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METHOD DEVELOPMENT AND VALIDATION OF ATORVASTATIN AND CLOPIDOGREL BISULFATE IN MARKETED FORMULATION USING HYDROTROPY PHENOMENA

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ABSTRACT

Method was developed and validated for the estimation of atorvastatin and clopidogrel bisulfate using hydrotropy phenomena. Firstly both standard drugs were characterized to check quality by melting point, solubility and FT-IR. The maximum absorbance of ATV and CLP was observed at 244.0 nm and 228.0 nm, respectively. ATV and CLP showed linearity in the concentration range of 5- 25□g/ml and 10-50□g/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration. The overlain spectra also showed isoabsorptive points at 233.0 nm. Both drugs were estimated by using simultaneous equation method. Based on the solubility, stability and spectral characteristics of the drugs, 2M Sod. Citrate was selected as hydrotropic agent. Presence of hydrotropic agent do not shows any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method. Simultaneous equation method was validated by using different parameters like linearity, accuracy and precision. One marketed formulation was also estimated by novel developed method found 99.92 and 99.80 for ATV and CLP respectively.

KEYWORD: Simultaneous Estimation, Atorvastatin, clopidogrel bisulfate, hydrotropy phenomena.

INTRODUCTION

The term "hydrotropy" has been used to designate the increase in solubility of various substances due to the presence of large amounts of additives.[1] Various hydrotropic agents such as sodium salicylate, sodium benzoate, urea, nicotinimide, sodium citrate and sodium

acetate have been used to enhance the aqueous solubility of a large number of drugs.[2] Maheshwari and his associates have analyzed a large number of poorly water-soluble drugs by titrimetric and spectrophotometric analyse.[3] Increasing the aqueous solubility of insoluble and slightly soluble drugs is of major importance.[4] Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs. Hydrotropic solubilization is one of them. In the hydrotropic solubilization phenomenon, addition of large amount of second solute results in an increase in the aqueous solubility of another solute.[5] Concentratedaqueous hydrotropic solutions of urea, nicotinamide, sodium benzoate, sodium salicylate, sodium acetate and sodium citrate have been observed to enhance the aqueous solubility of poorly water soluble drugs.[6] The class of compounds that normally increase the aqueous solubility of sparingly- soluble solutes is called hydrotropes.[7] The hydrotropes are a special class of compounds that exhibit distinct solution properties.[8] They may self associate in aqueous medium, comparable to amphiphile self-association or micellization.[9] They are efficient solubilizers and can influence the formation of micelle and micro emulsion.[10]

Hydrotropic salts are essentially the same as low molecular weightamphiphiles with marked hydrophilic solvent affinity and proposed a mechanism as a supplement to the theory of micellization.[11] Studies on the effect of hydrotropes on the phase behaviors of mixed systems of oil/water/hydrotrope have helped arrive at the above conclusion.[12]

The concept of drug treatment, which was earlier “right drug for right person” is now changing from “right does for the right person” to “right time of the does for the right person”. The scope of developing and validating and analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise.[13]

The main objective is to improve condition and parameters which followed in the development and validation. The present work is to develop new simple, sensitive and validated method for estimation of atorvastatin and clopidogrel in marketed formulation using hydrotropy phenomena.

MATERIAL AND METHODS

Standard drugs atorvastatin and clopidogrel bisulfate was obtained from Bioplus life science, Bangalore as gift. HPLC grade methanol, water and acetonitrile were purchased from Merck

specialties pvt, Ltd., Mumbai. The instruments, Labindia 3000 Plus UV Visible spectrophotometer and Bucker's alpha/opus were used.

Methods

Identification and characterization of drugs

Physical characterization of drug: The drugs ATV and CLP were physically characterized on the beginning of appearance, color and odor. All these parameter were recorded and compared with the literature.

Melting point determination: The melting point determined used for the strength of mind of melting point of ATV and CLP by the open capillary methods. The melting point of drug was recorded and compared with literature values.

Identification by IR: scanned the drugs atorvastatin and clopidogral in FT-IR using KBr palate and spectrogram obtained.

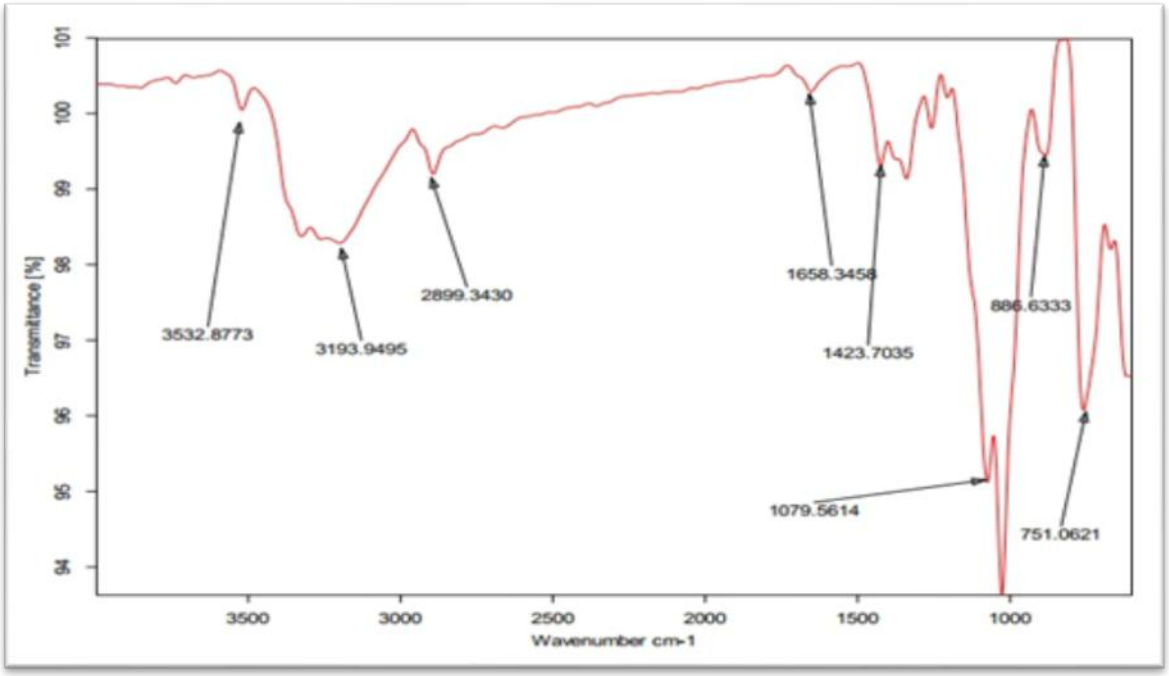

Figure 1: IR spectra of Sample Atorvastatin.

Table 1: FT-IR interpretation of Atorvastatin.

S. No.	Observed (wave number)	Functional Group
1.	3532.8773	-N-H strech
2.	1423.7035	-N-H bend
3.	1658.3458	C=O amide

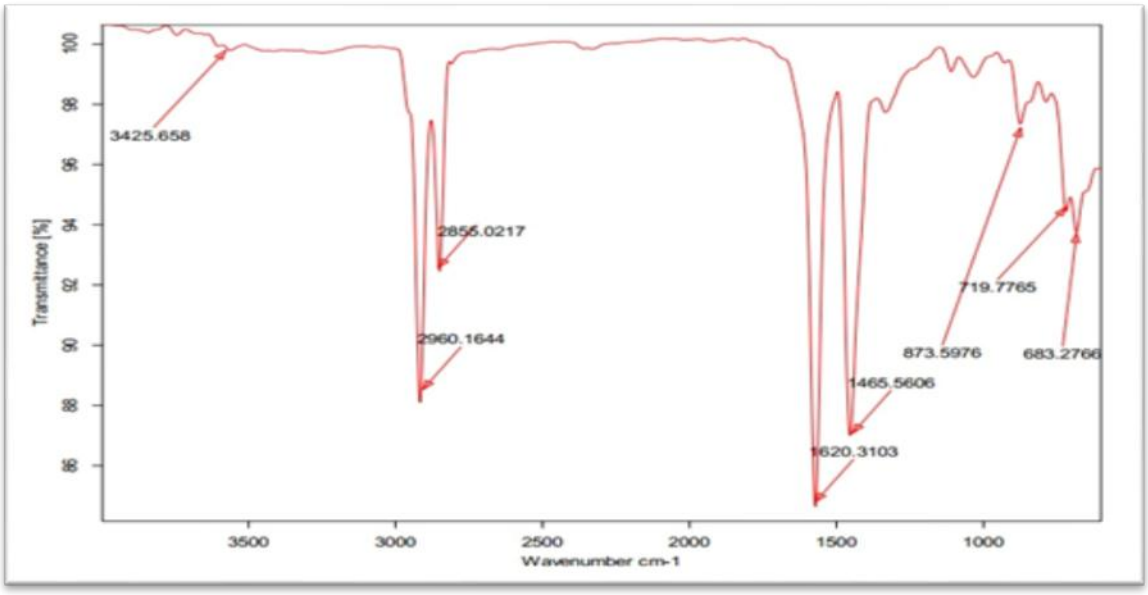

Figure 2: IR spectra of Sample CLP.

Table 2: Interpretation of CLP.

S. No.	Observed (wave number)	Functional Group
1.	3425.658	N-H stretching
2.	2960.1644	C-H stretching
3.	2855.0217	Sym C-H stretching
5.	1465.5606	Sym C-H wag
6.	719.7765	C-H bend

Solubility

Solubility of ATV and CLP was determined at 25±1°C. Accurately weighed 10 mg ATV and CLP was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed visually.

Table 3: Solubility of drug in different solvents.

S. No.	Solvents	Solubility	
		ATV	CLP
1	Water	-+	-+
2	Hot water	-+	-+
3	Cold water	-+	-+
4	2M Sodium acetate	+	+
5	8M Urea	+	+
6	8M Urea: 2M Sodium acetate	+	+
7	2M Sodium Benzoate	+	+
8	2M Ammonium Acetate	++	++
9	2M Sod. Citrate	+++	+++

(-) Insoluble, (-+) Slightly soluble, (+), Sparingly soluble (++) Soluble, (+++) Freely soluble Determination of solubility enhancement by UV VIS Spectroscopy Solubility studies were performed in distilled water 2M Sodium acetate, 8M Urea, 2M Sodium Citrate, 2M Sodium Benzoate, 2M Ammonium Acetate, 2M Sod Citrate at room temperature (25 ± 20C). An excess amount of drug was added to 100ml of solvent in screw-capped glass vials; these were mechanically shaken for 48 hours at 25°C until equilibrium was achieved. Aliquots were withdrawn, filtered through a membrane filter (0.45µ) and spectrophotometrically analyzed for solubility.

Table 4: Results of solubility enhancement by UV VIS. Spectroscopy.

S. No.	Solvents	Solubility Enhancement (folds)	
		ATV	CLP
1	2M Sodium acetate	2	3
2	8M Urea	2	4
3	8M Urea: 2M Sodium acetate	3	3
4	2M Sodium Benzoate	3	4
5	2M Ammonium Acetate	8	9
6	2M Sod. Citrate	15	13

Enhancement of solubility was more than 60 to 70 % for ATV and CLP respectively in 2M Sod Citrate, enhancement of solubility of ATV and CLP due to the hydrotropic solubilization phenomenon. Solubility in different solvent for both the drugs were shown in table 4.

Selection of solvent system

ATV and CLP were scanned in various hydrotropic agent in the spectrum mode over the UV range (200-400) and 2M Ammonium Acetate: 2M Sod. Citrate (1:1) was found to be most appropriate because.

- Both drugs are soluble in it.
- Both drugs are stable in it.
- Both drugs exhibit good spectral characteristics in it.
- 2M Sod. Citrate solutions have no interference with the λmax of both drugs.
- More than 15 folds solubility enhancement for ATV and more than 13 folds solubility enhancement for CLP.

Establishment of stability profile

Stability of both drugs was observed by dissolving ATV and CLP in 2M Sod. Citrate solution was used as solvent. Solution of ATV and CLP was prepared in the conc. of 5µg/ml and

10µg/ml respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A): Standard stock solutions were prepared by dissolving separately 10mg of each drug in 8`mL hydrotropic solution containing 2M Sod. Citrate and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to 10ml with mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B): Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of ATV and CLP and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 µg/ml.

Preparation of Working Standard Solution: 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for ATV.

Aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50 µg/ml respectively for CLP.

Selection of wavelength for linearity

Solutions of 10µg/ml of ATV and 20µg/ml CLP were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of ATV and CLP was observed at 244.0 nm and 228.0 nm, respectively. ATV and CLP showed linearity in the concentration range of 5- 25µg/ml and 10-50µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

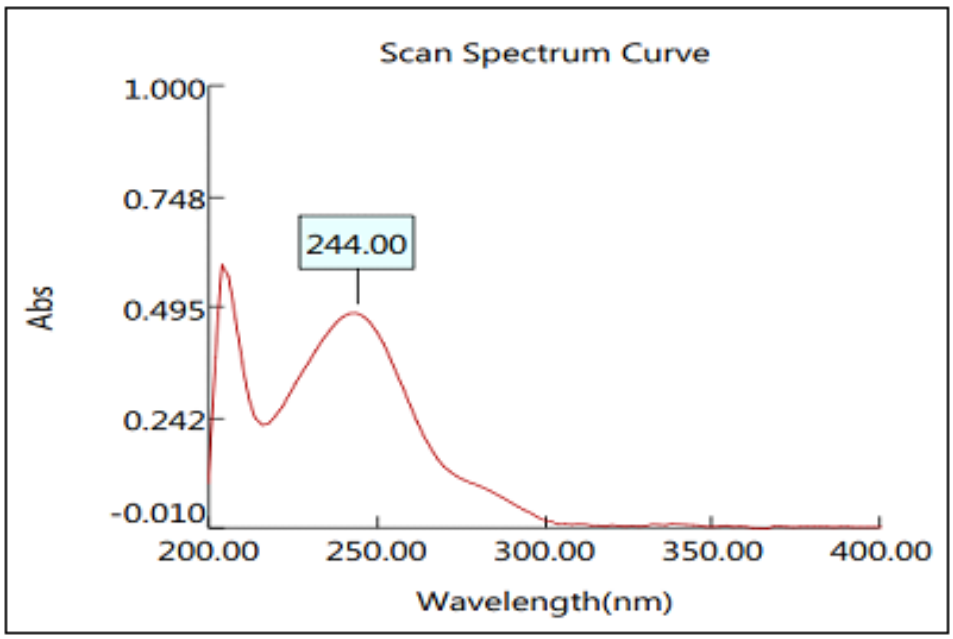

Figure 3: Determination of λmax of ATV.

Table 5: Linearity of ATV At λmax = 244.0 nm.

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
5	0.148	0.147	0.148	0.147	0.148	0.148
10	0.286	0.285	0.286	0.284	0.284	0.285
15	0.421	0.422	0.423	0.425	0.421	0.422
20	0.567	0.567	0.568	0.568	0.566	0.567
25	0.708	0.709	0.707	0.708	0.706	0.708

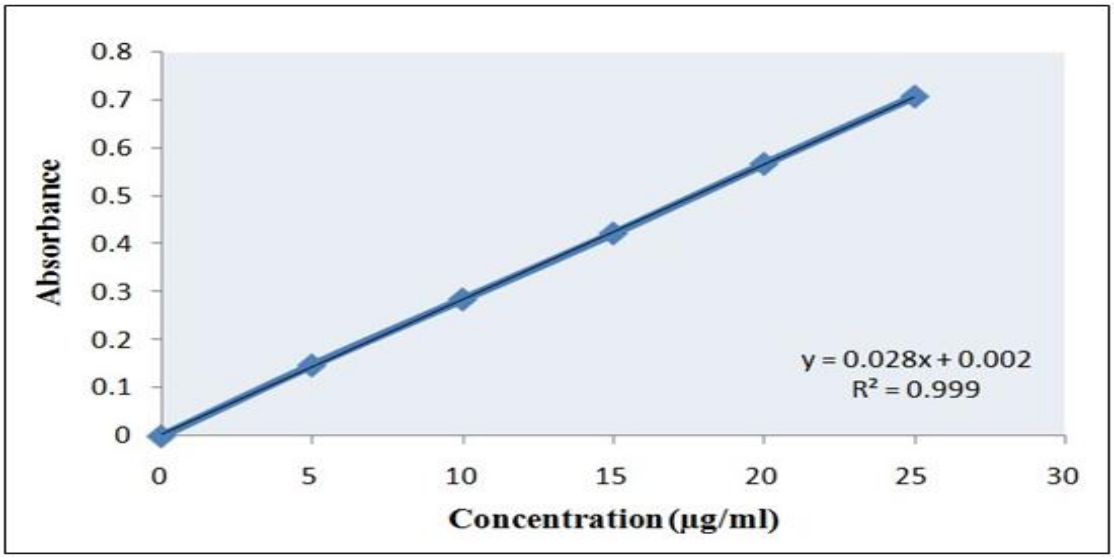

Figure 4: Calibration Curve of ATV.

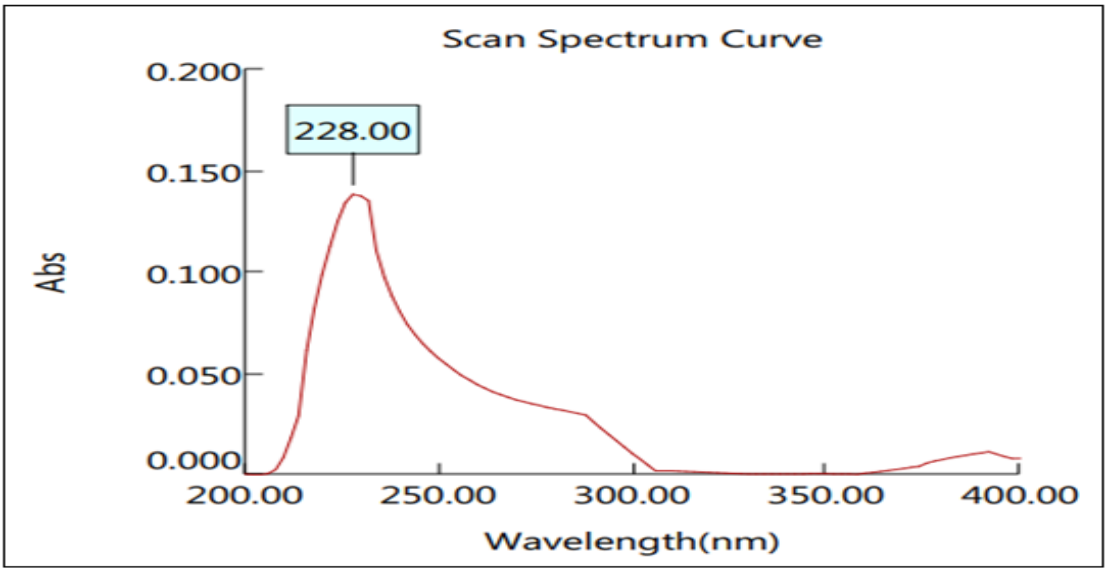

Figure 5: Linearity of CLP At λmax = 228.0 nm.

Table 6: Linearity of CLP At λmax = 228.0 nm.

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
10	0.078	0.079	0.078	0.079	0.078	0.078
20	0.145	0.146	0.145	0.146	0.145	0.145
30	0.212	0.213	0.212	0.213	0.212	0.212
40	0.291	0.292	0.291	0.292	0.292	0.292
50	0.378	0.377	0.378	0.375	0.377	0.377

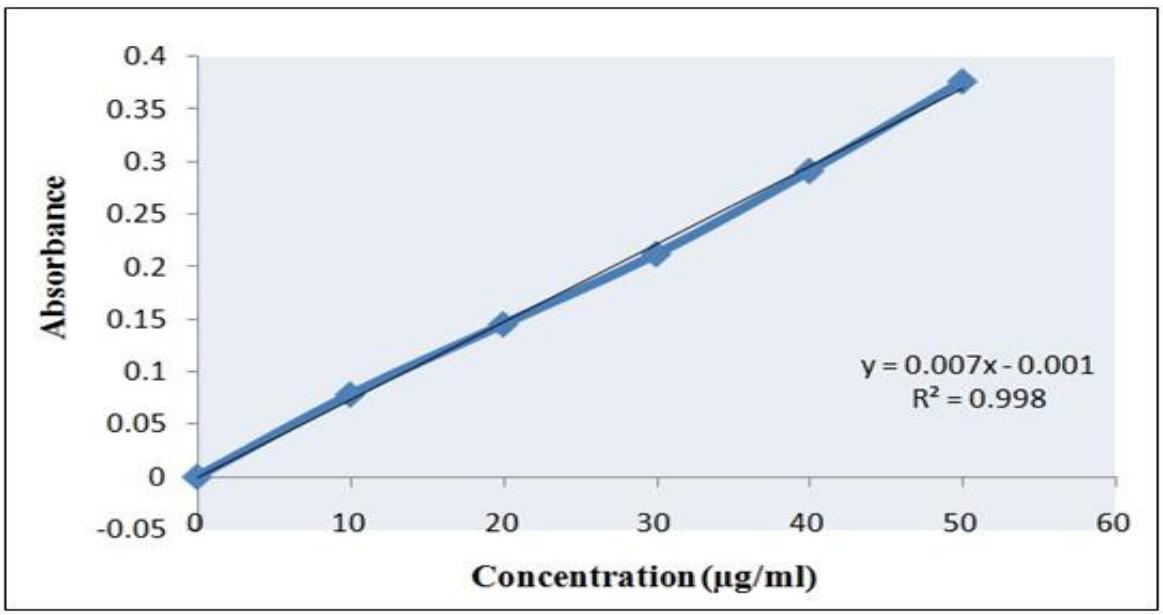

Figure 6: Calibration Curve of CLP.

Method development using Simultaneous equation method

Study of overlay spectra: Working standard solution from the standard stock solution prepared in concentration 10µg/ml of ATV and 20µg/ml of CLP were prepared in hydrotopes and scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were recorded. ATV showed an absorbance peak at 244.0 nm, whereas CLP at 228.0 nm. The overlain spectra also showed isoabsorptive points at 233.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

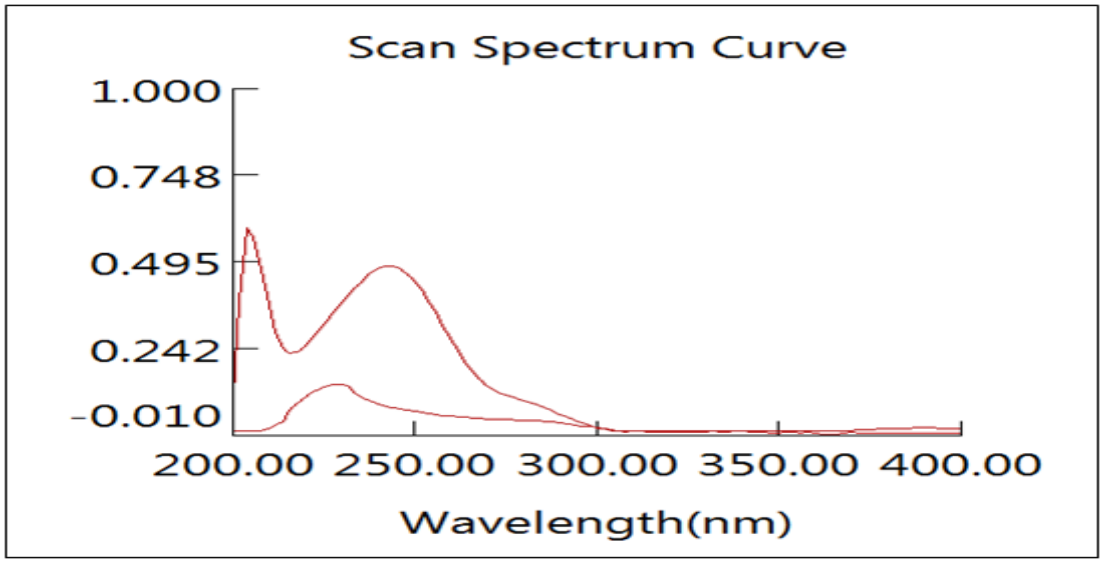

Figure 7: Overlay Spectra of ATV and CLP.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 242.0 nm and 228.0 nm that are λmax of ATV and CLP respectively. The absorbances were measured at the selected wavelengths and absorptivities (A1%, 1cm) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$\mathbf{C\,ATV} = \frac{\mathbf{A1ay2 - A2ay2}}{\mathbf{ax1ay2 - ax2ay1}}$$

$$\mathbf{C\,CLP} = \frac{\mathbf{A1ax2 - A2ax2}}{\mathbf{ax1ay2 - ax2ay1}}$$

Where, A1 and A2 are absorbances of mixture at 242.0 nm and 228.0 nm respectively, ax1 and ax2 are absorptivities of ATV at λ1 (242.0 i.e. λmax of ATV) and λ2 (228.0 i.e. λmax of

CLP) respectively and ay1 and ay2 are absorptivities of CLP at λ1 and λ2 respectively. CCLP and CATV are concentrations of ATV and CLP respectively. The overlain spectra of both the drugs in 10:75 ratio and the criteria for obtaining maximum precision [i.e.absorbance ratio (A2/A1)/ax2/ax1 and ay2/ay1] by this method were calculated and found to be outside the range of 0.1- 2.0 which is satisfied for both the ATV and CLP.

Validation of simultaneous equation method

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug was calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Table 7: Response Ratio of ATV and CLP.

S. No.	ATV			CLP		
	Conc. (µg/ml)	ABS	Response Ratio	Conc. (µg/ml)	ABS	Response Ratio
1	0	0	0	0	0	0
2	5	0.148	0.0296	10	0.078	0.0078
3	10	0.285	0.0285	20	0.145	0.00725
4	15	0.422	0.02813	30	0.212	0.00707
5	20	0.567	0.02835	40	0.292	0.0073
6	25	0.708	0.02832	50	0.377	0.00754

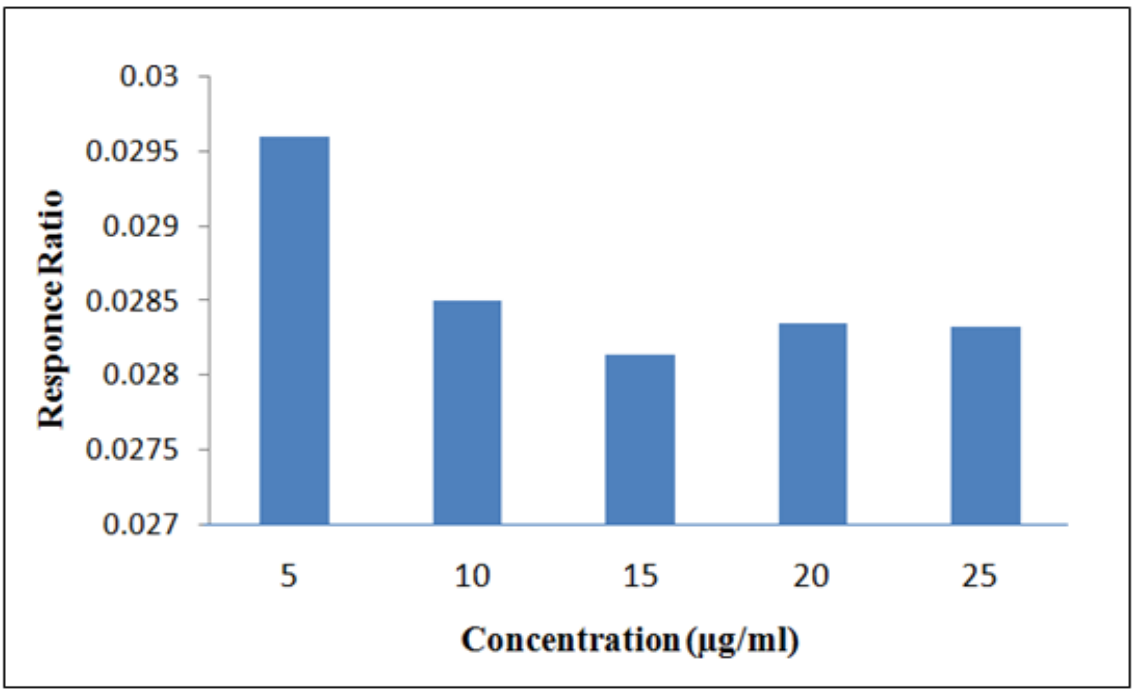

Figure 8: Graph of Response ratio graph for linearity for ATV.

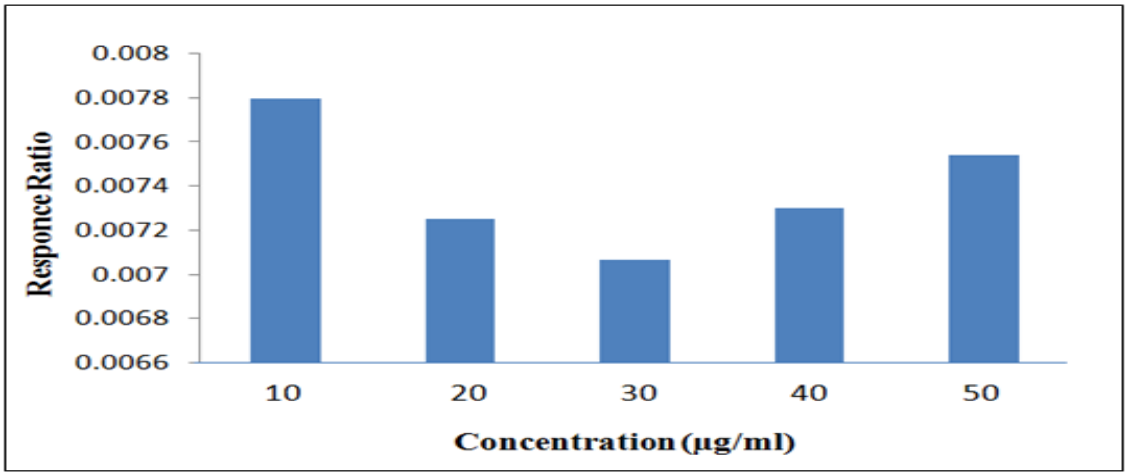

Figure 9: Graph of Response ratio graph for linearity for CLP.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of ATV and CLP to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Table 8: Recovery study of ATV (80% level).

ATV tablet (mg)	Std. ATV Added (mg)	Rep-1		Rep-2		Rep-3		ATV % Mean
		ATV Found	% Found	ATV Found	% Found	ATV Found	% Found	
5	**4**	3.85	96.25	3.98	99.50	4.01	100.25	98.67
10	**8**	7.95	99.38	7.85	98.13	7.78	97.25	98.25
15	**12**	11.78	98.17	11.92	99.33	11.85	98.75	98.75
20	**16**	15.69	98.06	15.84	99.00	15.65	97.81	98.29
25	**20**	19.84	99.20	19.99	99.95	19.95	99.75	99.63
							Mean*	98.72
							SD*	0.557
							% RSD*	0.565

*Mean of 3 replicate and 5 concentrations.

Table 9: Recovery study of ATV (100% level).

ATV tablet (mg)	Std. ATV Added (mg)	Rep-1		Rep-2		Rep-3		ATV % Mean
		ATV Found	% Found	ATV Found	% Found	ATV Found	% Found	
5	5	4.95	99.00	4.85	97.00	4.99	99.80	98.60
10	10	9.95	99.50	9.78	97.80	9.87	98.70	98.67
15	15	14.85	99.00	14.65	97.67	14.82	98.80	98.49
20	20	19.96	99.80	19.84	99.20	19.98	99.90	99.63
25	25	24.85	99.40	24.85	99.40	24.78	99.12	99.31
							Mean*	98.94
							SD*	0.502
							% RSD*	0.508

*Mean of 3 replicate and 5 concentrations.

Table 10: Recovery study of ATV (120% level).

ATV tablet (mg)	Std. ATV Added (mg)	Rep-1		Rep-2		Rep-3		ATV % Mean
		ATV Found	% Found	ATV Found	% Found	ATV Found	% Found	
5	6	5.98	99.67	5.88	98.00	5.95	99.17	98.94
10	12	11.85	98.75	11.65	97.08	11.82	98.50	98.11
15	18	17.95	99.72	17.85	99.17	17.95	99.72	99.54
20	24	23.74	98.92	23.74	98.92	23.78	99.08	98.97
25	30	29.95	99.83	29.96	99.87	29.85	99.50	99.73
							Mean*	99.06
							SD*	0.633
							% RSD*	0.639

*Mean of 3 replicate and 5 concentrations.

Table 11: Recovery study of CLP (80% level).

CLP Tablet (mg)	Std. CLP Added (mg)	Rep-1		Rep-2		Rep-3		CLP % Mean
		CLP Found	% Found	CLP Found	% Found	CLP Found	% Found	
10	**8**	7.85	98.13	7.82	97.75	7.87	98.38	98.08
20	**16**	15.65	97.81	15.65	97.81	15.87	99.19	98.27
30	**24**	23.78	99.08	23.78	99.08	23.95	99.79	99.32
40	**32**	31.74	99.19	31.85	99.53	31.85	99.53	99.42
50	**40**	39.96	99.90	39.95	99.88	39.82	99.55	99.78
							MEAN*	98.97
							SD*	0.749
							% RSD*	0.757

* Mean of 3 replicate and 5 concentrations.

Table 12: Recovery study of CLP (100% level)

CLP Tablet (mg)	Std. CLP Added (mg)	Rep-1		Rep-2		Rep-3		CLP % Mean
		CLP Found	% Found	CLP Found	% Found	CLP Found	% Found	
10	**10**	9.98	99.80	9.78	97.80	9.85	98.50	98.70
20	**20**	19.95	99.75	19.82	99.10	19.95	99.75	99.53
30	**30**	29.96	99.87	29.92	99.73	29.87	99.57	99.72
40	**40**	39.78	99.45	39.85	99.63	39.78	99.45	99.51
50	**50**	49.82	99.64	49.74	99.48	49.95	99.90	99.67
							MEAN*	99.43
							SD*	0.417
							% RSD*	0.419

* Mean of 3 replicate and 5 concentrations

Table 13: Recovery study of CLP (120% level).

CLP Tablet (mg)	Std. CLP Added (mg)	Rep-1		Rep-2		Rep-3		CLP % Mean
		CLP Found	% Found	CLP Found	% Found	CLP Found	% Found	
10	**12**	11.98	99.83	11.78	98.17	11.85	98.75	98.92
20	**24**	23.74	98.92	23.95	99.79	23.74	98.92	99.21
30	**36**	35.69	99.14	35.85	99.58	35.69	99.14	99.29
40	**48**	47.95	99.90	47.95	99.90	47.85	99.69	99.83
50	**60**	59.98	99.97	59.87	99.78	59.77	99.62	99.79
							MEAN*	99.41
							SD*	0.392
							% RSD*	0.395

*Mean of 3 replicate and 5 concentrations.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Repeatability

Table 14: Repeatability of ATV.

Replicate	Concentration Found				
	5	10	15	20	25
Replicate-1	4.95	9.98	14.85	19.96	24.78
Replicate-2	4.98	9.89	14.78	19.98	24.65
Replicate-3	4.85	9.78	14.65	19.96	24.77
Replicate-4	4.88	9.96	14.96	19.78	24.85
Replicate-5	4.96	9.97	14.78	19.68	24.96
Mean	4.924	9.916	14.804	19.872	24.802

% Mean	98.48	99.16	98.69	99.36	99.208	98.980
S.D.	0.056	0.084	0.113	0.135	0.114	0.100
% R.S.D.	0.057	0.085	0.115	0.135	0.115	0.101

Table 15: Repeatability of CLP.

Replicate	Concentration Found					
	10	**20**	**30**	**40**	**50**	
Replicate-1	9.95	19.95	29.98	39.98	49.95	
Replicate-2	9.96	19.85	29.78	39.85	49.98	
Replicate-3	9.98	19.92	29.65	39.96	49.85	
Replicate-4	9.85	19.67	29.85	39.95	49.98	
Replicate-5	9.74	19.99	29.84	39.85	49.78	
Mean	9.90	19.88	29.82	39.92	49.91	
% Mean	98.96	99.38	99.4	99.795	99.816	99.470
S.D.	0.101	0.126	0.120	0.063	0.089	0.100
% R.S.D.	0.102	0.127	0.121	0.063	0.089	0.100

Intermediate Precision

Day-to-Day Variation

Table 16: Day-to-Day Variation of ATV.

Replicate	Concentration Found					
	5	**10**	**15**	**20**	**25**	
Day – 1	4.95	9.95	14.75	19.95	24.96	
Day – 2	4.85	9.98	14.85	19.98	24.56	
Day – 3	4.65	9.85	14.65	19.95	24.65	
Mean	4.82	9.93	14.75	19.96	24.72	
% Mean	96.33	99.27	98.33	99.80	98.89	98.525
S.D.	0.153	0.068	0.100	0.017	0.210	0.110
% R.S.D.	0.159	0.069	0.102	0.017	0.212	0.112

Table 17: Day-to-Day Variation of CLP.

Replicate	Concentration Found					
	10	**20**	**30**	**40**	**50**	
Day – 1	9.95	19.95	29.95	39.96	49.95	
Day – 2	9.98	19.98	29.91	39.78	49.78	
Day – 3	9.85	19.65	29.78	39.85	49.95	
Mean	9.93	19.86	29.88	39.86	49.89	
% Mean	99.27	99.30	99.60	99.66	99.79	99.522
S.D.	0.068	0.182	0.089	0.091	0.098	0.106
% R.S.D.	0.069	0.184	0.089	0.091	0.098	0.106

Analyst to analyst variation

Table 18: Analyst-to-Analyst Variation of ATV.

Replicate	Concentration Found					
	5	10	15	20	25	
Analyst -1	4.95	9.95	14.78	19.95	24.78	
Analyst -2	4.85	9.85	14.96	19.85	24.65	
Mean	4.90	9.90	14.87	19.90	24.72	
% Mean	98.00	99.00	99.13	99.50	98.86	98.899
S.D.	0.071	0.071	0.127	0.071	0.092	0.086
% R.S.D.	0.072	0.071	0.128	0.071	0.093	0.087

Table 19: Analyst-to-Analyst Variation of CLP.

Replicate	Concentration Found					
	10	20	30	40	50	
Analyst -1	9.95	19.95	29.87	39.74	49.95	
Analyst -2	9.82	19.87	29.65	39.81	49.87	
Mean	9.89	19.91	29.76	39.78	49.91	
% Mean	98.85	99.55	99.20	99.44	99.82	99.372
S.D.	0.092	0.057	0.156	0.049	0.057	0.082
% R.S.D.	0.093	0.057	0.157	0.050	0.057	0.083

Reproducibility

Table 20: Reproducibility of ATV.

Replicate	Concentration Found					
	5	10	15	20	25	
Replicate-1	4.85	9.95	14.78	19.95	24.78	
Replicate-2	4.78	9.89	14.65	19.98	24.65	
Replicate-3	4.95	9.85	14.85	19.78	24.85	
Replicate-4	4.92	9.78	14.92	19.65	24.74	
Replicate-5	4.82	9.65	14.77	19.95	24.65	
Mean	4.86	9.82	14.79	19.86	24.73	
% Mean	97.28	98.24	98.62	99.31	98.936	98.479
S.D.	0.070	0.115	0.101	0.142	0.086	0.103
% R.S.D.	0.072	0.117	0.102	0.143	0.087	0.104

Table 21: Reproducibility of CLP.

Replicate	Concentration Found					
	10	20	30	40	50	
Replicate-1	9.96	19.85	29.98	39.68	49.98	
Replicate-2	9.98	19.78	29.98	39.96	49.89	
Replicate-3	9.87	19.69	29.65	39.78	49.78	
Replicate-4	9.87	19.85	29.89	39.65	49.69	
Replicate-5	9.89	19.99	29.78	39.98	49.85	
Mean	9.928	19.86	29.88	39.842	49.865	
% Mean	99.283	99.30	99.60	99.604	99.730	99.504
S.D.	0.052	0.110	0.142	0.154	0.110	0.114
% R.S.D.	0.053	0.111	0.142	0.155	0.110	0.114

Analysis of tablet sample

Twenty marketed tablets of ATV and CLP were weighed and ground to a fine powder; amount equal to 10mg of ATV was taken in 10 ml volumetric flask. The CLP present in this amount of tablet powder was 75mg. Then 8 ml of 2M Sod. Citrate solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

Table 22: Analysis of Tablet Formulation of ATV and CLP.

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
ATV	10	9.92	99.20	0.125	0.132
CLP	75	74.85	99.80	0.124	0.125

DISCUSSION

Based on the solubility, stability and spectral characteristics of the drugs, 2M Sod. Citrate was selected as hydrotropic agent. Presence of hydrotropic agent do not shows any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method. The developed methods were found to be linear. The values of mean percent recoveries were found. The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method.

Table 23: Results of Linearity of ATV and CLP.

Parameter	Method	
	ATV	CLP
Working λmax	244nm	228 nm
Beer's law limit (µg/ml)	5-25	10-50
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.028	0.007
Intercept (c)*	0.002	0.001

*Average of five determination.

Table 24: Results of Recovery Studies.

Recovery Level %	% Recovery (Mean±SD)*	
	ATV	CLP
80	98.72±0.557	98.97±0.749
100	98.94±0.502	99.43±0.417
120	99.73±0.633	99.41±0.392

Table 25: Results of validation (% Mean±SD)

Parameter		Method	
		ATV	CLP
Precision (%R.S.D.)*	Repeatability	98.980±0.100	99.470±0.100
	Intra-day Precision	98.525±0.110	99.522±0.106
	Inter-day Precision	98.899±0.086	99.372±0.082
	Reproducibility	98.479±0.103	99.504±0.114

*Average of five determination.

Table 26: Analysis of Tablet Formulation of ATV and CLP.

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
ATV	10	9.92	99.20	0.125	0.132
CLP	75	74.85	99.80	0.124	0.125

CONCLUSION

The proposed U.V. Spectrophotometer method enables simultaneous determination of ATV and CLP. This is the first reported method for simultaneous quantitative analysis of ATV and CLP and is a significant advance in spectroscopic analysis of such pharmaceutical mixtures. The method is suitable for qualitative and quantitative analysis of these pharmaceutical products. The results obtained are in a good agreement with the declared contents. Statistical analysis showed the method isaccurate and precise.

There was no interference of 2M Sod. Citrate solution in the estimation and hence the UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of ATV and CLP in bulk and tablet dosage forms. The proposed method can be successfully employed for the routine analysis of ATV and CLP containing dosage forms.

CONFLICTS OF INTEREST

There are no conflicts of interests.

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