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A REVIEW ON ANTI-VIRALS BY LC-MS

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ABSTRACT

The main aim of this review article is to enlighten the various anti-viral drugs in single and combination of drugs estimation by LC-MS technique. Anti-viral drugs are classified like Nucleoside Reverse Transcriptase Inhibitors, Non-Nucleoside Reverse Transcriptase Inhibitors, Protease Inhibitors, Fusion Inhibitors and Integrase strand transfer inhibitors and are Zidovudine, ganciclovir, lamivudine, acyclovir, valacyclovir, ribavirin, efavirenz, etravirenz, rilpivirine, ritonavir, saquinavir, tipranavir, simeprevir, parpitaprivir, sunaprevir, indinavir, lopinavir, Dolutegravir, Elvitegravir, Raltegravir were estimated by using most powerful analytical technique called LC-MS. Chromatographic parameters like different Columns were used by various researchers for better elution and mobile phases in different ratios andin different polarity were used for fast elution and different flow rates for proper elution. Mass spectroscopic parameters like ionization by electron spray ionization was mostly used with quadrupole analyzer and multiple reaction transitions were used for detecting the compounds.

Keywords: Nucleoside Reverse Transcriptase Inhibitors, Non-Nucleoside Reverse Transcriptase Inhibitors, Protease Inhibitors, Fusion Inhibitors and Integrase strand transfer inhibitors, Liquid chromatography-Mass spectrometry

INTRODUCTION

Antiviral drugs are a class of medication used for treating viral infections. Most antivirals target specific viruses, while a broad-spectrum antiviral is effective against a wide range of viruses. Unlike most antibiotics, antiviral

drugs do not destroy their target pathogen; instead, they inhibit its development **[1]**.

CLASSIFICATION OF ANTIVIRALS:

Nucleoside Reverse Transcriptase Inhibitors (NRTI) **Eg:** Ganciclovir, Zidovudine, Stavudine, Lamivudine, Acyclovir, Valacyclovir, Ribavirin,

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) **Eg:** Efavirenz, Etravirenz, Nevirapine, Rilpivirine

Protease Inhibitors (PI) **Eg:** Amprenavir, Atazanavir, Boceprevir, Darunavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir, Telaprivir, Simeprevir, Parpitaprivir, Asunaprevir, Indinavir, Lopinavir

Fusion Inhibitors (FI) or **Entry Inhibitors Eg:** Enfuvirtide

Integrase strand transfer inhibitors (INI) **Eg**: Dolutegravir, Elvitegravir, Raltegravir **[20]**

ESTIMATION OF DIFFERENT ANTI-VIRAL DRUGS BY LC-MS;

Table 1: Methods for estimating different anti-viral drugs in single by LC-MS

S. No.	DRUG	CHROMATOGRAPHIC CONDITIONS	MASS CONDITIONS	REFERENCE
1	Amantadine	COLUMN: Shim-pack XRODS III, 2.0 mm (I.D.) x 150 mm (L) x 2.2 µm MOBILE PHASE: Mobile phase A: 0.1% formic acid and 5 mM ammonium acetate Mobile phase B: Acetonitrile FLOW RATE: 0.4 mL/min LINEARITY:0.1- 50µg/L	ANALYZER: Triple Quadrupole Mass Spectrometer DETECTION: Positive electron spray ionization mode MRM TRANSISTIONS: Amantadine -135.2 Amantadine standard -152	[2]
2	Acyclovir	COLUMN: C-18 (150 X 4.6mm, 3.5µm) MOBILE PHASE: 5 mM Ammonium acetate and methanol (70:30 v/v) FLOW RATE: 0.4 mL /min. LINEARITY: 1.5-13.5ng/ml	ANALYZER: API-4000 LC/MS/MS electrospray DETECTION: Negative mode, Selected ion monitoring (SIM) MRM TRANSISTIONS: P-Toulene sulfonic acid -171.2 Acyclovir - 224.2	[3]
3	Bictegravir	COLUMN: Kinetex EVO C_{18} column, 50 × 3.0 mm, 5 µm MOBILE PHASE: Acetonitrile–water with 0.1% formic acid. (80:20) FLOW RATE: 0.250 mL/min LINEARITY: 1 - 10,000 ng/mL	ANALYZER: AB Sciex API 5500 Q Trap mass spectrometer withEelectron spray ionizer DETECTION: Positive multiple reaction monitoring mode MRM TRANSISTIONS: Bictegravir - 450.1-289.1 Bictegravir (IS)- 420.1-277.1	[4]
4	Efavirenz	COLUMN: C18 analytical column (50 mm×2.1 mm, 3.5 µm 120 Å). MOBILE PHASE: Mobile phase A: water with 0.1% formic acid and Mobile phase B: Acetonitrile with 0.1% formic acid. FLOWRATE: 0.3 mL/min LINEARITY: 1.0–2,500 ng/ml	ANALYZER: AB Sciex 4000 triple quadropole mass spectrometer equipped with Turbo Ionspray DETECTION: Tandem mass spectrometry in negative ionization mode with multiple reaction monitoring (MRM). MRM transistions: m/zratio: Efavirenz- 314.20-243.90; 13C6- efavirenz (IS) - 320.20-249.90.	[5]
5	Rilpivirine	COLUMN: Gemini C18 (150 × 4.6 mm, 5 µm) analytical	ANALYZER: SCIEX API-4000 Triple quadrupole mass	[6]

		column MOBILE PHASE: 0.05% formic acid in water and acetonitrile (10:90, v/v) FLOW-RATE: 1.0 mL/min LINEARITY: 0.5–200 ng/mL.	spectrometer with electro spray ionization DETECTION: Multiple reaction monitoring positive ionization mode. MRM TRANSISTIONS: Rilpivirine -367.1 - 128.0 Rilpivirine D6-IS 373.2 - 134.2	
6	Rilpivirine	COLUMN: C(18) column MOBILE PHASE: Acetonitrile and 0.1% formic acid buffer (80:20, v/v) FLOW RATE: 0.5 mL/min. LINEARITY: 0.51-200 ng/Ml	ANALYZER: API-4000 mass spectrometer with Turboionspray interface DETECTION: Multiple reaction monitoring mode MRM TRANSISTIONS: Rilpivirine - 367.2-195.1 and Nevirapine (IS) -267.1-226.1	[7]

Table 2: Methods for estimating different anti-viral drugs in combination by LC-MS

S. No.	DRUGS	CHROMATOGRAPHIC CONDITIONS	MASS CONDITIONS	REFERENCE
1	Valacyclovir Acyclovir	COLUMN: Inertsil CN-3 (5 µm) MOBILE PHASE: 1 mM ammonium acetate buffer and methanol (50:50 v/v) FLOW RATE: 0.8 mL/min LINEARITY: valacyclovir - 4.09 to 725.63 ng/mL Acyclovir - 50.35 to 10017.29 ng/mL.	ANALYZER: API-3000 equipped with electrospray ionization DETECTION: positive polarity using multiple reaction monitoring (MRM). MRM TRANSISTIONS: Valacyclovir - 325.2-152.1 Acyclovir - 226.2-152.1, Valacyclovir D4 -329.3-152.1 Acyclovir D4- 230.2-152.1	[8]
2	Ganciclovir and Dipeptide monoester prodrug	COLUMN: Reversed phase Xterra C8 column (50 × 4.6 mm i.d, 5µm). MOBILE PHASE: 85 % v/v of acetonitrile in water containing 0.05 % v/v of formic acid. FLOWRATE: 0.2 mL/min. LINEARITY: Ganciclovir -0.50–1,000 ng/mL, Valine ganciclovir - 10.0–500 ng/mL	ANALYZER: API 3200 Triple Quadrupole linear QTrap mass spectrometry system interfaced by turbo ion spray (TIS) DETECTION MODE: Positive ion source in MRM mode MRM TANSISTIONS: Ganciclovir - 152.3; Valine ganciclovir - 338.3; Tyrosine valine ganciclovir -518.6	[9]
3	Acyclovir and Valacyclovir	COLUMN: C18 column MOBILE PHASE: 0.1% formic acid: methanol (30:70% v/v) FLOW RATE: 0.8 mL/min LINEARITY: Acyclovir -47–10,255 ng/ml and Valacyclovir- 5–1,075 ng/mL	ANALYZERS: API 4000 triple Quadra pole Mass Spectrometer, equipped with a Turbo Ion spray source DETECTION: positive ion detection mode. MRM TRANSISTIONS: Acyclovir -226.30 - 152.10, Valacyclovir - 325.40 - 152.10 Intenal standard - 307.06 - 220.20	[10]
4	Lamivudine and Zidovudine	COLUMN: C18 (50 mmx4.6 mm, 5p.m)	ANALYZER: API 2000 LC/MS/MS was a Turbo	[11]

		MOBILE PHASE: Acetonitrile, Methanol, and Formic acid LINEARITY: Lamivudine - 25.291 - 4046.621 ng/mL and Zidovudine - 23.357 - 4057.141 ng/mL	**ion spray. DETECTION: The Positive ions were measured in MRM mode**	
5	**Ritonavir and Lopinavir**	**COLUMN: Inertsil ODS column MOBILEPHASE: Acetonitrile and 5 mM ammonium acetate buffer. FLOW RATE: 0.8 mL/min LINEARITY: Lopinavir -50.67–10,008.82 ng/mL Ritonavir - 5.066–1,000.693 ng/mL**	**ANALYZER: API-3000 mass spectrometer DETECTION: MS–MS in positive ion mode MRM TRANSISTIONS: Lopinavir– 629.4 - 447.1 Ritonavir– 721.2 - 296.2 Fluoxetine -310.1- 148.3**	**[12]**
6	**Ledipasvir, Sofosbuvir**	**COLUMN: Xterra MS C_8 column (4.6×50mm,5µm) MOBILE PHASE: Ammoniumformate buffer (pH 3.5; 10mM), acetonitrile and methanol FLOW RATE: 0.7mL/min LINEARITY: Ledipasvir - 0.1-1000 ng/mL Sofosbuvir - 0.3-3000 ng/mL GS-331007 - 3.0-3000 ng/mL**	**ANALYZER: API4000 triple quadrupole tandem mass spectrometer DETECTION: multiple reaction monitoring (MRM) positive electrospray ionization interface MRM TRANSISTIONS: Ledipasvir - 637.7 Sofosbuvir - 243.2 GS-331007 -582.3**	**[13]**
7	**Rilpivirine and Dolutegravir**	**COLUMN: Phenyl-hexyl column (Phenomenex Luna 5µm Phenyl-Hexyl, 2 mm x 50 mm) MOBILE PHASE: Mobile Phase A: 100% water, 10 mM ammonium acetate, 0.1% acetic acid) and Mobile Phase B: 100% Acetonitrile FLOW RATE: 3000 µL/min LINEARITY: Rilpivirine – 25-2000 ng/ml and Dolutegravir – 25-2000 ng/ml**	**DETECTION: Multiple reaction monitoring mode with turbo ion source MRM TRANSITIONS: Dolutegravir – 420.1-277.1 Rilpivirine - 367.1-195.2**	**[14]**
8	**Lopinavir and Ritonavir**	**COLUMN: ZORBAX Eclipse XDB-C18 column MOBILE PHASE: methanol-0.1% formic acid in water (80:20). LINEARITY: Lopinavir -62.5 - 10000 ng/mL, and Ritonavir - 12.5 - 2000 ng/mL**	**ANALYZER: A tandem mass spectrometer equipped with electrospray ionization DETECTION: multiple reaction monitoring (MRM) operated in the positive ion mode. MRM TRANSISTIONS: Lopinavir - 629.6- 155.2, Ritonavir - 721.4 -268.2, Telmisartan -515.2 - 276.2**	**[15]**
9	**Ombitasvir, Paritaprevir and Ritonavir**	**COLUMN: Inertsil® ODS-3 (4.6 mm×60 mm, 5 µm) MOBILE PHASE: methanol and 10 mM ammonium acetate (79:21, v/v) FLOW RATE: 0.55 mL/min. LINEARITY: Ombitasvir -5–250 ng/mL, Parpitaprevir - 30–1,500 ng/mL, Ritonavir - 20–1,000 ng/mL**	**DETECTION: MS/MS detection with electron spray ionizer SELECTED-REACTION MONITORING (SRM)TRANSISTIONS: Ombitasvir –894 - 588 Ritonavir – 721-296 Parpitaprevir – 766-571**	**[16]**
10	**Sofosbuvir Velpatasvir**	**COLUMN: C18 Zorbox eclipse plus (100 × 4.6 mm, 5 µm).**	**ANLYZER: Triple quadrupole (model**	**[17]**

	Ledipasvir	**MOBILE PHASE: 0.1% formic acid in water: acetonitrile: methanol (30:60:10, v/v/v) FLOW RATE: 0.55 mL/min LINEARITY: Sofosbuvir - 5–5000 ng/ml and Velpatasvir - 10–1500 ng/mL**	**6410A) DETECTION: Multiple reaction monitoring transitions operating at positive ionization mode. MRM TRANSISTIONS: Sofosbuvir -530.3 Velpatasvir -883.4 Ledipasvir -889.2**	
11	**Daclatasvir, Asunaprevir, and Beclabuvir**	**EXTRACTION: Liquid-Liquid Extraction MOBILE PHASE: Acetonitrile: Water (50:50, v/v) COLUMN: YMC Basic S-5, 50 × 2.0 mm, 5 µmV MOBILE PHASE: MOBILE PHASE A: 10 mM ammonium acetate in water, MOBILE PHASE B: 10 mM ammonium acetate in methanol, MOBILE PHASE C: 10 mM ammonium acetate in acetonitrile**	**ANALYZER: Triple quadrupole 5000 mass spectrometer DETECTION: Positive electrospray multiple reaction monitoring (MRM) mode MRM TRANSISTIONS: Daclatasvir - 739.4 - 565.3 Asunaprevir - 748.4-648.3 Beclabuvir – 660.4 - 535.3**	**[18]**
12	**Ribavirin, Boceprevir, Telaprevir, Simeprevir, Daclatasvir, Sofosbuvir**	**COLUMN: Chromsystems Master Column® MOBILE PHASE: Mobile phase A: Water-Methanol solution 95–5% v/v and 0.2% (v/v) Acetic acid and 2 mM Ammonium acetate. Mobile phase B: Methanol-water solution 95–5% v/v and 0.2% (v/v) Acetic acid and 2 mM Ammonium acetate. FLOW RATE: 0.5 ml/min.**	**ANALYZER: API 4000 QTRAP mass spectrometer from Sciex equipped with an electrospray ionization (ESI)Turbo Ion Spray source. DETECTION: Multiple reaction monitoring (MRM) mode for positive ions. MRM TRANSISTIONS: Ribavirin - 245 Boceprevir - 521, Telaprevir - 680.1, Simeprevir- 750.3, Daclatasvir - 739.5, Sofosbuvir - 261.3**	**[19]**
13	**Amprenavir, Atazanavir, Darunavir, Dolutegravir, Etravirine, Indinavir, Lopinavir, Nelfinavir, Nelfinavir-M8, Nevirapine, Raltegravir, Ritonavir, Saquinavir and Tipranavir.**	**COLUMN: C18 analytical column (50 by 2.1 mm, 3 µm particle size) MOBILE PHASE: Purified water, acetonitrile, and an aqueous buffer (containing ammonium acetate [5 g/L], acetic acid [35 mL/L of water], and trifluoroacetic acid [2 mL/L of water]. FLOWRATE: 0.5ml/min**	**ANALYZER: Triple-quadrupole LC-MS/MS with an MS Pump and electron spray ionization. DETECTION: positive selected reaction monitoring (SRM) mode.**	**[20]**

CONCLUSION

Finally it is concluded that this review expresses the estimation of various anti-viral drugs in single and combination in various marketed formulations. Analytical C18 column was mostly used and mobile phase like methanol, acetonitrile, water, ammonium acetate buffer was used almost in all the methods. Electron spray ionization was mostly used by all the

researchers because of its sensitivity and reproducibility and quadrupole analyzer was used for efficient separation according to mass to charge ratio and multiple reaction transitions was used for better detection capabilities in all the published methods. In future analysis of anti-viral drugs by any researcher will be beneficiary by reading this article.

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REFERENCES

[1] https://en.wikipedia.org/wiki/Antiviral drug.

[2] Determination of Amantadine Residues in Chicken by LCMS-8040. Liquid Chromatography Mass Spectrometry. No. LCMSMS-167E.

[3] Noor Shabana, K. Dayananda Reddy**,** Development and Validation of LC-MS/MS Method for the Analysis of P-Toluene sulfonic acid, In Antiviral Drug Acyclovir. International Journal of Innovative Research in Science, Engineering and Technology. 2015, Vol. 4, Issue 3, 839-847.

[4] Pavan Kumar Prathipati, Subhra Mandal, Christopher J. Destache, Development and validation of LC–MS/MS method for quantification of bictegravir in human plasma and its application to an intracellular uptake study. Bio Med Central. 2018, https://doi.org/10.1002/bmc.4379.

[5] Praveen Srivastava, Ganesh S. Moorthy, Robert Gross, Jeffrey S. Barrett, A Sensitive and Selective Liquid Chromatography/Tandem Mass Spectrometry Method for Quantitative Analysis of Efavirenz in Human Plasma. journal. pone, 2013.

[6] Ajay Gupta , Swati Guttikar , Yogesh Patel, Pranav S. Shrivastav, Mallika Sanyal, Reliable LC-MS/MS assay for the estimation of rilpivirine in human plasma: application to a bioequivalence study and incurred sample reanalysis. Data Testing and Analysis. 2014, 7, 290–299.

[7] Laxminarayana Burugula *et al*, Liquid chromatography-tandem mass spectrometric assay for the non-nucleoside reverse transcriptase inhibitor rilpivirine in human plasma. Biomedical Chromatography. 2013, 27(2).

[8] Arabinda Saha, Ajay Kumar, Sanjay Jagannath Gurule, Arshad Khuroo and Pratika Srivastava, Role of RBC Partitioning and Whole Blood to Plasma Ratio in Bioanalysis: A

Case Study With Valacyclovir and Acyclovir. Mass Spectrometry & Purification Techniques, 2017 Volume 3, Issue 2, 1-12.

[9] Sriram Gunda, Ravinder Earla, Kishore Cholkar, Ashim K. Mitra, Pharmacokinetic studies and LC-MS/MS method development of ganciclovir and dipeptide monoester prodrugs in Sprague dawley. Eur J Drug Metab Pharmacokinet. 2015, 40(3), 325–334.

[10] Ravi Kanneti, Ramadass Rajesh, J. R. Aravinda Raj, An LC–MS–MS Method for the Simultaneous Quantitation of Acyclovir and Valacyclovir in Human Plasma. Chromatographia. 2009, 70, 407–414.

[11] C. Parthiban, M. Bhagavan Raju, M. Sudhakar, D. Sathish Kumar, Simultaneous Estimation and Validation for Determination of Lamivudine and Zidovudine in Human Plasma by LCMS/MS Method. E-Journal of Chemistry. 2012, 9(2), 598-607.

[12] Rajasekhar D, Jaswanth KI, Ravi K, Srinivasulu P, Simultaneous Determination of Ritonavir and Lopinavir in Human Plasma after Protein Precipitation and LC-MS-MS. Chromatographia, 2010, Volume 71, Issue 9–10, pp 815–824.

[13] Ola M. Abdallah , Ahmed M. Abdel-Megied, Development a validated highly sensitive LC–MS/MS method for simultaneous quantification of Ledipasvir, sofosbuvir and its major metabolite GS-331007 in human plasma: Application to a human pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis, 2017, 143 (J. Med. Chem. 53 2010), 305-310.

[14] M.Grégoire,G.Deslandes,C.Renaud,R.Bouquié,C.Allavena,F.Raffi,P. Jolliet, E. Daill, A liquid chromatography–tandem mass spectrometry assay for quantification of rilpivirine and dolutegravir in human plasma. Journal of Chromatography B. 2014, 971, 1-9.

[15] Ya-min Yao **,** Jian-jun Sun**,** Jun Chen**,** Xiao-qian Liu**,** Hong-zhou Lu**,** Li-junZhang, LC-MS/MS method for simultaneous quantification of lopinavir and ritonavir in human plasma. 2010, 45(2): 279-82.

[16] Faten Farouk, Dina Wahba, Sherif Mogawer, Shaimaa Elkholy, Ahmed Elmeligui, Reham Abdelghani & Salwa Ibahim ,

Development and Validation of a New LC–MS/MS Analytical Method for Direct-Acting Antivirals and Its Application in End-Stage Renal Disease Patients. European Journal of Drug Metabolism and Pharmaco-kinetics. 2019, 45, 89–99.

[17] Ehab F. Elkady, Ahmed A. Aboelwafa , Rapid bioanalytical LC-MS/MS method for the simultaneous determination of sofosbuvir and velpatasvir in human plasma-application to a pharmacokinetic study in Egyptian volunteers. Journal of Chromatography B. 2018, 1102-1103 (Expert Rev. Gastroenterol. Hepatol. 11 2017), 116-124.

[18] Hao Jiang, Hamza Kandoussi, Jianing Zeng, Jian Wang, Roger Demers, Timothy Eley, Bing He, Richard Burrell, John Easter, Pathanjali Kadiyala, Janice Pursley, Laura Cojocaru, Chanda Baker, John Ryan, Anne-Francoise Aubry, Mark E. Arnold, Multiplexed LC-MS/MS method for the simultaneous quantitation of three novel hepatitis C antivirals, daclatasvir, asunaprevir, and beclabuvir in human plasma. Journal of Pharmaceutical and Biomedical Analysis. 2015, 107, 409-418.

[19] M. Conti, T. Matulli Cavedagna , E. Ramazzotti , R. Mancini , Multiplexed therapeutic drug monitoring (TDM) of antiviral drugs by LC–MS/MS. Clinical Mass Spectrometry. 2018, 7 (World J. Gastroenterol. 22 2016), 6-17.

[20] Daskapan, Alper; van Hateren, Kai; Stienstra, Ymkje; Kosterink, Jos; van der Werf, Tjip; Touw, Daan; Alffenaar, Development and Validation of a Bioanalytical Method for the Simul- taneous Determination of 14 Antiretroviral Drugs using Liquid Chro-matography-Tandem Mass Spectrometry. Journal of Applied Bioanalysis. 2018, 4(2), 37-50.