**METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION
OF AMLODIPINE BESYLATE AND ATORVASTATIN CALCIUM BY UV
SPECTROPHOTOMETRIC METHOD USING MIXED HYDROTROPIC PHENOMENA**

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ABSTRACT

A novel, simple, fast and reproducible UV spectrophotometric method was developed using 2M Sodium Benzoate: 2M Sodium acetate as hydrotropic solubilizing agent for the estimation of poorly water soluble drug Amlodipine besylate (AMD) and Atorvastatin calcium (ATV) in bulk and in pharmaceutical dosage form. After solubilising the Amlodipine besylate and Atorvastatin calcium in the selected hydrotropic agent, it was scanned in spectrum mode and Amlodipine besylate and Atorvastatin calcium showed linearity in the concentration range of 2-10 µg/ml and 5-25µg/ml at their respective maxima was selected as wavelength for estimation, considering the reproducibility and variability of the obtained result. The developed method was found to be linear in the range of 2-10 µg/ml and 5 to 25µg/ml and with correlation coefficient (r^2) of 0.999 and linear equation was $Y=0.056X-0.002$ and $Y=0.002X-0.000$ for Amlodipine besylate and Atorvastatin calcium. The mean percent label claims of synthetic mixture of Amlodipine besylate and Atorvastatin calcium in formulation estimated by the proposed method were found to be 99.046 ± 0.284 to 99.828 ± 0.308 and 98.18 ± 0.386 to 98.40 ± 0.706 . These values are close to 100, indicating the accuracy of the proposed analytical method. Low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method. Also the values of standard deviation, percent coefficient of variation and standard error were satisfactorily low. Result of precision at different level were found be within acceptable limits ($RSD < 2$). Therefore, the methods can be used for routine monitoring of Amlodipine besylate and Atorvastatin calcium in industry in the assay of bulk drug and tablets.

Keywords: Amlodipine besylate and Atorvastatin calcium, Hydrotrophy, Sodium acetate, Sodium Benzoate, Spectrophotometry.

INTRODUCTION

In the formulation development fields and pharmaceutical analysis, increasing the aqueous solubility of insoluble and slightly soluble drugs is major importance. Most of the newly developed drug molecules are lipophilic in nature and poor solubility is one of the frequent problems encountered. It is well known that drug efficacy can be sternly limited by poor aqueous solubility. It is also known that the side effects of some drugs are the consequence of their poor solubility. The ability to increase aqueous solubility can thus be a valuable assist to increasing effectiveness and/or reducing side effects for certain drugs. Various organic solvents like methanol, chloroform, alcohol, acetone, dimethyl formamide and benzene have been employed for the solubilisation of poorly water soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution and error in analysis due to volatility¹⁻². There are various approaches for solubilisation of poorly water soluble drugs. Hydrotropy is a solubilisation technique in which addition of large amount of a second solute results in an increase in the aqueous solubility of another solute. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilising insoluble compounds. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilisation. Literature review shows that a large number of poorly water-soluble drugs like frusemide, cefixime, tinidazole, amlodipine besylate, pramipexole, torsemide using hydrotropic solubilising agents³⁻⁸. Concentrated aqueous solutions of a large number of hydrotropic agents like sodium benzoate, sodium salicylate, urea, sodium ascorbate, niacin amide and sodium citrate have been employed to enhance aqueous solubility of various poorly water-soluble drugs. The UV-Visible spectroscopy is a common analytical technique for quantitative and qualitative analysis of a sample which worked on Lambert-Beer's law. Amlodipine is a member of 1, 4-dihydropyridine class of calcium antagonist approved for the treatment of heart diseases like hypertension and angina pectoris. It is a long acting calcium channel blocker that inhibits the influx of calcium ions into the vascular smooth muscle and cardiac muscle^{8,9}. Chemically amlodipine is 3-ethyl-5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydropyridine-6-methyl-3,5-dicarboxylate fig. 1¹⁰. Atorvastatin is chemically (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid calcium salt trihydrate¹¹. Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. Nicotinic acid is chemically pyridine-3-carboxylic acid. Nicotinic acid lowers plasma triglyceride and cholesterol concentration fig. 2. Literature survey revealed that few UV spectrophotometric methods have been developed and reported for the estimation of Amlodipine Besylate with other drug in combined dosage form¹²⁻¹⁴, However no efforts have been

reported for the UV spectrometric method of Amlodipine besylate and Atorvastatin calcium in bulk form by using mixed hydrotropic agent as a solvent, which could be very economic and easily applicable as well. Hence an attempt has been made to develop and establish a novel, simple, rapid and sensitive UV spectrometric method in accordance with ICH guidelines for the estimation of Amlodipine besylate and Atorvastatin calcium in bulk drug and formulation by using 2M Sodium Benzoate: 2M Sodium acetate (50:50 w/v) as hydrotropic agent to increase the solubility of drug.

Fig. 1 Chemical structure of Amlodipine

Fig. 2 Chemical structure of Atorvastatin calcium

MATERIALS AND METHODS

Apparatus

The proposed work was carried out on a Lab India UV-visible spectrophotometer (model 3000+ series), having double beam detector configuration. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-800 nm.

Reagents and standards

The working standard of Amlodipine besylate and Atorvastatin calcium was provided as gift sample from pharmaceutical industries. Sodium acetate, urea, sodium benzoate of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house.

Experimental

Preliminary solubility studies

Solubility of Amlodipine besylate and Atorvastatin calcium was determined at 28 ± 1 °C. An excess amount of drug was added to a screw capped 25 ml of volumetric flask containing different aqueous systems viz distilled water, buffer of pH 6.4, buffer of pH 8.2, and 2M sodium acetate solution and urea. The volumetric flasks were shaken mechanically for 12 hrs at 28 ± 1 °C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 hrs and then centrifuged for 5 min at 2000 rpm. The

supernatant liquid was taken for appropriate dilution after filtered through Whatmann filter paper #41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of Amlodipine besylate and Atorvastatin calcium was found to be more than and 32 folds in 2M Sodium Benzoate: 2M Sodium acetate (50:50 w/v) solution as compared to solubility studies in other solvents. This enhancement of solubility is due to the hydrotropic solubilisation phenomenon.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80mL mixed hydrotropic solution containing 2M Sodium Benzoate: 2M Sodium acetate (1:1v/v) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml 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 Amlodipine besylate and Atorvastatin calcium and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

1) 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 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 2 µg/ml, 4 µg/ml, 6 µg/ml, 8µg/ml and 10 µg/ml respectively for Atorvastatin calcium.

2) Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 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 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for Amlodipine besylate.

Selection of wavelength for linearity

Solutions of 50 µg/ml of Amlodipine besylate and 2 µg/ml Atorvastatin calcium were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of Amlodipine besylate and Atorvastatin calcium was observed at 376.0 nm and 238.0 nm, respectively. Amlodipine besylate and Atorvastatin calcium showed linearity in the concentration range

of 2-10 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ at their respective maxima (Table 6.3 and 6.4). Calibration curve was plotted, absorbance versus concentration.

Fig.3: Determination of λ_{max} of Amlodipine besylate

Fig 4: Determination of λ_{max} of Atorvastatin calcium

Table 1 Optical characteristic and linearity data of Amlodipine besylate and Atorvastatin calcium

S. No.	Parameter	Amlodipine besylate	Atorvastatin calcium
1	Working λ	376nm	238nm
2	Beer's law limit ($\mu\text{g/ml}$)	2-10	5-25
3	Correlation Coefficient (r^2)*	0.999	0.999
4	Slope (m)*	0.056	0.024
5	Intercept (c)*	0.002	0.000

*Average of five determination

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 10 $\mu\text{g/ml}$ of Amlodipine besylate and 10 $\mu\text{g/ml}$ of Atorvastatin calcium were 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. Amlodipine besylate showed an absorbance peak at 238.0 nm, whereas Atorvastatin calcium at 376.0 nm. The overlain spectra also showed isoabsorptive points at 268.0 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.

Fig 5: Overlay Spectra of Amlodipine besylate and Atorvastatin calcium

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 238.0 nm and 376.0 nm that are $\lambda_{\max}$ of Amlodipine besylate and Atorvastatin calcium respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1\text{cm}}$) for both the drugs at both wavelengths were determined as mean of five independent determinations.

$$C_{\text{AMLO}} = \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \quad \text{Eq (1)}$$

$$C_{\text{ATR}} = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \quad \text{Eq (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 238.0 nm and 376.0 nm respectively, a_{x1} and a_{x2} are absorptivities of Amlodipine besylate at λ_1 (238.0 i.e. $\lambda_{\max}$ of Amlodipine besylate) and λ_2 (376.0 i.e. $\lambda_{\max}$ of Atorvastatin calcium) respectively and a_{y1} and a_{y2} are absorptivities of Atorvastatin calcium at λ_1 and λ_2 respectively. C_{AMLO} and C_{ATR} are concentrations of Amlodipine besylate and Atorvastatin calcium respectively. Figure 5 represent the overlain spectra of both the drugs in 1:2 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio (A_2/A_1)/ a_{x2}/a_{x1} and a_{y2}/a_{y1}] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the Amlodipine besylate and Atorvastatin calcium.

Analysis of tablet formulation

Mixed Blends of Amlodipine besylate and Atorvastatin calcium were weighed and ground to a fine powder; amount equal to 5mg of Atorvastatin calcium was taken in 10 ml volumetric flask. The present

in this amount of tablet powder was 10mg Amlodipine besylate. Then 4ml of 2M Sodium Benzoate: 2M Sodium acetate (1:1 v/v) 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 2: Analysis of Tablet Formulation of Amlodipine besylate and Atorvastatin calcium

Conc.		Replicate-1				Replicate-2				Replicate-3			
Present		Conc.		% Conc.		Conc. Found		% Conc.		Conc. Found		% Conc.	
(µg/ml)		Found		Found		(µg/ml)		Found		(µg/ml)		Found	
(µg/ml)		(µg/ml)											
AMD	ATV	AMD	ATV	AMD	ATV	AMD	ATV	AMD	ATV	AMD	ATV	AMD	ATV
2	5	1.99	4.99	99.50	99.80	1.95	4.99	97.50	99.80	2.01	4.99	100.50	99.80
4	10	3.98	9.95	99.50	99.50	3.95	9.98	98.75	99.80	3.99	9.95	99.75	99.50
6	15	5.85	14.85	97.50	99.00	5.95	14.89	99.17	99.27	5.89	14.98	98.17	99.87
8	20	7.98	19.98	99.75	99.90	7.99	19.99	99.88	99.95	7.98	19.65	99.75	98.25
10	25	10.01	24.78	100.10	99.12	9.95	24.98	99.50	99.92	10.01	24.85	100.10	99.40

Validation

Linearity & Range

The linearity of calibration curves (Absorbance Vs concentration) in pure solution was checked over the concentration ranges of about Amlodipine besylate and Atorvastatin calcium showed linearity in the concentration range of 2-10 µg/ml and 5-25µg/ml respectively [15].

Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. In preanalyzed tablet solution, a definite amount of drug was added and then its recovery was studied. These studies were performed in by adding fixed amount of pure drug solution to the final dilution while varying the concentration of tablet sample solution in the final dilution. The percentage recovery and percentage relative standard deviation of the recovery were calculated and shown in Table 3.

Table 3: Result of recovery studies of tablet formulation with statistical evaluation

Recovery Level %	% Recovery (Mean $\pm$ SD)*	
	Amlodipine besylate	Atorvastatin calcium
80	99.046 $\pm$ 0.284	98.22 $\pm$ 0.547
100	99.055 $\pm$ 0.400	98.40 $\pm$ 0.706
120	99.828 $\pm$ 0.308	98.18 $\pm$ 0.386

*Average of five determination

Precision

To evaluate precision at different parameter like repeatability, intermediate precision and reproducibility, five dilutions in three replicates were analyzed in same day, in two different days and by two analysts for day to day and analyst to analyst variation. The % RSD values for Intraday and Interday precision were < 2%, indicating that the method was sufficiently precise. The results were shown in the Table 4.

Table 4: Results of precision

Parameter		Amlodipine besylate	Atorvastatin calcium
Precision (%R.S.D.)*	Repeatability	0.033	0.062
	Day to Day	0.050	0.085
	Analyst to Analyst	0.033	0.067
	Reproducibility	0.071	0.085

*Average of five determination

RESULTS AND DISCUSSIONS

Based on the solubility, stability and spectral characteristics of the drug, 2M Sodium Benzoate: 2M Sodium acetate was selected as hydrotropic agent. After solubilising the Amlodipine besylate and Atorvastatin calcium in the selected hydrotropic agent, it was scanned in spectrum mode and Amlodipine besylate and Atorvastatin calcium showed linearity in the concentration range of 2-10 μ g/ml and 5-25 μ g/ml at their respective maxima was selected as wavelength for estimation, considering the reproducibility and variability of the obtained result. The developed method was found to be linear in the range of 2-10 μ g/ml and 5 to 25 μ g/ml and with correlation coefficient (r^2) of 0.999 and linear equation

was $Y=0.056X-0.002$ and $Y=0.002X-0.000$ for Amlodipine besylate and Atorvastatin calcium. The mean percent label claims of synthetic mixture of Amlodipine besylate and Atorvastatin calcium in formulation estimated by the proposed method were found to be 99.046 ± 0.284 to 99.828 ± 0.308 and 98.18 ± 0.386 to 98.40 ± 0.706 . These values are close to 100, indicating the accuracy of the proposed analytical method. Low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method table 2. Also the values of standard deviation, percent coefficient of variation and standard error were satisfactorily low table 3. Result of precision at different level were found be within acceptable limits (RSD <2) table 4.

CONCLUSION

It was thus, concluded that the proposed method is new, simple, accurate, safe, free from pollution, precise and can be successfully employed in the routine analysis. The simplicity, rapidity reproducibility and economy of the proposed methods completely fulfil the objective of this research work.

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