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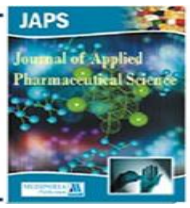

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Spectrophotometric method of estimation of Amlodipine besylate using hydrotropic solubilization

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ABSTRACT

A novel, simple, fast and reproducible UV spectrophotometric method was developed using 2M urea solution as hydrotropic solubilizing agent for the estimation of poorly water-soluble drug amlodipine besylate in bulk and in pharmaceutical dosage form. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solubilization may be a proper choice to preclude the use of organic solvents. It involve the addition of large amount of a second solute to increase the aqueous solubility of the first solute. Amlodipine exhibits absorption maximum at 243 nm. Urea did not show any absorbance above 225 nm and thus no interference in the estimation of drug was seen.Beer's law was found to be obeyed in the concentration range of 5-25µg/mL. In this method, there is no interference from any common pharmaceutical additives and diluents. The correlation co-efficient (' *r* ' value) for amlodipine was0.99863.The results of analysis have been validated as per ICH guidelines. The percentage recoveries obtained for amlodipine ranges from 99.94 to 99.96. The method is accurate, precise and economical.

Keywords: Amlodipine, Hydrotropy, Solubility, Urea.

INTRODUCTION

Amlodipine besylate (AML), 2-[(2-Aminoethoxy) methyl] -4-(2-chlorophenyl)-3ethoxy carbonyl-5- methoxycarbonyl-6-methyl- 1,4-dihydropyridine benzene sulfonate (Figure.1), is a dihydropyridine calcium-channel blocker that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and angina(Martindale., 2002 and Merck Index., 2008). Amlodipine has been determined by spectrophotometric methods(Sridhar et al., 1997,Khopade et al., 2000 and Prabhakar et al.,2002), HPTLC methods (Chandrashekhar et al., 1994 and Pandya et al.,1995) , HPLC methods(Patki et al., 1994 and Dongre et al., 2008), HPLC tandem mass spectrometric method(Feng et al., 2002) and adsorptive square wave anodic stripping voltammetry (Gazy et al.,2004). Hydrotropy is a solubilization phenomenon of increasing the solubility of a solute by the addition of fairly high concentrations of alkali metal salts of various organic acids such as concentrated aqueous solutions of sodium benzoate, sodium salicylate, urea, nicotinamide, sodium citrate and sodium acetate and have been observed to enhance the aqueous solubilities of many poorly water-soluble drugs. Maheshwari et al. has analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. ketoprofen, salicylic acid (Maheshwari et al., 2006), frusemide(Maheshwari et al., 2005), cefixime (Maheshwari et al., 2005), amoxicillin(Maheshwari et al., 2006), hydrochlorothiazide (Maheshwari et al., 2005) and aceclofenac (Maheshwari et al.,

2006). Aqueous solubility of AML was enhanced to a great extent in 2M Urea. The primary objective of the present investigation was to employ the hydrotropic solution to extract the drug from the dosage form and precludes the use of corrosive organic solvents.

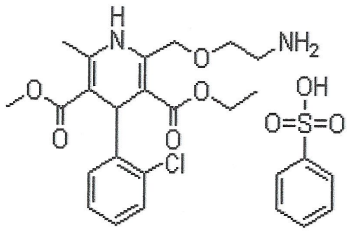

Figure 1: Chemical Structure of amlodipine besylate

MATERIALS AND METHODS

Instrument

Spectrophotometric analysis was carried out by using a double beam UV-visible Spectrophotometer (Shimadzu model UV-1700, Japan) with 1cm matched quartz cells .

Reagents and Chemicals

Reference standard of amlodipine besylate was generous gift from Cadila Healthcare Ltd.Ankleshwar (India). All chemicals were analytical grade obtained from SD fine chemicals. Water was purified by glass distillation apparatus.

Methods

Preliminary Solubility Study of the Drug

Solubility of amlodipine besylate was determined at 28±1°. An excess amount of drug was added to 2M urea solution in vials. The vials were shaken mechanically for 12 h at 28±1° in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hours and then centrifuged for 5 minutes at 2000 rpm. The supernatant of each vial was filtered through Whatmann filter paper No. 41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

Preparations of Standard Drug Solutions

For hydrotropic solubilisation, 50 mg of pure AML was dissolved in 50 ml of 2M urea solution and stirred for 15 minutes and the final volume was made up to 100 ml with distilled water (Deepti et al., 2008).The solution was filtered through Whatmann filter paper No. 41 and was diluted with distilled water to prepare working concentrations of 500 µg/mL of AML.This stock solution was further diluted suitably with distilled water to get a concentration of 25µg/ml and was then scanned in UV range 200-350nm.The spectrum showed an absorption maximum at 243 nm (Figure 2). From the spectra of the drug AML and that of 2M urea (Figure 3), it was found that the 2 M urea used does not interfere with the sampling wavelength. Therefore 2 M urea is used for the solubilization of drug. Aliquots of stock solutions corresponding to 5-25 µg/mL were prepared and absorbance measurements were carried out at 243 nm against the blank prepared in the same manner omitting the drug. Calibration curve was prepared by plotting absorbance against concentration (Figure.4) and the data is given in Table.1.

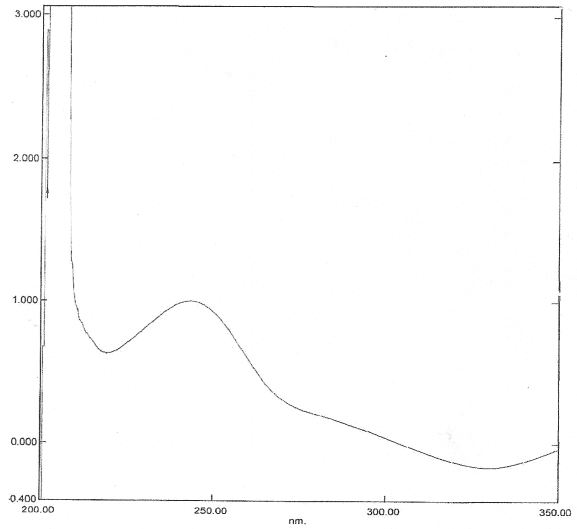

Figure 2: UV Spectrum of amlodipine besylate

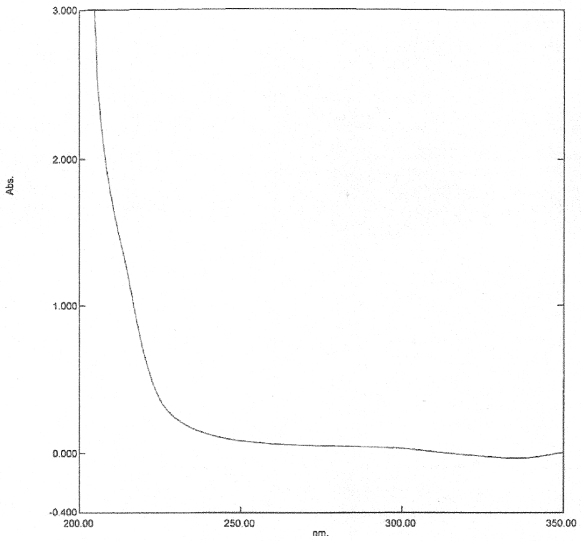

Figure 3: UV Spectrum of 2M Urea

Procedure for analysis of tablet formulation

Two commercial formulations, Amdepin (Cadila) and Amlopres (Cipla Ltd)were purchased from local market. The average weight of each tablet was calculated by weighing 20 tablets and were powdered finely in a glass mortar. Powder equivalent to 50 mg each of AML was weighed and transferred to two 100 ml volumetric flasks, 70 ml of 2M urea solution was added to both the flask and stirred for 15 min to dissolve the drug and the final volume was made up to 100 ml with distilled water. The solutions were filtered through Whatmann fitter paper No. 41 and the first few ml were rejected. The filtrates were diluted

suitably with distilled water to get 10 µg/mL and 15 µg/mL each of amlodipine besylate. The absorbance at 243 nm was measured and the amount of drug present in the sample solutions were obtained from the slope and intercept values obtained from the calibration curve (Table 1). The experiments were repeated three times to check its reproducibility. The results of analysis of tablet formulations are recorded in Table 3.

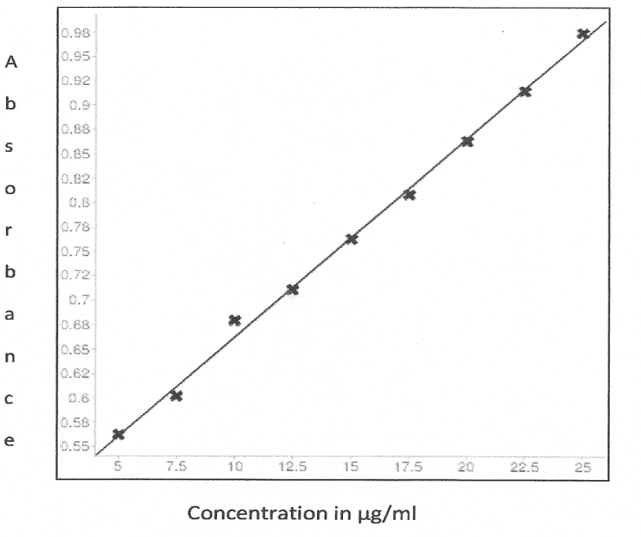

Figure 4: Beer's Law plot

Table 1: Data for Beer's Law plot

Sl No:	Volume of AML stock solution(ml)	Concentration of AML in final solution (µg/mL)	Absorbance at 243 nm
1	1.0	5	0.562
2	1.5	7.5	0.602
3	2.0	10	0.679
4	2.5	12.5	0.710
5	3	15	0.762
6	3.5	17.5	0.808
7	4.0	20	0.863
8	4.5	22.5	0.914
9	5.0	25	0.974

The data reveals that Beer's law is obeyed from 5–25µg/ml.

Table 2: Optical Characteristics of amlodipine besylate for the developed method.

No	Parameters	
1	Beers law limit	5-25µg/mL
2	Correlation coefficient	0.99863
3	Y= ax + C	Y=0. 0.02033333X+ 0.45877777
4	Molar absorptivity	2.0333 x 10^4.L/mol.cm.
5	Sandell's sensitivity	0.027890 (µg/cm^2/ 0.001/ absorbance unit).

Table 3: Result of Analysis of tablets.

Brand Name	Conc. (µg/mL)	Absorbance	*% Label claim	Active content per tablet (mg)	Mean% Label claim	Standard deviation	Standard Error
Amdepin	10	0.683	100.58	10.06	100.20	0.001732	0.0010
	15	0.758	99.82	9.98		0.002000	0.0012
Amlopres	10	0.672	98.97	9.89	99.88	0.002000	0.0012
	15	0.768	100.78	10.08		0.00400	0.0023

*Mean of three determinations

Method Validation

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, robustness and accuracy for the analyte (ICH Q2B., 1994). To evaluate the validity and reproducibility of the method, known amount of pure drug was added to the analyzed sample of tablet powder and the mixture was analyzed for the drug content using the proposed method. For this recovery study the tablet powder equivalent to 50 mg each was taken in two 25 ml volumetric flask. 10 mg of pure drug (spiked drug) was transferred to each flask and 20 ml of 2 M urea solution was added and the flasks were shaken for about 10 min. Then, volume was made up to the mark with distilled water and filtered through Whatmann filter paper No. 41. The solutions were diluted appropriately with distilled water and analyzed for drug content against blank. The percentage recovery was found to be within range (Table 4).

Table 4: Data of Recovery studies.

No	Brand Name	Drug added (spiked) (mg)	Amount found, mg*	% Recovery estimated (Mean±S.D) (n=6)
1	Amdepin	10	59.960	99.94±0.0023
2	Amlopres	10	59.976	99.96±0.0019

*Average of six determinations

Repeatability expresses the precision under the same operating conditions over a short interval of time. The precision of an analytical procedure is usually expressed as the standard deviation of a series of measurements. The reproducibility of the method was studied using three different concentrations of AML (10, 15 and 20µg/ml) which were prepared from stock solution. The absorbance was measured at 243nm against 2 M urea as blank for three times and their mean values were calculated and the data is given in table.5. The intra-day and inter-day precision studies of AML were carried out by estimating the corresponding responses three times on the same day and on three different days (1st, 2nd and 5th day) for three different concentrations of AML (10, 15 and 20µg/ml) and the results are reported in terms of relative standard deviation in table.6 and table.7

Table 5: Data for repeatability.

No	Concentration (µg/mL)	Absorbance at 243nm	Mean value	Standard deviation	Standard Error	Coefficient of variation
1	10	0.679 0.673 0.674	0.675	0.0032	0.0019	0.00388
2	15	0.761 0.764 0.761	0.762	0.0017	0.0010	0.001855
3	20	0.866 0.861 0.865	0.864	0.0026	0.0015	0.002500

Table 6: Intra-day precision.

No.	Concentration (µg/ml)	Absorbance at 243 nm			RSD %
		0 hr	1.5 hr	3hr	
1	10	0.676	0.670	0.658	1.37
2	15	0.760	0.749	0.740	1.34
3	20	0.860	0.851	0.839	1.24

Table 7: Inter-day precision.

Sl. No.	Concentration (µg/ml)	Absorbance at 243 nm			RSD%
		1st day	2nd day	5th day	
1	10	0.678	0.670	0.659	1.43
2	15	0.760	0.745	0.740	1.39
3	20	0.861	0.851	0.842	1.12

Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD of AML by the proposed method was found to be 2µg/ml.

Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample, which can be quantitatively determined with a suitable level of precision and accuracy.The LOQ of AML by the proposed method was found to be 5µg/ml.

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are proportional to the concentration of analyte in the sample. The calibration curve of AML was linear over the range of 5 - 25µg/ml.

RESULTS AND DISCUSSION

Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubility of poorly water-soluble drugs like amlodipine besylate in tablet dosage forms. The results of solubility studies indicated that enhancement in aqueous solubility of amlodipine besylate in 2M urea solution was more than 7 folds as compared to their solubility in distilled water. Therefore, this solution was employed to extract amlodipine besylate from the fine powder of tablet formulation. By proper choice of hydrotropic agents, the use of organic solvents in analysis may be discouraged to a large extent. It is evident that there is good agreement between the amounts estimated and those claimed by the manufacturers. The mean percentage label claims 100.20 and 99.88 for Amdepin and Amlopres respectively (Table 3) are very close to 100 with low values of standard deviation and standard error which confirms the accuracy of the proposed method. Accuracy, reproducibility and precision of the proposed method were further confirmed by the mean percentage recovery values (99.94 to 99.96), which were close to 100 with low values of standard deviation (Table.4). The proposed method for determination of AML showed molar absorptivity of 2.0333L x 10^4/mol.cm and Sandell's sensitivity of 0.027890 (µg/cm^2/ 0.001/ absorbance unit).Linear regression of absorbance on concentration gave the equation Y=0. 0.02033333 X + 0.45877777 with a correlation coefficient (r) of 0.99863 (Table 2).

CONCLUSION

The developed method is economic, simple, precise and rapid and hence can be employed for routine analysis for the estimation of amlodipine from marketed formulations and in biological fluids.

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REFERENCES

Chandrashekhar, T. G., Rao, P. S. N., Smrita, K.,Vyas, S. K. and Dutt, C. Analysis of amlodipine besylate by HPTLC with fluorimetric detection: a sensitive method for assay of tablets. J. Planar. Chromatogr. Mod. TLC. 1994;7: 458-460.

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.

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.

ICH Q2B, Text on Validation of Analytical Procedures, Geneva, 1994, 1–5

Khopade, S. A. and Jain, N. K. Difference spectrophotometric estimation of amlodipine besylate.Indian Drugs. 2000; 37: 351-353.

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.

Maheshwari R K. Analysis of frusemide by application of hydrotropic solubilization phenomenon. The Indian Pharmacist. 2005; 4(38): 55-58.

Maheshwari R K. Application of hydrotropic solubilization in the analysis of aceclofenac. Asian J. Chem. 2006; 18 (2):1572-1574.

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.

Maheshwari R K, Chaturvedi S C, Jain N K. Application of hydrotropic solubilization phenomenon in spectrophotometric analysis of hydrochlorothiazide tablets. Indian Drugs 2005; 42(8): 541-544.

Maheshwari R K, Chaturvedi S C, Jain N K. Application of hydrotropy in spectrophotometric determination of pharmaceutical dosage forms. Indian Drugs. 2005; 42(11):760-763.

Maheshwari R K. Spectrophotometric determination of cefixime in tablets by hydrotropic solubilization phenomenon. The Indian Pharmacist. 2005; 4(36):63-68.

Maheshwari R K. Spectrophotometric analysis of amoxycillin in tablets using hydrotropic solubilization technique. Asian J. Chem. 2006; 18 (4): 3194-3196.

Patki, R. V., Tamhankar, C. P. and Tipnis, H. P. Simple and rapid high performance liquid chromatographic estimation of amlodipine from pharmaceutical dosages. Indian Drugs. 1994; 31: 560-561.

Prabhakar, A. H. and Giridhar, R. A spectrophotometric method for the determination of amlodipine besylate in pure form and in tablets. Indian Drugs. 2002; 39:204-208.

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.

Sweetman S C. Martindale: The complete drug reference, Royal Pharmaceutical Society of Great Britain, London, (2002) 862.

The Merck Index, 23rd edition, Whitehouse Station, New jersey (2008); 516,6235.

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.

Y. Feng, L. Zhang, Z. Shen, F. Pan, Z. Zhang, Analysis of Amlodipine in Human Plasma by Liquid Chromatography–Mass Spectrometry. J. Chrom. Sci.2002; 40:49-54.