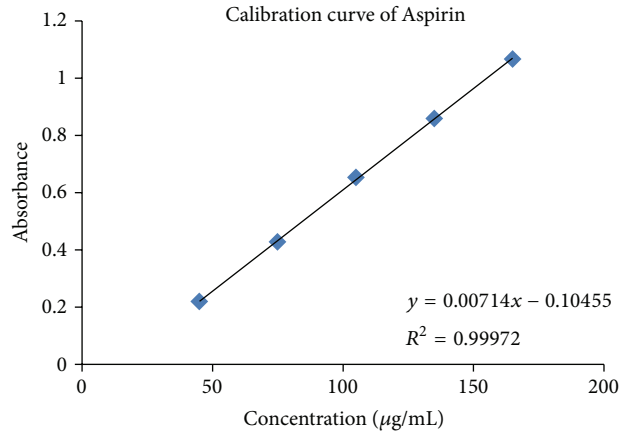


Figure 12: Calibration curve of Aspirin (45–165 $\mu$g/mL) by ratio first order derivative method.

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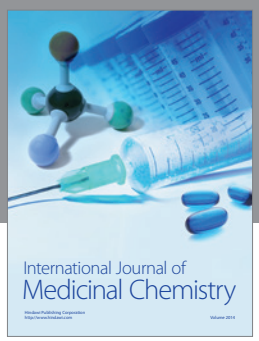

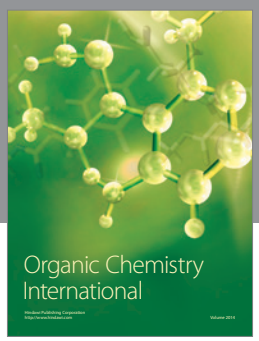

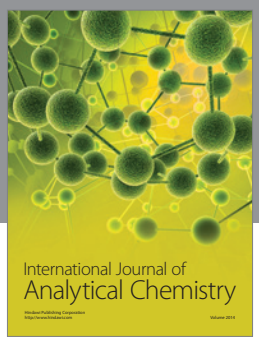

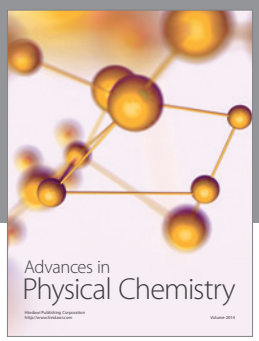

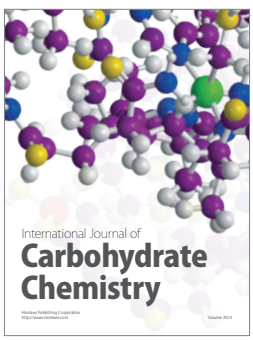

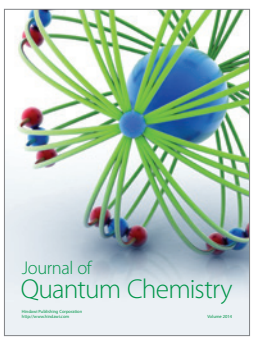

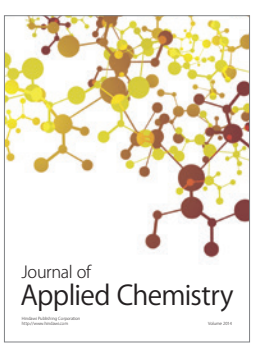

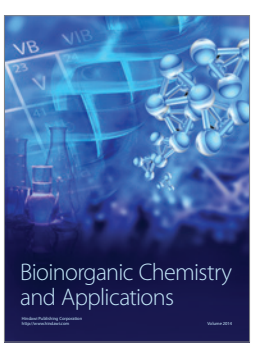

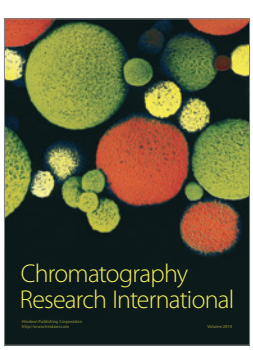

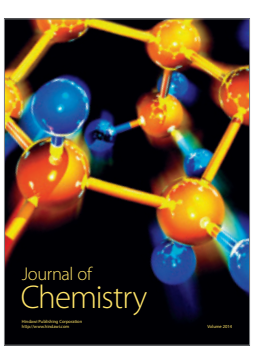

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