Journal of
Chromatography and Spectroscopy Techniques

J Chromatogr Spectrosc Tech. 2023;1(1):25-27.
https://doi.org/10.5281/jcst.11205425

Short Communication

A Sensitive and Straightforward High-Performance Liquid Chromatography Method for Estimating Transitmycin Levels in Plasma

Hemanth Kumar AK*

Department of Clinical Pharmacology, National Institute for Research in Tuberculosis, Chennai, India

***Correspondence:** Hemanth Kumar AK, Department of Clinical Pharmacology, National Institute for Research in Tuberculosis, Chennai, India. E-mail: akhemanth20@gmail.com

Received: June 11, 2023; **Accepted:** June 20, 2023; **Published:** June 27, 2023

Citation: Hemanth Kumar AK. A Sensitive and Straightforward High-Performance Liquid Chromatography Method for Estimating Transitmycin Levels in Plasma. J Chromatogr Spectrosc Tech. 2023;1(1):25-27.

ABSTRACT

A high performance liquid chromatographic method for estimation of Transitmycin (Tr) in human plasma was developed. The analyte was extracted using solid phase cartridges and the analysis of the eluent was carried out using Atlantis T3 column (150 cm × 4.6 mm ID) and photo diode array detector set at wavelength of 214 nm. The assay was specific for Tr and linear from 0.5 µg/mL to 20.0 µg/mL. The relative standard deviations for intra-day and inter-day assays were less than 10%. The method yielded a recovery for Tr that ranged from 94% to 107% and could be used in toxicology and pharmacokinetic studies.

Keywords: Transitmycin; HPLC Method; Plasma.

INTRODUCTION

Tuberculosis (TB) is the greatest infectious killer in India as well as in other developing countries and its high incidence worldwide is an important global health concern. Although several drugs are available for treatment of TB, resistance to existing anti-TB drugs necessitates the development of newer drug molecules and testing them in TB treatment. Thus, novel anti-TB drug development assumes significance.

Transitmycin (Tr) is a novel antibiotic isolated from the producer strain Streptomyces sp. MTCC 5597. It has a high potential to treat HIV and TB at the same time. The physico-chemical properties of this compound have been established [1]. This molecule was found to be active against Mycobacterium tuberculosis, Bacillus subtilis, Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, and Acinetobacter baumanii. In addition, it was found to be active against drug resistant Mycobacterium tuberculosis [2].

Since Tr is a new compound, there is no existing literature on methods for estimation of Tr. Shreevalli et al. developed a method using auto-luminescent Mycobacterium tuberculosis to determine the potency of compounds [3]. We aimed to develop a HPLC-based bio-analytical method for the estimation of Tr in human plasma.

MATERIALS AND METHODS

Pure Tr powder was obtained from an ongoing ICMR project on Tr; the crude extract from novel Streptomyces sp. was prepared at Periyar University, Salem and was subsequently purified upto >98% purity at the Indian Institute of Technology, Chennai. Acetonitrile (Merck, Germany), Methanol, Ammonium Acetate, Acetic Acid, DMSO and Ortho Phosphoric acid were purchased from Qualigens (India). Deionized water

was processed through a water purification system (Siemens, Germany). Pooled human plasma was obtained from a Blood Bank, Chennai, India.

Chromatographic System

The HPLC system – Prominence - i(Shimadzu Corporation, Kyoto, Japan) consisted of two pumps (LC-2030-3D), photo diode array detector (SPD-20AV) with built-in auto sampler and system controller. Lab solution software was used for data collection and acquisition. The analytical column used was Atlantis T3 (15 cm × 4.6 mm ID, 3.0 µm particle size) from Waters, USA.

An isocratic mobile phase was a mixture of 10 mM ammonium acetate solution and acetonitrile in the ratio of 20:80 (v/v). The solvents were degassed separately using a Millipore vacuum pump prior to preparation of the mobile phase. The run time of the chromatogram was 10 minutes at a flow rate of 0.8 mL/min at 40 °C, with the PDA detector set at a wavelength of 214 nm. Unknown Tr concentrations were derived from linear regression analysis vs concentration curve. Linearity of the calibration curve was verified using estimates of correlation coefficient (r).

Preparation of Standard Solution

A stock standard solution of Tr (1 mg/mL) was prepared in DMSO. Working standard solutions of Tr ranging from 0.5 to 20 µg/mL concentrations were prepared using pooled plasma.

Sample Preparation

To 200 µL of calibration standards and test samples, 200 µL of 4% Orthophosphoric acid in water was added and the contents were vortexed vigorously and centrifuged at 10,000 rpm for 10 minutes. The analyte was extracted using solid phase extraction cartridges MCX 30 mg/1CC. The eluted solution was evaporated to dryness under nitrogen. The dried residue was reconstituted in 100 microliters of the mobile phase, mixed and centrifuged. Fifty microliters of the supernatant was loaded into the HPLC column maintained at 10°C.

Precision

The precision of the analytical method was determined by processing plasma samples containing different concentrations of Tr in duplicate on three consecutive days.

Accuracy and linearity

A set of Tr standard concentrations ranging from 0.5 to 20.0 µg/mL were used to evaluate the accuracy and linearity of the calibration curve. The within-day and between-day variations were estimated by testing each standard Tr concentration in duplicate for six consecutive days.

Specificity

The specificity of the analytical method was assessed by analyzing blank plasma samples for interference from endogenous compounds. Interference from certain anti-TB drugs (rifampicin, isoniazid, pyrazinamide, ethambutol, ethionamide, levofloxacin, ofloxacin, moxifloxacin, rifabutin, rifapentine), anti-retroviral drugs (nevirapine, efavirenz, zidovudine, didanosine, stavudine, didanosine, lamivudine, ritonavir, tenofovir) and anti-diabetic drugs (metformin and sulphonyl ureas) at a concentration of 10 µg/mL was tested.

Recovery

Pooled human plasma not containing Tr was used to prepare Tr concentrations of 0.75, 3.75, 10.5 and 15 µg/mL. These samples were spiked with known concentrations of Tr. Percent recovery of Tr from plasma samples was calculated by dividing the difference in Tr concentrations by the added concentration. Recovery experiments were carried out on three different occasions.

Limits of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ of the method were determined mathematically from the standard curve equations. The LOD and LOQ were calculated using the formulae 3.3 × α/S and 10.0 × α/S respectively, α being the standard deviation of Y-axis intercepts and S being the slope of the calibration curve.

RESULTS AND DISCUSSION

When the above described chromatographic conditions were applied, we observed Tr to get well separated and viewed as a discrete peak of Tr calibration standards 20.0 and 0.5 µg/mL at the retention time of 4.7 minutes. Blank plasma sample did not give any peak at the retention time of Tr. Representative chromatograms of lowest (0.5 µg/mL) and highest (20.0 µg/mL) of Tr. Specificity experiments in the presence of certain drugs used in the treatment of TB, HIV and diabetes mellitus did not interfere in the analytical method of Tr.

The precision of the analytical method was also determined by analyzing three plasma samples containing different concentrations of Tr. The RSD from these experiments were 102%, 98% and 100% respectively. The LOD and LOQ of the method were calculated as 0.25 and 0.5 µg/mL respectively. The percent recovery of Tr ranged from 94% to 107% from experiments conducted on three occasions. The mean plasma Tr concentrations measured on days 1 and 15, in two samples were 20.77, 0.46 and 20.52, 0.48 µg/mL respectively.

We describe a simple, sensitive and specific high performance liquid chromatographic method for estimation of Tr in plasma. Method validation was carried out in accordance with FDA

guidelines, and the results were within the acceptable limits. The calibration curve of Tr for concentrations ranging from 0.5-20.0 µg/mL was observed to be linear, which spans the concentrations of clinical relevance. The LOQ was calculated as 0.5 µg/mL which denotes that the method described here is highly sensitive and significant in patients with TB or HIV during treatment with Tr. Furthermore, the method yielded satisfactory recovery of the desired analyte, and at the same time eliminated interfering materials from plasma.

Since Tr possesses potent anti-mycobacterial and anti-retroviral activity, it is likely that the drug would be used in the treatment of TB and HIV along with other medications. Hence it is essential to rule out interference of other anti-TB and anti- retroviral drugs in the analytical method of Tr, and establish the specificity of the method. We observed that no plasma endogenous substances or anti-TB drugs (rifampicin, isoniazid, pyrazinamide, ethambutol, ethionamide, levofloxacin, ofloxacin, moxifloxacin, rifabutin, rifapentine), anti-retroviral drugs (nevirapine, efavirenz, zidovudine, didanosine, stavudine, didanosine, lamivudine, ritonavir, tenofovir) and anti-diabetic drugs (metformin and sulphonyl ureas) interfered in the method.

Stability experiments showed no degradation (<10%) of Tr occurred in human plasma when stored at -20°C up to a period of 15 days.

CONCLUSION

During the resolution processes the enantiomers tend to form a more stable, more symmetrical conformation, according to their own code. In course of interactions they tend to reproduce themselves enforcing their own code. While the self-reproduction of racemic compounds is encoded by their eutectic composition, the resolving agent pursues to reproduce itself from the enantiomers of racemic compound but in the ratio of its eutectic composition, of its stoichiometry.

The molecular structure of the single enantiomer is the code for reacting with other (foreign) chiral molecules.

REFERENCES

1. Kumar V, Ramasamy B, Doble M, Manikkam R, Elizabeth HL, Arumugam SK, et al. Discovery of novel antibiotic, Transitmycin from marine Streptomyces species R2 against Mycobacterium tuberculosis and Human Immunodeficiency Virus. Scientific Reports. 2020. (in Press)
2. Kumar V, Doble M, Ramasamy B, Ganesan S, Manikkam R, Hanna L, et al. A Compound, Transitmycin, Effective Against Bacterial And Viral Pathogens. 2015;WO2015/022698Al.
3. Sharma S, Gelman E, Narayan C, Bhattacharjee D, Achar V, Humnabadkar V, et al. Simple and rapid method to determine antimycobacterial potency of compounds by using autoluminescent Mycobacterium tuberculosis. Antimicrob Agents Chemother 2014;58(10):5801-5808.