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Simultaneous estimation of acetylsalicylic acid and clopidogrel bisulphate in pure powder and tablet formulations by first-derivative spectrophotometric method

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ABSTRACT

This paper describes validated first-derivative spectrophotometric method for simultaneous estimation of acetylsalicylic acid (ASA) and clopidogrel bisulphate (CLP) in pure powder and formulations. ASA and CLP in combined preparations were quantitated by using the first-derivative responses at 229.11nm for ASP and 252.74nm for CLP in spectra of their solution in ethanol. The linearity ranges are 4-24μg/ml for both the drugs with mean recovery of 100.10±0.11 and 100.18±0.15% for ASA and CLP, respectively. This method is simple, precise, and sensitive and applicable for the simultaneous determination of ASA and CLP in pure powder formulations.

KEYWORDS

Acetylsalicylic acid;
Clopidogrel bisulphate;
Derivative spectroscopy.

INTRODUCTION

Acetylsalicylic acid(ASA) is a non steroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesics, antipyretics and platelet aggregation inhibitory activity[1,2]. Clopidogrel (CLP) (αS)-α-(2-chlorophenyl) -6, 7-dihydrothieno [3, 2-c] pyridine-5-(4H)-acetic acid methyl ester is an anti-platelet agent which belongs to the class of thienopyridines[3, 4]. The combination of ASA and CLP has been shown to be effective in the management of coronary syndrome such as unstable angina and myocardial infarction. The combination of ASA and CLP inhibits clotting and completely prevents vascular events with dual blockade of ADP inhibition and cyclo-oxygenase pathway inhibition[5,6]. A literature survey revealed that different analytical methods involving HPLC for determination of ASA in biological fluids[7-14] and in pharmaceutical preparations[15-31] have been developed. Literature reports concerning HPLC and HPTLC determination of CLP in pharmaceutical dosage forms[32-34] as well as enantiospecific HPLC method to determine the impurities and to perform the assay have been published[33]. Also thermal (DSC, TGA, HSM), crystallographic (XRD) and spectrophotometric (FTIR) methods for characterization and quantification of two enantiomers (R and S) of CLP have been reported[35]. Spectrophotometric method[36] and HPLC method[37] are reported in literature for estimation of ASA and CLP in their combined formulations. Because of the absence of an official pharmacopoeial method and none of these reported methods really meets our needs to have a simple and sensitive method for the simultaneous determination of ASA and CLP in pharmaceutical formulations, efforts were made to develop

analytical method for the estimation of ASA and CLP in their combined dosage form using first-derivative spectrophotometric (D^1) method.

EXPERIMENTAL

Apparatus

Absorbance was measured, and derivative spectra were recorded over the wavelength range 200-400 nm in two matched quartz cells with a 1 cm light path using a double beam Perkin Elmer Lambda 19 (Perkin Elmer, Norwalk, CT) and Chemito (Chemito Instruments Ltd., Nasik, India) 2600 UV-Visible spectrophotometer.

Reagents and materials

ASA and CLP pure powder were procured as gratis samples from Sun Pharmaceuticals (Vadodara, India). Analytical grade ethanol (Baroda Chemical Ind. Ltd, Dabhoi, India) was used for the sample preparation. Tablets containing ASA (75 mg) and CLP (75 mg) of the following 2 brands: Torrent Pharmaceutical Ltd (Gujarat, India) and Ajanta Pharma Ltd (Mumbai, India) were purchased from local market.

Preparation of standard stock solutions

Stock solution was prepared by weighing ASA (10 mg) and CLP (10 mg). Weighed powder of both drugs were accurately transferred to a same volumetric flask of 100 mL and dissolved in and diluted to the mark with ethanol to obtain a mixed standard stock solution of ASA (100µg/mL) and CLP (100µg/mL).

Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A mass equivalent to 75 mg of both ASA and CLP was weighed and transferred in a 100 mL volumetric flask and ethanol (80 mL) was added. The solution was sonicated for 15 min, and the final volume was diluted to the mark with ethanol to obtain solution of ASA (750µg/mL) and CLP (750µg/mL). This solution (0.4 mL) was further diluted to 25 mL with ethanol to obtain solution of ASA (12µg/mL) and CLP (12µg/mL).

Method validation

1. Calibration curve (Linearity of the method)

The calibration curves were plotted over the six different concentration range 4-24µg/mL for both drugs. Accurately measured mixed standard solution of ASA and CLP were transferred to a series of 10mL volumetric flasks and diluted to the mark with ethanol (n= 6).

2. Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of ASA and CLP by the standard addition method. Known amounts of mixed standard solution of ASA and CLP were added to prequantified sample solutions of tablet dosage forms. The amounts of ASA and CLP were estimated by applying values of absorbance to the regression equations of the calibration curve.

3. Precision (Repeatability and reproducibility)

The standard solutions of ASA and CLP were prepared and analyzed six times within the same day to obtain the repeatability and six times over different days to obtain the reproducibility. The results are reported in terms of relative standard deviation (RSD).

4. Limit of detection and limit of quantification

The limit of detection (LOD) with signal to noise ratio of 3:1 and the limit of quantification (LOQ) with signal to noise ratio of 10:1 were calculated for both drugs using the following equations as per International Conference on Harmonization (ICH) guidelines[38].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response and S = the standard deviation of y-intercept of regression line.

5. Specificity

The excipients hydroxypropylcellulose, mannitol, microcrystalline cellulose, polyethylene glycol 6000, lactose monohydrate (Signet Ltd., Mumbai, India); Methocel E5 Premium LV EP (Colorcon Asia Pvt. Ltd., Goa, India) were spiked into a preweighed quantity of drugs to assess the specificity of the methods. The absorbance was measured to determine the quantity of the drugs.

6. Robustness

Solutions of both the drugs in 0.1N HCl were studied for their stability at ambient temperature for 24h.

Analysis of ASA and CLP in tablet dosage forms

Tablets containing ALP (75 mg) and CLP (75 mg) of two different brands were purchased from local market. The absorbance of sample solutions were measured for quantification at analytical wavelength of ASP and CLP, respectively, by using D^1 method as described above. The amounts of ASP and CLP present in sample solution were determined by applying values of absorbance to the regression equations of the calibration curve.

RESULTS AND DISCUSSION

The derivative spectra of different orders were obtained from the zero order spectra using digital differentiation. The principle advantages of derivative spectroscopy are the improvement of resolution of overlapping absorption bands, accuracy, precision and greater sensitivities compared to UV absorption methods; therefore, derivative spectroscopy has been used in quantitative analysis when the analyte to be determined present in admixture with other components[39,40]. The first-derivative spectrum of ASP has zero absorption at 252.74 nm, where CLP gives significant derivative response, while the first-derivative spectrum of CLP has zero absorption at 229.11 nm, where ASP gives the significant derivative response. Therefore, 229.11 nm and 252.74 nm were selected for the estimation of ASA and CLP, respectively. (Figure 2)

Validation of proposed method

1. Linearity

Linear correlation was obtained between absorbance and concentration of ASA and CLP in the range of 4-24μg/mL for both the drugs, respectively. Data of regression analysis are summarized in TABLE 1.

2. Accuracy

The recovery experiments were carried out by the standard addition method. The recoveries obtained were 100.10 ± 0.11 and 100.18 ± 0.15 % for ASA and CLP, respectively. The high values indicate that both methods are accurate.

3. Method precision

The RSD values for ALP and FXT were found to be 0.228 and 0.296 %, respectively. The RSD values were found to be below 1% which indicates that the proposed methods are repeatable.

TABLE 1: Regression analysis of calibration curves for ASA and CLP for the proposed first-derivative spectrophotometric (D^1) method

Parameter	D^1 Method	
	ASA	CLP
Concentration range	4.24 μg/mL	4.24 μg/mL
Slope	0.01135	0.007234
Standard deviation of the slope	2.80×10^{-5}	2.30×10^{-5}
Intercept	0.0037	0.0072
Standard deviation of the intercept	5.29×10^{-4}	2.55×10^{-4}
Correlation coefficient	0.9998	0.9999

TABLE 2: Summary of validation parameters for the proposed First-Derivative spectrophotometric (D^1) method

Parameter	(D^1) Method	
	ASA	CLP
LOD[a]	0.14 μg/ml	0.11 μg/ml
LOQ[b]	0.47 μg/ml	0.35 μg/ml
Accuracy, %	99.66-101.05	99.58-100.84
Repeatability (RSD[c], %, n=6)	4.61×10^{-3}	7.26×10^{-3}
Precision (RSD, %)		
Interday (n=3)	0.06-0.78	0.44-0.71
Intraday (n=3)	0.17-0.77	0.50-0.90

[a]LOD = Limit of detection, [b]LOQ =Limit of quantification, [c]RSD= Relative standard deviation

4. Intermediate precision

The RSD values were found to be below 2% which indicates that the proposed methods are reproducible (TABLE 2).

5. LOD and LOQ

LOD for ASA and CLP were found to be 0.14 and 0.11μg/mL respectively. LOQ for ASA and CLP was found to be 0.47 and 0.35μg/mL respectively. These data show that microgram quantity of both drugs can be accurately determined.

6. Specificity

Excipients used in the specificity studies did not interfere with the estimation of either of drugs by the proposed methods. Hence, method was found to be specific for estimation of ASA and CLP.

7. Robustness

Absorbance variation was found to be less than 1%. Also, no significant change in absorbance was observed during 24 h. No decomposition was observed

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TABLE 3 : Assay results for the combined dosage form using the proposed first-derivative spectrophotometric (D^1) method

Parameter	(D^1) Method	
	ASA ± SD[a] (n[b] = 5), %	CLP ± SD[a] (n[b] = 5), %
A	99.77 ± 0.28	100.07 ± 0.66
B	100.04 ± 0.63	100.16 ± 0.77

[a]SD = Standard deviation, [b]n = Number of determination

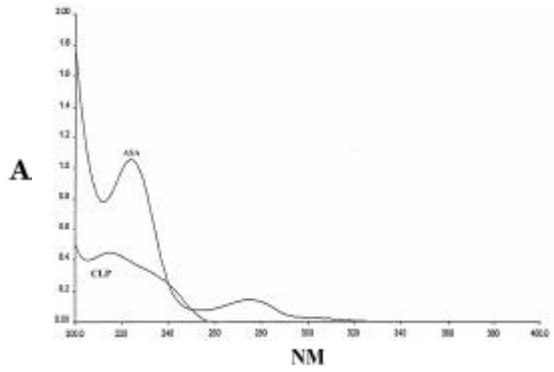

Figure 1: Overlain zero order spectrum of ASP and CLP in ethanol

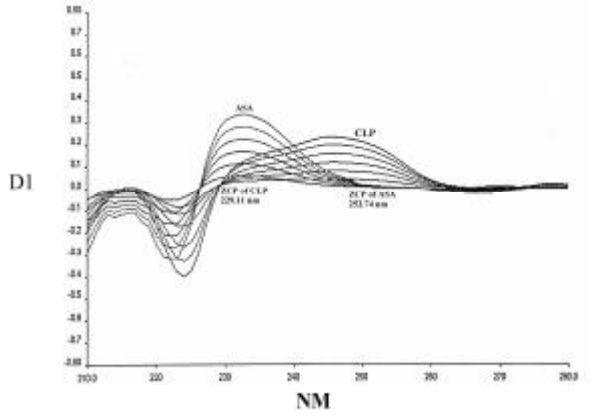

Figure 2: Overlain first derivative spectrum of ASP and CLP in ethanol

after 24h. Hence, methods were found to be robust for estimation of ASA and CLP.

Assay of the tablet dosage form (ASA 75 mg and CLP 75 mg/tablet)

The proposed validated method was successfully applied to determine ASA and CLP in their tablet dosage forms (tablet A and B). The results obtained for ASA and CLP were comparable with the corresponding labeled amounts (TABLE 3).

Comparison of the proposed method

The literature describes HPLC[36] and UV Spectrophotometry[37] methods for determination of ASA and for determination of CLP in tablet dosage forms. The assay results obtained by these methods were used for statistical comparison to evaluate the validity of developed D^1 Method. For ASA the calculated F value was found to be 1.58 (for HPLC) which is less than the tabulated F value (5.05) at 95% (P = 0.05) confidence interval. For CLP the calculated F value were found to be 1.35 which is less than the tabulated F value (3.48) at 95% (P = 0.05) confidence interval. Therefore, there was no significant difference among the methods.

CONCLUSION

The results were compared statistically and method was found to be simple, precise and accurate. Moreover, the proposed method has the advantages of simplicity and convenience for the sample preparation and quantification of ASA and CLP in combination and can be used for the routine simultaneous analysis of ASA and CLP in pharmaceutical preparations.

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