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Design of dissolution media for *in-vitro* bioequivalence testing of Lamivudine

Nagiat T Hwisa[1], Shanta Kumari Adiki[2], Prakash Katakam[1*], Babu Rao Chandu[1]
[1]Faculty of Pharmacy, University of Zawia, Al-Zawia, Libya.
[2]Nirmala College of Pharmacy, Mangalagiri, Guntur 522503, India.

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ABSTRACT

The present investigation is aimed at developing the stability indicating dissolution media for the determination of lamivudine (3TC) in pharmaceutical dosage forms. The stability of 3TC was tested in various dissolution media maintained at ambient temperature and 37 ^{o}C for 48 hrs. Stability studies of 3TC in various media indicated that the drug was stable in 0.1M HCl, pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers. The λ_{max} were found to be 280.0, 278.8, 273.0 and 271.5nm for 0.1M HCl, pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively with low CV of <4.44%. The linearity of standard plots in optimized media was 0.5-40 µg/ml for 0.1M HCl and pH 1.2 KCl-HCl buffer. Similarly it was 0.5-60 µg/ml in pH 6.2 buffer and 0.2-40 µg/ml in pH 7.0 phosphate buffer. The validated methods were applied to determine 3TC concentration in formulations. In-vitro dissolution testing indicated that the 3TC was stable and drug release is uniform from tablet dosage forms. The optimized media could be employed to study the dissolution profiles of 3TC in bioequivalence studies.

INTRODUCTION

Dissolution of drugs from solid dosage forms is an important parameter in assessing the product quality and uniformity at the formulation stage and during the shelf-life of the product. The significance of a dissolution test is based on the fact that for a drug to be absorbed and available to the systemic circulation, it should be in solution form. Therefore, an *in vitro* dissolution test was introduced not only for quality control to assess batch-to-batch consistency of release from a drug product, but also in an attempt to identify potential problems of *in vivo* drug release and absorption (Qureshi and McGilveray, 1999), Dissolution medium is used for the regular *in-vitro* determination of various drugs. It is often desirable to have a dissolution medium that is stable and selective based on the formulation used. Dissolution media were usually developed in the past to improve the solubility in dissolution media for poorly water soluble drugs such as for nimodipine (Zhonggui *et al.*, 2004), cefixime trihydrate (Madhura *et al.*, 2009), rifampicin (Rao and Murthy, 2001) and valdecoxib (Subramanian *et al.*, 2006). In light of the FDA's recent guidance there is an increased awareness of the potential relevance of dissolution tests (Guidance, 1996; Guidance for Industry, 1997; Martin *et al.*, 2003). The FDA provides guidelines for dissolution tests for oral modified release dosage forms, but also realizes the need for individualizing the method on a case by case basis leaving the justification of a given methodology up to the scientist. As a result of patent expiry for many drugs, there is increase in rise of formulating the dosage forms from conventional to extended release products. The authorized USP pending monograph for lamivudine tablets specifies 0.1N HCl as dissolution medium (Lamivudine tablets, 2011). In our earlier works, we have developed stability indicating dissolution media of abacavir sulphate and didanosine and proposed their application for extended release formulations (Awen *et al.*, 2011; Prakash *et al.*, 2011). Therefore there is a tremendous scope for pharmaceutical scientists to develop suitable dissolution testing media for bioequivalence studies of newly developed formulations. Lamivudine is 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one (Fig. 1). Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination (Lamivudine, 2006; Flexner, 2006; Steven *et al.*, 2009).

** Corresponding Author*
Faculty of Pharmacy, University of Zawia, Al-Zawia, Libya.Phone: +218-926855775. Email: pkatakam9@gmail.com

Lamivudine (3TC) was selected as drug in the present study because more varieties of generic formulations are coming up in the market both as conventional and extended release dosage forms. The present investigation is aimed at designing the stability indicating dissolution media for the determination of lamivudine in pharmaceutical dosage forms such as conventional and extended release tablets in bioequivalence studies.

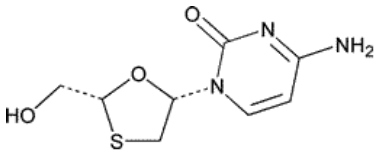

Fig. 1: Structure of lamivudine

MATERIALS AND METHODS

Materials

Pure lamivudine (3TC) was obtained as gratis sample from Matrix Laboratories, Hyderabad, India. Potassium dihydrogen phosphate of AR grade and other chemicals of AR grade were purchased from E. Merck® (India) Ltd., Mumbai. Water used was triple distilled grade and prepared by all glass distillation apparatus (quartz distillation unit, Borosil®). Four brands of lamivudine 100mg tablets, i.e., Hepitec (P), Heptavir (Q), Lamidac (R) and Hepitec(S) were purchased from local market.

Stability studies

Nine dissolution media were selected and prepared such as distilled water, 0.1M HCl, pH 1.2 KCl-HCl buffer and pH 5.8, 6.2, 6.6, 7.0, 7.4 and 7.8 phosphate buffers as per the standards of USP (United States Pharmacopoeia, 2007). The pH of the buffers was measured and adjusted using pH Analyzer (Elico®, Model No. LI612). Stock solutions of 3TC were prepared by dissolving accurately weighed (Afcoset® electronic balance) 25 mg of 3TC in 25 mL of distilled water, 0.1M HCl, pH 1.2 KCl-HCl buffer and pH 5.8, 6.2, 6.6, 7.0, 7.4 and 7.8 phosphate buffers separately to obtain 1mg/mL solutions. All the solutions were sonicated using ultrasonic bath (Enertech®) to dissolve the drug. From these solutions 2.5 mL was pipetted out (Genie® micropipettes) into 25 mL volumetric flask and diluted with the same solvent system to obtain 100 µg/mL solutions.

The stability of 100 µg/mL solutions of 3TC was tested in the above prepared dissolution media at room temperature (RT) and 37°C in an incubator (Thermolab®) for 48 hrs separately. Two sets of these solutions were prepared and maintained at RT and 37°C in an incubator. All the samples were centrifuged (Remi®) for 5 min before scanning. The samples were scanned at 0, 24 and 48 hr intervals using a double-beam UV-visible spectrophotometer (Elico®, India, model SL 169) connected to computer loaded with Spectral Treats® software. The λ_{max} and absorbance were measured to verify any deviations in the values. The above procedure was followed for all the media. The dissolution media that have shown stability of the drug were selected for further evaluation.

Development and validation of analytical methods

Standard graphs of 3TC were constructed for the selected dissolution media after optimizing the conditions based on stability studies. Absorbances were determined for 3TC at selected λ_{max} values using UV-visible spectrophotometer (Elico®, India, model SL 169) for each of the above selected stable media after making dilutions to obtain 0.1 – 100 µg/mL concentrations from the stock solutions. The beer's limit was determined from the constructed plots of wavelength *vs* absorbance. The proposed methods were validated for accuracy, precision and robustness. The methods were tested for intra and inter-day variations. The recovery studies were carried out by adding known amounts of (10 µg and 20 µg) of 3TC to the pre-analyzed samples and subjecting them to the proposed UV spectrophotometric methods. Replicates of six samples were tested for the above studies.

Assay of lamivudine in commercial formulations

The estimation of 3TC content in commercial formulations was carried out in the developed analytical methods using selected dissolution media. Contents of ten tablets containing 3TC were pooled and powdered. The powder equivalent to 25 mg of 3TC was extracted into selected medium and the volume was adjusted to 25 mL, mixed by sonication and filtered through a 0.45 µm Whatman filter paper. From the filtrate 0.1 mL was pipetted into a 10 mL graduated test tube and then the volume was adjusted to 10 mL with the dissolution medium and was assayed for 3TC content using selected methods. The above procedure was followed for remaining tablet brands and for all the selected methods in replicates of six.

In-vitro dissolution rate testing

The *in-vitro* test was conducted to verify the stability of 3TC in the optimized and selected dissolution media during the dissolution testing. The dissolution testing was carried out in a six-stage dissolution rate testing apparatus USP XXI (Labindia, Mumbai, India). A 900ml of the selected dissolution medium was taken separately and dissolution test was performed using paddle method at 37°C and 75 rpm. Aliquot volumes of 5ml each were withdrawn from the dissolution bowl at various time intervals, i.e., 5, 10, 15, 20, 30, 35, 45, 60, 90 and 120 min. The samples were replaced by equal volume of media and analyzed at selected λ_{max} using UV-visible spectrophotometer (Elico®, India, model SL 169) against blank solution containing dissolution medium after suitable dilution. The dissolution test was performed on the tablets of brand 'P' employing the selected dissolution media.

RESULTS AND DISCUSSION

The stability of 100 µg/mL solutions of lamivudine was successfully tested in nine dissolution media such as distilled water, 0.1M HCl, pH 1.2 KCl-HCl buffer and pH 5.8, 6.2, 6.6, 7.0, 7.4 & 7.8 phosphate buffers as per standards of USP, maintained at RT and 37°C for 48 hrs separately. Stability studies of 3TC in various media at RT and 37°C indicated that the drug is stable in 0.1M HCl, pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate

Table I: Stability of 3TC in various media at 37 ^{o}C.

Medium	0 Hrs		24 Hrs		48 Hrs		% CV[a]
	λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance	
Distilled Water	270.0(±0.1)	1.338(±0.003)	270.1(±0.1)	1.419(±0.001)	270.1(±0.1)	1.407(±0.002)	3.15
0.1M HCl	280.0(±0.1)	0.428(±0.002)	279.4(±0.1)	0.432(±0.006)	279.4(±0.1)	0.449(±0.005)	2.55
pH 1.2	278.8(±0.1)	1.311(±0.004)	278.7(±0.1)	1.265(±0.002)	278.7(±0.1)	1.292(±0.004)	1.79
pH 5.8	271.0(±0.1)	0.265(±0.006)	271.0(±0.1)	0.290(±0.004)	271.2(±0.1)	0.313(±0.007)	8.30
pH 6.2	273.0(±0.1)	0.108(±0.003)	273.0(±0.1)	0.111(±0.002)	273.0(±0.1)	0.132(±0.003)	1.18
pH 6.6	273.5(±0.1)	0.337(±0.002)	273.4(±0.1)	0.349(±0.005)	273.4(±0.1)	0.368(±0.003)	4.45
pH 7.0	271.5(±0.1)	0.371(±0.004)	271.4(±0.1)	0.382(±0.004)	271.4(±0.1)	0.396(±0.004)	3.27
pH 7.4	272.7(±0.1)	1.049(±0.002)	272.6(±0.1)	0.930(±0.005)	272.4(±0.1)	0.953(±0.006)	6.46
pH 7.8	273.5(±0.1)	1.167(±0.003)	273.5(±0.1)	1.273(±0.002)	273.4(±0.1)	1.284(±0.001)	5.20

Values in parenthesis are ±standard deviation (n=6). [a]%CV = percent coefficient of variation of absorbances of 0, 24 and 48 hrs.

Table II: Optical characteristics and regression analysis of proposed analytical methods in selected dissolution media.

Parameter	**Method A 0.1M HCl**	**Method B pH 1.2**	**Method C pH 6.2**	**Method D pH 7.0**
Optical characteristics:				
Beer's Law limit (µg/ml)	0.5 - 40	0.5 – 40	0.5 - 60	0.2 - 40
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.014135	0.018385	0.015684	0.023090
Molar Extinction coefficient (1 mole^{-1}.cm^{-1})	0.95 x 10^6	0.99 x 10^6	0.64 x 10^6	0.96 x 10^6
Regression analysis:				
Slope (m)	0.042	0.0445	0.0257	0.0433
Intercept (c)	0.0222	0.0077	0.033	0.0036
Standard error	0.014135	0.018385	0.015684	0.02309
Regression coefficient (r^2)	0.9996	0.9993	0.9993	0.9989

y = mx + c, where 'x' is concentration in µg/ml and 'y' is absorbance unit.

Table III: Precision of the proposed methods

Method	**Selected λ_{max} (nm)**	**3TC concentration (µg/mL)**	**Concentration of 3TC (µg/ml) found on**			
			Intra-day		**Inter-day**	
			Mean (n=6)	**% CV**	**Mean (n=6)**	**% CV**
A	280.0	10	10.08	1.75	10.18	2.49
		30	30.14	1.68	29.89	1.97
B	278.8	10	10.16	1.36	10.27	1.89
		30	30.09	2.08	30.28	2.19
C	273.0	10	10.16	0.99	9.98	1.13
		30	30.16	1.86	30.04	2.04
D	271.5	10	10.11	1.07	10.02	1.26
		30	30.18	2.18	29.97	2.27

%CV = percent coefficient of variation

Table IV: Recovery studies of 3TC.

Method	**Selected λ_{max} (nm)**	**Amount of drug added (µg)**	**Mean (±s.d.) amount (µg) found (n=6)**	**Mean % recovery**
A	280.0	10	9.996 (±0.06)	99.96
		20	19.984 (±0.18)	99.92
B	278.8	10	10.026 (±0.08)	100.26
		20	19.976 (±0.16)	99.85
C	273.0	10	9.997 (±0.11)	99.97
		20	20.169 (±0.21)	100.08
D	271.5	10	9.989 (±0.07)	99.89
		20	19.927 (±0.19)	96.63

buffers in the UV region for a period of 48 hr. The results are summarized in **Table I** and **Figs. 2-5**.

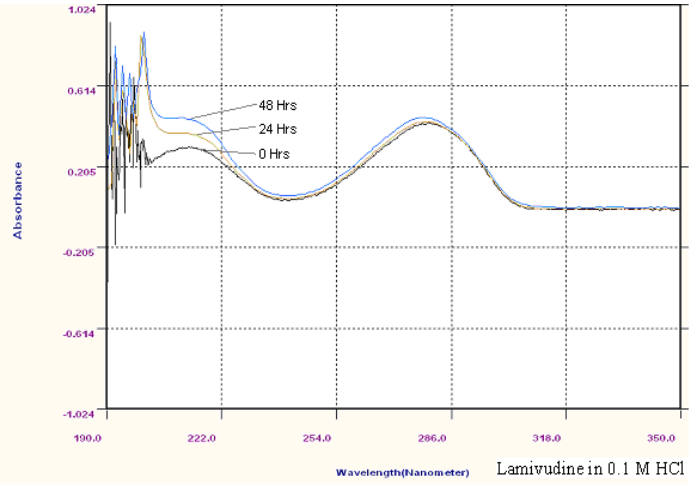

Fig. 2. Scanning curves of 3TC in 0.1 M HCl at time intervals of 0, 24 and 48 hrs and temperature 37 ^{o}C

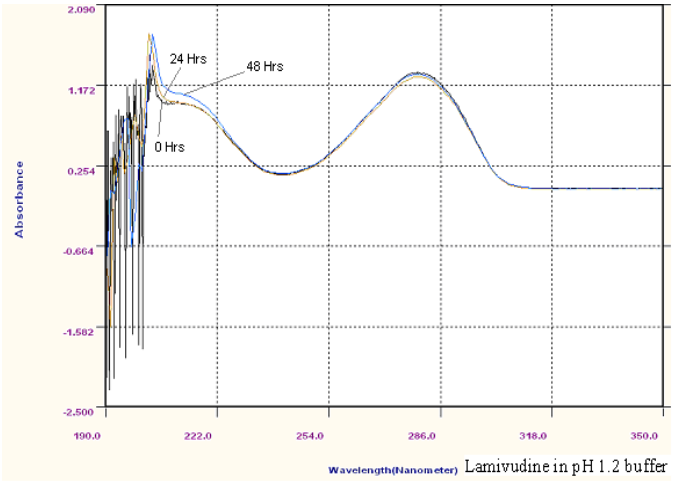

Fig. 3 Scanning curves of 3TC in pH 1.2 acid buffer at time intervals of 0, 24 and 48 hrs and temperature 37 ^{o}C.

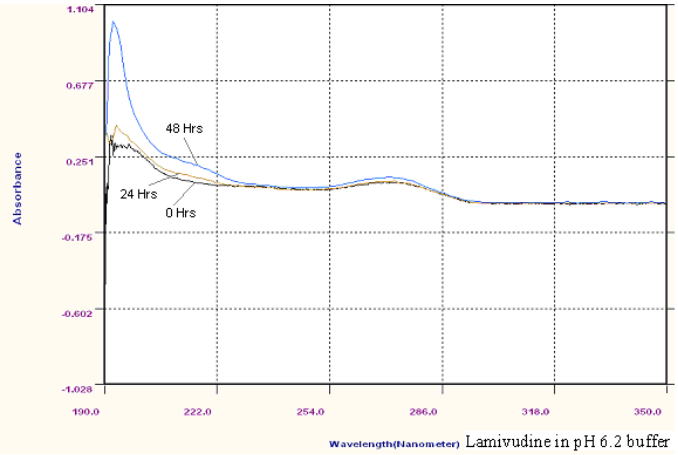

Fig. 4. Scanning curves of 3TC in pH 6.2 phosphate buffer at time intervals of 0, 24 and 48 hrs and temperature 37 ^{o}C.

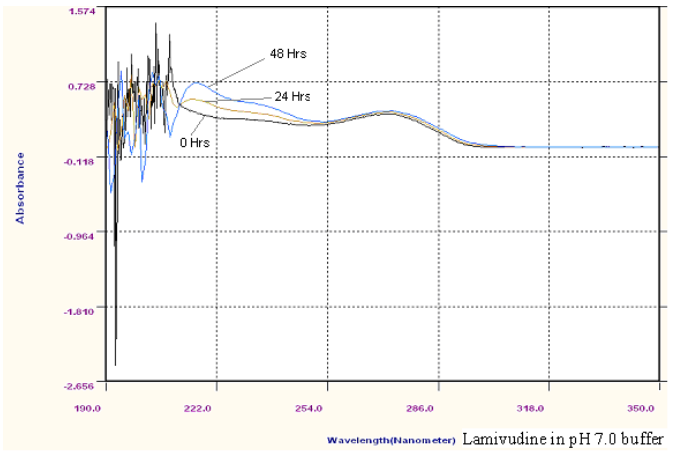

Fig. 5. Scanning curves of 3TC in pH 7.0 phosphate buffer at time intervals of 0, 24 and 48 hrs and temperature 37 ^{o}C

The λ_{max} were found to be 280.0, 278.8, 273.0 and 271.5nm for 0.1M HCl, pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively with an observed low coefficient of variation of < 3.27 %. The drug was unstable in other dissolution media. Hence they are selected as dissolution media to estimate 3TC in formulations.

The analytical methods for 3TC in the four selected dissolution media were developed and named as method A, B, C and D for 0.1M HCl, pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively. Standard graphs were constructed in the above four media and linearity of the graphs were found to be same in the range of 0.5-40 µg/ml for 0.1M HCl and pH 1.2 KCl-HCl buffer. Similarly it was 0.5-60 and 0.2-40 µg/ml for pH 6.2 and pH 7.0 phosphate buffers respectively. The regression equations from calibration graphs were found to be y = 0.042x - 0.0222 (R^2 = 0.9996), y = 0.0445x - 0.0077 (R^2 = 0.9993), y = 0.0257x + 0.033 (R^2 = 0.9993) and y = 0.0433x + 0.0036 (R^2 = 0.9989) for media stated in the above order. The optical characteristics and regression analysis of proposed analytical methods in selected dissolution media were summarized in **Table II**. The methods were validated for precision, accuracy and robustness. A low coefficient of intra-day and inter-day variation of 2.04-2.49 showed that the four methods are highly precise (Table III). About 99.96, 100.26, 100.08 and 99.89% of 3TC could be recovered from the preanalyzed samples using these methods indicating that the proposed methods are accurate (Table IV).

Assay of four brands (P, Q, R and S) of 3TC was determined using the developed methods. The mean amount of 3TC determined was 98.94-100.21, 99.85-100.63, 100.11-100.97 and 98.92-100.27% of the labeled amount for methods A, B, C and D respectively (Table V). The low percent of coefficient of variation (2.27-2.68%) indicated that the reproducibility of the assays of 3TC in the tablet dosage forms. The commonly used excipients and additives in the pharmaceutical formulations did not interfere in the proposed methods. The *in-vitro* dissolution test was conducted to verify the stability of 3TC in the optimized and selected dissolution media during the dissolution testing. The

dissolution test was performed on the tablets of brand-P employing the four dissolution media which were optimized. The results of *in-vitro* dissolution testing indicated that the drugs were stable simulated gastric fluid (0.1M HCl and pH 1.2 KCl-HCl buffer) and simulated intestinal fluids (pH 6.2 and pH 7.0 phosphate buffers) and drug release was uniform for all brands of 3TC (Fig. 6). The results show that the optimized dissolution medi\a can be employed to conduct dissolution testing of 3TC tablets.

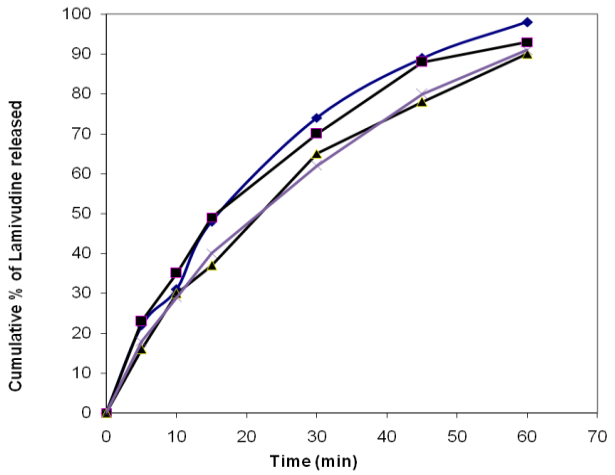

Fig. 6: Cumulative % of 3TC (brand P) released vs time plots in 0.1M HCl (♦) and pH 1.2 HCl-KCl buffer (■) pH 6.2 phosphate buffer (Δ) and pH 7.0 phosphate buffer (×).

Table V: Assay of different brands of 3TC tablets.

Method	Brand	Labeled amount of drug (mg)	Mean % of labeled amount (n=6)	% CV
	P	100	99.86	2.64
A	Q	100	100.21	1.94
	R	100	98.94	2.18
	S	100	101.63	2.68
B	P	100	100.19	1.07
	Q	100	99.85	2.11
	R	100	100.97	1.73
C	S	100	100.11	2.59
	P	100	101.16	2.37
	Q	100	98.92	1.96
D	R	100	100.27	2.27
	S	100	100.07	2.07

% CV= percent coefficient of variation.

CONCLUSIONS

It is concluded that the four stable dissolution media were optimized for the first time to test *in-vitro* bioequivalence studies of lamivudine. Analytical methods using optimized media were developed and they can be used for routine assay of 3TC in various dosage forms. The developed dissolution media could be employed as simulated gastric fluid (0.1 HCl and pH 1.2 KCl-HCl buffer) and simulated intestinal fluids (pH 6.2 and pH 7.0 phosphate buffers) to study the *in-vitro* dissolution profiles of tablets of 3TC. Further these methods can be extended to bioequivalence studies of newly developed formulations of 3TC in the selected liquid media for both conventional and extended release dosage forms.

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