Journal of Chromatography A, 1218 (2011) 2540–2545

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Stability-indicating micellar liquid chromatography method for three novel derivatives of zidovudine in aqueous and simulated gastric and intestinal fluids matrices

Mónica A. Raviolo[a], Josep Esteve-Romero[b,*], Margarita C. Briñón[a]

[a] Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[b] Química Bioanalítica, QFA, ESTCE., Universitat Jaume I, Campus Riu Sec, 12071 Castelló, Spain

ARTICLE INFO

*Article history:*
Received 19 November 2010
Received in revised form 7 February 2011
Accepted 9 February 2011
Available online 16 February 2011

*Keywords:*
Zidovudine derivatives
Gastric and intestinal matrices
Stability-indicating HPLC methods
Micellar liquid chromatography

ABSTRACT

This work studies the stability of three new anti-HIV agents which were obtained by the association of zidovudine (AZT) with different amino acids, such as leucine (AZT-Leu) and valine (AZT-Val), and one with an acid group (AZT-Ac). Before commercialisation, their stability in different matrices – simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8), both as the USP 32 Guideline indicates, and buffers (pH 1.2 and 6.8) – must be studied. To this end, a new stability-indicating micellar liquid chromatography (MLC) method has been optimised and validated. Measurements were based on the disappearance of reagents and the appearance of the only degradation product (AZT). This optimised and validated method used a C18 column and a mobile phase containing 0.05 M sodium dodecyl sulphate–1% (v/v)1-butanol–0.01 M $NaH_2PO_4$ (pH 3.0) at 30 °C, and a flow rate of 1 mL $min^{-1}$. Under these conditions, retention times were 1.4, 3.6, 6.3 and 9.5 min for AZT-Ac, AZT, AZT-Val and AZT-Leu, respectively. Calibrations better than 0.9995, intra- and inter-day precisions below 1.08% and good recoveries (94.47–116.52%) and robustness (RSD less that 1.08%) were obtained and were adequate to analyse the four compounds. Finally, this MLC method was applied to achieve stability studies which resulted in the evidence that all the compounds followed a pseudo-first-order kinetics, and in the determination of their kinetic constants and half-life time. A reference method, applied in the same studies, validated the MLC method reported herein.



## 1. Introduction

In the last few years, biological active drugs molecules discovery has undergone important changes by employing appropriate analytical technologies and strategies, thus providing more opportunities for acquiring and integrating information to enhance discovery and success, and efficiency in developing novel chemical entities with biological activity. Generally, physicochemical properties can be used as predictors of ADMET (absorption, distribution, metabolism, excretion and toxicity) characteristics, and to reduce time, expense and the use of animals [1,2]. Earlier stability screening provides important information about possible structural modifications to improve drug molecules discovery.

The Food and Drug Administration (FDA) Guidance for Industry [3] concerning *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms includes a discussion on gastrointestinal stability, along with an appropriate methodology to help classify a drug based on its intrinsic solubility, intestinal permeability and drug product dissolution. The stability of a drug substance in gastric and intestinal fluids evidences whether drug loss from the gastrointestinal tract takes place by intestinal permeation or by a degradation process in the gastrointestinal fluids prior to membrane absorption. Stability in the gastrointestinal tract may be confirmed by incubating the drug substance in gastric and intestinal fluids that are representative of *in vivo* drug exposure to these fluids; e.g., 1 h in simulated gastric fluids (SGF) and 3 h in simulated intestinal fluids (SIF). Significant degradation (>5%) of a drug assessed in this manner could suggest potential instability in the gastrointestinal tract [3]. A validated stability-indicating assay is then utilised to measure drug concentrations.

The rapid worldwide spread of acquired immunodeficiency syndrome (AIDS) has prompted intense research efforts to discover compounds that effectively inhibit the human immunodeficiency virus (HIV-1) [4–10], the aetiologic agent of AIDS [11]. Despite worldwide attempts underway to develop chemotherapeutic agents that are effective against HIV, 3′-azido-2′,3′-dideoxythymidine, or zidovudine (AZT) (Fig. 1), the first drug approved for the treatment of AIDS patients [12], is still one of

* Corresponding author. Tel.: +34 964728093; fax: +34 964728066.
*E-mail address:* josep.esteve@qfa.uji.es (J. Esteve-Romero).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.02.018

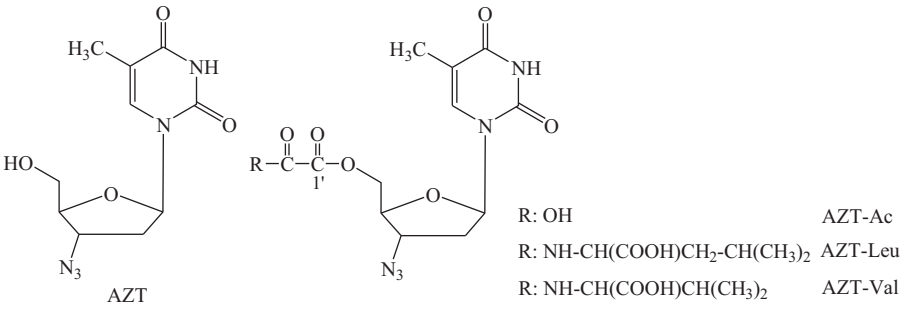


**Fig. 1.** Chemical structure of zidovudine (AZT) and its derivatives AZT-Ac, AZT-Leu and AZT-Val.

the most potent active agents against HIV, and is used as a primary option in AIDS treatment in combination with other HIV inhibitors. Nevertheless, utility of AZT is limited by its toxic effect on bone marrow [13], hepatic abnormalities [14], limited brain uptake [15], short half-life time in plasma [16], high susceptibility to catabolism [17], general myopathy [18], lipoatrophy [19] and the rapid progress of resistance by HIV-1 [20]. For these reasons, numerous chemical strategies have been developed by medicinal scientists to design analogues or prodrugs of AZT to increase its therapeutic efficacy [21]. Most of these have been prepared by derivatising the 5′-OH of AZT, whose mechanism of action is based on the hydrolysis and/or the enzymatic cleavage of the 5′-*O*-bonds between the drug (AZT) and its attached moiety [22].

As part of our continuing efforts to discover novel and effective antiviral agents, we have reported the synthesis and antiviral activity of novel AZT analogues at the 5′-position [23–25]. In addition, these derivatives have demonstrated bactericide effects against bacteria which can produce opportunistic infections in AIDS patients [23]. Amino acids have been employed to improve the physicochemical properties of different compounds, such as aqueous solubility [26] and intestinal permeation [27]. Among them, those associated with an acid (AZT-Ac), or with the leucine (AZT-Leu) and valine (AZT-Val) groups, have been selected because of not only their biological activity [23,25], but also their physicochemical [24] and pharmacokinetic [28–30] properties (Fig. 1). It is important to point out that AZT is the parent compound of these derivatives.

New compounds like these three novel AZT derivatives need to pass certain trials before their commercialisation, one of which is the evaluation of their stability behaviour in the gastrointestinal tract. Depending on the results, the new pharmaceutical can be formulated as an oral administration form, or it is necessary to look for alternative dosages. In addition, and according to the Guidance for Industry [3], it is necessary to employ an appropriate analytical method to correctly evaluate the intact drug molecules in the presence of their degradation products. Micellar liquid chromatography (MLC) is an alternative method to traditional high performance liquid chromatography (HPLC) [31,32], where the mobile phase is composed of a surfactant at a higher concentration than the critical micellar concentration. An organic modifier, such as 1-propanol, 1-butanol or 1-pentanol, which lower retention times and improve efficiency is usually added to the mobile phase. One of the main advantages of MLC is the possibility of quantifying drugs molecules in complex matrices without a previous extraction process [31]. In addition, MLC mobile phases are non-toxic, not flammable, biodegradable and relatively inexpensive in comparison to HPLC solvents [31,32].

Thus, this work aimed to evaluate the stability behaviour of the AZT derivatives AZT-Ac, AZT-Leu and AZT-Val, firstly in aqueous matrices and later in SGF and SIF using MLC, and then to compare the results with HPLC to establish our MLC method as a stability-indicating HPLC method.

## 2. Experimental

### 2.1. Chemicals and reagents

AZT was generously provided by Filaxis (Buenos Aires, Argentina). AZT-Ac, AZT-Leu and AZT-Val were synthesised as previously reported [23]. Monobasic potassium phosphate, sodium dihydrogen phosphate, sodium hydroxide and phosphoric acid were purchased from Anedra (San Fernando, Argentina). Sodium dodecyl sulphate (SDS) was purchased from Biopack (Zárate, Argentina). Sodium chloride was obtained from Baker Co. (New Orleans, USA). Porcine pepsin and pancreatin enzymes were American Chemical Society (ACS) reagent grade and were bought from Sigma (St. Louis, MO, USA). Dimethylsulphoxide (DMSO), hydrochloric acid, 1-butanol, all of analytical grade, were purchased from Cicarelli (San Lorenzo, Argentina). Methanol (MeOH) and tetrahydrofurane (THF) both HPLC grade, were acquired from Sintorgan (Villa Martelli, Argentina). The water used in the HPLC and MLC analyses and in all the studies was of Milli-Q grade (Millipore®), and solutions and mobile phases were filtered through Millipore filters Type FH (4.5 μm) (Millipore S.A.S., Molsheim, France) and degassed under vacuum. pH was measured using a Crison GLP 21 pHmeter (Modena, Italy).

### 2.2. Preparation of the SGF and SIF solutions

The SGF was prepared according to USP specifications [33]; 2.0 g of sodium chloride and 3.2 g of pepsin (obtained from porcine stomach mucosa) were dissolved in 7.0 mL of hydrochloric acid (37%) and then diluted to 1000 mL. The pH of SGF was approximately 1.2.

On the other hand, the SIF was also prepared according to the USP guide; 6.8 g of monobasic potassium phosphate were dissolved in 250 mL water, and then 77 mL of 0.2 M sodium hydroxide and 500 mL of water were added and mixed along with 10.0 g of pancreatin (obtained from porcine pancreas). The SIF solution was adjusted to pH $6.8 \pm 0.1$ with either 0.2 M sodium hydroxide or 0.2 M hydrochloric acid and then diluted with water to 1000 mL. To perform the stability studies in an aqueous medium at pH 1.2 and 6.8, the above-mentioned buffer components were used but without enzyme substances.

### 2.3. Preparation of stock and working sample solutions

The stock solutions ($2 \times 10^{-4}$ M) of each compound were prepared in DMSO prior to use. Then, 100 μL of the stock solution were added to a vial containing 1900 μL of buffer or matrix (gastric or intestinal fluid) to obtain the work solutions. Afterwards, the phials containing the samples were placed in a water bath at 37 °C throughout the experiment. At the appropriate time, aliquots of 200 μL were taken and added to a solution of 4800 μL of the mobile phase. Each sample was immediately stored at −18 °C until use. At this time, and after samples had reached ambient temperature,