Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Review Article

Received on: 21-09-2015
Accepted on: 27-09-2015
Published on: 15-10-2015

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Zidovudine: A Review of Analytical Methods

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ABSTRACT

Zidovudine (AZT) is a NRTI type of antiretroviral drug used for the treatment of human immunodeficiency virus (HIV) infection / acquired immunodeficiency syndrome (AIDS), metabolized in lever. It is an analog of thymidine. It not easy to get whole data of zidovudine within a short time, it was reported that it mostly prescribed used in combination with other agents in the therapy and prophylaxis of HIV infection and the AIDS. This article provides published analytical methods reported so far in the literature for the determination of AZT in bulk drug, pharmaceutical formulation and biological samples. In that they include various techniques like spectroscopic chromatographic methods like RP-HPLC, HPTLC, LC-MS, LC-MS/MS and electroanalytical methods.

Key-words: Zidovudine, Analytical Methods, Anti HIV, Antiretroviral

Cite this article as:
Ashish S. Patil, Gaurav Patil, Zidovudine: A Review of Analytical Methods, Asian Journal of Pharmaceutical Technology & Innovation, 03 (14); 2015. www.asianpharmtech.com

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Introduction

Zidovudine (AZT) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV/AIDS, metabolized in lever. It is an analog of thymidine. AZT is chemically is 1-[(2R, 4S, 5S) -4-azido-5- (hydroxymethyl) oxolan-2-yl] -5-methyl- 1,2,3,4-tetrahydropyrimidine -2,4-dione.[1,2] AZT also has been referred to as 3′aAZTo-3′-deoxythymidine. It has a molecular formula of $C_{10}H_{13}N_5O_4$ and a molecular weight of 267.24 g/mol. It has the structural formula as shown in Fig.1. AZT is a white to beige, odorless, crystalline solid and it is soluble in ethanol (95%), sparingly soluble in water. The drug is official in Indian Pharmacopoeia, British Pharmacopoeia, United State Pharmacopoeia and European Pharmacopoeia. AZT is a structural analog of thymidine, is a prodrug that must be phosphorylated to its active 5′-triphosphate metabolite, AZT triphosphate (AZT-TP). It inhibits the activity of HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue. It competes with the natural substrate dGTP and incorporates itself into viral DNA. It is also a weak inhibitor of cellular DNA polymerase α and γ.[3,4] There are several formulations of AZT either alone or in combinations with one or two drugs such as Lamivudine (3CT), Nevirapine (NVP), Efavirenz (EFV) and Abacavir (ABV). In the present review we have compiled the published analytical methods reported so far in the literature for determination of AZT in biological samples and pharmaceutical formulations. Techniques like electroanalytical (voltammetric, amperometric), spectrophotometry, capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), liquid chromatography and tandem mass spectroscopy, Ultra pressure liquid chromatography (UPLC) and high-performance thin-layer chromatography (HPTLC) have been used for analysis, from which HPLC methods are used most extensively. Overview of these methods for determination of AZT is shown in Fig.2.

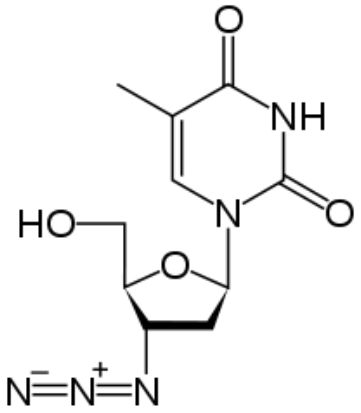

Fig.1 Structure of Zidovudin

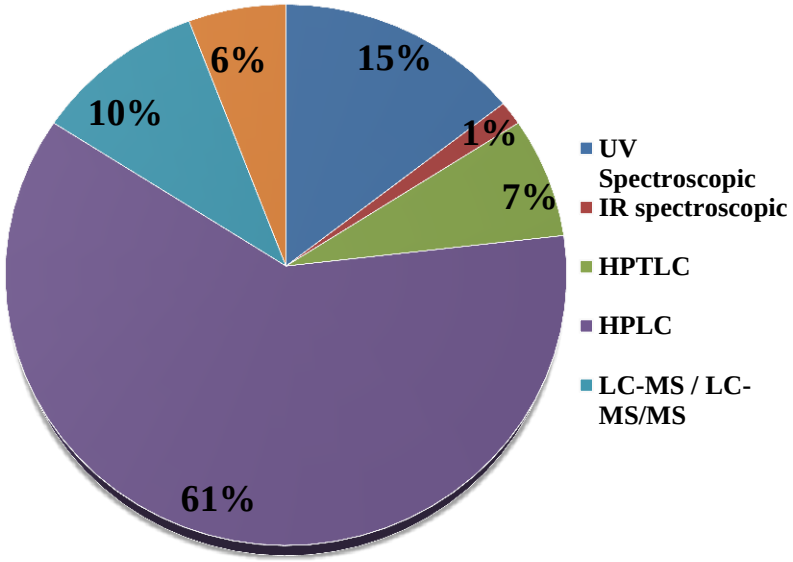

Fig. 2 Overview of analytical methods for the estimation of zidovudine in biological and pharmaceutical samples

UV-visible spectrophotometric method

According to the literature about 10 methods were reported for the estimation of AZT using spectrophotometry of which 4 methods are for determining AZT alone, while the others are for quantifying AZT in combination with other drug substances. Table.1 shows the summary of the reported spectrophotometric methods indicating the basic principle, λmax, solvent, Linearity, limit of detection and quantification (LOD and LOQ).

Table No. 1 Spectroscopic Method For Analysis of AZT in alone or in combination With other Drugs such as 3TC, NVP, EFV.

Sr. No	Method	Comp	Solvent	Linearity	λ max	LOD	LOQ	Ref
1	Quantitative estimation	AZT	0.1 N HCl		267	0.243	0.735	5
2	Simultaneous estimation by zero and first order	3TC, AZT, NVP	Methanol: Water 60:40		310, 270, 271	0.0938, 0.0012, 0.0129	0.2841, 0.0035, 0.0391	6

	derivative							
3	Quantitative estimation	AZT	Methanol: Water		266	0.6	1.8	7
4	Absorbance Method	AZT	Methanol		266	-	-	8
5	Multivariate Calibration by first and second order Derivative Method	AZT, 3TC	Water		First order 267.3, 249.3 Second Order 250.7, 229.0	0.6, 0.18	0.12, 0.03	9
6	Simultaneous estimation	ABA, 3TC, AZT	Acetronitrile Water Mixture		295.6, 279.8, 262.2	1, 1, 2	3, 2, 5	10
7	First order method for Antiretroviral	3TC, AZT	0.1 N HCl		279, 300	-	-	11
8	Extractive Spectroscopic method	3TC, AZT	Ion-pair complex extraction with chloroform		414, 414	0.106, 0.352	0.217, 0.722	12
9	Absorption Correction Method	EFV, 3TC, AZT	Methanol		305, 250, 254	0.08	0.2664	13
10	Absorbance Method	AZT	Distilled water		266	6.45	21.79	14

FT-IR Spectroscopic Method[15]

Ranju Bansal *et al.,*worked on New, simple and cost effective infrared spectroscopic (FT-IR) method developed for the quantitative estimation of AZT in bulk and tablet dosage form. The quantitative analysis of AZT was carried out in solid form using KBr pellet method and in liquid form using quartz cuvette. Linearity range was found to be 0.8–1.6% w/w in KBr pellet method and 250–1500 µg/ml in solution. The proposed methods were successfully applied for the determination of AZT in pharmaceutical formulation (tablets). The results demonstrated that the proposed methods areaccurate, precise and reproducible (relative standard deviation<2%), while being simple, economical and less time consuming than other available methods and can be used for estimation of AZT in different dosage forms.

HPTLC Method

As per literature survey 5 High Performance Thin Layer Chromatographic methods are reported for simultaneous estimation of AZT with other drugs. It was observed that in all methods methanol is used as solvent for the preparation stock solution. Table No. 2 shows the summary of the reported HPTLC methods indicating the basic principle, Stationary Phase, Mobile Phase, λmax, R_f, Linearity, limit of detection and quantification (LOD and LOQ).

Table 2 High Performance Thin Layer Chromatographic Methods for the estimation of AZT in combination with other drugs such as 3TC, NVP, ABA.

Sr. No	Method	Comp	Stationary Phase	Mobile Phase	Linearity	λmax	Rf	LOD	LOQ	Ref
1	Simultaneous Densitometric Measurement	3TC, AZT	Merck HPTLC Silica gel 60 F254 Plate	Toluene: Chloroform: Methanol (1: 6: 3)		271, 276	0.28, 0.57	3.06, 3.34	9.28, 10.13	16
2	Simultaneous estimation by Normal phase HPTLC	3TC, AZT	Merck HPTLC plates pre-coated with silica gel GF aluminum TLC plate	Toluene: Ethyl acetate: Methanol (4: 4: 2)		276	0.41, 0.60	2.90, 2.23	8.85, 7.90	17

3	HPTLC Densitometric method for simultaneous estimation	3TC, AZT, NVP	Pre-coated Silica gel GF aluminum TLC plate	n-Haxane: Chloroform: Methanol (1: 7: 2)		275	0.22, 0.55, 0.73	4.36, 3.68, 13.28	13.21, 11.15, 40.23	18
4	Simultaneous estimation by using QBD	3TC, AZT	Merck pre-coated Silica gel 60 F254 Plate	Ethyl acetate: Hexane: Methanol: Acetic acid (4: 4: 2: 0.1)		278	0.25, 0.73	5.09, 10.51	15.43, 31.85	19
5	Stability-indicating TLC-Densitometric Determination	ABA, 3TC, AZT	TLC aluminum plates pre-coated with silica gel 60F254	Toluene: Ethyl acetate: Methanol (8: 1: 1)		279	0.28, 0.46, 0.65	13.99, 7.33, 7.23	42.40, 22.32, 15.23	20

HPLC Method

There are 42 High Performance Liquid Chromatographic methods have been reported for AZT either alone or in combination with other drugs for estimation of AZT of which 12 literatures are for estimation of AZT in biological samples and 30 literatures are for estimation of AZT in pharmaceutical dosage forms.

Pharmaceutical samples

Analytical methods for the determination of AZT in pharmaceutical dosages forms using HPLC are shown in Table No. 3 Several mobile phase combinations are observed in this paper for estimation of AZT which are described with detail.

Table No. 3 Reported analytical HPLC methods for determination of AZT either alone or in combination with other drugs like Lamivudine (3TC), Nevirapin (NVP), Efavirenz (EFV) and abacavir (ABA) in pharmaceutical dosage forms

Sr. No	Aim of Study	Comp	Column	Mobile Phase	Linearity µg/mL	λ max (nm)	Flow Rate mL/min	Rt	Ref
1	HPLC method for stability studies	AZT	Merck C-18 column (250mm×4.6mm i.d. with particle size of 5µm)	Water : Methanol (77:23)	25–500	265	1	11.5	21
2	Simultaneous Determination	3TC, ABA AZT	HiQ Sil C 18 V column	0.01 M potassium dihydrogen ortho-phosphate (pH 3.0) and methanol (55:45 v/v)	0.5-10	272	0.8	3.8, 6.3, 8.1	22
3	Simultaneous Determination	3TC, AZT, NVP	HiQ Sil C 18 V column	0.015 M potassium dihydrogen ortho-phosphate (pH 5.0) and ACN (45:55 v/v)		270	1	2.45, 2.93, 3.72	23
4	Simultaneous Determination	3TC, AZT	Phenomenex Luna C18 (250mm×4.6mm i.d. with particle size of 5µm) column	0.1 M ammonium acetate buffer : Methanol (50:50v/v)		270	1.5	3.55, 5.08	24
5	Development of a liquid chromatographic assay for an anti-HIV tablet.	3TC, AZT	Hypersil BDS C18 (250mm×4.6mm i.d. with particle size of 5µm) column	ACN, 0.2M potassium dihydrogen phosphate and water (1:1) mixture		270	1	3.55, 5.08	25
6	Simultaneous Estimation of	3TC, AZT	Hypersil SS C18	Methanol : Phosphate buffer		270	1	4.64, 3.27	26

	Lamivudine and Zidovudine		(250mm×4.6mm i.d. with particle size of 5µm) column						
7	QbD approach to RP-HPLC method	AZT	Phenomenex Luna C18 250mm×4.6mm i.d. with particle size of 5µm) column	Potassium dihydrogen ortho-phosphate (pH 3.0) and methanol (50:50v/v)		266	1	4.4	27
8	RP-HPLC method for determination	AZT	Zodiac 100-5 C18 (250mm×4.6mm i.d. with particle size of 5µm) column	Methanol : ACN (60:40)	0.1-0.6	270	1	2.51	28
9	Development and Validation of Antiretrovirals like by HPLC	AZT, 3TC	Thermo Hypurity C18 (50×4.6mm, 5µm) column	50mM potassium dihydrogen phosphate : Methanol (65:35)	10-50, 10-30	221	1.2	4.96, 2.64	29
10	Development of and Validation from sustained release tablet	AZT	Waters RP-18 XTerra column	Water : Methanol (80:20)	40-220	266	1	9.1	30
11	HPLC Determination of tablet dosage form	AZT	Phenomenex Luna C18 (250mm×4.6mm i.d. with particle size of 5µm) column	ACN : 0.02M potassium dihydrogen phosphate	10-60	270	1	4.5	31
12	Estimation of fixed dose combination in Lamivudine and Zidovudine	3TC, AZT	RP C18 column	Water : ACN (pH 3.5 adjusted with OPA)		272	1	2.6, 3.2	32
13	Stability indicating HPLC for	3TC, AZT, ABA	Inertsil ODS-3V (250mm×4.6mm i.d. with particle size of 5µm) column	Ammonium dihydrogen phosphate and diammonium hydrogen phosphate buffers (pH 3.9) and Methanol		270	1	19.17,	33
14	Simultaneous Determination	3TC, AZT, NVP	Qualisil BDS C8 (250mm×4.6mm i.d. with particle size of 5µm) column	Water : ACN (70:30) pH 5 adjusted with OPA		250	1	3.1, 4.4, 7.0	34
15	Quantitation of Antiviral Drugs in presence of Impurities By LC	3TC, AZT, NVP	Water symmetry C18 (250mm×4.6mm i.d. with particle size of 5µm) column	0.025M ammonium acetate buffer (pH 4.2 adjusted with glacial acetic acid) and Methanol	15-45, 15-90, 20-60	270	1	13.4, 24.0, 33.0	35
16	Simultaneous Determination of three Antiretrovirals	3TC, AZT, NVP	Luna C18 (150mm×4.6mm i.d. with particle size of 5µm) column	50mM ammonium acetate buffer (pH 6.8) and Methanol		265	1	7.9, 12.8, 15.7	36
17	New HPLC analysis without using salt buffers	AZT	Zodiac C18 (250mm×4.6mm i.d. with particle size of 5µm) column	ACN : Water (90:10) pH to 4.8		240	1	2.47	37

18	Simultaneous Estimation of Tablet Dosage form	3TC, AZT	Inertsil ODS C18 (250mm×4.6mm i.d. with particle size of 5µm) column	ammonium acetate buffer (pH 4.0) and ACN : THF (60:30:10)		240	1	3.79, 2.54	38
19	Development and Validation	3TC, AZT	XTera (150mm×4.6mm i.d. with particle size of 5µm) column	Potassium dihydrogen phosphate buffer : ACN (55:45)		271	0.5	3.556, 5.364	39
20	Simultaneous Estimation in API and Dosage Form	3TC, AZT	Thermo Hypersil BDS C18 (150mm×4.6mm i.d. with particle size of 5µm) column	Potassium dihydrogen ortho phosphate buffer : Methanol : ACN (70:5:25)		267	0.8	2.21, 3.22	40
21	Simultaneous Estimation	3TC, AZT, NVP	NucleodurC 18 (150mm×4.6mm i.d. with particle size of 5µm) column	Ammonium acetate Buffer : Methanol (60:40)		270	0.8	4.01, 7.50, 22.57	41
22	Simultaneous Estimation	3TC, AZT, NVP	Phenomenex C18 (250mm×4.6mm i.d. with particle size of 5µm) column	0.1% OPA buffer (pH 6) :ACN (70:30)		268	1	3.44, 4.34, 6.12	42
23	Stability-indicating Simultaneous Estimation	3TC, EFV, AZT	XTerra C18 (150mm×4.6mm i.d. with particle size of 5µm) column	Water : Methanol pH 2.1 Adjusted with OPA (70:30)		275	1	1.91, 2.90, 7.52	43
24	Simultaneous Estimation	3TC, AZT, EFV	Symmetry C18 (250mm×4.6mm i.d. with particle size of 5µm) column	Methanol : Water (65:35)		250	1	2.519, 3.015, 24.103	44
25	Simultaneous Estimation in Combined Dosage Form	3TC, ABA, AZT	Eurosphere 100-5 C18 (250mm×4.6mm i.d. with particle size of 5µm) column	Methanol: potassium hydrogen phosphate buffer : ACN :THF (35:60:5:0.4)		271	0.6	4.87, 6.61, 8.21	45
26	Simultaneous Estimation	EFV, 3TC, AZT	Enable C18G (250mm×4.6mm i.d. with particle size of 5µm) column	ANC: 0.02M potassium dihydrogen ortho phosphate buffer pH 3.2 (30:70)		275	1	2.01, 2.94, 7.52	46
27	Simultaneous Estimation	3TC, AZT, NVP	Waters Symmetry C18 250mm×4.6mm i.d. with particle size of 5µm) column	ACN: Methanol: phosphate buffer pH 0.3 (20:30:50)		246	1	3.085, 3.680, 4.803	47
28	Fast analysis by UHPLC	AZT	Synoronis C18 Column 250mm×4.6mm i.d. with particle size of 5µm) column	Methanol: Water (20:80)		265	0.8	2.416	48

29	UPLC Method For Simultaneous Estimation	3TC, ABA, AZT	Symmetry C18 100mm×2.1mm i.d. with particle size of 1.7µm) column	Phosphate buffer(pH 3) : Methanol (60:40)		280	0.25	1.276, 1.010, 1.641	49
30	Simultaneous Estimation By UPLC	3TC, ABA, AZT	Symmetry C18 100mm×2.1mm i.d. with particle size of 1.7µm) column Make BEH	Phosphate buffer(pH 3) : Methanol (60:40)		280	0.25	1.019, 1.271, 1.617	50

Bioanalytical Sample

Various methods for the determination of AZT in biological samples like plasma, serum and urine are listed in Table No.4

Sample preparation techniques used for extraction of AZT from biological samples:

Sample preparation is an integral part of analytical methodology, and it was reported that about approximately 30% error generated in sample analysis was due to sample preparation. [51] Fig. 3 shows various diluents used for the analysis of AZT in biological samples. In major cases methanol was used as a diluent. The sample preparation techniques for the extraction of AZT from biological matrices (plasma, serum and urine) include protein precipitation with methanol, liquid–liquid extraction using ethyl acetate, methanol, acidified acetonitrile and dichloromethane ethyl acetate and isopropyl alcohol mixtre and solid phase extraction with water-methanol and methanol.

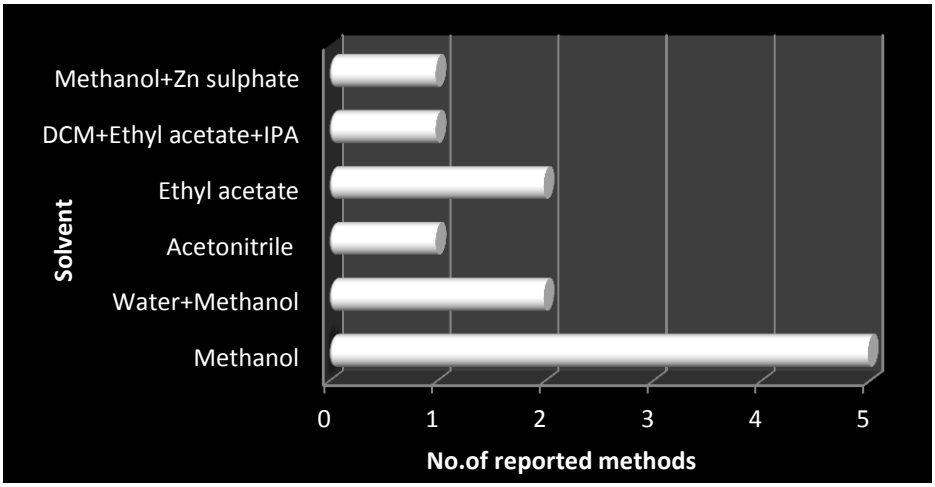

Fig. 3 Various solvents used for the extraction of zidovudine from biological samples.

Table No.4. Reported Bioanalytical methods of zidovudine (AZT) either alone or in combination with other drugs like 3TC, NVP

Sr. No	Method	Matrix	Internal Standerd	Sample preparation	Column	Mobile Phase	λ max (nm)	Flow Rate mL/min	Rt	Ref
1	Bioanalysis of AZT	Human plasma and urine	-	Liquid-liquid with ethyl acetate	Lichrosorb RP8 (300x3.9 i.d., 10µm)	1% acetate buffer (pH 5): Methanol (82:18)	265	1.5	-	52
2	Simultaneous quantification of AZT and its Glucoronide	Human serum	β-aAZTo stereoisomer of AZT	Solid Phase Extraction with	Analytical column (5 ,um, 250x4.6 mm i.d.) guarded by	15% ACN buffered to pH 2.7 with ammonium	267	1		53

				methanol	Adsorbosphere C18 guard column	phosphate				
3	Quantification fo AZT and AZT phosphate in peripherial blood	Human Blood	A22U	Solid Phase Extraction with methanol	RP C18 Column (Novapak RP C18 300x3.9mm i.d.), guarded with an Adsorbosphere C18,5µ cartridge	Sodium Dihydrogen phosphate: Disodium hydrophosphate buffer (140mM): Tetrabutyl ammonium dihydrogen phosphate (pH 7.5): ACN (957.3:7.7:35)	254	1	14.64	54
4	Determinatin of AZT, 3TC and NVP	Human plasma	Apobarbital	Solid Phase Extraction with water-methanol	Octylsilane (150x3.9mm i.d.)	20mM sodium phosphate buffer: ACN (86:14)	265	1	28.8, 14.8, 13.3	55
5	Simultaneous Determinatio n of NRTI	Plasma	-	Solid Phase Extraction with water-methanol	Symmetry Shield RP 18 column (150mm×4.6mm i.d./ 3.5µm particle) protected by a Symmetry Shield RP18 guard column	Acetate buffer (20mM potassium acetate adjusted to pH 4.6 with acetic acid and CAN	260	1		56
6	Simultaneous Estimation AZT, NVP	Human plasma	3-isobutyl-1-methyl xanthine	Liquid-liquid Extraction with Ethyl acetate	C18(150mm×4.6 mm I.D., 5µ particle size) column protected by (Lichrospher 100 RP-18e) guard column	Potassium dihydrogen phosphate (15mM, pH7.5): ACN (80:20)	260	1.5	2.4, 6.0	57
7	Determinatio n of 3TC, AZT and NVP in Capillary Blood Sampled on Filter Paper	Human blood	-	Solid phase Extraction on oasis HLB column with methanol-4mM Zn sulphate	Zorbax SB C8 (250 × 4.6 mm) column	Methanol: 0.05 mol/L acetic acid: Sodium acetate buffer	260	1	-	58
8	Simultaneous determination of AZT and NVP using isocratic RP-HPLC	Human plasma	-	Protein precipitation by using methanol	Phenomenex LUNA C18, column (250×4.6 mm i.d., 5µm) protected by Phenomenex HPLC guard cartridge	Water : ACN (pH 3.0) (73:27)	246	0.9	5.26, 9.50	59
9	Simultaneous Estimation of 3TC, AZT and NVP	Human plasma	Ciprofloxacin	Solid phase Extraction with	Analytical R.P.18e (250×4 mm, 5.0µm particle size)	20 mM ammonium acetate buffer (pH5.0):	265, 285		4.8, 13.4, 17.5	60

				methanol	Merck column	Methanol				
10	Simultaneous determination of AZT, 3TC, NVP	Human plasma	Esomeprazole	Liquid-liquid extraction DCM-ethyl acetate-IPA	Hypersil BDS C18 (250×4.6 mm i.d., 5µm) protected by Phenomenex column	0.1mM ammonium acetate buffer in 0.5% acetic acid: Methanol (40:60)	270	0.85	4.15, 4.81, 5.93	61
11	Quantification of AZT	Rat plasma		Liquid-liquid extraction with acidified ACN	RP-C18 column	ACN: Methanol: 0.1% Formic acid (20:20:60)	266	0.8	-	62
12	Fluorescence derivatization for chromatographic determination of Zidovudine based on the Huisgen reaction	Rat plasma		Liquid-liquid extraction with methanol	Cosmosil 5 C18 AR-II (250×4.6 mm i.d., 5µm) column	ACN 0.5mM Tris HCl buffer (pH 7.4) (50:50)	310 and 400	1	-	63

LC-MS

K. B. Kenney *et al.,* Developed HPLC method with MS/MS for Simultaneous determination of AZT and 3TC in human serum. Samples were extracted by solid-phase extraction. Elution of two compounds was done isocratically on Keystone Aquasil C18 (150x2mm, i.d, 5µm) column with a mobile phase comprised of ACN: Water (15:85v/v). Detection (LC-MS/MS) was performed on a PE-Sciex API III with mass spectrometer equipped with a turbo-ion spray (TISP) source for electrospray ionization. Ionization mode was switched from positive or negative ion mode within the same run.[64]

A.S. Pereira *et al.,*worked on a HPLC-MS/MS method was developed and validated to measure 3TC and AZT simultaneously in small volumes of human seminal plasma. Sample preparation was simple and rapid, requiring 25 µl of sample, the use of isotopically labeled 3TC and AZT as internal standards and ultrafiltration through a molecular mass cut-off membrane. 3TC and its internal standard were separated from AZT and its internal standard isocratically on Keystone Aquasil C18 (150x2mm, i.d, 5µm) column with a mobile phase comprised of ACN: Water. Detection was carried out using a PE-Sciex API III with mass spectrometer equipped with a turbo-ion spray (TISP) source for electrospray ionization. This validated method was used to analyze seminal samples obtained from six HIV-positive patients prescribed 3TC and AZT.[65]

S. Compain *et al.,* Developed a LC-MS/MS method to separate, detect and quantify with high sensitivity for the nucleoside analogues used in multitherapies (Zidovudine, stavudine, zalcitabine, didanosine, Lamivudine and abacavir) in plasma and in the intracellular medium. Sample preparation was done by solid phase extraction only for plasma sample. LC separation was carried out on Zorbax stable Bond C18 column with a mobile phase mixture solvent A (5% Methanol and 95% water) and solvent B (40% ACN and 60% water) followed by MS/MS analysis after electrospray ionization in either negative and positive mode for detection [66]

Ya-min Yao *et al.,* developed A quick and high-through LC-MS/MS method and applied to simultaneously quantify Lamivudine (3TC), stavudine (d4T), Zidovudine (AZT), Efavirenz (EFV), Nevirapin (NVP), and Lopinavir/Ritonavir (LPV/RTV) plasma concentrations. A combination of protein precipitation and liquid–liquid extraction was used to extract all compounds. LC separation was carried out on Eclipse XDB-C18 (150 mm × 4.6 mm, 5µm) analytical column with a mobile phase consisted of A(1%formic acid in water): B(1% formic acid in methanol) (20:80 v/v). The column oven temperature was maintained at 30° C and flow was maintained at 0.5mL/min. The method showed a good accuracy between 85% and 115% for all seven analytes. The whole run is 13 minutes. This method has been successfully used for analyzing 133 samples from 84 HIV-positive patients being treated with the combination therapy in China. To our knowledge, it is the first time to

use LC-MS/MS method to simultaneously quantify 3TC, d4T, AZT, EFV, NVP, LPV and RTV in China for investigating the relationship between blood drug concentrations and efficacy and/or toxicity.[67]
J. E. Rower *et al.,* explained a sensitive LC-MS/MS assay for determining AZT and 3TC in human plasma with a labeled isotopic AZT and 3TC as an internal standard, a solid-phase extraction was performed with an Oasis HLB 1 cm3 cartridge. LC separation was carried out on Phenomonex Synergi Hydro-RP (2.0 × 150 mm) with a mobile phase consisted of an aqueous solution of 15% acetonitrile and 0.1% acetic acid. Detection was accomplished by ESI/MS/MS in the positive ion mode with a TSQ quantum triple quadrapole MS/MS single reaction monitoring.[68]

M. K. Matta *et al.,*., developed rapid and sensitive LC–MS/MS method for the simultaneous quantitation of lamivudine, AZT and nevirapine in human plasma using abacavir as internal standard has been developed and validated. The analytes and IS were extracted from plasma by solid phase extraction using Oasis HLB cartridges and separated on a Hypurity Advance C18 column using a mixture of acetonitrile: 0.1% formic acid (76:24, v/v) at a flow rate of 0.8mL/min. Detection involved an API-4000LC–MS/MS with electrospray ionization in the positive ion mode and multiple-reaction monitoring for analysis. The method was successfully applied to a pharmacokinetic study involving a single oral administration of a combination tablet to human male volunteers.[69]

V.R. Kumar *et al.,* Worked to developed and validated a selective and sensitive high performance liquid chromatography–tandem mass spectrometry method for simultaneous determination of AZT, 3TC and NVP in human plasma. After Solid phase extraction, analytes and internal standard were run on Peerless Basic C18 column with a mobile phase 0.1% formic acid in water:methanol (15:85, v/v) was used with positive mass spectrometric detection. The method was validated over a concentration range of 5–1500 ng/mL for ZDV and 3TC and over the concentration range of 10–3000 ng/mL for NVP.[70]

Other Methods
Besides the spectroscopic and chromatographic methods there are also some electrochemical methods such as voltammetric and amperometric methods have been reported for determination of AZT and other anti-HIV drugs.

K. C. Leandro *et al.,*Determine AZT in Pharmaceuticals by Differential Pulse Voltammetry. The quality control of this substance in drugs is of outstanding importance to public health because of its undesirable effects and short therapeutic interval. For the determination of AZT in pharmaceuticals, an alternative analytical methodology using voltammetry was developed. The reduction of AZT at a hanging mercury drop electrode (HMDE) is at −0.96 V at pH 8.0. The influence of electroanalytical parameters, such as scan rate (20 mV/s), amplitude (50 mV), nature of the support electrolyte (phosphate buffer), and pH (8.0) on the voltammetric signal, was optimized. Under these optimized conditions, the AZT peak current varied linearly with its concentration from 0.25 to 1.25 mg/L (1 to 5 μM). Detection and determination limits of 0.0025 and 0.025 mg/L, respectively, and recovery of 99.88% with a relative standard deviation of 0.95% were obtained. The results obtained using this methodology in analyzing drugs containing AZT were compared favorably with those obtained by technique liquid chromatography as recommended by The United States Pharmacopoeia.[71]

A. A. Castro *et al.,*Determine AZT in Diluted Alkaline Electrolyte by Adsorptive Stripping Voltammetry at the Mercury Film Electrode. He describes a stripping method for the determination of AZT at the submicromolar concentra- tion levels. This method is based on the controlled adsorptive accumulation of AZT at the thin-film mercury electrode, followed by a linear-sweep stripping voltammetry measurement of the surface species. Optimal experimental conditions include a NaOH solution of 2.0×10^{-3} mol·L^{-1} (supporting electrolyte), an accumulation potential of −0.30 V and a scan rate of 100 mV·s−1. The response of AZT is linear over the concentration range 0.01 - 0.08 ppm. After an accumulation time of 5 minutes, the detection limit was found to be 0.67 ppb (2.5×10^{-9} mol·L^{-1}). More convenient methods to measure AZT concentration in the presence of the didanosine, acyclovir, NVP, 3TC, and EFV, were also investigated. The presence of AZT together with ATP or ssDNA demonstrates the utility of this method. [72]

K. Peckova *et al.,*Work on volumetric Voltammetric Determination of AZT Using Silver Solid Amalgam Electrodes. It is based on differential pulse voltammetry at silver solid amalgam electrode with polished surface (p-AgSAE) or surface modified by mercury meniscus (m-AgSAE). The electro-reduction of AZT in basic media at these electrodes gives rise to one irreversible cathodic peak. Its potential in 0.05 mol L_1 borate buffer, pH 9.3 at ca. _1050 mV is comparable to that using hanging mercury drop electrode (HMDE). Achieved limits of

quantitation are in the 10_7 mol L_1 concentration range for both amalgam electrodes. According to the procedure based on the standard addition technique, the recoveries of known amounts of AZT contained in pharmaceutical preparations available in capsules were 101.4_1.8% (m-AgSAE), 100.3_3.5% (p-AgSAE) and 102.0_1.0% (HMDE) (n¼10). There was no significant difference between the values gained by proposed voltammetric methods and the HPLC-UV recommended by the United States Pharmacopoeia regarding the mean values and standard deviations. [73]

A.A. Rafati, A. Afraz *et al.,proposed* Amperometric sensing of anti-HIV drug AZT on Ag nanofilm-multiwalled carbon nanotubes modified glassy carbon electrode. A new AZT sensor was fabricated on the basis of nanocomposite of silver nanofilm (Ag-NF) and multiwalled carbon nanotubes (MWCNTs) immobilized on glassy carbon electrode (GCE). The modified electrodeswere characterized by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), cyclic voltammetry (CV), and linear sweep voltammetry (LSV) techniques. Results showed that the electrodeposited silver has a nanofilm structure and further electrochemical studies showed that the prepared nanocomposite has high electrocatalytic activity and is appropriate for using in sensors. The amperometric technique under optimal conditions is used for the determination of AZT ranging from 0.1 to 400 ppm (0.37 μM–1.5mM) with a low detection limit of 0.04 ppm (0.15 μM) (S/N = 3) and good sensitivity. The prepared sensor possessed accurate and rapid response to AZT and shows an average recovery of 98.6% in real samples. [74]

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