A COMPREHENSIVE ASSESSMENT OF ANALYTICAL TECHNIQUES FOR QUANTIFYING THE NUCLEOSIDE ANALOGUE REVERSE TRANSCRIPTASE INHIBITOR (NRTIS) STAVUDINE

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Abstract

HIV/AIDS should always be treated for the remainder of their lives with influential life-saving drugs such as nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors. HIV/AIDS remains a major global public health problem, having claimed the lives of 40.1 million [33.6-48.6 million] people to date. In 2021, 650 000 [510 000-860 000] people may have died from HIV-related causes, and 1.5 million [1.1-2.0 million] will have become infected. HIV infection has no cure exists. There is currently no cure for HIV/AIDS. Once infected, your body is unable to rid itself of it. There are, however, innumerable medications that can control HIV and prevent complications. These drugs are known as antiretroviral therapy (ART). Based on their molecular mechanism and resistance profiles, these drugs are separated into six distinct classes: (1) Reverse transcriptase inhibitors predicated on nucleoside aptamers. Just before to 1996, there had been few antiretroviral treatment options for HIV-1 infection. Prophylaxis against common opportunistic pathogens and management of AIDS-related illnesses composed the major part of HIV-1 clinical management. The development of inhibitors of reverse transcriptase and protease, two of HIV-1's three essential enzymes, and the introduction of drug regimens that combined these agents to increase overall efficacy and durability of therapy reinvented the treatment of HIV-1 infection in the mid-1990s. A timeline for both the development and approval of antiviral drugs for human use.

Keywords: ‘analysis’ ‘NRTIs’ and STA

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1. Introduction

IUPAC name on stavudine as 1-[(2R,5S) -5-(hydroxymethyl)-2,5-dihydrofuran-2-yl] -5-methyl -1,2,3,4-tetrahydropyrimidine-2,4-dione. Formula Molecular Its racemic mixture C10H12N2O4, also termed as 2',3'-didehydro-3'-deoxythymidine, is a white, crystalline solid or powder with little to no smell. Stavudine appears to be 83 mg/mL dissolved in water and 30 mg/mL accessible in propylene glycol at 23° C.

Approaches such as emission spectra, chromatography, critical assessment, capillary electrophoresis, and chemometrically assisted methods can be used to calculate the amount of ketones existing in dosage forms and biological fluids, etcetera.Figure 2 is a summary of the analytical methods used to figure out STA levels. Figure 3 shows how the number of ways to test for STA changed from 1978 to 2022.

This objective seeks to simplify, contextualise, and discuss the many analytical procedures that can be used to measure STA in a variety of compositions and biological matrices. This will fulfil assignment criteria (Figure 4). Volumetric analysis, optical methods, chromatographic, electro analysis tools, linear electrochemical techniques, bioanalytical methods, and chemometric analysis were frequent studies. Capillary electrophoretic and electro analytical tests are many other.

Volumetric Approaches

Evaluation via Titrimetry

In the field of science, titration is implemented as a quantitative method of analysis. How much of a renowned chemical must be introduced to a solution whose concentration has been established in order to validate the reaction The method was evaluated in light of the ICH recommendations

Integrating Optical Approaches

Spectroscopic techniques in the UV/visible area

A spectrophotometer is an external device of light that somehow a sample emanates at a multitude of wavelengths. Measurement tool that is extremely exact. Spectrophotometry is a testing tool that is designed when other, more complex and costlier methods, like GLC or HPLC, are inaccessible. These speedy, minimal, and simple methods come with lots of pros

"Table 1 :Spectrophotometry"

method	matrices	solvent	lambda max	linearity	lod	loq	ref
UV	bulk and pharmaceutical dosage forms	N/A	250nm	4-20 mcg / ml.	N/A	N/A	[1]
UV	bulk and pharmaceutical dosage forms	phosphate buffer pH 6.8	266 nm	3-24 µg/mL	N/A	N/A	[2]
UV	bulk and pharmaceutical dosage forms	Methanol, Distilled water	250 nm	2-20 µg / ml	0.45	1.35	[1]
UV	bulk and pharmaceutical dosage forms	Bromate-bromide mixture	A520 B610 C550nm	A 0.125–1.75 B 1.0–10.0 C 1.0–9.0	A 0.03 B 0.15 C0.11	A 0.11 B 0.44 C 0.34	[3]

Spectrophotometry combined

analyte	method	matrices	solvent	lambda max	linearity	lod	loq	ref
(STA), (LMV) and (NVP)	UV spectroscopy method	tablet formulations	Phosphate buffer pH – 6.8	t 266, 271 and 315 nm	10 to 30 µg / ml	N/A	N/A	[4]
STA and chloranilic acid	uv spectroscopy method	bulk and dosage forms.	1,4-dioxan	526 nm	N/A	N/A	N/A	[5]
METHOD A								

STA	uv spectroscopy method	bulk and pharmaceutical formulations	1,2-naphthaquinone-4-sulfonic acid	464 nm	2.0-12.0 µg/mL	N/A	N/A	6
METHOD B								
STA	uv spectroscopy method	bulk and pharmaceutical formulation	1,10-phenanthroline	480 nm	5.0-30.0 µg/mL	N/A	N/A	
(NVP), LMV and STA	HPLC and spectrophotometric methods	tablet/capsule	N/A	300, 285, and 270 nm		N/A	N/A	7
NVP,LMV,STA	UV visible spectrophotometer	bulk and dosage forms.	Water 0.01 N HCl pH 4.5 Acetate buffer pH 6.8 phosphate buffer	N/A	N/A	N/A	N/A	8
LMV,NVP,STA	HPLC and n UV spectrophotometric	pharmaceutical dosage forms	HPLC grade methanol, acetonitrile and isopropyl alcohol					9
METHOD 1 HPLC						0.17 ± 0.07, 0.03 ± 0.01, 0.12 ± 0.1	0.52 ± 0.22, 0.09 ± 0.05, 0.37 ± 0.05	
METHOD 2 UV						0.19, 0.29, 0.25	0.19, 0.29, 0.26	
STA, LMV,NVP	UV spectroscopy, reverse phase HPLC, and HPTLC	tablets	sodium phosphate buffer ,acetonitrile, with pH adjusted to 3.5 using phosphoric acid	t 265 nm	s 0.8–6.4 g/ml (r = 0.996), 4–32g/ml (r = 0.9962) and 5.33–42.64g/ml (r = 0.9843)	N/A	N/A	10

Chromatographic protocols

Optimal Liquid Chromatography

It is the most extensively used chromatographic separation technique medication compounds. Lc is an effective strategy and for its sensitivity, persistence, and efficiency. Sridhar, N. Y. et.al used UPLC for analysis of LMV, NVP,STA, using methanol as solvent and column used is acquity x-bridge column 9(50 x4.6mm, 3.5 um) using phosphate buffer of ph2.2 is better compared to other reported method

"Table 2: Chromatography combined"

analyte	method	matrices	mobile phase	stationery phase	detection	flow rate	ct	linearity	lod	loq	ref
STA, LMV,NVP	UPLC	TABLET FORMULATION	gradient elution of 0.1%perchloric acid in water adjusted the ph2.6 with dil sodium hydroxide solution and acetonitril	Acquity UPLC HSS T3 (2.1 × 100 mm), 1.8 μ column	UV detection at 265nm	flow rate of 0.2ml/min		values of correlation coefficients greater than 0.995	%w/w for LA-Imp-1 to, LA-Imp-6, ST-Imp-1 to ST-Imp-3, NE-Imp-1, to NE-Imp-2 was 0.010, 0.010, 0.010, 0.011, 0.013, 0.009, 0.024, 0.020, 0.019, 0.015, 0.010 and 0.011	%w/w for LA-Imp-1 to, LA-Imp-6, to ST-Imp-3, NE-Imp-1, to NE-Imp-2 is 0.020, 0.019, 0.020, 0.021, 0.026, 0.018, 0.049, 0.040, 0.039, 0.030, 0.021 and 0.022	11
STA		CAPSULES									12
STA	RR-LC	bulk drug	water andmethanol in the ratio of 85:15 (v/v)	C18	UV detection at 265 nm	f 0.5 mLmin-1	f 0.5 mL min-1	he correlation coefficient obtained was greater than 0.9999	N/A	.500 Ïg mL -1	8
STA	Isocratic, simultaneo	bulk drug	mixt. of Et acetate with 4% (vol./vol.) of ethylene glycol	common silica column	phase separat	n/a	n/a	N/A	N/A	N/A	13

	us normal-phase LC separation				ion						
zidovudine (AZT), LMV, didanosine (ddA) and its administered form (ddl), stavudine (d4T) and hivid (ddC)	electrochromatography	e reverse transcriptase inhibitors in a pool of endogenous nucleosides		β-cyclodextrin-bonded silica	UV DETECTION		N/A	5 to 25 gmL–1	0.5 gmL–1 (ddI) and 0.9 gmL–1 (AZT)	ranged from 1.7 gmL–1 for ddI to 3.2 gmL–1 for AZT	14
STA + LMV + NVP	Thin Layer Chromatography and Self-Reported Adherence										15
AZT, STA,LMV,NVP	Micellar Electrokinetic Capillary Chr	capsules	A buffer consisting of 5 mM sodium tetraborate at pH 9.8, containing 50 mM SDS, 30% methanol and 5% ethanol	c18	phase separation		N/A	N/A	N/A	0.037, 0.051, 0.029 and 0.028 mg/L	16

	omatography										
LMV, STA, tenofovir, AZT and efavirenz	micellar liquid chromatography	TABLET FORMULATION	Four aq. micellar mobile phases buffered at pH 7 sodium dodecyl sulfate as the tensioactive, and 1-propanol or 1-pentanol as the org. modifier	C18 apolar column	UV detection set at 214 nm	1 mL min-1	NO TEMPERATURE CONTROL	0.5 and 50 ppm (r2 > 0.9995)	lower than 0.25 ppm	N/A	17
LMV, NVP,STA	UPLC	TABLETS	METHANOL	acquity x-bridge column 9(50 x4.6 mm, 3.5 um) using phosphate buffer og ph2.2	UV detection was performed at 266 nm	1.0 mL/min-1	30°	3.2-19.2, 4.4-26.4, and 8.8-52.8 µg/min-1	N/A	N/A	18
abacavir sulphate, valaciclovir hydrochloride, acyclovir sodium, ganciclovir sodium , didanosine, efavirenz, indinavir sulphate, LMV, NVP anhydrous, zalcitabine and AZT , ritonavir AND STA	micellar liquid chromatography	tablets	SDS, which was buffered with disodium hydrogenphosphate/sodium dihydrogenphosphate 10 mM at pH 7, and lastly 1-propanol, 1-butanol or 1-pentanol was added to achieve the desired concentration		n/a	n/a	n/a	f 0-10 µg mL -1	m 6 to 30 ng	10s criterion	19

LMV,STA	Gas chromatography	liposomal formulations	n2 carrier gas	BP 624 column	GC 17A Shimadzu with FID	N/A	N/A	0.9988	100 ppb	N/A	20
amivudine, STA, and NVP	micellar electrokinetic chromatog	pharmaceutical formulations	buffer consisting of pH 9.8 10 mM sodium tetraborate, 100 mM sodium dodecyl sulfate (SDS) and 15% 2-propanol.	a 75 µm i.d. uncoated fused-silica capillary	N/A	N/A	N/A	f 20–200, 5–50 and 25–250 lg mL 1	0.6, 0.8, and 0.6 lg mL1	1.8, 2.0, and 1.6 µg/mL-1	21

"Table 3: C HPLC Single Component"

analyte	method	matrices	mobile phase	stationery phase	detection	flow rate	ct	linearity	lod	loq	ref
Lamuvidine and Stavudine	RP-HPLC	tablet dosage form	0.1M disodium hydrogen phosphate anhyd. buffer: methanol, adjusted to pH 5 with glacial acetic acid in the ratio of 50:50, vol./vol	REVERSED PHASE BDS HYPERSIL C18 COLUMN	UV detector at 238 nm	flow rate of 1 ml/min	N/A	good ($r^2 > 0.999$)	N/A	N/A	22

β-stavudine	RP-HPLC	TABLET dosage form	0.01 mmol·L-1 ammonium acetate soln.-acetonitrile (96.5:3.5) and 0.01 mmol·L-1 ammonium acetate soln.-acetonitrile (75:25) with gradient elution	SUPELCOSIL LC-18-DB column (4.6 mm × 250 mm, 5 µm) with Agilent 1100 liq. chromatog	UV DETECTION 254 nm	N/A	25 °C.	0.51-26 µg·mL-1 (r = 1.000) for β-stavudine, 0.13-27 µg·mL-1 (r = 1.000) for thymine, 0.50-25 µg·mL-1 (r = 1.000) for βthymidine and 1.7-6.3 µg·mL-1 (r = 1.000) for 5-O'-benzoyl-stavudine.	2.5 ng · mL-1	N/A	23
stavudine	RP-HPLC	bulk and pharmaceutical dosage forms	0.01M ammonium acetate buffer:methanol (60: 40).	Venusil XBP C-18, 5 µm column in iso cratic mode	uv detection 265 nm	1.0 mL/min	ambient	f 25-75 mcg / ml			24
STAVUDINE	RP-HPLC	bulk and capsule dosage forms	methanol: acetate buffer pH 6.0 (40:60)	A Phenomenex Gemini C- 18, 5 µm column having 250 × 4.6 mm i.d. in isocratic mode	UV DETECTION AT 265 NM	1.0 mL/min	N/A	25-75 mcg/mL			25
stavudine	HPLC	capsules	methanol-water (15:85)	Attach C18 column (150 mm × 4.6 mm, 5 µm)	UV DETECTION AT 266 NM	N/A	N/A	9.63-77.04 µg/mL (r = 1.0000)			26

STAVUDINE	HPLC	bulk and pharmaceutical dosage forms	acetonitrile and 0.05M potassium dihydrogen phosphate (pH 4.2) in the ratio of 50:50	RPC-18 column using an isocratic HPLC system	UV DETECTION AT 266 NM	1 mL/min	N/A	0.1-140 µg/Ml	N/A	N/A	27
STAVUDINE	HPLC	pharmaceuticals or suspected products		C18 column	UV DETECTION				N/A	N/A	28
METHOD A			Ammonium acetate buffer 0.025 M pH 4.0 AND METHANOL	YMC pack ODS-AM, 250 × 4.6 mm, 5m	λ = 270 nm	1.0 ml/min	ambient				
METHODB			Potassium phosphate buffer 50 mM pH 5.65 AND acetonitrile	Symmetry C18 250 × 4.6, 5m	λ = 260 nm	1.5 ml/min	30 °C				
Zalcitabine (ddC), lamivudine (3TC), didanosine (ddI), stavudine (d4T), carbovir (CBV), zidovudine (AZT), tenofovir (PMPA) (tenofovir diisopropyl fumarate, TDF),	HPLC	PHAARMACEUTICALS	acetic acid-hydroxylamine buffer (ionic strength 5 mM, pH 7)-acetonitrile elution gradient	Atlantis dC18 column	UV at 260 nm	N/A	AMBIENT	30-10,000 ng/mL plasma concn	N/A	30-90 ng/mL concn	29
stavudine	HPLC as	PHARMACEUTI	water-methanol in the ratio of 90:10	C18 column	UV DETE	N/A	N/A	25-500 µg mL-1	N/A	N/A	30

	say method	CAL DOSAGE FORM			CTION AT 265 NM						
											358
STAVUDINE	RP-HPLC	TABLET	water-methanol (34:66)	ODS C18 column	UV DETECTION 266 nm	N/A	N/A	8-600 µg mL-1 (r = 0.9999)	N/A	N/A	31

"Table 4: HPLC Combained"

analyte	method	matrices	mobile phase	stationery phase	detection	flow rate	ct	linearity	lod	loq	ref
LMV, STA and efavirenz	RP-HPLC	bulk and pharmaceutical for mulation	0.2% Triethylamine: Acetonitrile (40:60 pH: 2.0).	reversed-phase L10 column (ZORBAX SB CN Column, 5µ, 150 mm × 4.6 mm)	uv detection at 258 nm	0.6 mL/min	ambient				32

LMV, STA and Nevirapinein	RP-HPLC	pharmaceutical dosage forms	0.1%OPA:Acetonitrile(50:50)	Zorbax C8(250 mm × 4.6mm; 5 µm)	PDA detector at 290nm	N/A	room temperature	37.5-112.5µg/mL (r2 = 0.999), 7.5-22.5µg/mL (r2 = 0.999), 50-150µg/mL (r2 = 0.999)	0.146, 0.1705 and 1.215	0.486, 0.5682 and 4.049	33
LMV, STA	RP-HPLC	Tablet dosage form	MeOH, acetonitrile, and 0.05 M phosphate buffer (adjusted to pH 4.5 with ortho-phosphoric acid) at a ratio of 60:20:20 vol./vol./v	C 18 column grace smart RP18 (250 × 4.6 mm, 5 µm)	ASPD detector at 254 nm	1.0 mL/min	n/a	10-602 2 µg/mL (r = 0.9992) for lamivudine and 10-60 µg/mL (r = 0.999) for stavudine	0.16µg/mL and 0.14µg/mL	0.49µg/mL and 0.41µg/mL	34
STA,LMV	RP-HPLC	pharmaceutical dosage forms	HPLC grade water and methanol in the ratio of 90:10 vol./vol.	reverse phase C18, 4.5µ (250 × 4.6 mm)	UV DETECTION 257nm	1.0 mL/min	N/A	20-100 µg/spot	3.52µg/ml/0.825µg/ml	10.61µg/ml/2.	35

										501μg/ml	
STA,LMV	RP-HPLC	tablet dosage form by RP-HPLC	ammonium formate buffer (pH 3.5) and acetonitrile in the ratio of 15:85 vol./vol.	Phenomenex C18 column (250 × 4.6 mm id, 5 µm particle size utilizing Shimadzu HPLC LC-10AT VP)	UV-VISIBLE AND PDA DETECTOR-AT266NM	1.0ML/MIN	N/A	1 - 15 µg/ml for Stavudine and 2 – 30 µg/ml for Lamivudine	0.0963µg/ml,0.4101µg/ml	0.2917µg/ml,1.2428µg/ml	36
LMV,STA	RP-HPLC	TABLET DOSAGE FORMS	methanol, acetonitrile and 0.05 M phosphate buffer (adjusted to pH 4.5 with ortho-phosphoric acid) at a ratio of 60:20:20 vol./vol./v	C 18 column grace smart RP18 (250 × 4.6 mm, 5 µm) in isocratic mode	ASPD detector at 254 nm	1.0ML/MIN	N/A	10-602 2 µg/mL (r = 0.9992) for lamivudine and 10-60 µg/mL (r = 0.999) for stavudine.	0.16 µg/mL and 0.14 µg/Ml	0.49 µg/mL and 0.41 µg/mL	37

LMV,STA	RP-HPLC	tablet dosage form	94: 6 v/v (buffer: acetonitride)	hypersil ODS C18 150×4.6 mm i.d, 5µm size was fixed	270mm	N/A	N/A	37.5 to 225 ppm and 10 to 60 ppm	N/A	N/A	38
LMV,STA	RP-HPLC	tablet dosage form	0.1 M disodium hydrogen phosphate anhyd. buffer: methanol, adjusted to pH 5 with glacial acetic acid in the ratio of 50:50, vol./vol	reversed phase BDS HYPERSIL C18 column	238nm	1.0 ml/min	N/A	15-19(µg/ml) ,3-18(µg/ml)	N/A	N/A	39
LMV,STA	RP-HPLC	combined dosage forms	acetonitrile-0.02 M ammonium acetate buffer (pH 4.5) in a gradient elution mode	Kromasil C8 (250 mm × 4.6 mm i.d.; 5 µm) column	265 nm	1.oml/min	N/A	2.5-50 µg/mL for 3TC and 0.5-10 µg/mL for d4T	0.82 µg/mL and 0.33 µg/mL	2.5(µg/ml),0.5(µg/ml)	40
LMV,STA	HPLC method development and validation	bulk and combined tablet dosage form	Phosphate buffer pH 2.5:methanol (80:20 vol./vol.)	symmetry C-18, Thermo (100 × 4.6,), i.d, and 3.5 µm particle size in isocratic mode	UV detector dual i.e. wavelength of 262 nm for stavudine and 271 nm for lamivudine	0.8ML/MIN	N/A	25-125 µg/mL			41

LMV,STA, NVP	RP-HPLC	combined dosage form	mixt. of phosphate buffer:acetonitrile (pH-3.5, adjusted with 1% Glacial acetic acid) in the ratio of 85:15% vol./vol	A C8 Intersil column [4.6 (i.d.) × 250 mm, 10 μm]	266.00 nm	1.0 ML/MIN	N/A	15-75 μg/ml, 4-20 μg/ml and 20-100 μg/ml	15μg/ml, 4μg/ml and 20μg/ml	45μg/ml, 12μg/ml and 60 μg/ml	42
(NVP), LMV and STA	Comparison of HPLC & spectrophotometric methods	single tablets/capsules	15mM phosphate buffer pH 7.5 and acetonitrile (80:20 v/v)	C18 column	UV detector set at 260 nm.	1.0 ML/MIN	ambient	6.25(μg/ml),12.5(μg/ml),25.0(μg/ml),50.0(μg/ml)	N/A	N/A	7
LMV,STA	RP-HPLC	TABLETS	methanol: 0.1% wt./vol. of ammonium acetate, adjusted to pH 3.8 with glacial acetic acid in the ratio of 15:85, vol./vol.	Sunfire C18 250 × 4.6 mm, 5 μm particle size column in isocratic mode	UV detection at 266 nm	1.2 ML/MIN	N/A	75-225 μg/mL and 20-60 μg/mL	N/A	N/A	43
LMV,STA	RP-HPLC	tablet dosage form	methanol and water (80:20 vol./vol.)	reversed-phase C-18 SYMMETRY column	UV detection at 266nm	N/A	n/a	0.999,0.999	0.9μg 0.8 μg	2.8 μg 2.5	44

										µg	
STA,LMV	HPLC	human plasma	10 mM acetate buffer pH 6.5 and acetonitrile	C18 column	UV detection at 265 nm			50-3,000 ng/mL for stavudine and 50-5,000 ng/mL for lamivudine			45
LAM, AZT and abacavir	RP-HPLC	tablet dosage form	0.01 M potassium dihydrogen ortho-phosphate (pH 3.0) and methanol (55:45 vol./vol.)	HiQ Sil C 18 V column	272 nm	0.8 mL/min	N/A	5-250 µg/mL for both zidovudine and abacavir and 5-140 µg/mL for lamivudine	N/A	N/A	46
STA	HPLC	BULK DRUG	isocratic elution with methanol/water (20:80)	LiChrospher C18 column (250 × 4.6, 5 µm)	UV detection at 266 nm			0.5-5.0 µg/mL	N/A	0.021 µg thymine/mL and 0.134 µg	47

											thymidine/mL	
STA,LMV	RP-HPLC	combined dosage form	acetonitrile-methanol-water at 10:1:1 ratio	isocratic HPLC with a single tunable absorbance detector and µ-Bondapack C18 column	280 nm							48
STA,LMV,NVP	RP-HPLC	multicomponent pharmaceuticals			UV detection at 265 nm				0.1-75.0 µg/mL-1, 0.6-375.0 µg/mL-1, and 0.8-500.0 mg/mL-1			49
LMV,STA, NVP	HPLC	TABLETS	phosphate buffer pH 3.0 with acetonitrile (75:25),	LiChrospher ODS column	UV DETECTION AT 266NM	1.oml/min	30oC	15-135 µg/mL (lamivudine), 4-36 µg/mL (stavudine), and 20-180 µg/mL (nevirapine)	N/A		N/A	50

LAM,STA, NVP	HPLC	antiretroviral fixed dose combinations	mobile phase (A) comprising of 80% of 10 mM acetate buffer adjusted to pH 3.5 with glacial acetic acid and 20% MeOH, and mobile phase (B) comprising of 50% acetonitrile with 50% iso-Pr alc	C-18 column	UV DETECTION 270 nm	0.6 ML/MIN	N/A	5–100 g ml−1, 5–100 g ml−1, 5–100 g ml−	N/A	N/A	51
LMV,STA	first derivative spectrophotometry and high performance liquid chromatography	antiretroviral fixed dose combinations							0.13g/m	0.40g/m	51
METHOD 1	UV-spectrophotometry				UV DETECTION AT 280 and 300 nm			2–20 g/ml, 2–14 g/ml			
METHOD 2	HPLC		MeOH:water (20:80) as the mobile phase	reverse phase column	UV DETECTION 270 nm			1–10 g/ml, 1–10 g/ml			
LMV,STA, NVP	RP-HPLC and ultraviolet spectrophotometric method	pharmaceutical dosage forms	mobile phases	reversed-phase C-18 SYMMETRY column	uv detection			2.5–20, 2–20 and 4–30 g/ml			9
hplc method									0.17 ± 0.07 ,0.03 ± 0.01, 0.12 ± 0.1	0.52 ± 0.22	

										0.09 ± 0.05 0.37 ± 0.05	
uv method									0.19 0.29 0.25	0.58 0.92 0.76	
			Lamivudine: Methanol:water (70:30)		270 nm	0.75ML/MIN					
			Stavudine : Methanol:water (20:80)		270 nm	0.80ML/MIN					

			Nevirapine :Solvent A: methanol:water (20:80) 0.18 27 5 Solvent B: acetonitrile:isopropyl alcohol (50:50)		27 nm	0.18 ML/MIN					
STA,LMV,NVP	UV spectroscopy, reverse phase HPLC, and HPTLC	tablets			266, 271, and 315 nm						52
uv method								0.8–6.4 g/ml (r = 0.996), 4–32g/ml (r = 0.9962) and 5.33–42.64g/ml (r = 0.9843)			
rp hplc			20 mM sodium phosphate buffer (contg. 8 mM 1-octanesulfonicacid sodium salt):acetonitrile (4:1, vol./vol.) with pH adjusted to 3.5 using phosphoric acid	C18-ODS-Hypersil (5 μm, 250 mm × 4.6 mm) column in isocratic mode				10–50g/ml (r = 0.9968), 50–250g/ml (r = 0.9999) and 66.6–333.2g/ml (r = 0.9996)			

hptlc			chloroform:methanol (9:1, vol./vol.)	precoated plate of silica gel 60 F254	densitometric absorbance mode at 265 nm			10–50g/ml (r = 0.997), 50–250g/ml (r = 0.998) and 66.6–333.2g/ml (r = 0.9993)			
STA ,LMV,NVP	rp- hplc	tablets	0.01M sodium octane sulfonate buffer (pH 2.8) and methanol (60: 40 vol./vol.)	inertsil ODS - 3V (250mm × 4.6mm, 5u)	UV detection 270 nm			7.5 µg/mL to 30.0 µg/mL, 28.0 µg/mL to 112.0 µg/mL and 37.5 µg/mL to 150.0 µg/mL			53
zalcitabine, LMV, didanosine, STA, AZT, Abacavir	liquid chromatography		human blood plasma	new Polarity dC C18 silica column	1.0ml/min	n/a		0.781760.078, 1.087960.044, 1.653360.022, 1.396060.025, 1.228160.033, 1.871960.035and 2.145260.011	n/a	10.0 ng/ml	54

"Table 5: HPTLC"

analyte	method	matrices	mobile phase	stationery phase	detection	flow rate	linearity	lod	loq	ref
sulfamethoxazole and trimetho	hptlc	combination								55

prim, LMV,STA,NVP		tablets								
STA,NVP	normal-phase HPTLC method	combination tablets	ethylacetate, methanol, toluene and concd. ammonia (38.7:19.4:38.7:3.2, v:v:v:v)	silica gel 60F 254 plates	densitometric detection 254 nm		0.284, 0.276 and 0.327	N/A	N/A	[56]
STA, LMV,NVP	UV spectroscopy, reverse phase HPLC, and HPTLC	TABLETS								[52]
	uv-spectroscopy				266, 271, and 315 nm		0.8–6.4 g/ml (r = 0.996), 4–32 g/ml (r = 0.9962) and 5.33–42			

							.64g/ml (r = 0.9843)			
	RP-HPLC		20 mM sodium phosphate buffer (contg. 8 mM 1-octanesulfonicacid sodium salt):acetonitrile (4:1, vol./vol.) with pH adjusted to 3.5 using phosphoric acid	C18-ODS-Hypersil (5 µm, 250 mm × 4.6 mm) column			10–50 g/ml (r = 0.9968), 50–250g/ml (r = 0.9999) and 66.6–333.2g/ml (r = 0.9996)			

	HPTLC		chloroform:methanol (9:1, vol./vol.)	precoated plate of silica gel 60 F254	densitometric absorbance mode at 265 nm		10–50 g/ml (r = 0.997), 50–250g/ml (r = 0.998) and 66.6–333.2g/ml (r = 0.9993)			
LMV, STA	HPTLC		toluene:n hexane:tetrahydrofuran (7:1.5:1.5 vol./vol.)	Silica gel 60 F254 plate	uv detection at 253nm		2.5-7.4ug, 0.5-1.4ug			57
hptlc sin										

STA	HPTLC assay method	bulk drug and formulation	toluene-methanol-chloroform-acetone (7.0:3.0:1.0:1.0, vol./vol./vol./vol.)	TLC silica gel 60 F 254 on aluminium, 10- × 20-cm (cut from a 20- × 20-cm) 200-µm thickness column	Densitometric analysis at 270 nm		30-1000 ng/spot	10 ng/spot	30 ng/spot	58

STA-STAVUDINE,LMV-LAMIVUDINE,NVP-NEVIRAPINE

Hyphenation

To optimize the efficiency between both methods, experts often adopt coupled chromatographic and spectral strategies. Chromatography can then be used to isolate a pure or substantially pure fraction of a chemical component from the a mixture. If you really need to identify something precise, you could use spectroscopy, which is a technique that uses either benchmark spectra or a catalog of spectra to generate selected information. Compain et.al reported analysis of AZT, STA, zalcitabine, didanosine, LMV and abacavir using liquid chromatographic/tandem mass spectrometric assay with linearity 0.5-200 ng ml-1is the better than other reported method

"Table 6: Hyphenation"

analyte	method	matrices	extraction tech	extraction solvent	mobile phase	stationery phase	detection	flow rate	IS	linearity	lod	loq	Ref
STA	HPLC-UV		liq.-liq. extn	tert-BuMe ether	10mM ammonium acetate pH 3.5, methanol, acetonitrile and glacial acetic acid	Hypercil, C18 column	UV detector at 265nm	1 ml/min	Hydrochlorothiazi	0.05 to 2µg/mL	N/A	N/A	59

									de				
STA	Liquid chromatography-tandem mass spectrometric assay		extd. by liq.-liq. Extn	ethyl acetate and pH 6 phosphate buffer (50 mmol/L)	methanol-water 1% ammonia (50 : 50 : 0.5, v/v/v)	a Zorbax Extend C18 column , SecurityGuard C18 guard column		0.5 ML/MIN	β-thymidine	5.00-1000 ng/mL	N/A	5.00 ng/mL	58
(AZT), LMV, STA, (NVP), (EFV), (LPV) (RTV)	LC-MS/MS		liq.-liq. extn	Et acetate	methanol/0.1% formic acid aq. soln. (80:20)	Agilent ZORBAX Eclipse XDB-C18 column	electrospray ionization (ESI) source, and multiple reaction monitoring (MRM) mode to perform cation detection						60
(LMV),	LC-MS/MS		protei	1.0 mL	A (0.1% formic acid in water): B	Eclipse XDB-C18 (150 mm ×	tripleq uad	0.5	tel	20-3200 μg/L for 3TC,	AZT 0.25 (μg·L-	AZT 1 (μg·	61

(STA), (AZT), (EFV), (NVP), (LPV/RTV)			n ppt n. and liq.-liq. extn	ethyl acetate	(0.1% formic acid in methanol) (20:80, v/v)	4.6 mm, 5mm) analytical column	rupole mass spectrometer, API-3200	ML/MIN	misartan	d4T, AZT, 40-6400 μg/L for EFV and NVP, 62.5-10000 μg/L for LPV, and 12.5-2000 μg/L for RTV	1),3TC 0.5 (μg·L-1),d4t 1.0 (μg·L-1),EFV 1.O (μg·L-1),NVP 0.2 (μg·L-1), LPV 0,015 (μg·L-1), RTV 0.008 (μg·L-1)	L-1),3TC 2 (μg·L-1), D4t 3 (μg·L-1),EFV 2 (μg·L-1),NVP 0.5 (μg·L-1),LPV 0.05 (μg·L-1),RTV 0.015 (μg·L-1)	
STA	LC/MS/MS					Reverse phase micro bore column with ion pairing	multiple reaction monitoring	0.3 ml/min	2H8ATP	n/a	9.2,5.6fmol/10 6 cell and 3.1fmo;/10 6 cells for d4T-mp,D4t-dp, D4t-tp	0.30 pmol,0.24pmol,0.14pmol for d4T-MP, d4T-DP ,D4t-tp	62
STA and DNS	CE-ESI-MS/MS						triple quadrupole electrospray (ES) MS (M					50 mg/L (ddATP, dATP) to 110 mg/L (d4TTP,	63

							S/MS)					dTTP)	
AZT, STA, zalcitabine, didanosine, LMV and abacavir	liquid chromatographic/tandem mass spectrometric assay		solid-phase extn	MeOH	two mixed solutions A and B, where A was composed of 5% MeOH and 95% ultrapure water and B was composed of 40% acetonitrile (ACN) and 60% ultrapure water	Zorbax Stable Bond C18 column followed by MS/MS anal				0.5-200 ng ml-1		LOQ for the NRTIs was set at 0.5 ng ml1 for plasma samples and 0.025 ng per pellet for intracellular samples	64
(STA), (AZT) and (EFV)	liquid chromatography-tandem mass spectrometry				NH4OAc	Supelco LC-18-DB (3.3 mm x 3.0 mm, 3.0 m ID) chromatographic column equipped with Supelco Discovery C-18 (3.0 mm) guard column (Supelco, St. Louis, MO, USA)	multiple reaction monitoring (MRM)	1 ml/min	cimetidine				65
(AZT) (STA)	CZE-MS/MS				formic acidammonia (pH 10, 50 mM ionic strength)	fused-silica capillary of 47 (or 67) cm50 m ID375 m OD	uv detection	at 254 nm					66

(AZT) (STA), (LMV) , (DNS)	capillary electrophoresis-electrospray ionization mass spectrometry					fused-silica capillary of 37 cm× 75 µm i.d × 375 µm o.d	uv detection at 254nm				2.5 ppb for AZT and 20 ppb for d4T, 2 ppb for ddA and 5 ppb for 3TC		67
STA	liquid chromatography/tandem mass spectrometry				solvent A comprised DMH (10MM) AMMONIUM FORMATE (3MM)BPH 11.5, SOLVENT B comprised DMH (20mm) and amonium formate (6mm)/acetonitril 1:1 (v/v)	SMT-C18 O.d. 5micrometer 100 A (150x2.1mm) column	multiple reaction monitoring (MRM) mode		2[H8]-ATP			d4T-TP was 138 fmol per 7 mL blood (9.8 fmol per 106 cells)	62
STA or DNS	capillary electrophoresis-electrospray ionization tandem mass spectrometry				0.5% formic acid added to methanol–water (95:5V/V)	fused-silica separation capillary has 70 cm350 mm I.D.3150 mmO.D. geometrical dimensions	uv detection at 254nm				2 µg l -1 in a formic acid ammonia buffer (pH 2.5, 10 mM ionic strength)		68

2. Discussion

STA has been utilized in drug manufacturing, UV/VIS spectroscopy, and HPLC since 1976. STA insolubility makes bioanalytical or capillary electrophoretic studies tricky. Sample solution consisted MeOH and ACN.Recent advances in STA determination have been hindered by the need to upgrade sophisticated equipment to strengthen sensitivity and tackle issues such the cost-effective use of organic solvent in sample preparation.

3. Conclusion

This research is aimed at spectrophotometric and spectrofluorometric chromatographic characterization of STA in both standalone and in combination with other drugs, following its

evolution and development through time. Liquid chromatography is frequently used for both solitary and combined STA analysis. Though there are established protocols for determining and managing STA levels, most procedures still do not adhere to environmentally benign principles. Therefore, efforts will be made to create biological matrices and dosage forms that limit negative impacts on the environment. As a result, less potentially harmful organic effluents are needed

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