International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 988-991, April-June 2011

Spectrophotometric determination of Lamivudine in Bulk and Pharmaceutical Formulation using hydrotropic Solubilization

M.C.Sharma*, S.Sharma[1]

***School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India**

[1]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) India

Corres.author: mukeshcsharma@yahoo.com

Abstract: Ultraviolet absorption spectrophotometric method for the estimation of poorly water soluble drug like Lamivudine in pharmaceutical formulations has been developed. Aqueous solubility of this selected model drug was to a great extent (21 to 243fold) in 5.0 M sodium benzoate. Lamivudine shows maximum absorbance at 315 nm. Beer's law was obeyed in the concentration. Results of analysis were validated statistically and by recovery studies.
Keywords: Lamivudine, Hydrotropic Solubilization, Sodium benzoate.

Introduction

Lamivudine (2'-deoxy-3'-thiacytidine: 3TC) is a pyrimidine analog reverse transcriptase inhibitor that is active against HIV-1, HIV-2, and HBV. The molecule has two chiral centers and is manufactured as the pure 2*R*, *cis* (-)-enantiomer. The racemic mixture from which lamivudine originates has antiretroviral activity but it has less potency and is substantially more toxic than the pure (-)-enantiomer. Compared with the (+)-enantiomer, the phosphorylated(-)-enantiomer is more resistant to cleavage from nascent RNA/DNA duplexes by cellular 3'-5' exonucleases, which may contribute to its greater potency. [1] Nucleoside reverse transcriptase inhibitor (NRTIs) was the first class of drug those was introduced as antiretroviral agents for the treatment of infection with human immunodeficiency virus (HIV). There have been several publications describing analytical methods for the determination of lamuvidine [2-5]. Moreover, lamivudine is active against zidovudine-resistant HIV [6]. The US Department of Health and Human Services' current guideline for the treatment of established HIV infection strongly recommends 3-TC in combination with another NRTI and either a protease inhibitor or efavirenz [7]. The mechanism of action of lamivudine seems to be similar to that of zidovudine [8]. 3-TC has approximately 80% oral bioavailability in human and its usual dosage is 150 mg twice daily or 300 mg once daily in combination with other antiretroviral agents [9].The term "hydrotropy" has been used to designate the increase in aqueous solubility of various poorly water-soluble compounds due to the presence of a large amount of additives. Sodium benzoate, sodium salicylate, niacinamide, sodium hydroxide, and urea have been employed to enhance the aqueous solubility of poorly water-soluble drugs [10-11]. Most of these organic solvents are toxic, costlier and sources of pollution. Inaccuracy in spectrophotometric estimations due to volatility of organic solvents is another drawback of these solvents. There was tremendous increase in aqueous solubility of atenolol in 5M Sodium benzoate solution. Sodium benzoate does not show absorbance above 325 nm. The Beer's law was obeyed in the range of 50 to 300 mcg/ml at 315 nm for Lamivudine in presence of Sodium benzoate. In the present investigation Lamivudine tablets have been estimated by BP method (spectrophotometric) which involved use of an organic solvent, methanol and also by the hydrotropic solubilization technique which involved

use of Sodium benzoate as hydrotropic solubilizing agent. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form by hydrotropic agents [12-23]. The proposed method is new, accurate, simple and economic. Statistical data proved the accuracy, reproducibility and precision of the proposed method.

Experimental

Instruments and chemicals

UV/Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). HPLC Grade solvents (water and methanol) were obtained from S.D.Fine chemicals Ltd., India, and Lamivudine tablets were procured from the local market lamivir-HBV 100 mg. In the preliminary it was found that there was considerable enhancement in the aqueous solubility of Lamivudine in 1 M sodium benzoate, 1 M sodium acetate, 1 M sodium bicarbonate, 1M sodium chloride, 1 M sodium gluconate, 1M thiourea, 1M trisodium citrate and 1 M urea solutions. Since these solutions do not absorb above 306 nm, it was thought to use these agents' hydrotropic agents, to extract out the drugs having _max above 315 nm, from their corresponding solid dosage forms. Recovery studies and statistical analysis were used to validate the methods.

Calibration curve

In a 100 ml volumetric flask, about 100 mg lamivudine (accurately weighed) was transferred. To this flask 10 ml of 5M Sodium benzoate solution was added and drug was dissolved in it. Distilled water was used to make up the volume up to the mark to give a stock solution (1mg/ml). This stock solution was diluted suitably with distilled water to produce various standard solutions containing 50, 100, 150, 200, 250 and 300 mcg/ml of drug. Absorbances of these solutions were observed at 315 nm against corresponding reagent blank.

Analysis of lamivudine in tablets

Twenty tablets (formulation I and II) were weighed and powdered finely. A portion of this powder containing 100 mg lamivudine was accurately weighed and transferred to a 500 ml volumetric flask. Methanol (300 ml) was added and the suspension was heated to 40^{o}C and shaken for 20 min. After cooling, it was diluted to 500 ml with methanol and filtered through a sintered glass funnel. The filtrate was diluted suitably with methanol to produce a solution containing 0.01% w/v of lamivudine. The absorbance of this solution was noted at 315 nm. The drug content was calculated using 23.1 as the value of A [1%,1 cm].

Analysis of lamivudine in tablets by the proposed method –

Tablets powder equivalent to 100 mg lamivudine was transferred to a 100 ml volumetric flask containing 10 ml of 5 M sodium benzoate solution. Flask was shaken for about 10 minutes to solubilize the drug present in tablet powder and volume was made up to the mark with distilled water. After filtration through sintered glass funnel, the filtrate was appropriately diluted with distilled water and absorbance was noted at 315 nm against reagent blank. Using the calibration curve, the drug content was computed. Recovery studies were performed by spiking the preanalyzed tablet powder with lamivudine bulk drug sample at three levels and determining the drug content by the proposed method. Each type of analysis was performed six times.

Preliminary solubility studies of drugs:

Solubility of lamivudine were determined at 25±1°c. An excess amount of drug was added to screw capped 30 ml glass vials containing different aqueous systems viz. distilled water, buffer of pH 9.4, buffer of 5M Sodium benzoate. The vials were shaken mechanically for 24 hr at 25±1° in a mechanical shaker. These solutions were allowed to equilibrate for the next 35 hr, and then centrifuged for 15 min at 2500 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. From the preliminary solubility studies of drugs the hydrotropic agent selected was sodium benzoate.

Recovery studies:

For recovery studies, tablet powder (formulation I), equivalent to 100 mg drug was taken in a 25 ml volumetric flask. In this flask, 20 mg of pure drug (corresponding spiked drug) was transferred, 20 ml of 5.0 M sodium benzoate solution was added, and the flask was shaken for about 10 min. The volume was made up to the mark with distilled water, and filtered through Whatman filter paper No. 41. The solution was diluted appropriately with distilled water, and analyzed for drug content. A similar procedure was repeated using 1.0 M other hydrotropic solutions, in place of 5.0 M sodium benzoate solution, in all the cases. The results of analysis of recovery studies are presented in Table 2.

Table 1. Results of analysis of commercial tablet formulations.

S No.	Hydrotropic Solu.	T F	LC (mg/tab)	% LC estimated* (mean±S.D.)	Coeff of variation	S.E.
1	5 M sodium benzoate	I	100	101.0+0.36	0.24	0.13
2	1 M sodium acetate	I	100	99.7±0.69	0.69	0.38
3	1 M sodium bicarbonate	I	100	101.5±0.45	1.03	0.65
4	1M sodium chloride	I	100	100.6±0.97	0.31	0.83
5	1 M sodium gluconate	I	100	99.4±0.67	0.76	0.25
6	1M thiourea	I	100	100.1±0.78	0.54	0.28
7	1M trisodium citrate	I	100	99.3±0.45	1.12	0.47
8	1 M urea	I	100	100.4±0.34	0.42	0.59

TF (I)- Tablet formulation, LC- Label claim, SE- Standard error,
*Mean of three determinations,I- lamivir-HBV 100 mg

Table 2: Recovery study for spiked concentration of drugs added to the preanalyzed dosage form

S No	Hydrotropic Solu.	T F	LC (mg/tab)	Drug Added (spiked mg)	%LC estimated* (mean±S.D.)	Coeff of variation	S.E.
1	5 M sodium benzoate	I	100	40	99.64±0.11	0.21	0.34
2	1 M sodium acetate	I	100	40	100.17±0.25	0.13	0.26
3	1M sodium bicarbonate	I	100	40	99.45±0.58	0.45	0.65
4	1M sodium chloride	I	100	40	101.20±0.02	0.16	0.44
5	1 M sodium gluconate	I	100	40	99.53±0.5	0.62	0.76
6	1M thiourea	I	100	40	100.7±0.37	0.66	0.87
7	1M trisodium citrate	I	100	40	101.7±0.04	1.03	0.95
8	1 M urea	I	100	40	101.4±0.21	0.19	0.67

TF- Tablet formulation, AD- Amount of drug, LC- Label claim, SE- Standard error,
*Mean of three determinations, I- lamivir-HBV 100 mg

Table 3: Analysis Data of Tablet Formulations with Statistical Evaluation

Tablet Formulation	Label Claim (mg/Tablet)	%Label Claim Estimated* (Mean±S.D.)	% Coeff. of Variation	Standard Error
I	100	100.15±0.08	0.849	0.74
II	100	99.94±0.13	0.438	0.210

* Mean (n = 6)

Results and discussion

The mean percent label claims of tablet formulations I estimated by British Pharmacopoeial method (a standard analytical method) were 100.75 and 99.82 respectively. The mean percent label claims estimated by proposed method for tablet formulations I were 99.02 which are very close to 100, indicating the accuracy of the method. The values of the mean percent label claims obtained in case of the proposed method are very comparable with those obtained by use of British Pharmacopoeial method. This also indicates that there was no interference of urea and the commonly used additives present in the tablet formulation in the estimation by the proposed method. Validation of the proposed method is further confirmed by the low values of standard deviation, percent coefficient of variation and standard error.Results of solubility studies indicated that, enhancements in aqueous solubilities in 1 M sodium benzoate, 1 M sodium acetate, 1 M sodium bicarbonate, 1M sodium chloride, 1 M sodium gluconate, 1M thiourea, 1M trisodium citrate and 1 M urea solution, as compared to solubility in distilled water, were more than 21, 43, 77,104,132,155,195 and 243 fold in case of lamivudine study proves that increase in solubilities of these three drugs in hydrotropic solutions are not due to alteration in pH, but are due to hydrotropic phenomenon. This indicates that the enhancement in the aqueous solubility of lamivudine in 5.0 M hydrotropic solutions was largely

due to hydrotropy.Part A solution of drug was kept at room temperature for 48 h. There was no precipitation of drug in Part A solutions within 48 h. In addition, drug contents of Part A solutions (after 48 h) were same as those of Part B solutions (fresh solutions). This study reveals that the estimations can be done within 48 h at least, without having any detrimental effect on drug stability. From Table 1, it is evident that there is good agreement between the amounts estimated, and those claimed by the manufacturers. Percent label claims are very close to 100, with low values of standard deviation, % coefficient of variation, and standard error. Accuracy, reproducibility, and precision of the proposed methods, were further confirmed by percent recovery values, which were close to 100 with low values of standard deviation, % coefficient of variation, and standard error (Table 2).The mean percent recovery values ranged from 99.98 to 101.05 and were very close to 100. Also, the values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error were significantly low. Thus, the proposed method of analysis was very well validated.

Conclusion

Thus, it may be concluded that the proposed method of analysis, using urea as the hydrotropic solubilizing agent is new, simple, cost-effective, environmentally friendly, safe, accurate and reproducible. Urea and the commonly used tablet excipients did not interfere in spectrophotometric estimation at 315 nm. Decided advantage is that organic solvent (methanol) is precluded but not at the expense of accuracy. The proposed method is worth adopting in pharmacopoeia. By proper choice of hydrotropic agents, the use of organic solvents in analysis may be discouraged to a large extent.

Acknowledgement

Authors are grateful to Head, School of Pharmacy, DAVV, Indore for providing necessary facilities. Authors also grateful to thanks Prof.R.K. Maheshwari Department of Pharmacy SGSITS Indore for given valuable suggestion to related experimental.

References

[1] Goodmann and Gilman's, The Pharmacological Basis of Therapeutics,11th Edition. Mc-HILL Medical Publishing Division, New York, 1288-1294(2006)
[2] Y Alnouti, C.A. White, MG. Bartlett., Biomed. Chromatogr, 18, 641-647(2004)
[3] R.M. Lopez,L. Pou, M.R. Gomez, I. Ruiz, J. Monterde., J. Chromatogr,75, 371-376(2001)
[4] J.W. Pav, L.S. Rowland, D.J .Korpalski., J. Pharm Biomed. Anal, 20, 91-98(1999)
[5] T.L. Laurito, V. Santagada, G. Caliendo, C.H. Oliveira, R.E.B. Astigarraga, G.D. Nucci., J. Mass Spectrom, 37, 434(2002)
[6] G.A.Balint., Pharmacol. Ther, 89, 17–27 (2001)
[7] A.S.Pereira, R.R. Tidwell., J. Chromatogr. B 764, 327–347 (2001)
[8] G.J. Hart, D.C.Orr, C.R. Penn, H.T. Figueiredo, N.M. Gray, R.E. Boehme, J.M. Cameron., Antimicrobial. Agents. Chemother. 36, 1688–1694(1992)
[9] Eunice Kazue Kano, Cristina Helena dos Reis Serra., International Journal of Pharmaceutics. 297, 73–79(2005)
[10] Jain NK, Singhai AK, Jain S., Pharmazie, 51: 236-239(1996)
[11] Maheshwari RK. ,Asian J. Chem, 18, 640-644 (2006)
[12]SharmaS, Sharma MC, Sharma R, Sharma AD.J. Pharm.Res. 2010; 3(5):1074-1076
[13] Sharma S, Sharma MC, Sharma R,Sharma AD.J. Pharm.Res. 2010, 3(5), 1063-1067
[14] Sharma S, Sharma MC. Jou. Curr.Pharmac. Rese. 2010; 01: 26-30
[15] SharmaMC,SharmaS,SharmaAD.Jour. Chem. Pharma.Res., 2010, 2(2):411-415
[16] Sharma MC, Sharma S, Sharma A D. Jour. Chem. Pharma.Res., 2010, 2(2): 416-420
[17] Sharma S, Sharma MC.A Inte.Jour.Chem.Tech. Res.2010; 2(4): 2487-2491.
[18] Sharma S, Sharma MC. Journal of Optoelectronics and Biomedical Materials.2010; 2(4) 223 – 225.
[19] Sharma S, Sharma MC. Journal of Optoelectronics and Biomedical Materials.2010; 2(4) 227 – 229.
[20] SharmaR, PathodiyaG, MishraGP, SharmaM.. Pharm.Res. 2010, 3(12), 2953-2955.
[21]Sharma S, Sharma MC. International Journal of Pharm Tech Research, 3(1), 244-247; 2011
[22] Sharma S, Sharma MC. International Journal of Chem Tech Research,3(1), 230-233;2011
[23]]Sharma S, Sharma MC.International Journal of Pharm Tech Research,3(1), 253-255;2011

*****