A Review on Analytical Techniques Used for Tilorone Determination

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Abstract - Tilorone is an oral, Antiviral – immunomodulatory drug. This Review demonstrate various analytical techniques like Ultra fast Liquid Chromatography, HPLC- MS/MS, LC- Tandem mass Spectrometry, 1H-NMR used for the determination of Tilorone in drug substance and simultaneous quantitation of Tilorone with Tiloronoxim in biological fluids. This review highlights advanced techniques used for the determination of Tilorone. Analytical method development and validation plays important role in development process starting from Formulation step to the final product. While developing a method active pharmaceutical ingredients contains or may arise Impurities which leads to side effects . The purpose of this literature review is to give a systematic survey of Analytica techniques used for the Tilorone until today.

Key words: Tilorone, Antiviral, Immunomodulator, Inducer.

Introduction:

Tilorone is chemically named as 2,7- Bis [2- diethylamino) ethoxy] – 9H – fluorine-9 one with Molecular formula C_{25} H_{36} Cl_2 N_2 O_3.2HCl. Molecular weight of Tilorone is 410.549 Da. It was originally synthesised and developed by Merrill DOW which is now part of Sanofi. FDA Guidelines and regulations are not readily available for Tilorone. It was available under trade name AMIXIN and LAVOMAX manufactured by OAO Pharmstandard Tomskkhimpharm and NIZHFARM pvt. Ltd respectively. It was available in film coated tablets. Tilorone was used to treat several viral diseases like influenza, Accute Respiratory Viral Infection, viral Hepatitis, viral encephalitis. Side effects are Possible allergic reactions, dyspeptic events, short term chills. [1]

Tilorone is a synthetic inducer of interferon, stimulates the body's interferon (alpha, beta, gamma, lambda). The cells which produce interferon in response to the induction of Tilorone are intestinal epithelium, hepatocytes, T-lymphocytes, neutrophils, granulocytes. Interferon lambda induction in pulmonary tissue helps increasing the antiviral protection of respiratory tract infections.

The mechanism of antiviral action is associated with inhibition of translation of virus specific proteins in infected cells, resulting in suppressed reproduction of viruses. [2,3]

Pharmacokinetics

After oral administration, the drug is rapidly absorbed form the gastrointestinal tract. Bioavailability is 60%. About 80% of the drug is bound with plasma proteins. Excreted almost intact through intestines (70%) and kidneys (9%). Elimination half- life ($T_{1/2}$) – 48 hours. [4]

Analytical techniques used for Tilorone determination:

Ultra fast Liquid Chromatographic method

Gujju Hima Bindu et al. Developed a precise, Accurate, sensitive and reproducible UFLC method for the Estimation of Tilorone in tablets.. The Chromatographic separation was performed using C18 Agilent column (150 mm × 4.6 mm, 3.5µm particle size). The mobile phase used is a mixture of Acetonitrile: 0.1% Triethylamine (pH was adjusted to 3.2 with orthophosohoric acid). The method was performed on isocratic mode, with injection volume 20 µl, flow rate 0.5 ml/min and total run time was 10 minutes. The study was observed at ambient temperature (25±2°C) with retention time 2.440 ±0.02 minutes. The UFLC system was maintained at 270 nm. The Tilorone has obeyed Beer- Lambert law with the concentration range of 0.1 – 20 µg/ml with correlation coefficient 0.9999. Tilorone is subjected to various stress conditions like acid, oxidative, alkali, thermal and hydrolysis. The Method was validated as per ICH guidelines , LOD and LOQ was found to be 0.0104 & 0.0316 µg/ ml respectively.[5]

Spectrophotometric Methods:

Gujju Hima Bindu et al. has been developed a Derivative Spectrophotometric Method for the Determination of Tilorone. The spectrophotometric techniques were proposed for the determination of Tilorone in different buffer solutions such as sodium Acetate Buffer (pH-4) Borate buffer (pH -9) Phosphate buffer (pH – 2.0) & phosphate

buffer (pH 5.0). The instrument used for this method is shimadzu UV-1800 model Ultraviolet- visible spectrophotometer with double beam. This method presents four zero order, four first order derivative, four second order derivative Methods for the assay of Tilorone in various Buffer solutions. Tilorone has shown maximum absorption at 270 nm and Linearity range was maintained from 0.4 to 14µg/ml in all the three Methods & in all the four buffer solutions. All three Methods follow Beer-Lambert law and they were validated as per ICH guidelines. In zero order spectroscopy, first order derivative spectroscopy, second order derivative spectroscopy the % RSD values are precise and accurate.[6]

O V Karpov et al. Developed a spectrophotometric method to study the interactionof single stranded RNA with Tilorone. This study gives a data about the formation of complex between phage MS2 single stranded RNA and Tilorone. With the concentration of components forms a strong linkage between RNA and Tilorone. The absorption spectra of RNA - Tilorone complex in UV – region shows Hypochromic & Bathochromic shifts and emerges maximum absorption at 280 nm.This method also detects the changes of spectral properties in the long waved region of the complex spectra. This method was concluded that the interaction between single stranded RNA and Tilorone forms a complex due to the interaction between voluntarily occurred two stranded regions in the single stranded RNA and Tilorone.[7]

Spectroscopic Method:

Allan V. Yegorova et al. Was Studied on the interaction between Tilorone and Human Serum Albumin by using spectroscopic methods. This method was performed by combining the fluorescence method with UV – visible Spectroscopy distance between donor and acceptor molecules were found to be 1.63 nm. [8]

Bio Analytical Methods:

X Zhang et al. Developed a Acute ,selective and sensitive HPLC-MS/MS Method to Observe the Performance of Tiloronoxim and Tilorone in Human Blood. An aliquot of 200 µl human blood was extracted with chloroform/ethyl ether (1/2, v/v) mixture and metoprolol is used as internal standard. Separation was carried out by using an Xterra MS C18 column (150 mm × 2.1 mm, 5 Micron) with a gradient mobile phase of methanol and water containing 15 mM ammonium bicarbonate (pH- 10.5) . Detection was performed by using TurboIonspray source on MRM (Multiple reaction mode). The mass transmission for tiloronoxim, tilorone and internal standard were m/z 426.3--> 100.0,m/z 411.3 --> 100.0 & m/z 268.3 -- > 116.1 respectively. The method was validated using total error theory. It is based on beta- expectation tolerance intervals and include accuracy & internal precision. The method was found to be Acute with concentration range of 1- 100 mg/ml for both blood.[9]

Zhang, L Yang et al. Was Simultaneously Quantify the Tiloronoxim and Tilorone in Human Urine by Liquid Chromatography-Tandem mass spectrometry . The tiloronoxim and tilorone together with metoprolol which was used as an internal standard were extracted with mixture of solvents of chloroform/ ethyl ether (1/2 v/v). The Chromatographic separation was achieved by a narrow- bore RP-HPLC (Reverse phase - High Pressure Liquid Chromatography) column and gradient mobile phase of methanol, water containing 15 mM ammonium bicarbonate (pH 10.5). The API 3000 Mass spectrometer with TurboIonspray was monitored on positive ion , multiple reaction mode. The mass transmission for tiloronoxim, tilorone and internal standard were found to be m/z 426.3--> 100.0,m/z 411.3 --> 100.0 & m/z 268.3 -- > 116.1 respectively **.** The assay shows a linear range of 1-100 ng/ml for both tiloronoxim and tilorone based on the Analysis of 0.2 ml aliquot of urine . The total run time is 8 minutes for each injection made it possible to analyse LOD was found to be 1 mg/ml for both tiloronoxim and tilorone. The Method is Acute and precise.[10]

Tomoki Nishimura et al. was developed1H NMR and calorimetry Method to study binding of DNA with Tilorone. This method presents two dimensional (2-D) NMR & isothermal titration calorimetry (ITC) , for the Tilorone, DNA complex, coupled with circular Dichroism (CD) Spectroscopy and viscosity measurements. NMR (Nuclear Magnetic Resonance) studies imply that Tilorone binds to DNA through intercalation, shows more affinity for insertion between AT (Adenine , Thymine) base pairs than between CG (Cytosine, Guanine) base pairs. Circular Dichroism spectral changes were observed for Tilorone/ DNA Base pair molar ratios higher than the stoichiometric ratio. By comparing NMR and other measurements, this method states that the circular Dichroism changes below the plateau should be related to the intercalation and the later is related to electrostatic interactions. Because of the side chains located in the groove obstruct the further intercalation to the neighbour – Exclusion principle. [11]

Discussion

Presented review covers the current analytical methods for Tilorone & its combination in pharmaceutical and biological samples like blood and urine.

CONCLUSION

This review summarizes different analytical and bio analytical techniques used for determination of Tilorone. General approach for the method development and validation was described . The sensitive, specific, and better separation analytical techniques were used for quantitative determination of Tilorone. The presented article is

useful for further researchers involved in Method Development.

References

[1] Sea Ekins, Thomas R. Lane and Peter B. Modrid et al. Tilorone: A Broad spectrum Antiviral invented in the USA and Commercialized in Russia and Pharmaceutical Research, 2020: 37 (4); 71.

[2] Gerald D. Mayer, Russell F. Krueger et al. Tilorone Hydrochloride: Mode of action, American association for the Advancement of science. 1970: 169 ; 3951, 1214-1215.

[3] National Centre for Advancing Translational Sciences, Tilorone. [Accessed December 29,2020. Online] Available : https://drugs.ncats.io/drug/O6W7VEW6KS

[4] https://en.m.wikipedia.org/wiki/Tilorone

[5] Gujju Hima Bindu, Mukthinuthalapati Mathrusri Annapurna, Duvvi Sri Valli, et al. New stability Indicating Ultra fast liquid chromatographic Method for the determination of Tilorone tablets. Research Journal of Pharmacy and Technology., 2018,11 : 9, 3950-3956.

[6] Gujju Hima Bindu, Mukthinuthalapati Mathrusri Annapurna, Duvvi Sri Valli et al. Derivative spectrophotometric Method for the determination of Tilorone -An antiviral Drug. Asian Journal of Pharmaceutics., 2018, 12 ;1, S288-299.

[7] O V Karpov. Ukr Biokhim Zh et al. Spectrophotometric study of the interaction of single – stranded RNA with Tilorone. National Library of Madicine,Pub med.gov., 1997, 69;3, 122- 125.

[8] Allan V. Yegorova, Inna I .Leonenko , Yulia V . Scrypynets, Georgy V. Maltsev Valery P. Antonovich, Sergey N. Kashutskyyer et al. Spectroscopic Studies on the interaction between Tilorone and Human Serum Albumin. French – Ukraine journal of chemistry, 2017, 05 ;01.

[9] X Zhang, J Duan, S Zhai, Y Yang ,L Yang et al. performance of tiloronoxim and tilorone determination in human blood by HPLC-MS/MS: Method Validation, Uncertainty Assessment and its application to a pharmacokinetic study. Journal of Chromatography B Analytical Technologies in the Biomedical &Life sciences. 2010, 1;878(3-4), 492-496.

[10] X Zhang, L Yang, S Zhai, J Dian et al. Simultaneous quantification of tiloronoxim and tilorone in human urine by liquid chromatography – tandem mass spectrometry. Journal of chromatography B ,2008 , 875 ; 2, 349- 357.

[11] Yoichi Takeda and Kazuo Sakurai et al. DNA Binding of Tilorone : 1 H NMR & Calorimetric studies of the intercalation. American Chemistry Society publications, 2007, 46; 27, 8156- 8163.