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Original Article

An Inclusive Review on Analytical Methods for Ritonavir in Various Pharmaceutical and Biological Matrix

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ABSTRACT

Background: Ritonavir (RTV) is an anti-retroviral drug used in the treatment of HIV/ AIDS. Many times, RTV is used alone and in combination with many anti retroviral drugs. So far, around seventy nine analytical methods have been reported for various studies on analysis of RTV in bulk, pharmaceutical formulations and biological fluids. **Aim:** To summarize the various analytical techniques such as Chromatography, Spectrophotometry; Capillary electrophoresis and also hyphenated techniques such as LC- MS/MS for estimation of Ritonavir. **Method:** The present article deals with the comprehensive details about the type of various analytical techniques such as Chromatography, Spectrophotometry; Capillary electrophoresis and also hyphenated techniques such as LC- MS/MS, and their applicability in analysis of RTV. These techniques are either explored for the quantification, detection of metabolite and also for stability-studies of the RTV. **Conclusion:** The present studies revealed that HPLC technique along with the spectroscopic have been most widely explored for the analysis. The investigatory review may provide the comprehensive details to the researchers who are working in the area of analytical research of RTV.

Key words: Ritonavir, Analytical Methods, Validation, Stability Studies, HPLC, HPTLC, UV-spectrophotometry.

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INTRODUCTION

The therapeutic class of HIV protease inhibitors (PI) belongs to an important constituent of existing very active antiretroviral therapy (HAART) regimens.[1]

Ritonavir (RTV), chemically is 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl] carbamate shown in Figure 1. RTV is solid in nature with melting point in the range of 126 - 132^{o}C; It is practically insoluble in water.[2,3]

RTV, is a Cytochrome P450 3A and Protease Inhibitor.[4] RTV is available in 100 mg reported to be tolerable and safe. The most common side effects of RTV include nausea, vomiting, loss of appetite, diarrhea, and numbness of the hands and feet. Serious side effects include liver problems, pancreatitis, allergic reactions, and arrythmias.[5-8] The RTV is official in Indian Pharamcopoeia (IP) and United States Pharmacopoeia (USP).[9,10]

IP depicted HPLC assay method using C18 (15 cm × 4.6 mm, 5µm) column as a stationary phase and mobile phase consisting of acetonitrile, acetate buffer (45:55 v/v) with a flow rate of 1 mL/min. Column effluent was monitored at 239 nm.[9]

Analytical Particulars on RTV

Literature revealed, many analytical techniques viz UV/Visible- Spectrophotometry, HPLC, UPLC, HPTLC and LC-MS for the estimation of RTV in bulk and pharmaceutical formulations and also in biological samples. The present review described about the estimation of RTV in various dosage forms as single constituent and in combination with many antiretroviral drugs such as Lopinavir, Valacyclovir, Atazanavir, Ombitasvir, Paritaprevir, Dasabuvir, Saquinavir, Abacavir, Darunavir, Efavirenz, Nevirapine, Amprenavir, Indinavir, Nelfinavir, Raltegravir, Etravirine, Tenofovir, and Clozapine. The details about the several analytical methods implemented for the analysis of RTV is shown in Figure 2.

Spectrophotometry Methods[11,12]

Five UV- Spectrophotometry methods have been reported for the estimation of RTV in bulk and in pharmaceutical formulations. In developed methods, methanol was used as solvent for dissolving drug and for extracting RTV from pharmaceutical formulations. Most of the authors have studied linearity range of RTV from 10 µg/mL to maximum 50 µg/mL and reported correlation coefficient (r^2) > 0.999. The detection limit (DL) was found to be 1.1 µg/mL and the quantization limit (QL) was found to be 3.3 µg/mL; hence the method reported to be sensitive.

Similarly, six UV- Spectrophotometry methods have been reported for the simultaneous estimation of RTV in combined in bulk and in pharmaceutical formulations. In developed methods methanol, acetonitrile and hydrotropic reagent (SLS) were used as solvent for preparation of stock solution for simultaneous estimation of RTV and in combination with other drugs. The reported Spectrophotometry methods showing λ_{max}, sample matrix, and solvent and linearity range; the details are given in Table 1. These methods are simple, economical and less time consuming since it involves less calculation. The amount of drug estimated in these methods was found to be in good agreement with label claimed.

UPLC Method

G. D. Kill *et al.* reported a novel validated UPLC method for quantitation of lopinavir and RTV in bulk drug and pharmaceutical formulation with its impurities. The method was performing on C18 (Acquity UPLC BEH C18, 50 × 2.1 mm, 1.7 µm) column thermo stated at 30^{o}C using triethylamine (pH 2.2): 0.1% H_3PO_4 in acetonitrile and methanol (85:15 v/v) as mobile phase for development. Quantitation was performed with photo Diode Array (PDA) detector at 215 nm with flow rate 0.4 mL/min.[22]

HPLC Methods[23-40]

Many RP-HPLC methods have been studied for estimations of RTV in pharmaceutical formulations. The detail about reported RP- HPLC methods is depicted in the Table 2.

HPTLC Methods [41-46]

A simple, precise and sensitive normal phase HPTLC method has been established for the determination of RTV in bulk and pharmaceutical formulation. The separation of the RTV was achieved on pre-coated silica gel $60F_{254}$ using toluene: ethyl acetate: methanol: glacial acetic acid) (7.0:2.0:0.5:0.5% v/v/v/v) as a mobile phase. The amount of RTV in tablet matrix was found to be in good agreement with label claimed. The method showed good recovery in the range of 98.00-101.11% for RTV.[41] The method implemented complex mobile phase containing three solvents and a modifier as for separation of RTV in pharmaceutical formulation.

Five, HPTLC methods have been studied for the simultaneous determination of RTV in combination with LPV. The detailed account on development and validation of HPTLC methods for the combination of RTV with LPV is depicted in Table 3.

Stability-Indicating Methods (SIM) for Determination of RTV[47-54]

Eight stability-indicating methods have been studied so for determination of RTV in bulk substances and pharmaceutical formulations implementing different analytical techniques like viz HPLC, UPLC and HPTLC. Amongst all these stability-indicating methods, three methods

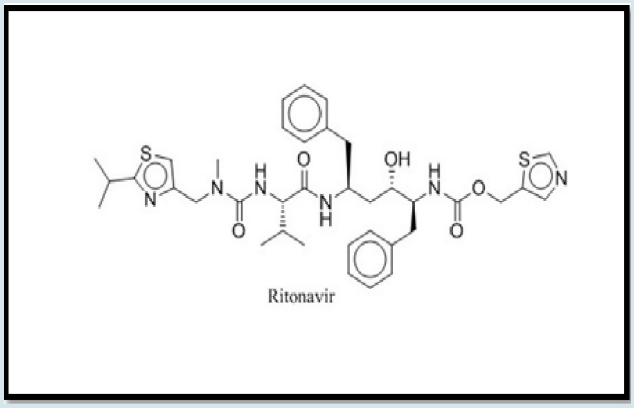

Figure 1: Chemical Structure of RTV.

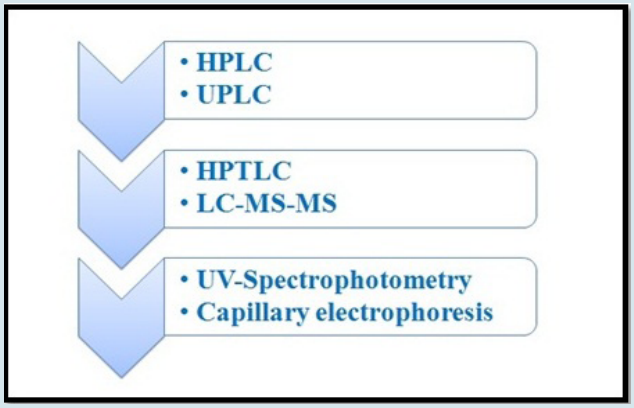

Figure 2: Various Analytical Methods employed for Estimation of RTV.

Table 1: Spectrophotometric methods used for determination of RTV alone and in Combined dosage form.

Sr. No	Name of Drug	Sample matrix	Methods	Detection (nm)	Linearity	Comments	Ref.
1.	RTV	Capsule	Second order	RTV-223.3	10-30 µg/mL	The method is simple, economical and validated	11
2.	RTV	Tablet	First order	RTV-266	10-30 µg/mL	The method is validated.	12
3.	RTV	Tablet	Zero order	RTV-242	10-20 µg/mL	The linearity was studied in lower range.	13
4.	RTV	Tablet	Zero order First order	RTV- 239 RTV- 232	10- 50 µg/mL	The method is simple and economical	14
5.	RTV	Tablet	Zero order AUC	RTV- 238 RTV- 230-246	10-50 µg/mL	The method is simple and coefficient correlation value reported to be greater than 0.99.	15
6.	RTV+ LPV	Tablet	First order	RTV- 278.10 LPV- 246.70	5-30 µg/mL 2-20 µg/mL	The method is validated	16
7.	RTV + LPV	Tablet	Zero 0rder	RTV- 239 LPV- 259	4-24 µg/mL 1-6 µg/mL	The method is simple and economical and validated	17
8.	RTV + VCV	Tablet	ARM	RTV- 256.75 VCV-237.52	10-20 µg/mL	The method is simple and economical and validated	18
9.	RTV + ATZ	Tablet	SEM First order	RTV- 239 ATZ- 249 RTV-264 ATZ-254	10-90 µg/mL 15- 90 µg/mL	The method is simple and economical and validated	19
10.	RTV + ATZ	Tablet	Zero order	RTV- 240 ATZ- 279	10-30 µg/mL 30-30 µg/mL	The method is validated	20
11.	RTV + LPV	Tablet	Zero order AUC	RTV-238 LPV-260 RTV-228-248 LPV-250-270	10-35 µg/mL 100-500 µg/mL	The method is simple and economical and validated	21

Table 2: HPLC methods for RTV

Sr.No	Name of drug/ Formulation	Mobile phase composition	Discussion	Comments	Ref
1	RTV (Tablet)	Phosphate buffer : Acetonitrile (50:50 v/v)	The analysis of RTV was carried out on symmetry C18 column with dimension (250×4.6, 5µm) with flow rate 1 mL/min. Retention time was 5.1 min and UV-detection was carried out at 239. RTV obeyed linearity in the range of 100-300 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Simple, sensitive and accurate. Buffer is used for development of mobile phase	23
2	RTV (Capsule)	Methanol : Water (67:33 v/v)	The analysis of RTV was carried out on LiChrospher C18 column with dimension (125 × 4.0, 5µm) with flow rate 1 mL/min. Retention time was 7.8 min and UV-detection was carried out at 210. RTV obeyed linearity in the range of 100-300 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Simple, precise and specific. Very simple mobile phase is established for separation	24
3	RTV (Tablet)	Ammonium acetate buffer: Acetonitrile (85:15 v/v)	The analysis of RTV was carried out on HiQSil C18 column with dimension (250 × 4.6, 5µm) with flow rate 1 mL/min. Retention time was 5.9 min and UV-detection was carried out at 239. RTV obeyed linearity in the range of 5-30 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Rapid, simple and sensitive. Buffer is used for development of mobile phase	25
4	RTV (Tablet)	Potassium dihydrogen phosphate: Acetonitrile (70:30 v/v)	The analysis of RTV was carried out on Hypersil BDS C18 column with dimension (100 × 4.6, 5µm) with flow rate 1 mL/min. Retention time was 2.55 min and PDA-detection was carried out at 237. RTV obeyed linearity in the range of 25-150 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Accurate and simple. Buffer is used for development of mobile phase.	26
5	RTV+ LPV (Tablet)	Methanol + Phosphate buffer (78:22 v/v)	The analysis of RTV and LPV was carried out on Eurosphere C18 column with dimension (250 × 4.6, 5µm) with flow rate 1 mL/min. Retention time was 6.10 and 7.15 min and UV-detection was carried out at 230. RTV and LPV obeyed linearity in the range of 10-50 and 40-200 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Selective and sensitive. The method is validated.	27
6	RTV + ATV (Tablet)	Methanol: Water (90:10 v/v)	The analysis of RTV and ATV was carried out on Phenomenex Gemini C18 column with dimension (150 × 4.6, 5µm) with flow rate 1 mL/min. Retention time was 2.6 and 5.4 min and UV-detection was carried out at 249. RTV and LPV obeyed linearity in the range of 5-25 and 15-75 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Rapid and precise. The method is studied in lower part of linearity range.	28
7	RTV + ATV (Tablet)	Acetonitrile: Methanol: Phosphate buffer (44:11:45 v/v/v)	The analysis of RTV and ATV was carried out on Nucleodur C18 column with dimension (150 × 4.6, 5µm) with flow rate 1.5 mL/min. Retention time was 6.13 and 3.13 min and UV-detection was carried out at 210. RTV and LPV obeyed linearity in the range of 10-30 and 34-102 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Robust and accurate. Mobile phase containing three solvents.	29
8	RTV + LPV (Tablet)	Potassium hydrogen phosphate buffer: Acetonitrile: Methanol (50:35:15 v/v/v)	The analysis of RTV and LPV was carried out on Phenomenex Gemini C18 column with dimension (250 × 4.6, 5µm) with flow rate 1 mL/min. Retention time was 3.7 and 6.0 min and UV-detection was carried out at 254. RTV and LPV obeyed linearity in the range of 100-150 and 400-600 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Accurate, sensitive and robust. The mobile phase is consisting of three solvents.	30
9	RTV + VCV (Tablet)	Methanol: Acetonitrile : Water (35:41.5:23.5 v/v/v)	The analysis of RTV and VCV was carried out on Agilent TC-C18 column with dimension (250 × 4.6, 5µm) with flow rate 1.3 mL/min. Retention time was 5.64 and 2.61 min and UV-detection was carried out at 222. RTV and LPV obeyed linearity in the range of 12.5-125 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Accurate and precise. The mobile phase consisting of three solvents. The total run time was less than 15 min	31

10	RTV + LPV (Tablet)	Acetate buffer: Acetonitrile (55: 45 v/v)	The analysis of RTV and LPV was carried out on Phenomenex Luna C18 column with dimension (250 × 4.6, 5µm) with flow rate 1.5 mL/min. Retention time was 6.10 and 7.15 min and UV-detection was carried out at 255. RTV and LPV obeyed linearity in the range of 10-50 and 40-200 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Accurate and sensitive. Retention time was 6.10 min for RTV and 7.15 min for LPV	32
11	RTV + DAS + OMB + PAR (Tablet)	Phosphate buffer : Acetonitrile (35 : 65 v/v)	The analysis of RTV, DAS, OMB, and PAR was carried out on RP C18 column with dimension (150×4.5, 3.5µm) with flow rate 1 mL/min. Retention time was 3.49, 1.47, 6.38 and 1.47 and DAD-detection was carried out at 254. RTV, DAS, OMB and PAR obeyed linearity in the range of 1.7 - 40, 1.25 - 30, 0.42 - 10 and 2.5 - 60 µg/ml. Correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Robust and accurate. The lower proportion of the buffer is used for mobile phase.	33
12	RTV + LPV (Tablet)	Acetonitrile : Phosphate buffer (60:40 v/v)	The analysis of RTV and LPV was carried out on Agilent ODS UG 5 column with dimension (250×4.6, 5µm) with flow rate 1.5 mL/min. Retention time was 6.9 and 8 min, and UV-detection was carried out at 220. RTV and LPV obeyed linearity in the range of 100-200 µg/ml. Correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Precise, rapid and economical Lower proportion of buffer for mobile phase development	34
13	RTV + LPV (Tablet)	Acetonitrile : Potassium Dihydrogen Phosphate buffer (50:50 v/v)	The analysis of RTV and LPV was carried out on Zorbax SB-C18 column with dimension (150 × 4.6, 3.5µm) with flow rate 1.2 mL/min. Retention time was 6.9 and 8 min and UV-detection was carried out at 260. RTV and LPV obeyed linearity in the range of 20-100 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Simple, rapid and accurate	35
14	RTV + LPV (Tablet)	Buffer: Methanol: Acetonitrile (30:60:10 v/v/v)	The analysis of RTV and LPV was carried out on Inertsil-C18 column with dimension (150 × 4.6, 3.5µm) with flow rate 0.6 mL/min. Retention time was 6.9 and 8 min and UV-detection was carried out at 226. RTV and LPV obeyed linearity in the range of 5-60 and 20-240 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Simple, linear and accurate. Total run time was reported to be less than 10 min.	36
15	RTV + LPV (Tablet)	Water and Acetonitrile (70:30 v/v)	The analysis of RTV and LPV was carried out on Hypresil BDS C18 column with dimension (250 × 4.6, 5µm) with flow rate 2 mL/min. Retention time was 10.16 and 8.45 min and UV-detection was carried out at 210. RTV and LPV obeyed linearity in the range of 50-150 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Simple, sensitive.	37
16	RTV + LPV (Tablet)	Ammonium acetate : Acetonitrile (50:50 v/v)	The analysis of RTV and LPV was carried out on Hypresil BDS C18 column with dimension (250 × 4.6,5 µm) with flow rate 1 mL/min. Retention time was 12.5 and 14.7 min and UV-detection was carried out at 245. RTV and LPV obeyed linearity in the range of 0.4- 4.4 and 2-18 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Simple and accurate. Linearity was studied at lower concentration.	38
17	RTV + LPV (Tablet)	Methanol; Phosphate Buffer (90:10 v/v)	The analysis of RTV and LPV was carried out on Phenomenex C18 column with dimension (250 × 4.6,5 µm) with flow rate 1 mL/min. Retention time was 4.7 and 3.7 min and UV-detection was carried out at 215. RTV and LPV obeyed linearity in the range of 100-600 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Accurate and precise. Buffer proportion is very small.	39
18	RTV + ATV (Tablet)	Acetonitrile: Acetate buffer (60:40 v/v/v)	The analysis of RTV and ATV was carried out on Eclipse C18 column with dimension (100 × 4.6, 5 µm) with flow rate 1 mL/min. Retention time was 2.95 and 2.49 min and UV-detection was carried out at 210. RTV and ATV obeyed linearity in the range of 1-50 and 3-150 µg/ml. correlation of coefficient was 0.999. The method has been validated as per ICH guidelines.	Sensitive, precise. Both these drugs are separated before 4 min.	40

Table 3: HPTLC methods for determination of RTV

Sr. No	Name of drug	Formulation	Stationary Phase plates	Mobile phase Composition	Detection (nm)	Linearity (ng/band)	Rf	Ref
1	RTV	Tablet	Silica gel 60 F 254	Toluene: Methanol: Ethyl acetate: Glacial Acetic acid` (7:0.5:0.2:0.5 v/v/v/v)	263	RTV-200-1000	RTV- 0.73	41
2	RTV + LPV	Tablet	Silica gel 60 F 254	Ethyl acetate: ethanol: toluene: diethylamine (7:2.0:0.5:0.5, v/v/v/v)	266	RTV- 200-1000 LPV- 800-2000	RTV- 0.41 LPV- 0.62	42
3	RTV + LPV	Capsule	Silica gel 60 F 254	Toluene: Methanol: Ethyl acetate: Glacial Acetic acid (7:0.5:0.2:0.5 v/v/v/v)	263	RTV- 160 - 500 LPV- 660 - 2000	RTV- 0.39 LPV- 0.33	43
4	RTV + LPV	Tablet	Silica gel 60 F 254	Chloroform: 1, 4 - Dioxane (7:3 v/v)	210	RTV- 40-240 LPV-160-960	RTV- 0.78 LPV- 0.74	44
5	RTV + LPV	Tablet	Silica gel 60 F 254	Toluene: ethyl acetate: methanol: glacial acetic acid 6.5:2.5:0.5:0.5 (v/v/v/v)	266	RTV- 400-2000 LPV- 1600- 8000	RTV- 0. 24 LPV- 0.41	45
6	RTV + LPV	Tablet	Silica gel 60 F 254	Ethyl Acetate: n-Hexane: Acetic Acid (7.0: 2.5: 0.5 (v/v/v)	190	RTV- 100- 700 LPV- 100- 600	RTV- 0. 53 LPV- 0.81	46

Table 4: Stability-indicating HPLC, UPLC and HPTLC methods for determination of RTV

Sr. No	Name of drug	Sample Matrix	Mobile phase	Detection (nm)	Linearity (µg/ml)	Rt/Rf (min)	Ref.
			HPLC Methods				
1.	RTV + LPV	Tablet	Potassium dihydrogen Phosphate buffer: Acetonitrile (pH 4.6) (60:40 v/v)	250	RTV- 12.5 - 75 LPV- 50 - 300	RTV- 2.27 LPV- 3.74	47
2.	RTV + ATV	Tablet	Ammonium acetate buffer :Acetonitrile (pH 4) (60:40 v/v)	205	RTV- 5 - 14 ATV- 18 - 42	RTV-5.7 ATV- 2.7	48
3.	RTV + LPV	Tablet	Acetonitrile: phosphoric acid (55:45 v/v)	240	RTV- 2 - 12 LPV- 8 - 48	RTV- 4.35 LPV- 6.68	49
4.	RTV + LPV	Capsule	Acetonitrile-water-methanol (53:37:10 v/v/v)	210	RTV- 10-90 LPV- 40- 360	RTV- 8 LPV- 9.8	50
5	RTV	Capsule	Acetonitrile : phosphoric acid (55 : 45, v/v)	210	RTV- 1-500	RTV- 4.82	51
			HPLTC Method				
6	RTV	Capsule	Acetonitrile – water (1:2 v/v)	240	RTV- 0.8- 12.5 ng/band	RTV- 0.41	51
7.	RTV + DNV	Tablet	Toluene: ethyl acetate: methanol (6:2.5:1.2 v/v/v)	250	RTV- 200-1000 ng/band	RTV- 0.50 DNV- 0.29	52
8	RTV	Tablet	Toluene: Ethyl acetate: methanol (6: 4.5 : 0.6 v/v/v)	240	RTV- 500-4000 ATV- 1000- 8000	RTV- 0.41 ATV- 0.25	53
			UPLC Method				
9	RTV	Tablet	Potassium di hydrogen phosphate: acetonitrile (20:80 v/v) (pH 3.5)	240	RTV- 75 - 450	RTV- 3.12	54

are reported for the RTV alone and five analytical methods for RTV in combination with other drugs. The reported stability-indicating methods for RTV, illustrating sample matrix, λ max, linearity range and retention time/factor are presented in Table 4.

Accounts on Bio-analytical Method for Determination of RTV [56-83]

Bioanalysis is a term generally used to describe the quantitative measurement of a compound (drug) or their metabolite in biological fluids, primarily blood, plasma, serum, urine or tissue extracts.[55] HPLC and LC-MS/MS methods are predominantly used for bio analysis of RTV, alone and in combination in most of these methods. Twenty eight bioanalytical methods are reported for the determination of RTV. In these methods various extraction techniques such as liquid-liquid extraction, solid phase extraction and protein precipitation extraction techniques have been implanted for extraction of RTV from biological fluids. The most commonly used solvents for extraction of analytes were ethyl acetate, methanol, n-hexane, tert-butyl methyl and diethyl ether. Bioanalytical methods for determination of RTV are summarized in Table 5.

Table 5: Bioanalytical determination of RTV

Drug	Sample Matrix	Method	Colum	Detection	Internal Standard	Ref
RTV	Human Plasma	HPLC-UV	ODS-AQ	205 nm	A-86093	56
RTV + ATV + LPV + DNV + EFV +NVP	Blood Spot	LC-MS	Phenomenex Gemini C18	-	Dibenzepine, 13C6-efavirenz and D5-saquinavir	57
RTV + APV + IDV + NFV + SQV	Human Plasma	HPLC-UV	Zorbax SB-C18	239 nm	-	58
RTV + RTG + ETV + DNV	Human Plasma	LC–MS/MS	-	-	-	59
RTV + SQV	Human Plasma	HPLC-PDA	Luna C18	210 nm 240 nm	-	60
RTV + LPV + TNF	Human Plasma	LC-MS	Synergi column	-	cyheptamide	61
RTV + SQV + NFV + LPV + ATV + EFV + NVP	Human Plasma	LC-MS	SymmetryShield	-	clozapine	62
RTV + RTG + ETV + ATV +LPV + NVP + EFV + SQV + IDV + NFV + M8 + APV + DNV	Human Plasma	HPLC-DAD	Luna 5m C18	210-320 nm	quinoxaline	63
RTV + APV +ATV + IDV + LPV + NFV + SQV	Human Plasma	LC-MS	HyPURITY C18	-	Ro31-9564	64
RTV + LPV	Human Plasma	LC-MS/MS	Phenomenex	-	saquinavir	65
RTV + LPV	Human Plasma	LC-MS	LiChrocart	-	A886093.0	66
RTV + SQV	Human Serum	HPLC- UV	Kromasil C8	240 nm	diazepam	67
RTV + APV + IDV + LPV + NFV + SQV + NVP + EFV	Human Plasma	HPLC-UV	Stability RP-C18	240 nm 215 nm	A886093.0	68
RTV	Human Plasma, Saliva, Cerebrospinal fluid	HPLC-UV	Zorbax SB-C18	239 nm	-	69
RTV + EFV + LPV	Human Hair	LC-MS	BDS-C18	-	Ritonavir-d6 and celecoxib	70
RTV + IDV + SQV + NFV	Human Plasma	HPLC-UV	Chrompack Inertsil ODS-2 C 18	215 nm	A 86093.0	71
RTV + SQV + IDV	Human plasma	HPLC-UV	Phenomenex C18	240 nm 258 nm	A 86093.0	72
RTV + SQV + ABV	Human Plasma	HPLC-UV	Luna C18	210 nm 240 nm 285 nm	-	73
RTV + SQV + APV + IDV + EFV + NFV	Human Plasma	HPLC-UV	Nucleosil 100	201 nm	Clozapine	74
RTV + LPV	Human Plasma	LC-MS/MS	Agilent Zobax Extend	-	RTV IS LPV IS	75
RTV + DNV + ETV	Human Plasma	LC-MS	Agilent Zorbax- XBD-C8	-	Alprazolam	76
RTV + IDV + SQV + M8 + LPV + NFV + APV + EFV	Human Plasma	HPLC-PDA	Luna C18	215 nm 235 nm 248 nm 265 nm	-	77
APV + LPV + RTV + SQV + EFV	Human Plasma	LC-MS	X-TERRA TM MS	-	A-86093	78
RTV + IDV + APV + SQV + NFV	Human Plasma	HPLC-UV	Nova Pak C18	210 nm 240 nm 220 nm	Verapamil	79
RTV + IDV + SQV + LPV + ATV + NFV + M8 NFV + APV	Human Plasma	HPLC-UV Fluorescence	Allsphere hexyl	215 nm 280 – 340 nm	PR-25 AR- 86093	80
RTV + LPV	Human Plasma	LC-MS/MS	Symmetry C18	-	A-86093	81
RTV + LPV	Human Plasma	UPLC-MS/MS	Acquity UPLC BEH	-	d-6 ritonavir d8-lopinavir	82
RTV + LPV	-	LC-MS-MS	Atlantis T3	-	-	83

Capillary Electrophoresis (CE) Method[84-88]

A. Z. Carvalho *et al*. developed micellar electro kinetic chromatography method development for estimation of impurities in RTV. Experiment was performed on P/ACETM MDQ equipment with diode array detector (DAD). The optimized separation was performed in a background electrolyte composed of sodium tetraborate (pH 9.6; 15 mM) containing sodium dodecylsulfate (30 mM) and acetonitrile (18 %, v/v). Mass spectrometry was used to confirm the identity of the tested substances. UV normalized absorbance detected for RTV and its impurities at 195, 205, 210, 230, 240 and 265.[84]

W. Gutleben *et al*. reported simultaneous separation of IDV, NFV, APV, SQV, RTV, ABV, LMV, DDI, NVP, DVD and ZCB protease and reverse transcriptase inhibitors for human immunodeficiency virus therapy by-co-electroosmotic capillary zone electrophoresis. The experiments were performed on waters quanta on 4000 instrument.[85]

E. A. Pereira *et al*. established a simple and rapid electrokinetic chromatography method for the simultaneous separation of different protease inhibitors (IDV, RTV, SQV, and NVF), nucleoside reverse transcriptase inhibitors (STD, ZVD, DDI) and non-nucleoside reverse transcriptase inhibitors (NVP, EFV). The analyses were performed in a 75m i.d. uncoated fused-silica capillary with 48.5 cm length (effective length of 40 cm) using a running buffer consisting of 20 mmol/L sodium dodecyl sulfate, 10 mmol/L sodium tetra borate, 30% acetonitrile and 5% ethanol. Samples were injected hydrodynamically by applying 50 mbar pressure during 6 s. All analytes were separated within 10 min with a voltage of 20 kV.[86]

N. D. Tuan *et al*. reported simultaneous separation of fifteen approved protease and reverse transcriptase inhibitors for human immunodeficiency virus therapy by capillary electrophoresis. The method employed an acidic background electrolyte with sodium polyanethol sulfonate (SPAS) as polyanionic electroosmotic flow (EOF) modifier to establish a strong cathodic EOF, sodium dodecyl sulfate (SDS) as pseudostationary selector, and acetonitrile and ethanol as organic modifiers. Separation of the analytes was depend upon two different mechanisms; basic analytes are protonated at the prevailing pH conditions and thus migrate in front of the cathodic EOF, whereas the less basic and neutral analytes interact with the SDS and are retained after the EOF. [87]

Bin Fan *et al*. described validated Micellar electrokinetic chromatography methods for separation and quantitative analysis of anti-HIV drug mixtures containing ZVD/ DDI/ NVP (mixture A) and ZVD/ DDI/ RTV (mixture B) in human serum. Serum samples were prepared using a solid-phase extraction procedure methanol was used as a solvent. The optimized resolution achieved with a run buffer containing 18 mM sodium dodecylsulfate in 15 mM phosphate and borate buffer (pH 9.0). An uncoated 52 cm (effective length 30 cm)/50mm ID fused-silica capillary operated at 308 ^{O}C was used in the analysis with UV detection at 210 nm. [88]

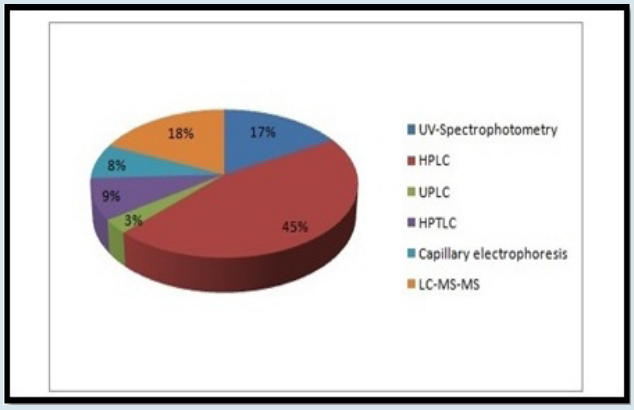

Figure 3: Percentage Utility of Analytical Approaches used for estimation of RTV.

Others Analytical Methods

K. Gambhir *et al*. reported thermal stability and hydration behavior of RTV: A vibrational spectroscopic approach. Competency of vibrational (infrared and Raman) spectroscopy were assessed to identify structural changes of the RTV symbolizing its stress degradation. High- Performance Liquid-Chromatography (HPLC) was used as a confirmatory technique for both thermal and hydration stress study, while thermo gravimetric analysis/differential thermal analysis and atomic force microscopy substantiated the implementation of vibrational spectroscopy in this framework. The results was exhibited high thermal stability of the RTV as significant variations were observed in the diffuse reflectance infrared Fourier transform spectra only after the drug exposure to thermal radiations at 100 °C. Hydration behavior of ritonavir sulfate was evaluated using Raman spectroscopy and the value of critical relative humidity was found to be 467%. [89]

DISCUSSION AND CONCLUSION

The present review discussed about different analytical approach employed for the assessment of RTV. Profuse examinations have been accomplished including, Bio-analytical, HPLC, UPLC, HPTLC, UV/ Vis-Spectroscopy, capillary electrophoresis, LC-MS, LC-ESI-MS etc. for evaluation of RTV in bulk and in its combination with other drugs from pharmaceutical formulations and also biological fluids.

The percentage utility of analytical approaches used for estimation of RTV is shown in Figure 3. Liquid chromatography with UV detection has been found to be most studied for estimation of RTV in bulk as well as pharmaceutical dosage forms, while hyphenated LS-MS, LS-MS/MS methods reported for determination of RTV and its metabolite in plasma and other biological fluids. Few chromatography approaches like HPTLC and Stability-indicating HPLC, UPLC and HPTLC are also reported. Few simple UV -Spectrophometric methods may be used for routine analysis of RTV alone and in combination with other drugs. These compiled data may of use for research for further studies in analysis of RTV.

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CONFLICT OF INTEREST

Authors do not have conflict of interest for this manuscript.

ABBREVIATIONS USED

RTV: Ritonavir; **LPV:** Lopinavir; **VCV:** Valacyclovir; **ATV:** Atazanavir; **OMB:** Ombitasvir; **PAR:** Paritaprevir; **DAS:** Dasabuvir; **SQV:** Saquinavir; **ABV:** Abacavir; **DNV:** Darunavir; **EFV:** Efavirenz; **NVP:** Nevirapine; **APV:** Amprenavir; **IDV:** Indinavir; **NFV:** Nelfinavir; **RTG:** Raltegravir; **ETV:** Etravirine; **TNF:** Tenofovir; **ARM:** Absorption ratio method; **AUC:** Area under curve; **HPLC:** High- Performance Liquid Chromatography; **HPTLC:** High Performance Thin-Layer Chromatography; **CE:** Capillary Electrophoresis; **UPLC:** Ultra: Pressure Liquid -Chromatography.

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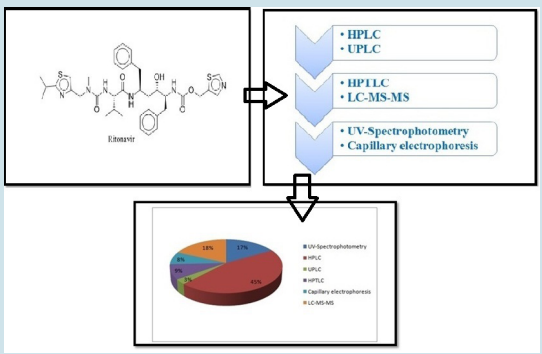
PICTORIAL ABSTRACT



SUMMARY

- Ritonavir (RTV) is an anti-retroviral drug used in the treatment of HIV/AIDS.
- The review article deals with the comprehensive details about the type of various analytical techniques such as Chromatography, Spectrophotometry; Capillary electrophoresis and also hyphenated techniques such as LC- MS/MS, and their applicability in analysis of RTV.
- The present studies revealed that HPLC technique along with the spectroscopic have been most widely explored for the analysis.
- The investigatory review may provide the comprehensive details to the researchers who are working in the area of analytical research of RTV.

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