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Simultaneous Estimation of Aspirin, Atorvastatin and Clopidogrel in Combined Capsule Dosage Form Using Reverse Phase High-Performance Liquid Chromatography

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ABSTRACT

Simple, accurate and precise reversed phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of aspirin (ASP), atorvastatin (ATO) and clopidogrel (CLO) in combined capsule dosage form have been developed and validated. The RP-HPLC method uses a Shimadzu LC 10 ATVP system with a Luna C_{18} column and acetonitrile: methanol: water (pH adjusted with ortho phosphoric acid) at pH 3.5 (50: 30: 20, v/v/v) as the mobile phase. The detection was carried out using a diode array detector set at 250 nm. Linearity of chromatographic method was found in the concentration range of 5-100, 2-24 and 5-100 µg/mL in methanol at 238, 247 and 220 nm for ASP, ATO and CLO respectively. The recoveries were in the range of 101.25 ± 0.60 for ASP, 100.34 ± 0.62 for ATO and 100.36 ± 0.60 for CLO using HPLC. These methods may be used for routine analysis of the drugs in a pharmaceutical formulation. Results of analysis were statistically validated.

Keywords: RP-HPLC, validation
Abbreviations: **ASP**, aspirin; **ATO**, atorvastatin; **CLO**, clopidogrel; **RP-HPLC**, reversed phase high performance liquid chromatography

INTRODUCTION

ASP is chemically known as 2-acetoxybenzoic acid. Aspirin also known as acetylsalicylic acid, and belongs to the class of compounds known as is a salicylate drug and also having analgesic, antipyretic action and anti-inflammatory medication. It is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia (Anonymous 1998, 2004, 2007). The analgesic action is mainly due to obtunding of peripheral pain receptor and prevention of PG (prostaglanding). Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damage of the walls within blood vessels (Panchal *et al.* 2009; Patel *et al.* 2009).

Atorvastatin is chemically known as (3R,5R)-7-[2-(4-fluorophenyl)- 3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)- 1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.

Atorvastatin is a member of drug class known as statins. It is official in Indian Pharmacopoeia (2007). Atorvastatin is a HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitor (Gupta *et al.* 2009; Hirave *et al*. 2010; Kolsure *et al.* 2010).

Clopidogrel is chemically known as (+)-(S)-methyl 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate. Clopidogrel is an oral antiplatelet agent thienopyridine class. Clopidogrel acts as an antithrombotic drug, which altes ther surface receptor on platelets and inhibits ADP (Adenosine diphosphate) as well as fibrinogen-induced platelets aggregation (Saber *et al* 2008; Renapurkur *et al.* 2010). Chemical structures of ASP, ATO and CLO are shown in **Fig. 1**.

A literature survey revealed that several analytical methods have been reported for the determination of ASP in pure and dosage forms in the official Indian Pharmacopoeia (Anonymous 1996, 2007) and apart from Pharmacopeias, several analytical methods have been used to analyse human plasma and urine (Buskin *et al.* 1982; Kees *et al.* 1996).

HPLC for determination of ATO from capsule formulation is official in Indian Pharmacopoeia (2007). Several analytical methods that have been reported for the determination of ATO in biological fluids and in bulk as well as pharmaceutical formulations include HPLC, UV absorption spectrophotometry (Bahramia *et al.* 2005; Sonawane *et al.* 2006; Stanisz *et al.* 2006; Hirave *et al.* 2010).

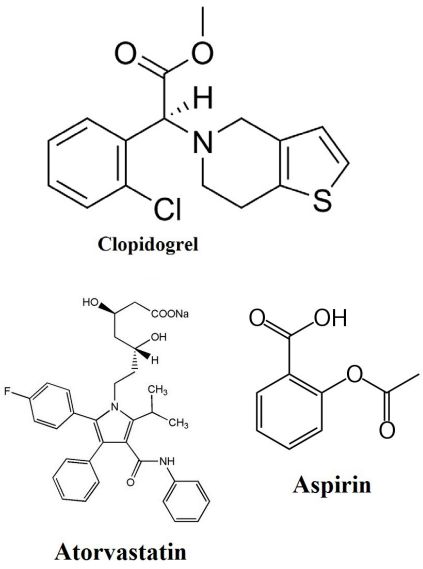

Fig. 1 Chemical structures of aspirin (ASP), atorvastatin (ATO) and clopidogrel (CLO).

This paper describes simple, accurate, precise, and sensitive reversed-phase (RP)-HPLC methods for simultaneous determination of ASP, ATO and CLO in a combined capsule dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines (ICH Harmonized Tripartite Guideline 2005; Mandhanya *et al.* 2011).

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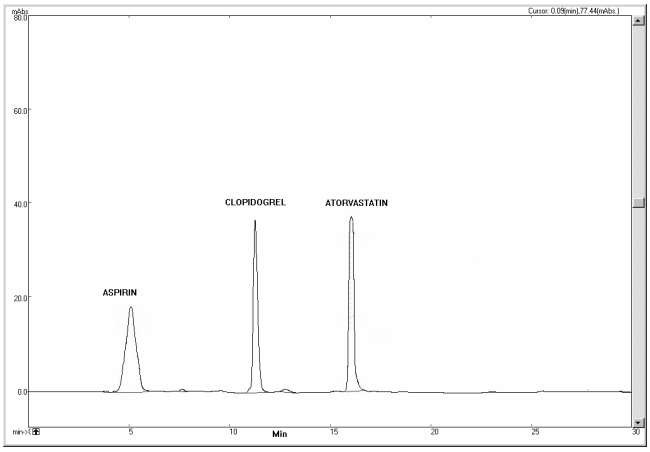

Fig. 2 Chromatogram of ASP, ATO and CLO in capsule dosage form.

MATERIALS AND METHODS

Drugs and chemicals

Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Merck (Mumbai, India) and water (HPLC grade) was prepared in the institute. All other reagents used were of analytical grade. Standard bulk drug samples of ASP (98.92% pure), ATO (99.81% pure) and CLO (99% pure) were provided by Ipca laboratories Ltd. (Ratlam, India) as gratis samples. The pharmaceutical dosage form used in this study was Deplatt-CV labeled to containing ASP 75 mg, ATO 10 mg and CLO 75 mg/capsule (Suren Pharmaceutical, Villiyanur, Commune Punuchery India) were purchased from the local market.

Instrumental

The HPLC method, an HPLC system consisting of an LC 10 ATVP pump equipped with a diode array detector (Shimadzu, Japan) and a Luna C18 (4.6 mm id) column and class M10A software version 1.6 was used. A Rheodyne (Rohnert Park) injector with 20 µL loop was used for injecting the sample.

Method: RP-HPLC method

In the RP-HPLC method, separation and analysis of ASP, ATO and CLO were carried out on a Luna C18 column (4.6 mm id) with the diode array detector set at 250 nm. Mobile phase consisting of methanol: acetonitrile: water (pH 3.5 adjusted with ortho phosphoric acid) (50: 30: 20, v/v/v; filtered through a 0.2 µm membrane filter, degassed and sonicated) was used at flow rate of 1.0 mL/min.

(a) Standard stock solutions: Standard stock solutions containing 100 µg/mL ASP, 100 µg/mL ATO and 100 µg/mL CLO were prepared by dissolving the pure drugs separately in the mobile phase (Steward *et al* 2000; Jalalizadeh *et al* 2006; Qutab *et al* 2007).

(b) Preparation of the calibration curves: Aliquots of 5, 10, 15, 20, 25 and 30 mL stock solution of ASP and 2, 4, 6, 8, 10 and 12 mL stock solution of ATO and 5, 10, 15, 20, 25 and 30 were transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with the mobile phase. Each solution was injected, and chromatogram was recorded. Mean retention times for ASP, ATO and CLO were found to be 4.98, 16.43 and 11.21 min, respectively. The peak area of ASP, ATO and CLO were noted, and respective calibration curves were plotted as peak area against concentration of each drug (Steward *et al.* 2000; Jalalizadeh *et al.* 2006; Qutab *et al.* 2007).

(c) Procedure for analysis of capsule formulation: Twenty capsules of commercial capsules were taken and their average weight was determined. Then powder equivalent to 10 mg of ASP (respective quantity of ATO and CLO) was placed in a 50 ml volumetric flask and dissolved with mobile phase. The supernatant liquid was transferred to 100 ml of volumetric flask through a Whatman #41 filter paper. After that 10 ml of the above solution was diluted up to 100 ml with mobile phase. The sample solution was injected, and the peak areas were recorded. A representative chromatogram is given in **Fig. 2**.

From the peak area the drugs concentration of each drug/capsule was estimated from the respective calibration curves (Steward *et al.* 2000; Jalalizadeh *et al.* 2006; Qutab *et al.* 2007).

(d) Recovery studies: Accuracy of the method was analyzed by recovery studies carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and 120% of the target concentration (ICH guidelines).

(e) Precision: Precision of the method was checked by 3 replicate readings at 3 concentration levels of within range expressed as RSD values.

Statistical analysis

Means, standard deviation (SD), relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for ASP, ATO and CLO. Different mobile phases containing methanol, acetonitrile and water were examined (data not shown), and the mobile phase methanol: acetonitrile: water (pH adjusted with ortho phosphoric acid) (50: 30: 20, v/v/v) was selected as optimal for obtaining well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 250 nm, at which the best detector response for these substances was obtained. Linear calibration curves were obtained for ASP, ATO and CLO in the RP-HPLC methods. **Table 1** summarizes the linearity, precision, standard deviations (SD), limit of detection (LOD) and limit of quantitation (LOQ) values for methods. System suitability parameters for the RP-HPLC method are listed in **Table 2**.

Although chromatographic methods (Buskin *et al.* 1982; Kees *et al.* 1996; Anonymous 1998, 2004, 2007) have been reported for analysis of ASP, ATO and CLO in bulk drug, biological fluid and urine but no method has been reported for the analysis of ASP, ATO and CLO. The method reported analyzes all three components with accuracy (≤ RSD ± 2%) in combination in the bulk formulations as well as in combined dosage form without prior separation.

The proposed methods were also evaluated in the assay of commercially available capsule containing ASP, ATO and CLO. Six replicate determinations were performed on the accurately weighed amounts of capsule. For ASP, ATO and CLO recovery (mean, %, ± SD, n = 3) was found to be 101.25 ± 0.60, 100.34 ± 0.62 and 100.36 ± 0.60%, respectively (**Table 3**).

Table 1 Regression analysis of calibration curves of method.

Parameters	Method		
	ASP	ATO	CLO
Intercept	1443	1502	5279
Slope	3061	1195	1242
SD[c]	5988.04	5968.12	8860.32
Detection limit, µg/mL	3.22	0.82	0.94
Quantitation limit, µg/mL	2.1	1.1	2.3

[a] Detection wavelength for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL).
[c] SD = standard deviation.

Table 2 System suitability parameters for RP-HPLC method.

Parameters	ASP	ATO	CLO
Calibration range, µg/mL	5-100	2-24	5-100
Theoretical plate number	4747	4983	5231
HETP[a]	0.0032	0.0030	0.0028
Tailing factor	1.25	1.07	1.08
Capacity factor (k')	0	2.29	1.25
Resolution	-	6.73	13.68

[a] HETP = Height equivalent to theoretical plate, cm

Table 3 Recovery studies.

%	Drug	Mean	RSD
80	ASP	101.46	0.0035
	ATO	100.30	0.0093
	CLO	100.31	0.0053
100	ASP	100.94	0.0035
	ATO	100.41	0.0093
	CLO	100.39	0.0053
120	ASP	101.36	0.0035
	ATO	100.33	0.0093
	CLO	100.37	0.0053

CONCLUSIONS

The validated RP-HPLC method developed here proved to be simple, fast, accurate, precise and sensitive. Thus, they may be used for routine analysis of ASP, ATO and CLO in combined capsule dosage form without prior separation.

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