

Spectrophotometric Method Development and Validation for Quantitative Estimation of Amlodipine Besylate in Bulk Drug and Their Dosage Forms By Using Hydrotropic Agent

Nilesh Jain^{a*}, Ruchi Jain^a, Arti Jain^b, Sharad P. Pandey^c and Deepak Kumar Jain^c

^aSagar Institute of Research and Technology-Pharmacy, Bypass Road, Bhopal – 462036, (M.P), India.

^bSmriti College of Pharmaceutical Education, Indore – 452010, (M.P), India

^cTruba Institute of Pharmacy, Karond Bypass, Bhopal-462036, (M.P), India.

Received: 18 June 2009; Accepted: 05 December 2010

Abstract

A novel, safe, accurate and sensitive spectrophotometric method was developed using 2 mol L⁻¹ sodium acetate solution as hydrotropic solubilizing agent for the quantitative determination of poorly water-soluble drug amlodipine besylate in tablet dosage form. There was more than 75 fold enhancement in the solubility of amlodipine besylate in 2 mol L⁻¹ sodium acetate solution as compared to solubility in distilled water. Amlodipine besylate shows maximum absorbance at 365 nm. Sodium acetate did not show any absorbance above 240 nm and thus no interference in the estimation of drug was seen. The sample follows the Beer's law in the concentration range of 50 to 250 µg.mL⁻¹ ($r^2 = 0.9998$) with mean recovery ranging from 97.84 to 100.16%. Proposed method is new, simple, economic, safe, rapid, accurate and reproducible. The developed method was validated according to ICH guidelines and found to be in good accordance with the prescribed values.

Keywords:

Amlodipine besylate; hydrotrophy; sodium acetate; spectrophotometry

1. Introduction

Amlodipine besylate [1] (3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylate, monobenzenesulphonate) is a calcium channel blocker and widely used in the treatment of hypertension [2, 3]. Hydrotropic solubilization is the phenomenon by which aqueous solubility of poorly water-soluble drugs and insoluble drugs increases. Various techniques have been employed to enhance the aqueous solubility and hydrotrophy is one of them. Sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility of drug [4-16]. Maheshwari et al. has analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. ketoprofen, salicylic acid [4], frusemide [5], cefixime [6], tinidazole [7], amoxicillin [8]. Maheshwari et al. have developed various analytical techniques employing hydrotropic solubilization phenomenon to analyze poorly water-soluble drugs like hydrochlorothiazide [9], aceclofenac [10], ofloxacin [11], nefedepine [12]. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out

* Corresponding Author

E-mail: nilujain01@yahoo.co.in

ISSN: 1306-3057,

Moment Publication ©2010

spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents. Several analytical methods that have been reported for the estimation of amlodipine besylate in biological fluids and/or pharmaceutical formulations include UV spectrophotometric [13-21] and high-performance liquid chromatographic estimation (HPLC) [22]. In the preliminary solubility studies there were more than 75 fold enhancement in the solubility of amlodipine besylate in 2 mol L⁻¹ sodium acetate solution. Therefore, it was thought worthwhile to employ this hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation.

2. Experimental

2.1. Apparatus

The proposed work was carried out on a Shimadzu UV-visible spectrophotometer (model UV-1700 series), having double beam detector configuration with 1 cm matched quartz cells.

2.2. Reagents and standards

Reference standard of amlodipine besylate was generous gift from Matrix Laboratories, Mumbai (India). Sodium acetate was obtained from Merck Chemical Division, Mumbai. Commercial tablets of amlodipine besylate, Amdepin (Cadila), Amlopres (Cipla Ltd) and Amlovas (Macleods) were procured from the local drug market.

2.3. Preliminary solubility studies

Solubility of amlodipine besylate was determined at 28±1 °C. An excess amount of drug was added to screw capped 30 mL glass vials containing different aqueous systems viz. distilled water, buffer (pH 8.2) and 2 mol L⁻¹ sodium acetate solution. Enhancement of solubility of drug was more than 75 fold. This enhancement of solubility was due to the hydrotropic solubilization phenomenon.

2.4. Preparation of calibration curve

Accurately weighed 100 mg of the amlodipine besylate drug sample were transferred in to 100 mL volumetric flask containing 10 mL of 2 mol L⁻¹ sodium acetate solution and diluted up to 100 mL with distilled water. The Aliquots of 50, 100, 150, 200 and 250 µg.mL⁻¹ were prepared from stock solution (1000 µg.mL⁻¹) and absorbance were noted at 365 nm against respective reagent blank (Fig.1). Calibration curve was plotted between concentration verses absorbance.

2.5. Analysis of tablet formulation

Three marketed formulation Amdepin (Cadila), Amlopres (Cipla Ltd) and Amlovas (Macleods) were selected for tablet analysis. Twenty tablets of each formulation were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 10 mg of amlodipine besylate was transferred to a 100 mL of volumetric flask containing 10 mL of 2 mol L⁻¹ sodium acetate solution. The flask was shaken for about 10 min to solubilize the drug and then volume was made up to mark with distilled water. The solution was filtered through Whatmann filter paper No 41. The filtrate was diluted appropriately with distilled water and analyzed on UV spectrophotometer against reagent blank. Drug content of tablet formulation were calculated using calibration curve and value are reported in Table 1 and Table 2.

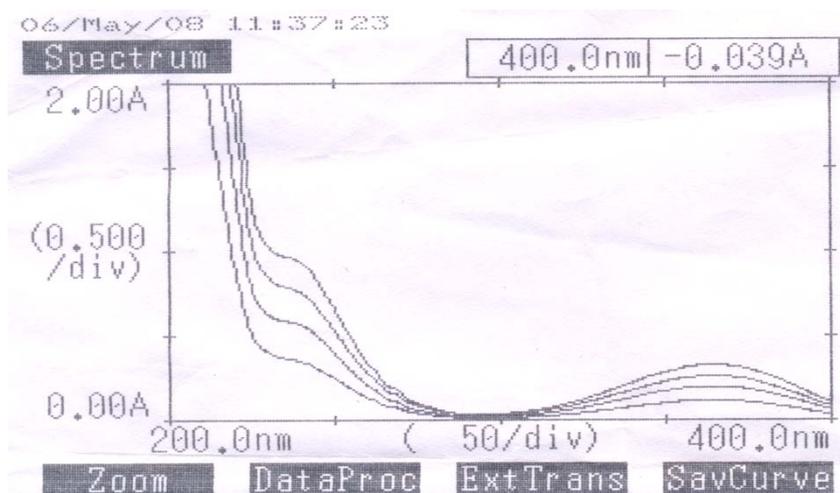


Fig 1. Overlay spectra of different concentration of amlodipine in 2 mol L⁻¹ sodium acetate solution

Table 1. Result of tablet analysis

Amount of drug claimed (mg)	Amount of drug found (mg) in formulation			Percentage estimated in formulation		
	I	II	III	I	II	III
10	9.82	9.89	10.08	98.20	98.9	100.8
10	10.02	9.98	9.92	100.20	99.8	99.2
10	9.87	9.94	9.83	98.7	99.4	99.83

Formulation: I = Amdepin, II = Amlopres and III = Amlovas

Table 2. Statistical evaluation of analysis of tablets

Parameter	Formulation		
	I	II	III
Mean % estimated	99.03	99.4	99.5
Standard deviation	1.004	0.451	0.806
% Coefficient of variation	1.013	0.453	0.810
*Standard error	0.409	0.184	0.328

Formulation: I = Amdepin, II = Amlopres and III = Amlovas

*n=6

2.6. Recovery studies

Recovery studies were performed by adding the definite amount of drug using pre-analyzed tablet solution. These studies were performed in two ways: one by adding fixed amount of pure drug solution to the final dilution while varying the concentration of tablet sample solution in the final dilution and second, by varying the amount of drug solution added to the final dilution keeping the concentration of sample solution in the final dilution constant and the calculate the recovery in both the cases and result of recovery studies are presented in Table 3.

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

Recovery method	Theoretical conc. ($\mu\text{g.mL}^{-1}$)	Amount added ($\mu\text{g.mL}^{-1}$)	Average conc. recovered	Percentage recovery (mean \pm SD) (n=6)	Coefficient of variation (%)	*Standard error
1	100	50	48.92	97.84 \pm 0.892	0.911	0.372
	150	50	49.77	99.54 \pm 0.512	0.514	0.210
	200	50	50.08	100.16 \pm 0.843	0.841	0.344
	250	50	49.23	98.46 \pm 0.734	0.745	0.297
2	100	100	98.73	98.73 \pm 0.619	0.626	0.252
	100	150	149.62	99.74 \pm 0.521	0.522	0.213
	100	200	200.02	100.01 \pm 0.398	0.397	0.162
	100	250	249.67	99.86 \pm 0.793	0.193	0.323

*n=6

2.7. Precision study

To evaluate precision at different parameter like repeatability, intermediate precision, and five dilutions in six replicates were analyzed in same day, in two different days and by two analysts for day to day and analyst to analyst variation. Results were shown in Table 4.

Table 4. Result of precision of amlo

	Validation Parameter	Percentage Mean \pm S.D*. (n=6)	Percentage RSD
With 2 mol L ⁻¹ sodium acetate	Repeatability	98.61 \pm 0.03	0.030
	Intermediate precision		
	Day to Day	97.22 \pm 0.07	0.072
	Analyst to Analyst	98.01 \pm 0.59	0.601

* Mean of thirty determinations (6 replicates at 5 concentration level)

3. Result and discussion

Based on the solubility, stability and spectral characteristics of the drug, 2 mol L⁻¹ sodium acetate was selected as hydrotropic agent. After solubilizing the amlodipine besylate in selected hydrotropic agent, it was scanned in spectrum mode and the working wavelength for the estimation, considering the reproducibility and variability was found to be 365 nm. The developed method was found to be linear in the range of 50-250 $\mu\text{g.mL}^{-1}$ with correlation coefficient (r^2) of 0.9998 and linear equation was $Y=0.003015X + 0.00181$. The mean percent label claims of tablets of amlodipine besylate in formulation-I, formulation-II and formulation III estimated by the proposed method were found to be 99.03 \pm 1.004, 99.4 \pm 0.451 and 99.5 \pm 0.806. 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 1 and Table 2. The values of mean percent recoveries were also found to show variability in ranged from 97.84 to 100.16% and standard deviation values were found to be 0.398-0.892. All these values were very close to 100. 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. 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.

4. Conclusion

It was thus, concluded that the proposed method is new, simple, cost effective, accurate, precise, safe, free from pollution and can be successfully employed in the routine analysis of amlodipine besylate in bulk drug and tablet dosage forms.

References

1. Budavari S (2001) The Merck index: An encyclopedia of chemicals, drugs and biologicals, Merck Research Laboratories, Division of Whitehouse Station, NJ: Merck and Co. Inc, p 86.
2. Hardman J G, Limbird L E and Gilman A G (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw Hill, New York, p. 871.
3. Sweetman S C (2005) Martindale: The complete drug reference, Royal Pharmaceutical Society of Great Britain, London, p. 862.
4. Maheshwari R K (2006) A novel application of hydrotropic solubilization in the analysis of bulk samples of ketoprofen and salicylic acid .Asian J. Chem. 18(1): 393-396.
5. Maheshwari R K (2005) Analysis of frusemide by application of hydrotropic solubilization phenomenon. The Indian Pharmacist 4(38): 55-58.
6. Maheshwari R K (2005) Spectrophotometric determination of cefixime in tablets by hydrotropic solubilization phenomenon. The Indian Pharmacist 4(36):63-68.
7. Maheshwari R K (2006) Novel application of hydrotropic solubilization in the spectrophotometric analysis of tinidazole in dosage form. Asian J. Chem.18 (1): 640-644.
8. Maheshwari R K (2006) Spectrophotometric analysis of amoxicillin in tablets using hydrotropic solubilization technique. Asian J. Chem.18 (4): 3194-3196.
9. Maheshwari R K, Chaturvedi S C, Jain N K (2005) Application of hydrotropic solubilization phenomenon in spectrophotometric analysis of hydrochlorothiazide tablets. Indian Drugs 42(8): 541-544.
10. Maheshwari R K (2006) Application of hydrotropic solubilization in the analysis of aceclofenac. Asian J. Chem.18 (2):1572-1574.
11. Maheshwari R K, Chaturvedi S C, Jain N K (2005) Application of hydrotropy in spectrophotometric determination of pharmaceutical dosage forms. Indian Drugs 42(11):760-763.
12. Jain N K, Agrawal R K, Singhai A K (1990) Hydrotropic solubilization of nifedipine. Pharmzie 45:221-5.
13. Jain N K, Patel V V (1986) Hydrotropic solubilization. The Eastern Pharmacist 29:51.
14. Etman M A, Hada A H (1999) Hydrotropic cosolvent solubilization of indomethacin. Acta Pharm 49:291.

15. Poochikian G D, Cradock J C (1979) Enhanced chartreusin solubility by hydroxyl benzoate hydrotrophy. *J Pharm Sci* 68:728.
16. Saleh A M, Daabis N A (1974) Study of interaction of menadione with hydrotropic salts. *Pharmazie* 29:525.
17. Gohil K., Trivedi P, Molvi K J (2005) Spectrophotometric method for amlodipine besylate in bulk and in tablet dosage forms. *Indian J Pharm* 67:376.
18. Surekha A, Jain N K (2000) Difference spectrophotometric estimation of amlodipine besylate. *Indian Drugs* 37:351.
19. Kasture AV and Ramteke M (2006) Simultaneous UV-spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form *Indian J Pharm Sci* 68(3): 394-6
20. Topale P R, Gaikwad N J, Tajane M R (2003) Simultaneous UV-spectrophotometric estimation of losarten potassium and amlodipine in tablet. *Indian Drugs* 40:119.
21. Jain N K, Agrawal R K (2000) Spectrophotometric methods for simultaneous estimation of amlodipine besylate and lisinopril in tablets. *Indian Drugs* 37:196.
22. Rao J R, Kadam S S, Mahadik K R (2000) RP-HPLC determination of amlodipine and benzepril hydrochloride in tablets. *Indian Drugs* 39:378.