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Simultaneous Estimation of *Atorvastatin Calcium* and *Amlodipine besylate* by UV Spectrophotometric method using hydrotropic solubilization

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Abstract

Plan: *An analytical method for the simultaneous estimation of Atorvastatin Calcium (ATV) and Amlodipine Besylate (AML) by UV spectrophotometry using hydrotropic solubilization is described.*
Prologue: *The method is simple, fast and accurate and can be used for the routine analysis of Atorvastatin and Amlodipine in combination tablets.*
Methodology: *The developed method uses the absorption ratio or Q-value which is based on the measurement of absorptivity at 293 nm (iso-absorptive point, both the drugs were found to have same absorbance at this wave length) and 247 nm (absorption maximum of one of the drugs, Atorvastatin). Both the drugs are insoluble in water and require corrosive organic solvents for solubilization. Therefore an attempt was made to preclude the use of corrosive solvents by the use of 2M Urea by hydrotropic solubilization method. The calibration curves for both drugs were found to be linear in a concentration range of 10-60µg/mL .*
Outcome: *The proposed method has been applied successfully for the simultaneous determination of Atorvastatin and Amlodipine in pharmaceutical dosage forms. No significant interference was observed from the tablet excipients and 2M Urea used for solubilization. The mean recovery of the drugs from the combination tablets was 100.65%.for Atorvastatin and 101.42% for Amlodipine respectively.*
Key Words: *Amlodipine, Atorvastatin, Spectrophotometry, Tablets.*

1. Introduction

Hypertension and dyslipidemia are the most commonly co-occurring cardiovascular risk factors. Amlodipine besylate, a dihydropyridine calcium channel blocker, is approved for the treatment of angina pectoris and is chemically2-[(2-Aminoethoxy) methyl] -4-(2-chlorophenyl)-3ethoxy carbonyl-5-methoxycarbonyl-6-methyl- 1, 4-dihydropyridine benzene sulfonate[1].

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Atorvastatin calcium, chemically [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3-phenyl-4- [phenylamino) carbonyl]-1H-pyrrole-1- heptanoic acid, calcium salt (2:1) trihydrate, is an inhibitor of HMG –CoA reductase, an enzyme involved in cholesterol biosynthesis[2,3].The fixed-dose combination containing Amlodipine Besylate and Atorvastatin Calcium improved the fibrinolytic balance more than either single agent in hypertensive, hypercholesterolemic patients with insulin resistance and could potentially improve medication compliance.

HPLC methods are official in IP [4] for the estimation of ATV while in IP [5], BP[6], EP[7] and USP [8] for the determination of AML, but they do not involve simultaneous determination of ATV and AML. Detailed survey of literature for AML revealed several methods based on different techniques, viz. Spectrophotometric methods[9,10,11] , HPTLC methods[12,13], HPLC methods[14,15] and Adsorptive Square Wave Anodic Stripping Voltammetry [16]. Similarly, literature survey for ATV revealed that HPLC[17,18], GC-MS[19],LC-MS[20], HPLC-Electron spray tandem mass spectrometry[21] and HPTLC [22] have been reported for the estimation of Atorvastatin Calcium and spectrophotometric methods[23],HPLC[24,25] and HPTLC[26] methods for simultaneous determination of ATV and AML. Both the drugs are insoluble in water. Hydrotropic solubilization involves the addition of large amount of a second solute to increase the aqueous solubility of the first solute.

Maheshwari et al. has analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. Ketoprofen, Salicylic acid [27], Frusemide [28], Cefixime [29] and Amoxicillin [30].No UV spectrophotometric method for the simultaneous estimation of ATV and AML 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 AML and ATV were enhanced to a great extent in 2M Urea.

2. Materials and Methods

2.1. Instrument:

The Spectrophotometric analysis was carried out by using a double beam UV-visible Spectrophotometer (JascoV-550, Japan) with 1cm matched quartz cells.

2.2.Reagents and Chemicals:

The reference standard of Amlodipine Besylate and Atorvastatin Calcium were gift samples from Cadila Healthcare Ltd and Intas Pharmacetical Industry,Dehradun respectively.All chemicals were analytical grade obtained from SD fine chemicals. Water was purified by glass distillation apparatus.

2.3.Preliminary solubility studies of drugs[31]

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 2.0 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 2M Urea solution separately and final volume was made up with distilled water in 100ml volumetric flask. From the above solution 10 ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 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 maxima at 247nm and 214 nm (Figure1) and Amlodipine showed absorption maximum at 243nm (Figure2).From overlain spectra (Figure3) it is evident that isoabsorptive point was obtained at 293 nm.

2.5. Method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one of which is an isoabsorptive point and the other being the absorption maximum of one of the two components. From the overlain spectra of two drugs, it is evident that Atorvastatin and Amlodipine show an isoabsorptive point at 293 nm. The second wavelength used is 247 nm, which is the absorption maximum of Atorvastatin. Appropriate aliquots from the stock solution of Atorvastatin and Amlodipine were used to prepare three different sets of dilutions, Series A, B and C as follows. Series A and B consisted of different concentrations (10-60 µg/ml) of Atorvastatin and Amlodipine respectively. Series C comprised of mixture of Atorvastatin and Amlodipine in the ratio of 60:30,50:25,45:22.5,40:20,35:17.5, and 30:15.The solutions were prepared in distilled water by diluting appropriate volumes of the respective standard stock solutions. The absorbances of solutions were then measured at 293 nm and 247 nm. The calibration curves were constructed by plotting absorbance versus concentration, the regression equations and absorptivity coefficients were calculated using calibration curve. The method employs Q-values and the concentrations of drugs in sample solution were determined by using the following formula**,**

Concentration of Atorvastatin: $C1=\{(QM-Q2) / (Q1-Q2)\} X (A/ a)$..... *(1)*

Concentration of Amlodipine: $C2 = \{(QM-Q1) / (Q2-Q1)\} X (A/ a)$……..*(2)*

Where, A is the absorbance of sample at isoabsorptive point, a is the absorptivity of Amlodipine and Atorvastatin respectively at isoabsorptive point 293nm. QM =A2/A1, Q1= ax2/ ax1, and Qy = ay2/ay1[32].QM, Q1 and Q2 are absorptivity ratio of mixture, Atorvastatin and Amlodipine at Iso-absorptive point (293nm) to the maximum wavelength of one of the component (247nm).

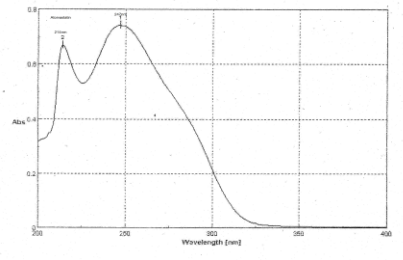

Figure.1. UV absorption spectrum of Atorvastatin in 2M Urea solution

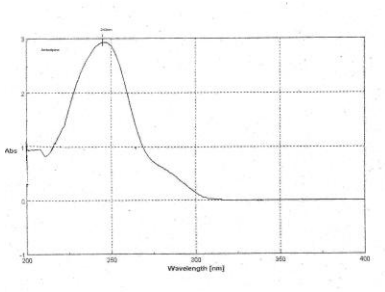

Figure.2. UV absorption spectrum of Amlodipine in 2M Urea solution

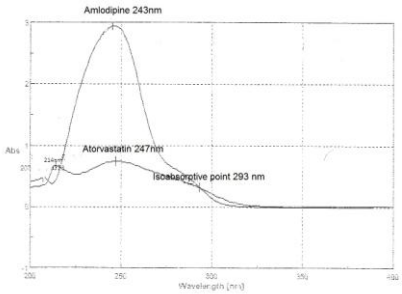

Figure. 3. Overlain absorption spectrum of Atorvastatin and Amlodipine in 2M Urea solution

Validation of the proposed method[33, 34]

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of the solutions (n = 6) of Atorvastatin and Amlodipine (20 µg/ml for both drugs) without changing the parameters of the proposed method.

Intermediate precision (reproducibility)

The intra-day and inter-day precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of Atorvastatin and Amlodipine (30, 40 and 50 µg/ml).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Atorvastatin and Amlodipine by the standard addition method. Known amounts of standard solutions of Atorvastatin and Amlodipine were at added at 50, 100 and 150 % level to the pre-quantified sample solutions of Atorvastatin and Amlodipine (40 µg/ml for both drug) and the recovery was calculated.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[34].

$$LOD = 3.3 \times \sigma/S \quad LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of Atorvastatin Calcium and Amlodipine Besylate in combined tablet

Twenty tablets were weighed (Brand Name: STACARD and CADUET) and the average weight were calculated. The sample of powdered tablets equivalent to 5mg of AMLand 10mg of ATV [equivalent to one tablet] were weighed and transferred to 100 ml volumetric flask. For analysis of drug Amlodipine Besylate, a standard addition method was used. An accurately weighed 5 mg of pure AML was added to the accuralely weighed samples in the volumetric flask to bring the ratio of AML and ATV to 1:1.The contents of the tablet was extracted by the addition of 50ml solution of 2M urea and sonicated for 15 min. The volume was adjusted up to the mark with distilled water. The solution was then filtered through Whatman no. 41 filter paper. and the filtrate was suitably diluted with distilled water to get a final concentration of 40 µg/ml of ATVand 40 µg/ml of AML. The absorbance of the sample solution i.e. A1 and A2 were recorded at 293 nm (isoabsorptive point) and 247 nm (Absorption maximum of ATV) respectively, and the ratios of absorbance were calculated, i.e. A2/A1.

Relative concentration of two drugs in the sample was calculated using above equation (1) and (2). The analysis procedure was repeated three times with tablet formulation.

Table.1.Data of Recovery Studies

Drug	*Level*	*Amount taken (µg/mL)*	*Amount added (%)*	*% Mean recovery ± S.D. (n = 3)*
ATV	I	40	50	100.01 ± 1.09
(Atorvastatin)	II	40	100	101.01 ± 0.98
	II	40	150	100.95 ± 0.49
AML	I	40	50	101.22 ± 1.19
(Amlodipine)	II	40	100	100.99 ± 1.12
	II	40	150	102.05± 1.092

S. D. is Standard deviation and 'n' is the number of replicate.

Table .2. Report of Statistical Analysis of Atorvastatin and Amlodipine combination in marketed formulations by UV Spectrophotometric method using hydrotropic solubilization

Brand name	*Label claim in mg*		*Concentration found in mg*		*/t[b] / value*		*F-value[b]*	
	ATV	*AML*	*ATV*	*AML*	*ATV*	*AML*	*ATV*	*AML*
Stacard	10	5	10.02 ±0534	4.98±0.00816	0.168	1.096	1.5	1.5
Caduet	10	5	9.98±.00816	4.98±0.0083	1.096	1.068		

[a] *Mean ± SD, n =* [b] *The tabulated value of t is 2.23 and of F is 6.38.*

Table .3.Optical Characteristics

Parameters	*ATV*	*AML*	*ATV & AML*	
	247nm	247nm	247nm	293nm
Beer's Law limit	10-60µg/mL	10-60µg/mL	10-60 µg/mL	10-60µg/mL
Molar absorptivity (L/mol/ cm)	1.43 x 10^4	2.74 x 10^4	1.73 x 10^4	0.58 x 10^4
Sandell's sensitivity (µg/cm2/0.001 absorbance unit.)	0.08457	0.02069		
	Regression equation	*Y=Mx + C*		
Slope(c)	82.1013206	54.68362	0.0808666	0.05165714
Intercept (m)	-6.310647	-17.4507150	0.08086666	0.0808666
Correlation coefficient (r)	0.99293	0.999886	0.9997777	0.999964
Limit of detection LOD µg/mL	2.5	1		
Limit of quantitation LOQ µg/mL	10	5		

Results and Discussion

In absorbance ratio (Q-value) method the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength , which was fulfilled in case of both these drugs. The two wavelengths used for the analysis of the drugs were 293 nm (isoabsorptive point) and 247 nm (Absorption maximum of Atorvastatin) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of Amlodipine (243 nm) and Atorvastatin (247 nm) showing isoabsorptive point (293) in 2M Urea is shown in Figure 3. The validation parameters were studied at 247nm and 293nm for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (Table1).

The method was successfully used to determine the amounts of Amlodipine and Atorvastatin present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labelled amount (Table 2). Precision was calculated as intra-day and inter-day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table 3.

Conclusion

By observing the validation parameters, the method is found to be simple, sensitive, accurate and precise. Hence the proposed method can be employed for the routine analysis for the simultaneous estimation of Atorvastatin and Amlodipine in bulk and combined dosage form.

References

1. *The Merck Index*, an Encyclopediaof Chemicals, Drugs, and Biologicals, Merck &Co. Inc., Whitehouse Station, NJ, 13th Edn, **2001**;86-87.
2. Poswar E L., Radulovic L L., Cilla D D., Whitfield L R., Sedman A J;Tolerance and pharmacokinetics of single-dose atorvastatin, a potent inhibitor of HMG-CoA reductase, in healthy subjects. *J. Clin. Pharmacol*. **1996**; 36: 728.
3. *British National Formulary*. September **2007**;54th Edition, Section 2.12.
4. *Indian Pharmacopoeia*, Govt. of India, Ministry of Health and Family Welfare, Vol. 2, Delhi: Publication by Controller of Publication, **2007**; 749-52.
5. *Indian Pharmcopoeia*, Govt. of India, Ministry ofHealth and Family Welfare, Vol. 2, Delhi:Publication by Controller of Publication, **2007**;714-16.
6. *British Pharmacopoeia*, International ed. Published on the Recommendation of the Medicines Comissions Pursuant to Medicines Act vol. 1, **2005**; 138.
7. *The European Pharmacopoeia*, Counsile of Europe, Codex,France, 4th Edn, **2002**; 639-40.*The 8.United States Pharmacopoeia Convention*, Inc., Rockville, MD, **2007**;3496-97, 1532.
8. Khopade, S. A. and Jain, N. K;Difference spectrophotometric estimation of amlodipine besylate.*Indian Drugs.* **2000**; 37: 351-353.
9. Sridhar, K., Sastry, C. S. P., Reddy, M. N., Sankar, D.G. and Srinivas, K; Spectrophotometric determination of amlodipine besylate in pure forms and tablets. *Anal. Lett.* **1997**; 30:121-133.
10. Prabhakar, A. H. and Giridhar, R; A spectrophotometric method for the determination of amlodipine besylate in pure form and in tablets, *IndianDrugs.* **2002**; 39:204-208.
11. Chandrashekhar, T. G., Rao, P. S. N., Smrita, K.,Vyas, S. K. and Dutt, C; Analysis of amlodipine besylate by HPTLC with fluorimetricdetection: a sensitive method for assay of tablets. *J. Planar. Chromatogr. Mod.TLC,* **1994**;7: 458-460.
12. K.K. Pandya, Milan Satia, T.P. Gandhi; Detection and determination of total amlodipine by high-performance thin-layer chromatography: a useful technique for pharmacokinetic studies. *J. Chrom. B.* **1995**; 667:315-319.
13. Patki, R. V., Tamhankar, C. P. and Tipnis, H. P; Simple and rapid high performance liquid chromatographic estimation of amlodipine frompharmaceutical dosages, *Indian Drugs.* **1994**; 31: 560-561.
14. V.G. Dongre, S.B. Shah, P.P. Karmuse, M. Phadke, V.K. Jadhav;Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC, *J. Pharm. Biomed. Anal*. **2008**; 46:583-587.
15. Gazy, A. A. K; Determination of amlodipine besylate by adsorptive square wave anodic stripping voltammetry on glassy carbon electrode in tablets and biological fluids,*Talanta*, **2004**; 62: 575-582.
16. Altuntas T G., Erk N; Liquid chromatographic determination of Atorvastatin in bulk drugs, tablets and human plasma ,*J. Liq. Chro*.**2004**;27 (1): 83.
17. Gowari Sankar D., Raju M S M., Sumanth., Kalyan S., Latha P V M; Estimation of Atorvastatin by high performance liquid chromatography in pure and pharmaceutical dosage form,*Asian J.Chem,**2005;*** 17 (4) : 2571.
18. McKenney J M., Mccormik L S., Weis S., Koren M., Kotonek S., Black D M; A Randomized Trial of the Effects of Atorvastatin and Niacin in Patients with Combined Hyperlipidemia or Isolated Hypertriglyceridemia,*Amer. J. Med*,**1998**; 104: 137.
19. Black A E., Sinz M W., Hayes R N., and Woolf T F; Metabolism and excretion studies in mouse after single and multiple oral doses of the 3–hydroxyl-3- methyl glutaryl- CoA reductase inhibitor atorvastatin ,*Drug Metab. Dispos*,**1998** ; 26: 755.
20. Bullen W W., Miller R A., Hayes R N;Development and validation of a high performance liquid chromatography tandem mass spectrometry assay for atorvastatin, orthohydroxy atorvastatin and parahydrxy Atorvastatin in human, dog and rat plasma, *Amer. Soc. Mass Spectrum* **1999**; 10: 55.
21. Yadav S S., Mhaske D V., Kakad A B., Patil B D., Kadam S S., Dhaneshwar SR; A simple and sensitive HPTLC method for the determination of content uniformity of Atorvastatin calcium tablets. *Indian J. Pharm. Sci* **2005**; 67(2): 182.
22. Sahu R. and Patel V.B;Simultaneous spectrophotometric determination of amlodipine besylate and atorvastatin calcium from their binary mixture by dual wavelength and zero absorbance measurement, *Indian Drugs* **2006**, 43, 160-161.

23. Chaudhari B.G. and Patel N.M.; Development and Validation of HPLC method for simultaneous estimation of Atorvastatin Calcium and Amlodipine Besylate, *J. Pharm. Research* **2006**; 5,141-144.
24. Chaudhari B.G., Patel N.M. and Shah P.B; Stability Indicating RP-HPLC for simultaneous determination of atorvastatin calcium and amlodipine besylate from their combination drug products, *Chem. Pharm. Bull* **2007**; 55, 241-246.
25. Chaudhari, B.G, Patel, N. M. and Shah, P. B;Simultaneous estimation of atorvastatin and amlodipine from formulation by HPTLC. *IndianDrugs* **2006**, 43, 649-655.
26. Maheshwari R K ;A novel application of hydrotropic solubilization in the analysis of bulk samples of ketoprofen and salicylic acid .*Asian J. Chem* **2006**;18(1): 393-396.
27. Maheshwari R K ;Analysis of frusemide by application of hydrotropic solubilization phenomenon. *The Indian Pharmacist* **2005**;4(38): 55-58.
28. Maheshwari R K;Spectrophotometric determination of cefixime in tablets by hydrotropic solubilization phenomenon. *The Indian Pharmacist* **2005** ;4(36):63-68.
29. Maheshwari R K ;Spectrophotometric analysis of amoxycillin in tablets using hydrotropic solubilization technique. *Asian J. Chem*, **2006**;18 (4): 3194-3196.
30. Deepti M., Jain A., Maheshwari R. K. and PatidarV; Simultaneous Spectrophotometric Estimation of Metronidazole and Norfloxacin in Combined Tablet Formulations using Hydrotropy, Research *J. Pharm. and Tech*. **2008**; 1(4), 357-361.
31. Beckett A.H. and Stenlake J.B; *Practical Pharmaceutical Chemistry*. 4th edn. – Part. 1:CBS Publishers, New Delhi, India ,**2005**; 133-136.
32. Guidelines on validation of analytical procedure: Definitions and terminology. Federal Register; *ICH Q2A*,**1995**; 60:11260.
33. Guidelines on validation of analytical procedure: methodology. Federal Register, *ICH Q2B*, **1996**; 60:27464.

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