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Development of Stability Indicating Media for *In-Vitro* Dissolution Testing of Didanosine in Pharmaceutical Dosage Forms

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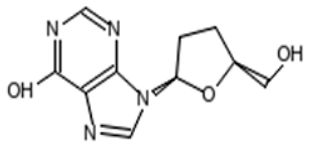
Abstract

The present investigation is aimed at developing the stability indicating dissolution media for the determination of didanosine (DDI, a HIV-1 Reverse Transcriptase inhibitor) in pharmaceutical dosage forms for the first time. The stability of didanosine was tested in various dissolution media, ie., 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. The stability was tested at room temperature and 37^{o}C for 48 hrs. The samples were scanned for stability and the optimized samples were selected for further study. Stability studies of DDI in various media at RT and 37^{o}C indicated that the drug is stable in pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers in the UV region for a period of 48 hr. The λ_{max} were found to be 248.4, 249.3 and 254.4 nm for pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively with observed low coefficient of variation of <2.81%. Standard graphs were constructed in the above three media and linearity of the graphs were found to be in the range of 0.2-60, 0.2-40 and 0.5 – 40 µg/ml in pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively. The methods were validated and found to be precise, accurate and robust. These methods can be used for routine assay of DID in various dosage forms. In-vitro dissolution testing indicated that the drugs are stable and drug release is uniform for all dosage forms. It is concluded that the three optimized media could be used as dissolution media as simulated gastric fluid (pH 1.2 KCl-HCl buffer) and simulated intestinal fluids (pH 6.2 and pH 7.0 phosphate buffers) to study the dissolution profiles of DDI. Further these methods can be extended to bioequivalence studies of newly developed formulations of DID in the selected liquid media for both conventional and extended release dosage forms.

Key Words: Didanosine, Stability indicating, Dissolution media, Dissolution testing

Introduction

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. In light of the FDA's recent guidance there is an increased awareness of the potential relevance of dissolution tests [1, 2]. 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 draft proposal of International Pharmacopoeia mentions methanol as dissolution fluid and recommends to develop a suitable dissolution medium for DID tablets and capsules [3, 4]. Therefore there is a tremendous scope for pharmaceutical scientist to develop suitable dissolution testing media for bioequivalence studies of newly developed formulations. Didanosine (DID) is chemically, 9-[5-(hydroxymethyl)oxolan-2-yl]-3H-purin-6-one, is a antiretroviral drug acts by inhibiting HIV-1 Reverse Transcriptase enzyme [5]. DID 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 developing the stability indicating dissolution media for the determination of didanosine in pharmaceutical dosage forms such as conventional and extended release tablets.



9-[5-(hydroxymethyl)oxolan-2-yl]-3H-purin-6-one

Materials and Methods

Materials

Pure samples of Didanosine 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®). Formulations of didanosine (Dinex-Brand 1, Dinosin-Brand 2 and Virosine-Brand 3) were purchased from local Indian 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 [6]. The pH of the buffers was measured and adjusted using pH Analyzer (Elico®, Model No. LI612). Stock solutions of didanosine (DDI) were prepared by dissolving accurately weighed (Afcoset® electronic balance) 25 mg of DDI 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 didanosine 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 are 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 absorbances were measured to verify any deviations in the values. The above procedure is 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 DID were constructed for the selected dissolution media after optimizing the conditions based on stability studies. Absorbances were determined for DDI 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-day and inter-day variations. The recovery studies were carried out by adding known amounts of (10 µg and 20 µg) of DDI 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 didanosine in commercial formulations

The estimation of drug content in commercial formulations was carried out in the developed analytical methods using selected dissolution media. Contents of ten tablets containing DDI were pooled and powdered. The powder equivalent to 25 mg of DDI was extracted into selected medium and the volume was adjusted to 25 mL, mixed by sonication and filtered through a 0.45 µm Whatnam 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 DDI 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 DID 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). 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 1 employing the selected dissolution media.

Results and Discussion

The stability of 100 µg/mL solutions of didanosine 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 and 7.8 phosphate buffers as per the standards of IP maintained at RT and 37°C for 48 hrs separately. Stability studies of DDI in various media at RT and 37°C indicated that the drug is stable in pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers in the UV region for a period of 48 hr. The results are summarized in Table 1 and Figures 1-3. The λ_{max} were found to be 248.4, 249.3 and 254.4 nm for pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively with observed low coefficient of variation of <2.81%. The analytical methods for DID in the three selected dissolution media were developed and named as method A, B and C for pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively. The selected dissolution media were used to develop analytical methods for DID and denoted as methods A, B and C for pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively. Standard graphs were constructed in the above three media and linearity of the graphs were found to be in the range of 0.2-60, 0.2-40 and 0.5-40 µg/ml in pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers respectively. The regression equations from calibration graphs were found to be $y = 0.026x + 0.0115$ ($R^2 = 0.9995$), $y = 0.0478x + 0.0117$ ($R^2 = 0.9995$) and $y = 0.0467x + 0.0055$ ($R^2 = 0.9994$) for pH 1.2 KCl-HCl buffer (method A), pH 6.2 phosphate buffer (method B) and pH 7.0 phosphate buffer (method C) respectively. The optical characteristics and regression analysis of proposed analytical methods in selected dissolution media were summarized in Table 2. The methods were validated for precision, accuracy and robustness. The variation for intra-day analysis was <1.87% and for inter-day

analysis was <2.31% which indicates that the proposed methods were precise (Table 3). Mean recovery of DID using the developed methods was in the range of 99.91-100.05 (Table 4) showed that the methods were accurate. The methods were validated and found to be precise, accurate and robust. Assay of three brands of DID was determined using the developed methods. The mean amount of DID determined was 98.9-101.24, 99.79-100.02 and 99.55-100.16 for the developed methods A, B and C respectively.

The in-vitro test was conducted to verify the stability of DID in the optimized and selected dissolution media during the dissolution testing. The dissolution test was performed on the tablets of Brand 1 employing the three dissolution media which were optimized. In-vitro dissolution testing indicated that the drugs were stable in pH 1.2 KCl-HCl buffer, pH 6.2 and pH 7.0 phosphate buffers and drug release was uniform for all dosage forms (Figure 4). The results show that the optimized dissolution media can be employed to conduct dissolution testing of DID tablets.

Table 1. Stability of Didanosine in various media at 37 °C

Medium	0 Hrs		24 Hrs		48 Hrs		% CV[a]
	λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance	
Distilled Water	250.9(±0.1)	0.592(±0.004)	250.9(±0.1)	0.669(±0.003)	250.9(±0.1)	0.731(±0.003)	10.48716
0.1M HCl	248.6(±0.1)	0.242(±0.002)	248.4(±0.1)	0.484(±0.004)	248.4(±0.1)	0.508(±0.003)	35.77078
pH 1.2	248.4(±0.1)	0.517(±0.002)	248.4(±0.1)	0.516(±0.006)	248.4(±0.1)	0.513(±0.004)	0.403946
pH 5.8	249.4(±0.1)	0.607(±0.004)	249.5(±0.1)	0.621(±0.004)	249.4(±0.1)	0.652(±0.005)	3.674835
pH 6.2	249.4(±0.1)	0.870(±0.003)	249.3(±0.1)	0.908(±0.002)	249.3(±0.1)	0.918(±0.002)	2.818015
pH 6.6	254.4(±0.1)	0.547(±0.006)	254.3(±0.1)	0.560(±0.004)	254.4(±0.1)	0.525(±0.004)	3.25217
pH 7.0	254.4(±0.1)	0.524(±0.005)	254.4(±0.1)	0.529(±0.003)	254.4(±0.1)	0.542(±0.006)	1.747631
pH 7.4	254.4(±0.1)	0.619(±0.002)	254.4(±0.1)	0.644(±0.005)	254.4(±0.1)	0.661(±0.004)	3.294169
pH 7.8	254.7(±0.1)	0.618(±0.007)	254.7(±0.1)	0.661(±0.004)	254.7(±0.1)	0.659(±0.003)	3.756861

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

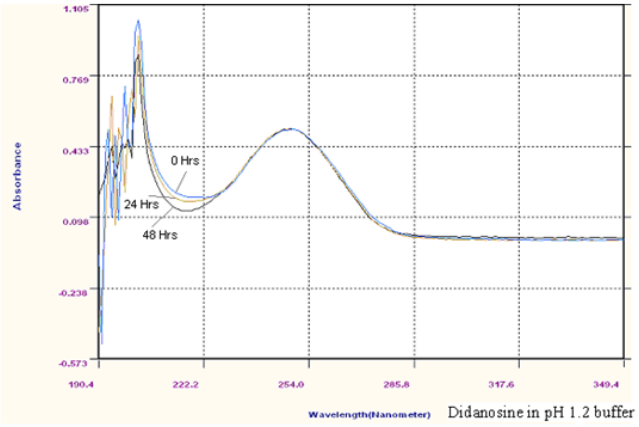


Figure 1. Scanning curves of didanosine in pH 1.2 acid buffer at time intervals of 0, 24 and 48 hrs and temperature 37 °C

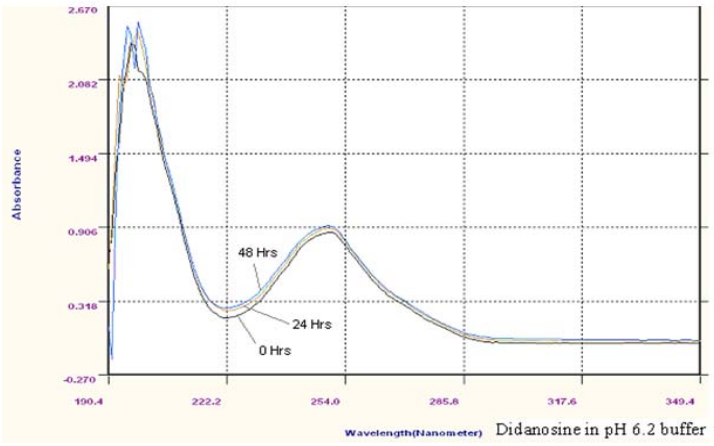


Figure 2. Scanning curves of didanosine in pH 6.2 phosphate buffer at time intervals of 0, 24 and 48 hrs and temperature 37 °C

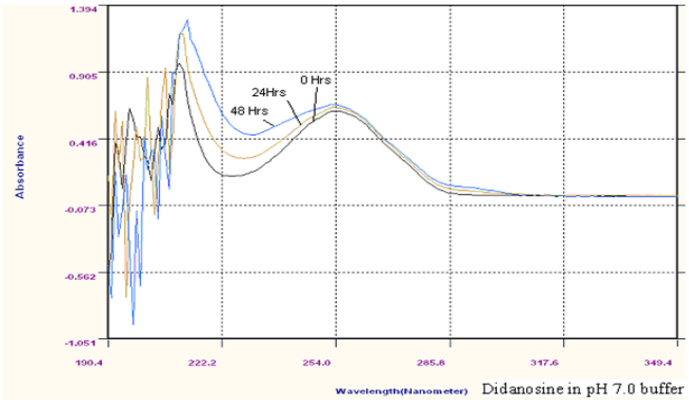


Figure 3. Scanning curves of didanosine in pH 7.0 phosphate buffer at time intervals of 0, 24 and 48 hrs and temperature 37 °C

Table 2. Optical characteristics, Regression analysis of proposed analytical methods in selected dissolution media

Parameter	Method A pH 1.2	Method B pH 6.2	Method C pH 7.0
Optical characteristics:			
Beer's Law limit (µg/ml)	0.2 – 60	0.2 - 40	0.5 - 40
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.02181	0.020366	0.020768
Molar Extinction coefficient (1 mole^{-1}.cm^{-1})	1.29 x 10^6	1.17 x 10^6	1.11 x 10^6
Regression analysis:			
Slope (m)	0.026	0.0478	0.0467
Intercept (c)	0.0115	0.0117	0.0055
Standard error	0.013176	0.016962	0.018411
Regression coefficient (r^2)	0.9995	0.9995	0.9994

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

Table 3. Precision of the Proposed Methods

Method	Selected λ_{max} (nm)	Didanosine concentration (µg/mL)	Concentration of didanosine (µg/ml)			
			Intra-day		Inter-day	
			Mean (n=6)	% CV	Mean (n=6)	% CV
A	248.4	10	10.17	1.87	10.14	2.31
		30	30.09	1.36	30.12	2.11
B	249.3	10	10.11	1.61	10.05	1.89
		30	30.14	1.13	30.09	1.95
C	254.4	10	10.09	1.76	10.10	1.99
		30	30.07	1.64	30.14	2.26

%CV = percent coefficient of variation

Table 4. Recovery studies of didanosine

Method	Selected λ_{max} (nm)	Amount of drug added (µg)	Mean (±s.d.) amount (µg) found (n=6)	Mean % recovery
		10	9.996 (±0.06)	99.92
A	248.4	20	20.015 (±0.08)	100.05
		10	10.022 (±0.04)	100.02
B	249.3	20	19.955 (±0.06)	99.91
		10	10.016 (±0.03)	100.01
C	254.4	20	20.008 ±0.09)	100.04

Table 5. Assay of different Brands of Didanosine tablets

Method	Brand	Labeled amount of drug (mg)	Mean % of labeled amount (n=6)	%CV
	Brand 1	100	101.24	2.84
A	Brand 2	100	99.84	1.76
	Brand 3	250	98.9	2.49
	Brand 1	100	99.79	2.72
B	Brand 2	100	99.81	2.16
	Brand 3	250	100.02	1.98
	Brand 1	100	100.16	2.51
C	Brand 2	100	100.09	2.36
	Brand 3	250	99.55	1.99

%CV= percent coefficient of variation

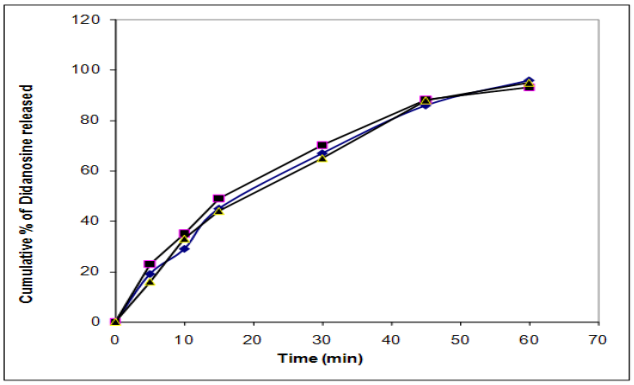


Figure 4. Cumulative % of DID released vs time plots in pH 1.2 KCl-HCl buffer (–♦–), pH 6.2 phosphate buffer (–■–) and pH 7.0 phosphate buffer (–▲ –).

Conclusions

It is concluded that the three stable liquid media were optimized for the first time. Analytical methods using optimized media were developed and they can be used for routine assay of DID in various dosage forms. The developed dissolution media could be employed as simulated gastric fluid (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 DDI. Further these methods can be extended to bioequivalence studies of newly developed formulations of DID in the selected liquid media for both conventional and extended release dosage forms.

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