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NEW SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ATORVASTATIN CALCIUM AND ASPIRIN USING UREA AS HYDROTROPIC SOLUBILIZING AGENT

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ABSTRACT

Plan: *An analytical method for the simultaneous determination of Atorvastatin and Aspirin in capsule dosage form using 1.5M urea as hydrotropic solubilizing agent is described.*

Prologue*: The method is simple, fast, & accurate and precludes the use of corrosive solvents and can be used for the routine analysis of commercial combinations of Atorvastatin and Aspirin.*

Methodology: *The developed method used the simultaneous equation method (method-A)using 243 nm and 233 nm as absorbance maxima for ATR and ASP respectively and Q-absorbance ratio method (method-B), which is based on the measurement of absorptivity at iso-absorptive point 239 nm and 243 nm (absorption maximum of Atorvastatin). The calibration curves for both drugs were found to be linear in the concentration range of 10-50µg/ml.*

Outcome: *The proposed method has been applied successfully for the simultaneous determination of Atorvastatin and Aspirin in capsule dosage form. The mean recovery of the drugs from the combination tablets was found to be 98.83 %for Atorvastatin and 97.77 % for Aspirin for method-A and 98.09 % and 98.06 % for method-B respectively. No significant interference was observed from urea and other excipients commonly used in the formulation.*

1. INTRODUCTION

Cardiovascular disease is one of the leading causes of death in the world and one of the most significant factors for these diseases is total/high density lipoprotein (HDL) cholesterol level.

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Atorvastatin calcium(ATV), chemically [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3phenyl-4- [phenylamino) carbonyl]-1H-pyrrole-1- heptanoic acid, calcium salt trihydrate, is an inhibitor of HMG –CoA reductase, an enzyme involved in cholesterol biosynthesis[1,2]. Aspirin (ASP); Acetylsalicylic acid; is approved for the treatment of minor aches, inflammations and a low dose treatment of aspirin is used to reduce the overall risk of heart attack. Aspirin is chemically 2-Acetoxybenzoic acid[3].The fixed-dose combination containing Atorvastatin Calcium and Aspirin improved the fibrinolytic balance more than either single agent in hypertensive, hypercholesterolemic patients with insulin resistance and could potentially improve medication compliance.

HPLC method is official in Indian Pharmacopoeia (2007)[4] for the estimation of ATV and titrimetric method for the determination of ASP[3], but they do not involve simultaneous determination of ATV and ASP. Detailed survey of literature for ATV revealed several methods based on different techniques, viz. HPLC[5] GC-MS[6],LC-MS[7], HPLC-Electron spray tandem mass spectrometry[8] and HPTLC [9]have been reported for the estimation of Atorvastatin Calcium . Similarly, literature survey for ASP revealed spectrophotometric method[10], and HPTLC[11, 12] methods. A UV spectrophotometric method is reported[13] for the combination and the method uses methanol as solvent. Both the drugs are slightly soluble in water. Maheshwari et al. has analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. Ketoprofen, Salicylic acid[14], Frusemide [15], Cefixime[16] and Amoxicillin[17]. No UV spectrophotometric method for the simultaneous estimation of ATV and ASP using hydrotropic solubilization is reported so far. The primary objective of the present investigation was to employ the hydrotropic solution to extract the drugs from the combined dosage form and precludes the use of corrosive organic solvents. The aqueous solubility of ASP and ATV were enhanced to a great extent in 1.5M Urea.

2. MATERIALS AND METHODS

2.1. Instrument

Agilent Cary-150 UV- Visible double beam spectrophotometer with matched quartz cells of 10 mm optical path length was used for all spectral and absorbance measurements. Schimadzu AX 200 Analytical balance was used for weighing purposes.

2.2. Reagents and chemicals

The reference standard of Aspirin and Atorvastatin Calcium were gift samples from Torrent Pharmaceuticals. All chemicals were analytical grade obtained from SD fine chemicals. The drug sample (capsules), Ecosprin-AV,manufactured by USV Pharma and Modlip ASP, manufactured by Torrent Pharmaceuticals(both brands contain Atorvastatin 10mg and Aspirin 75mg) were procured from market and utilized for the study.

2.3. Preliminary solubility studies of drugs[18]

Solubility of both drugs was determined at 27 ±1°C. An excess amount of drug was added to two screw capped 40 ml glass vials containing 1.5 M Urea solution. The vials were shaken for 12 hrs at 27±1°Cin a mechanical shaker. These solutions were allowed to equilibrate for the next 20 hrs and then centrifuged for 25 minutes at 1500 rpm. The supernatant of each vial was filtered through Whatman filter paper No.41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

2.4. Preparation of standard stock and binary mixture solutions

The standard stock solutions of each drug were prepared by dissolving 50 mg of each in 50ml of 1.5M Urea solution separately and final volume was made up with distilled water in 100ml volumetric flask. From the above solution20 ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 200 µg/ml of each drug. Working standard solutions were scanned in the entire UV range of 400-200 nm to determine the absorption maximum of both drugs. Atorvastatin showed absorption maximum at 243nm (Figure-1) and Aspirin showed absorption maximum at 233nm (Figure-2).From overlain spectra (Figure-3) it is evident that the iso- absorptive point was at 239nm. Five working standard solutions of concentration 10, 20, 30, 40 and 50 µg/ml each of Atorvastatin calcium and Aspirin were prepared. The absorbance of resulting solutions were measured at their respective wavelengths and plotted the calibration curves to get linearity and regression equations.

2.5. Marketed formulation

Twenty capsules were accurately weighed, and the average weight of the content per capsule was calculated. The contents of a capsule were reduced to fine powder. A quantity of capsule powder equivalent to 10mg of Atorvastatin calcium and 75mg of Aspirin were weighted accurately and taken in a 50 ml of volumetric flask containing 50ml of 1.5 M urea .The solution were shaken for 12 hrs at 27±1°Cin a mechanical shaker and was filtered through Whatman filter paper no.41. From this solution, appropriate aliquots of ASP and ATR were diluted with distilled water to get concentrations within the Beer's law limit. The absorbance values of resulting solutions were measured at 233 nm, 239nm and 243 nm. Values were substituted in the respective formulae to obtain the drug concentrations[19].

2.6. Method-A (Simultaneous equation method)

The simultaneous equation method of analysis is based on the absorption of the drugs Aspirin and Atorvastatin calcium at their absorption maxima. Two wavelengths selected for the development of Simultaneous Equation were 233 nm and 243 nm. Absorptivity values of both the drugs at both the wavelengths were determined. The equations obtained for the estimation of concentration were,

$$Cx = A2\ ay1 - A1\ ay2 / ax1.\ ayl.ax1.ay2$$
$$Cy = A1\ ax2 - A2\ ax1 / ax2.ay1.\ ax1.ay2$$

Where, A1 and A2 are absorbance of sample solution at 233nm and 243 nm respectively.
ax1= Absorptivity of ASP at 233 nm, ax2 = Absorptivity of ASP at 243 nm.
Ay1 = Absorptivity of ATR at 233 nm,
Ay2= Absorptivity of ATR at 243 nm,
Cx and Cy are concentration of ASP and ATR in sample solution.

2.7. Method-B (Q-Absorbance or Absorbance ratio method)

The absorbance ratio method of analysis is based on the absorbance at two selected wavelengths; one is the iso-absorptive point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra, wavelength 239nm (iso-absorptive point) and 243 nm (absorption maximum of ATR) are selected for Q-Absorbance equation.

$$C_x = [Qm\text{-}Qy] \times A\ 1/\ [Qx - Qy] \times axl$$
$$C_y = [Qm\text{-}Qx] \times A1\ /\ [Qy - Qx] \times ayl$$
Where Qm = A2/A1, QX = ax2/ax1, Qy = ay2 /ay1

Where, A1 and A2 are absorbance of sample solution at 243 nm and 239 nm respectively,
ax1 = Absorptivity of ASP at 243 nm,
ax2= Absorptivity of ASP at 239 nm.
ay1= Absorptivity of ATR at 243 nm,
ay2= Absorptivity of ATR at 239nm.
Cx and Cy are concentration of ASP and ATR in sample solution.

3. METHOD VALIDATION

The method was validated according to ICH Q2B guidelines for validation of analytic procedures [20, 21] in order to determine the linearity, sensitivity, precision and accuracy.

3.1. Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%). Percentage recovery of ASP for method-A and method-B were found to be 97.77 and 98.06 and the corresponding recovery values for ATR was found to be 98.83 and 98.09respectively.

3.2Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of ASP at 233nm, ATR at 243nm and both at 239nm (Figures 4, 5&6). The Beer- Lambert's concentration range for both ASP and ATR was found to be 5-50 µg/ml.

3.3. Limit of Detection (LOD) and Limit of Quantitization (LOQ)

The LOD and LOQ of ASP and ATR by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response.

4. RESULTS AND DISCUSSION

The drugs obey beer's law with good correlation coefficient of 0.9998 and 0.9996 for Aspirin and Atorvastatin respectively. The optical characteristics and summary of validation parameters fare given in Table 3. The results of commercial formulation analysis are presented in Table 1. The results of recovery studies are shown in Table 2. Precision was calculated as intra-day and inter-day variations (% RSD) for both the drugs and the results are produced inTable 4.From the literature review it is evident that, the reported spectrophotometric methods for the simultaneous estimation of Atorvastatin calcium and Aspirin uses solvents such as methanol or ethanol. The drawback of these solvents includes toxicity, error due to volatility, pollution and cost. Thus, the use of 1.5M Urea as hydrotropic solubilizing agent in the proposed spectrophotometric method preclude the use of such solvents and makes the method cost effective, safe, accurate, precise and environment friendly.

Table 1: analysis of capsule formulations

Brand Name	*Drug content & Label claim/tab.*	*Amount found (mg)*		*% Drug found ±SD*		*Standard Error*	
		Method A	Method B	Method A	Method B	Method A	Method B
Modlip ASP	ATR 10	9.93	9.92	99.30 ± 0.30	99.20± 0.28	0.55	0.39
	ASP 75	74.86	74.69	99.81 ± 0.18	99.59 ± 0.39	0.32	0.26
Ecosprin-AV	ATR10	9.96	9.93	99.60 ± 0.20	99.30± 0.18	0.45	0.49
	ASP75	74.66	74.79	99.55 ± 0.17	99.72 ± 0.29	0.22	0.28

Values expressed mean± SD (n=6), Student's t-test, *: P value is < 0.01**,** **: P value is < 0.001

Table 2: Recovery study of ATR and ASP

Brand Name	*Drug*	*Level of addition (%)*	*Amount added (mg)*	*Amount recovered (mg)*		*Drug % Recovery ± SD*	
				Method A	Method B	Method A	Method B
Modlip ASP	ATR 10	80	4	3.88	3.90	99.25± 0.058	97.50± 0.055
		100	6	5.98	5.93	99.66± 0.045	98.83± 0.055
		120	8	7.99	7.89	98.62± 0.035	98.62± 0.055
	ASP 75	80	4	3.89	3.90	97.25± 0.059	97.50± 0.035
		100	6	5.88	5.93	98.40± 0.046	98.83± 0.055
		120	8	7.89	7.89	98.62± 0.043	98.62± 0.039
Ecosprin-AV	ATR 10	80	4	3.98	3.90	98.50± 0.045	97.50± 0.035
		100	6	5.93	5.93	98.83± 0.045	98.83± 0.045
		120	8	7.93	7.89	99.13± 0.055	97.50± 0.062
	ASP 75	80	4	3.85	3.90	96.25± 0.055	97.50± 0.055
		100	6	5.94	5.93	99.00± 0.035	98.25± 0.055
		120	8	7.92	7.89	99.10± 0.035	98.63± 0.055

Values expressed mean± SD (n=3), Student's t-test, *: P value is < 0.01**,** **: P value is < 0.001

Table 3: Optical characteristics of linearity plot

Parameters	*Values for ASP*		*Values for ATR*	
Absorption maxima (λ max)	233nm	239nm	243nm	239nm
Beer's law limit (µg/ml)	5-50µg/ml	5-50µg/ml	5-50µg/ml	5-50µg/ml
Regression equation	Y = 0.02171X + 0.01170	Y = 0.01959X - 0.05610	Y =0.01912X - 0.08140	Y = 0.02079X + 0.01790
Correlation coefficient (R^2)	0.9998	0.9989	0.9992	0.9993
LOD (µg/ml)	2.75	3.41	3.25	3.97
LOQ (µg/ml)	8.33	10.33	9.85	12.03
Molar absorptivity	$2.171x10^4$ L/mol/cm	$1.959x10^4$ L/mol/cm	$1.912x10^4$ L/mol/cm	$2.079x10^4$ L/mol/cm

Table 4: Intra-day and Inter-day precision studies of ATR& ASP

Drug (µg/ml)	*Intra-day precision*						SD	
	10am		*1pm*		*4pm*			
	ATR	*ASP*	*ATR*	*ASP*	*ATR*	*ASP*	*ATR*	*ASP*
20	0.340	0.446	0.343	0.439	0.334	0.433	0.0046	0.0065
30	0.543	0.643	0.534	0.639	0.531	0.629	0.0062	0.0072
50	0.920	0.980	0.914	0.976	0.915	0.970	0.0032	0.0050
Drug in (µg/ml)	*Inter-day precision*						SD	
	1st day		*2nd day*		*5th day*			
	ATR	ASP	ATR	ASP	ATR	ASP	ATR	ASP
20	0.340	0.445	0.338	0.440	0.328	0.432	0.0064	0.0066
30	0.543	0.642	0.540	0.640	0.529	0.638	0.0061	0.0020
50	0.920	0.992	0.913	0.988	0.910	0.987	0.0513	0.026

Student's t- test, *: P value is < 0.01, **: P value is < 0.001

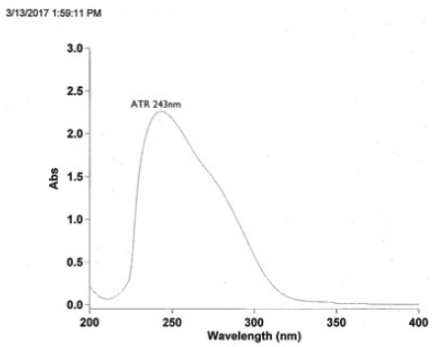

Fig.1 UV spectrum of Atorvastatin

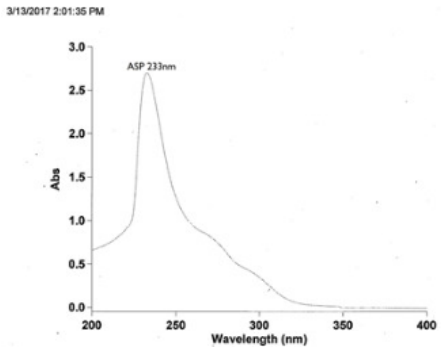

Fig.2 UV spectrum of Aspirin

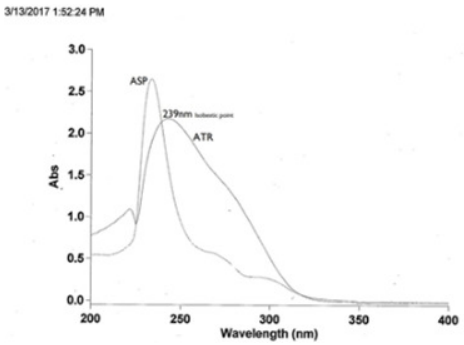

Fig.3 Overlain UV spectrum of Aspirin and Atorvastatin

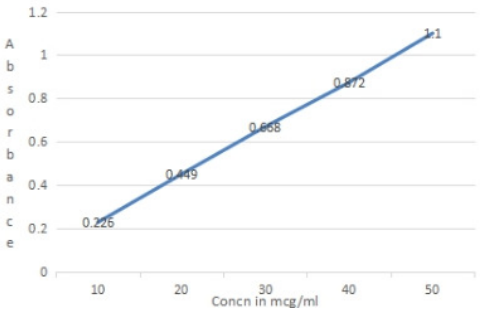

Fig.4 Linearity plot of Aspirin at 233nm

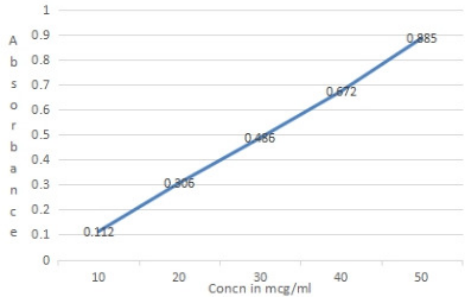

Fig.5 Linearity plot of Atorvastatin at 243nm

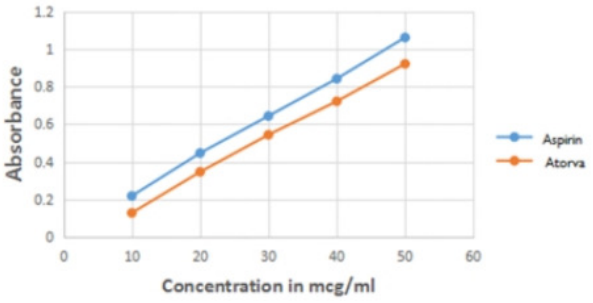

Fig.6 Linearity plot of Aspirin and Atorvastatin at 239nm

5. CONCLUSION

By observing the validation parameters, the proposed method is found to be simple, sensitive, accurate and precise and can be employed for the routine analysis for the simultaneous estimation of Atorvastatin calcium and Aspirin in bulk and capsule formulation.

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