IJBPAS, May, 2022, 11(5): 2332-2339

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A REVIEW ON ANALYTICAL METHODS OF RIBAVIRIN IN PHARMACEUTICALS

SAI TEJASWI A*, HARITHA CHOWDARY D, PRACHET P AND RAMA RAO N

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

***Corresponding Author: Dr. Sai Tejaswi Avala: E Mail: avalasaitejaswi@gmail.com**

Received 18th May 2020; Revised 17th June 2020; Accepted 24th July 2020; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.6077

ABSTRACT

The present review focuses on estimation of Ribavirin its combination of drugs by different analytical techniques. Ribavirin is very effectively used as an antiviral drug. It isused with an interferon medication such as peginterferon alfa-2a [Pegasys] or peginterferon alpha-2b [PEG-Intron]) to treat hepatitis C. In this review various analytical methods such as High-performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), Liquid Chromatography Mass Spectroscopy (LC-MS) for the estimation of Ribavirin in bulk and pharmaceutical dosage forms in various researchers' publications. This review enlightens the various solvents and conditions suitable for the estimation of Ribavirin and its combination of drugs. The techniques illustrated here may find application in the qualitative and quantitative estimation of Ribavirin and in analysing other related properties.

Keywords: Ribavirin, Antiviral agent, HPLC, HPTLC, Hyphenated Techniques

INTRODUCTION

Ribavirin (RBV) (1-β-D-ribofuranosyl – 1,2,4-triazole-3-craboxamide) is a purine nucleoside analog, structurally related to the endogenous ribonucleoside guanine and has demonstrated a broad-spectrum activity against a variety of DNA and RNA viruses. RBV is rapidly and extensively transported into almost all cell types in the body via a nucleoside transporter **[1]**. A combination of ribavirin with either interferon α-2a/2b was widely accepted for the treatment of chronic hepatitis C. It is a hydrophilic

molecule that does not bind to plasma proteins. It is considered by some physicians to be an effective and sometimes life-saving drug **[2]**.

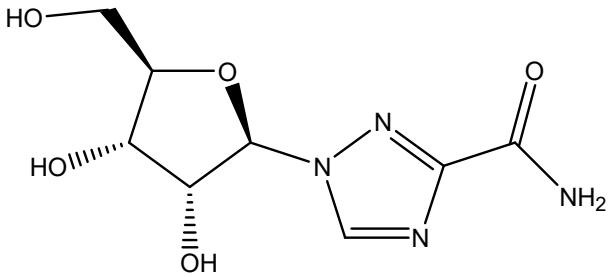

Figure 1: Structure of Ribavirin

Table 1: Information of drugs:

S. No	Drug Name	Information of drug
1.	Ribavirin	Category: Antiviral Molecular formula: $C_8H_{12}N_4O_5$ Molecular weight: 244.2 g/mol IUPAC name: 1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2,4-triazole-3-carboxamide Melting point: 166-168^{0}C pKa: 12.95 (acid) and 1.00 (base)
2.	Daclatasvir	Category: Antiviral Molecular formula: $C_{40}H_{50}N_8O_6$ Molecular weight: 738.9 g/mol IUPAC name: methyl *N*-[(2*S*)-1-[(2*S*)-2-[5-[4-[4-[2-[(2*S*)-1-[(2*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl]pyrrolidin-2-yl]-1*H*-imidazol-5-yl]phenyl]phenyl]-1*H*-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate Melting point: 224-226^{0}C pKa: 3.82
3.	Ledipasvir	Category: Antiviral Molecular formula: $C_{49}H_{54}F_2N_8O_6$ Molecular weight: 889 g/mol IUPAC name: methyl *N*-[(2*S*)-1-[(6*S*)-6-[5-[9,9-difluoro-7-[2-[(1*R*,3*S*,4*S*)-2-[(2*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl]-2-azabicyclo[2.2.1]heptan-3-yl]-3*H*-benzimidazol-5-yl]fluoren-2-yl]-1*H*-imidazol-2-yl]-5-azaspiro[2.4]heptan-5-yl]-3-methyl-1-oxobutan-2-yl]carbamate Melting point: 183^{0}C pKa: 3.8
4.	Sofosbuvir	Category: Antiviral Molecular formula: $C_{22}H_{29}FN_3O_9P$ Molecular weight: 529.5 g/mol IUPAC name: propan-2-yl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate Melting point: 120-125^{0}C pKa: 9.3
5.	Silybin	Category: Flavonoid Molecular formula: $C_{25}H_{22}O_{10}$ Molecular weight: 482.2 g/mol IUPAC name: (2*R*,3*R*)-3,5,7-trihydroxy-2-[3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one Melting point: Silybin A- 162-163^{0}C Silybin B- 158-160^{0}C pKa: 6.63 for 7-OH group 7.7-7.95 for 7-OH group 11.0 for 20-OH group
		Category: Antiviral Molecular formula: $C_8H_{11}N_3O_3S$

6.	**Lamivudine**	**Molecular weight: 229.26 g/mol IUPAC name: 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one Melting point: 160-162^{0}C pKa: 4.3**
7.	**Urodeoxycholic acid**	**Category: A bile acid Molecular formula: $C_{24}H_{40}O_4$ Molecular weight: 392.6 g/mol IUPAC name: (4*R*)-4-[(3*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,7-dihydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl]pentanoic acid Melting point: 203^{0}C pKa: 5.1**

METHODS FOR RIBAVIRIN

Chromatographic methods:

Several chromatographic methods like HPLC, HPTLC and UPLC were developed for estimation of Ribavirin in single and combination with other drugs. Methods for estimation of Ribavirin by HPLC and HPTLC are enlisted in the **Table 2-4**.

Table 2: Methods for the estimation of Ribavirin in single and combination with other drugs by HPLC method

S.NO	DRUGS	APPLICATION	DESCRIPTION	REF NO
1.	**Ribavirin**	**BiologicalSample**	**Detection wavelength: 235nm Column: Atlantis C $_{18}$ Mobile phase: KH_2PO_4 50Mm pH 3.2: Acetonitrile Retention time: 4.8±0.15min Flow rate: 1.0ml/min Linearity range:625 to 320,000 ng/mL**	**[3]**
2.	**Ribavirin, Daclatasvir, Ledipasvir and Sofosbuvir**	**Pharmaceutical dosage form**	**Detection wavelength: Ribavirin: 235nm Daclatasvir:315nm Ledipasvir: 332nm Sofosbuvir:260nm Column: Thermohypersil BDS C $_8$ Mobile phase: Phosphate buffer pH-7.5: Methanol Retention time: Ribavirin: 1.05min Daclatasvir: 13.68min Ledipasvir: 16.18min Sofosbuvir: 11.14min Flow rate:1.5 ml/min Linearity range: Ribavirin: 5-500 µl/ml Daclatasvir:0.5-75 µl/ml Ledipasvir 0.5-75µl/ml Sofosbuvir2-300 µl/ml**	**[4]**
3.	**Ribavirin**	**Pharmaceutical dosage form**	**Detection wavelength: 240nm Column: CPS Hypersil Cyano Mobile phase: Phosphate buffer pH 4 with OPA Retention time: 4.06±0.071min Flow rate: 0.8 ml/min Linearity range: 5-200 µg/ml**	**[5]**
4.	**Ribavirin, Daclatasvir and Sofosbuvir**	**Biological sample**	**Detection wavelength: Ribavirin: 207 nm Daclatasvir: 312nm Sofosbuvir: 260nm Column: Scharlau C_{18}**	**[6]**

			Mobile phase: Water:Acetonitrile Retention time: Ribavirin: 8min Daclatasvir: 11.21min Sofosbuvir: 9.55min Flow rate:1.0ml/min Linearity range: Ribavirin: 0.5-80 µl/ml Daclatasvir: 0.5-80 µl/ml Sofosbuvir: 0.1-40 µl/ml	
5.	**Ribavirin**	**Biological sample**	**Detection wavelength: 235nm Column: RP-C_{18} Mobile phase: 10Mm Ammonium phosphate buffer pH-6.5 Retention time: 6.5min Flow rate: 0.7ml/min Linearity range: 0.5 to 50µM**	**[7]**
6.	**Ribavirin**	**Biological sample**	**Detection wavelength: 207nm Column: Atlantis dC_{18} Mobile phase: 10Mm Potassium phosphate buffer pH-4 Retention time: 3.7min Flow rate: 1.0ml/min Linearity range: 0.05 to 10µg/ml**	**[8]**
7.	**Ribavirin**	**Biological sample**	**Detection wavelength: 207nm Column: C_{18} Mobile phase: A-Acetonitrile- 100% B-KH_2PO_4 pH 3.5 with OPA Retention time: 5.9± 0.10min Flow rate: 1.2ml/min Linearity range: 0.05 to 5.0 mg/mL**	**[9]**
8.	**Ribavirin, Silybin, Lamivudine and Urodeoxycholic acid**	**Biological sample**	**Detection wavelength: 214nm Column: VP-ODS Mobile phase: 0.1M Sodium dodecyl sulphate, 8% propanol, 0.3% triethylamine in 0.02M phosphoric acid (pH6) Retention time: Ribavirin: 3.0min Silybin: 3.4min Lamivudine: 8.6min Urodeoxycholic acid: 11.4min Flow rate: 0.8ml/min Linearity range: Ribavirin- 0.01-0.1µg/ml Silybin- 0.01- 0.2µg/ml Lamivudine- 0.1-1.0µg/ml Urodeoxycholic acid- 0.05- 1.0µg/ml**	**[10]**
9.	**Ribavirin**	**Biological Sample**	**Detection wavelength: 230 nm Column: C_{18} Mobile phase: A: 20mM KH_2PO_4 pH 3.0 with OPA B: 90% Acetonitrile with 10% HPLC grade water Retention time: 10.941min Flow rate: 0.5ml/min Linearity range: 0.1-8.0µg/ml**	**[11]**
10.	**Ribavirin, Sofosbuvir and Ledipasvir**	**Pharmaceutical dosage form**	**Detection wavelength: 220nm Column: Promosil CN Mobile phase: Methanol and 0.005M Heptane 1 sulphonic acid sodium salt pH 2.5 with phosphoric acid Retention time: Ribavirin: 3.7±0.005min Sofosbuvir: 8.526±0.039min Ledipasvir: 9.36±0.027min**	**[12]**

			Flow rate: 1.0ml/min Linearity range: Ribavirin- 0.2-500µg/ml Sofosbuvir- 5-500µg/ml Ledipasvir- 1-112µg/ml	
11.	**Ribavirin**	**Biological sample**	**Detection wavelength: 207nm Column: C_{18} Mobile phase: 10mM Ammonium phosphate pH 2.5 Retention time: 6.7min Flow rate: 1.0ml/min Linearity range: 0.2-5µg/ml**	**[13]**

Table 3: Methods for the estimation of Ribavirin by HPTLC method

S.NO	DRUGS	APPLICATION	DESCRIPTION	REF NO
1.	**Ribavirin**	**Tablet dosage form**	**Column: Precoated silica gel 60F 254 Mobile phase: Chloroform: Methanol: Water 6.0:3.5:0.5v/v/v/v Detection of spot: 254nm Retardation Factor: Ribavirin: 0.52±0.05 Degradation product: 0.02±0.05**	**[14]**
2.	**Ribavirin**	**Bulk and capsule dosage form**	**Column: Aluminum Plates precoated Silica gel 60F 254 Mobile phase: Chloroform: Methanol:Acetic acid 60:15:15v/v/v Linearity range: 5-40µg/ml Retardation factor: Ribavirin- 0.45±0.02 Degradation products: Boiling- 0.30, 0.60, 0.70, 0.85 Acid hydrolysis- 0.30, 0.60, 0.70, 0.85 Alkali hydrolysis- 0.30, 0.60, 0.85 Oxidation- 0.30, 0.60, 0.70, 0.85**	**[15]**

Table 4: Methods for estimation of Ribavirin in combination with other drugs by LC-MS method and UPLC-MS

S.NO	DRUGS	APPLICATION	DESCRIPTION	REF NO
1.	**Ribavirin and Sofosbuvir**	**Biological sample**	**Column: BEH C_{18} column Mobile phase: Gradient elution Acetonitrile:0.1% formic acid in water Flow rate: 0.4ml/min Ionizer: Electron spray Linearity: Ribavirin 5-1000ng/mL Sofosbuvir 10-2000ng/mL**	**[16]**
2.	**Ribavirin and Amantadine**	**Biological sample**	**Column: BEHHillic Mobile phase: 1% trichloroacetic acid:Acetonitrile(1:1v/v) Flow rate: 1mL/min Ionizer: Electron spray Linearity: 10-100µg/L Correlation coefficient: 0.999**	**[17]**

CONCLUSION

This review discussed the reported spectroscopic techniques like UV Spectroscopy and Chromatographic techniques like HPLC, HPTLC methods developed and validated for the estimation of Ribavirin in single and in combination with other pharmaceutical drugs. It can be

reviewed that out of all these techniques HPLC with UV detection was extensively with best suitable solvents like acetonitrile, potassium dihydrogen phosphate buffer, methanol, ammonium acetate buffer for better resolution. It was also observed that a flow rate of 1.0ml/min gives best compounds detection. Hence all these methods were simple, precise, accurate, and offers reproducibility with reliability with low in cost in compared with advanced technology.

REFERENCES

[1] Margarita, M., Osvaldo, R., Beatriz, Z., (2009) "HPLC-MS/MS method for the intracellular determination of ribavirin monophosphate and ribavirin triphosphate in CEM_{ss} cells", J. Pharma. & Bio. Analysis. 49: Pp. 1233-1240.

[2] Nawal, A., Alarfaj, Maha, F., & Tohamy, El., (2012) "Determination of the anti-viral drug ribavirin in dosage forms via micelle-enhanced spectrofluorimetric method", The J. of Bio. & Chem. Lumin.

[3] Antonio, D'A., *et al*., (2012) "Development and validation of a useful HPLC-UV method for quantification of total and phosphorylated-ribavirin in blood and erythrocytes of HCV+ patients", J. Pharma. & Bio. Analysis. 66: Pp. 376-380.

[4] Mostafa, M., Sherif, F.H., & Tarek. S., (2019) "Development and validation of a versatile HPLC-DAD method for simultaneous determination of the antiviral drugs daclatasvir, ledipasvir, sofosbuvir and ribavirin in presence of seven potential impurities. Application to assay dosage forms and dissolution studies", Talyor & Francis.

[5] Rim Said, H., Said Fathalla, B., Ismail Ibrahim, H., & Ola Ahmed El, R., (2031) "Stability-Indicating HPLC-DAD determination of ribavirin in capsules and plasma", J. of Chromatic. Sci. 52: Pp.493-500.

[6] Youssef, A., Magdy, N., Hussein, A., & El-Kosasy, A.M., (2019) "Validated RP-HPLC fro the simultaneous determination of ribavirin, sofosbuvir and daclatasvir in human plasma: A treatment protocol administered to HCV patients in Egypt", J. of Chromatic. Sci. Pp. 1-8.

[7] Masato, H., Anura, L., John, G., Aweeka, F., (1999) "Hihg-Performance Liquid Chromato-graphic determination of ribavirin in whoe blood to assess disposition in erythrocytes", Antimicrobial Agents & Chemo. 43: Pp. 271-2719.

[8] Arianna, L., Maria Christina, S., Silvana, P., Saverio Giuseppe, P.,

Giorgio, P., (2007) "Measurement of ribavirin and evaluation of its stability in human plasma by high-performance liquid chromatography with UV detection", J. of Chromato. B. 856: Pp. 358-364.

[9] Judit, M., *et al*., (2007) "Measurement of ribavirin in plasma concentrations by high-performance liquid chromatography using a novel solod-phase extraction method in patients treated for chronic hepatitis C", The Drug Monit. 29: Pp. 802-806.

[10] Mohie Sharaf El, D., Manal, E., & Wael, T., (2014) "Micellar liquid chromatographic determination of ribavirin, silybin, interferon α 2A, lamivudine and ursodeoxycholic acid in dosage forms and biological fluids", Taylor & Francis. 37: Pp. 1785-1804.

[11] Abdul Rafiq, K., *et al*., (2016) "Short and robust HPLC-UV method to determine serum ribavirin concentration without evaporation step", Mod. Chem. Appl. 4(3):

[12] Hanan, I., Fawzi, E., Sherin Hammad, F., Amina, M., (2020) "A green stability-indicating RP-HPLC-UV method using factorial design for determination of ribavirin, sofosbuvir and ledipasvir: Application to average content, acid degradation kinetics and in vitro drug interactions study" Microchem. J. 157:

[13] Sylvie, L., *et al*., (2003) "Ribavirin quantification in combination treatment of chronic hepatitis C", Antimicrobial Agents & Chemo. 47(1): Pp. 124-129.

[14] Sadanshio, P.P., Wankhede, S.B., Chaudhari, P.D., (2014) "A validated stability indicating HPTLC method for estimation of ribavirin in capsule in presence of its alkaline hydrolysis degradation product", Taylor & Francis. Pp. 343-358.

[15] Ibrahim Darwish, A., Hassan Askal, F., Alaa khedr, S., and Ramadan Mahmoud, M., (2008) "Stability-indicating thin-layer chromatographic method for quantitative determination of ribavirin", J. of Chromato. Sci. 46:

[16] Xiaojun, SHI., Dedong, ZHU., Jie, LOU., Bo, ZHU., Ai-rong, HU., Dongmei, GAN., (2015) "Evaluation of a rapid method for the simultaneous quantification of ribavirin, sofosbuvir and its metabolite in rat plasma by UPLC-MS/MS", J. Chromato. B.

[17] Huan, YUN., Fengyun, CUI., Xin, LIU., Zhaohui, ZHANG., (2013)

"Determination of ribavirin and amantadine in chicken by ultra-performance liquid chromato-graphy-tandem mass spectro-metry", Chinese J. of Chromato. 31(8): Pp. 724-728.