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Research Article

**CHARACTERIZATION OF BI-LAYERED TABLET FOR  
TREATING HEPATOTOXICITY CAUSED BY  
ANTITUBERCULAR DRUG FOR THE EFFECTIVE  
MANAGEMENT OF TUBERCULOSIS.****Shreya Tripathi \*, Sailesh kumar Ghatuary, Satkar Prasad, Satyawar Dangi**  
RKDF School of pharmaceutical Science, Bhabha University, Bhopal**Article Received:** December 2022    **Accepted:** December 2022    **Published:** January 2023**Abstract:**

*The proposed study aimed at development and characterization of bi-layered tablet for treating hepatotoxicity caused by antitubercular drug for the effective management of tuberculosis. Isoniazid is first line antitubercular drug which acts via inhibiting InhA and KasA genes. Based on its solubility and log P values, drug was found to be hydrophilic in nature. Isoniazid is first line drug due to its high efficacy but major disadvantage associated with this drug is hepatotoxicity. Due to this drawback there are various chances to discontinue the therapy. Hence, to prevent this discontinuation silymarin, a herbal hepatoprotective drug can be used in combination with isoniazid.*

*Isoniazid was obtained as white, crystalline powder and its melting point was found to be in 161°C-164° C range. It has maximum absorbance at 261nm and endothermic DSC peak at 163°C. Based on solubility profile and log P value, amphiphilic nature of Silymarin was determined. Silymarin was obtained as solid powder with maximum absorbance at 288 nm The drug content of bilayer tablet was estimated by simultaneous estimation method. Bilayer tablet was prepared by using HPMC, Carbopol, and cyclodextrin in optimum concentration. Prepared bilayer tablet was optimized for in vitro drug release in altered media. Then the formulation with higher sustained release was selected for bilayer tablet formulation.*

*Bilayer tablet was characterized on the basis of different parameters such as hardness, friability, weight variation, drug content and in vitro drug release. Drug release kinetics of silymarin from bilayer tablet was found to be Hixson Crowell mechanism whereas INH followed Higuchi diffusion model.*

**Key words:** hepatotoxicity, antitubercular, tuberculosis, Isoniazid, amphiphilic nature.

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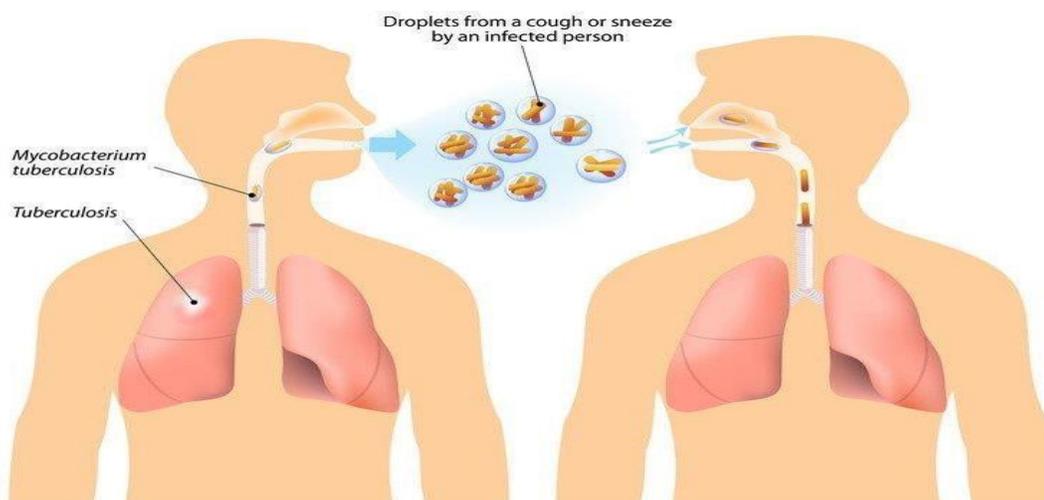


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**INTRODUCTION:**

The disease primarily affects lungs and causes pulmonary tuberculosis. It can also affect intestine, meninges, bones and joints, lymph glands, skin and other tissues of the body. The disease is usually chronic with varying clinical manifestations. The disease also affects animals like cattle; this known as "bovine tuberculosis", which may sometimes be communicated to man.

According to the most recent Global Tuberculosis Report (2019) edited by the World Health Organization (WHO), T.B. is considered the ninth cause of death worldwide and the leading cause of mortality by a single infectious agent, with the highest rate of infections and death toll rate mostly concentrated in developing and low-income countries.

**TUBERCULOSIS**

**Figure-1: Transmission of tuberculosis (Dua *et al.*, 2018)**

**Types of tuberculosis****a) Primary tuberculosis**

Primary tuberculosis is the form of the disease that develops in a previously unexposed and therefore, unsensitized patient. This complication occurs in patients who are overtly immune compromised or who have more subtle defects in host defences, as is characteristic of malnourished individuals. The incidence of progressive primary tuberculosis is particularly high in HIV-positive patients with significant immunosuppression. Immunosuppression results in an inability to mount a CD4+ T cell-mediated response that would contain the primary focus; because hypersensitivity and resistance are most often concomitant factors, the lack of a tissue hypersensitivity reaction results in the absence of the characteristic caseating granulomas (nonreactive tuberculosis) (Kumar *et al.*, 2018).

**(b) Secondary Tuberculosis**

Secondary tuberculosis should always be an essential consideration in HIV positive patients who present

with pulmonary disease. For example, patients with less severe immunosuppression (CD4+ T cell counts >300 cells/ $\mu$ L) present with "usual" secondary tuberculosis (an apical disease with cavitation) while those with more advanced immunosuppression (CD4+ T cell counts below 200 cells/ $\mu$ L) present with a clinical picture that resembles progressive primary tuberculosis (lower and middle lobe consolidation, hilar lymphadenopathy) immunosuppressed patients to greater than 50% in those with severe immune deficiency (Kumar *et al.*, 2018).

**Epidemiology**

A total of 1.5 million people died from TB in 2020 (including 214 000 people with HIV). Worldwide, TB is the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS). Tuberculosis (TB) is a major public health problem globally and is one of the major causes of death among adults in the world. As per the Global TB Report 2020, in 2019, it was estimated that 10

million people (range 8.9–11.0 million) developed TB disease; TB caused an estimated 1.2 million deaths (range 1.1–1.3 million) among human

immunodeficiency virus (HIV)-seronegative people and an additional 208,000 deaths (range 177,000–242,000) among HIV-seropositive individuals.

#### WHO Statistics of India- 2019

Estimates of TB Burden (WHO)	Number	Rate per 100,000 Population
Incidence of TB cases (includes HIV+TB)	2.640	193
Incidence (HIV+TB only)	71,000	5.2
Incidence (MDR/RR-TB)	124,000	9.1
Mortality (Deaths) (Excludes HIV+TB)	436,000	32
Mortality (Deaths) (HIV+TB only)	9500	0.69

The following epidemiological indices are generally used in tuberculosis problem measurement and programme strategy:

- 1. Incidence** is defined as the number of new and recurrent (relapse) episodes of T.B. (all forms) occurring in a given year.
- 2. Prevalence** is defined as the number of T.B. cases (all types) at a given point in time. It is the best available practical index to estimate the caseload in a community.
- 3. Mortality** from T.B. is defined as the number of deaths caused by T.B. in HIV- negative people, according to the latest revision of the International Classification of Diseases (ICD-10).
- 4. The case fatality rate** is the risk of death from T.B. among people with active T.B. disease.
- 5. The case notification rate** refers to new and recurrent episodes of T.B. notified to WHO for a given year, expressed per 100,000 population.
- 6. Case detection rate:** The case detection rate is calculated as the number of notifications of new and relapse cases in a year divided by the estimated

#### BILAYER TABLET

Bi-layer tablet is a unit compressed tablet dosage form intended for oral application. It contains two layers in which one layer having conventional or immediate release part of single or multiple active ingredients, another layer is sustained or controlled release part of single or multiple active ingredients.

Bi-layer tablets are novel drug delivery system where combination of two or more drugs in single unit having different release profiles improves the

patient compliance, prolongs the drug action, resulting in effective therapy along with better control of plasma drug level.

Bi-layer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances, and also for sustained release tablet in which one layer is immediate release as initial dose and second layer is maintenance dose (**Lachman and Liberman, 1987**).

#### ADVANTAGES

- IR and SR in the same tablet for chronic condition requiring repeated dosing.
- Promoting patient convenience and compliance because fewer daily doses are required compared to traditional delivery system.
- Two different drugs in same dosage form.
- Separation of incompatible components thus minimizes the physical and chemical incompatibilities.
- Solve degradation problem.
- Retain potency and ensure dose accuracy.

#### ADVANTAGES OF BI-LAYER TABLETS OVER CONVENTIONAL TABLETS

- Blood level of drug can be held at consistent therapeutic level for improved drug deliver, accuracy, safety and reduce side effects. Reduction of adverse side effects can be accomplished by targeting the drug release to the absorption site as well as controlling the rate of release, enabling the total drug content to be reduced.
- Patient convenience is improved by fewer daily doses are required compared to traditional system. Patient compliance is enhanced leading to improved drug regimen efficacy.

Bilayer tablets are readily lend themselves to repeat action products, where in one layer on layered tablet provides the initial dose, rapidly disintegration in the stomach, the layer are insoluble in gastric media but released in the intestinal environment.

- Separate physically and chemically incompatible ingredients.

### Etiology

Mycobacteria are slender rods that are acid-fast (i.e., they have a high content of complex lipids that readily bind the Ziehl-Neelsen [carbol fuchsin] stain and subsequently stubbornly resist decolourization). *M. tuberculosis* hominis is responsible for most cases of tuberculosis; the reservoir of infection typically is found in individuals with active pulmonary disease. Transmission usually is direct, by inhalation of airborne organisms in aerosols generated by expectoration or by exposure to contaminated secretions of infected individuals. Oropharyngeal and intestinal tuberculosis contracted by drinking milk contaminated with Mycobacterium Bovis infection is now rare except in those countries with tuberculous dairy cows and sales of unpasteurized milk. Other mycobacteria, particularly Mycobacterium avium complex, are much less virulent than *M. tuberculosis* and rarely cause disease in immunocompetent individuals. However, they cause disease in 10% to 30% of patients with AIDS (Kumar *et al.*, 2018).

The pathogenesis of tuberculosis in the previously unexposed immunocompetent individual is centred on the development of cell-mediated immunity, which confers resistance to the organism and results in the development of tissue hypersensitivity to tubercular antigens. The pathologic features of tuberculosis, such as caseating granulomas and cavitation results in the destructive tissue hypersensitivity that is a part and parcel of the host immune response. Because the effector cells for both protective immunity and damaging hypersensitivity are the same, the appearance of tissue hypersensitivity also signals the acquisition of resistance to the organism. The sequence of events from inhalation of the infectious inoculum to containment of the primary focus can be outlined as follows:

- **Entry into macrophages-** A virulent strain of mycobacteria gains entry to macrophage endosomes, a process mediated by several macrophage receptors, including the macrophage mannose receptor and complement receptors that recognize several components of the

mycobacterial cell walls.

- **Replication in macrophages-** Once internalized, the organisms inhibit normal microbicidal responses by preventing the fusion of the lysosomes with the phagocytic vacuole, allowing the Mycobacterium to persist and proliferate. Thus, the earliest phase of primary tuberculosis (the first three weeks) in the non-sensitized patient is characterized by bacillary proliferation within the pulmonary alveolar macrophages and air spaces, eventually resulting in bacteremia and seeding of the organisms to multiple sites. Despite the bacteremia, most individuals at this stage are asymptomatic or have a mild flu-like illness.
- **Development of cell-mediated immunity-** This occurs approximately three weeks after exposure. Processed mycobacterial antigens reach the draining lymph nodes and are presented to CD4 T cells by dendritic cells and macrophages. Under the influence of macrophage-secreted IL-12, CD4+ T cells of the TH1 subset are generated that are capable of secreting IFN- $\gamma$ .
- **T cell-mediated macrophage activation and killing of bacteria-** IFN- $\gamma$  released by the CD4+ T cells of the TH1 subset is crucial in activating macrophages. Activated macrophages, in turn, release a variety of mediators and up regulate expression of genes with critical downstream effects, including -
  - TNF, which is responsible for the recruitment of monocytes, which in turn undergo activation and differentiation into the "epithelioid histiocytes" that characterize the granulomatous response.
  - Inducible nitric oxide synthase (iNOS), which raises nitric oxide (NO) levels, helping to create reactive nitrogen intermediates that appear to be particularly important in killing of mycobacteria.
  - Antimicrobial peptides (defensins) that is also toxic to mycobacterial organisms.

### Granulomatous inflammation and tissue damage-

In addition to stimulating macrophages to kill mycobacteria, the TH1 response initiates the formation of granulomas and caseous necrosis. . Macrophages activated by IFN- $\gamma$  differentiate into the "epithelioid histiocytes" that aggregate to form granulomas; some epithelioid cells may fuse to form giant cells. In many individuals, this response stops the infection before significant tissue destruction or illness occurs. In other individuals with immune deficits due to age or immunosuppression, the disease progresses, and the ongoing immune response results in caseation necrosis. Activated macrophages also secrete TNF

and chemokines, which promote the recruitment of more monocytes.

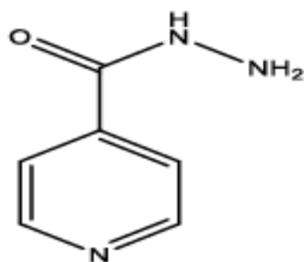
In summary, immunity to a tubercular infection is primarily mediated by TH1 cells, which stimulate macrophages to kill mycobacteria. This immune response, while largely effective, comes at the cost of hypersensitivity and the accompanying tissue destruction. Defects in any of the steps of a TH1 T cell response (including IL-12, IFN- $\gamma$ , TNF, or nitric oxide production) result in poorly formed granulomas, absence of resistance, and disease progression. Individuals with inherited mutations in any component of the TH1 pathway are extremely susceptible to infections with mycobacteria. Reactivation of the infection or re-exposure to the bacilli in a previously sensitized host results in rapid mobilization of a defensive reaction but also increased tissue necrosis (Kumar *et al.*, 2018).

### Research envisaged

Tuberculosis is an infectious chronic granulomatous illness caused by *Mycobacterium tuberculosis*. It is an airborne disease that primarily affects the lungs and other organs or tissue in the body, causing severe coughing, fever, and chest pain.

Tablets are one of the most commonly used conventional cost-effective dosage forms. Several bilayer tablet containing drugs are effectively used to treat several diseases. An anti-tubercular drug such as

### Drug profile



### □ Isoniazid

- **Structure**
- **IUPAC name** - pyridine-4-carbohydrazide
- **Molecular formula** - C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O
- **Molecular mass** - 137.14 g/mol
- **Physical appearance** - odorless, white crystalline powder
- **Melting point** - 170-173 °C
- **Solubility**- Freely soluble in water; sparingly soluble in ethanol (95 per cent); slightly soluble in chloroform; very slightly soluble in ether.
- **Biological half-life**- Fast acetylators: 0.5 to 1.6 hours. Slow acetylators: 2 to 5 hours.
- **Absorption** - Readily absorbed following oral administration; however, may undergo significant first pass metabolism. Absorption and bioavailability are reduced when isoniazid is administered with food.
- **Metabolism**- Primarily hepatic
- **Route of elimination** -From 50 to 70 percent of a dose of Isoniazid excreted in the urine within 24 hours.

isoniazid is widely used first-line drug for the treatment of tuberculosis. Isoniazid acts on InhA and KasA gene hence inhibiting the synthesis of mycolic acids leading to mycobacterium death.

The major disadvantage of isoniazid is idiosyncratic (host-dependent) drug-induced hepatotoxicity due to the induction of oxidative stress, mitochondria dysfunction peroxidation of endogenous lipid, reactive toxic metabolites such as caused acetyl hydrazine (AcHz), hydrazine (Hz), and acetyl isoniazid (AcINH). Due to this major drawback isoniazid is discontinued from the regimen.

Silymarin is a herbal drug which is most widely used for the treatment of hepatic disease due to its antioxidant, anti-inflammatory, and anti-fibrotic activities which acts via reduction of the free radicals formed by toxins that damages the cell membranes (LPO). Additionally, silymarin metabolically stimulates hepatic cells and activates the RNA synthesis of ribosomes to stimulate protein formation.

The combination of isoniazid and silymarin can be used in bilayer tablet for effective management of tuberculosis.

Hence, it is envisaged to develop a bilayer tablet of isoniazid and silymarin for the effective management of tuberculosis.

**MATERIALS AND METHODS:**

Isoniazid (INH) was obtained as gift samples from Amsal chem pvt. Ltd.(Ankleshwar).

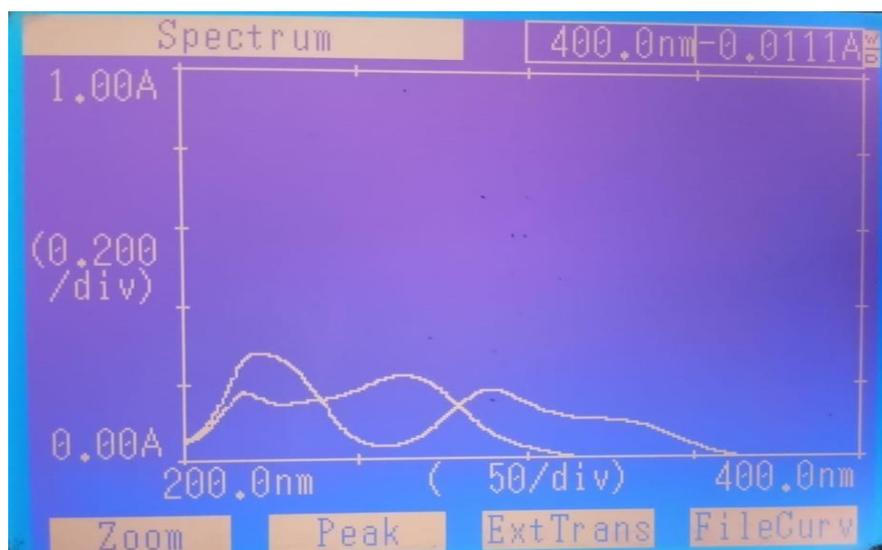
These were tested for impurities by determining melting point and IR spectra and no impurities were detected. All other chemicals were analytical grade.

**Apparatus**

Shimadzu - UV mini 1240 (Japan) single beam UV-Vis spectrophotometer was used. Absorption and overlain spectra of the test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell.

**Method of Estimation**

Stock solution of INH and Silymarin were prepared separately to obtain a final concentration of 100 µg/ml. Accurately weighed 10mg of INH was dissolved in methanol and the volume was made up to 100ml with methanol in a 100ml volumetric flask. Accurately weighed 10mg of Silymarin was dissolved in 100ml methanol in a 100ml



**Table 1: Absorbance and Absorptivity of INH at  $\lambda_{max}$  261 and 222 nm**

S.no.	Conc. (µg/ml)	Absorbance at $\lambda_{max}$		Absorptivity	
		261nm	222nm	261nm ( $a_{x1}$ )	222nm ( $a_{x2}$ )
1	2	0.0444	0.0177	22.2	8.85
2	4	0.0767	0.0496	19.17	12.4
3	6	0.1301	0.0905	21.68	15.08
4	8	0.1723	0.1322	21.53	16.525
5	10	0.244	0.1723	24.4	17.23
6	12	0.2916	0.2211	24.3	18.42
7	14	0.3233	0.2311	23.09	16.50
8	16	0.3666	0.2942	22.91	18.3
9	18	0.4094	0.3159	22.74	17.55
10	20	0.4521	0.351	22.60	17.55

The mean absorptivity of INH at 261 nm ( $a_{x1}$ ) = 22.4625 at 222 nm ( $a_{x2}$ ) = 15.848

Table 2: Absorbance and Absorptivity of Silymarin at  $\lambda_{\max}$  261 nm and 222 nm

S.no.	Conc ( $\mu\text{g/ml}$ )	Absorbance at $\lambda_{\max}$		Absorptivity	
		253.5nm	351nm	253.5nm (ay1)	351nm (ay2)
1	2	0.0124	0.0896	6.2	44.8
2	4	0.0247	0.1045	6.175	26.12
3	6	0.0385	0.1887	6.41	31.45
4	8	0.0552	0.2502	8.9	31.27
5	10	0.0824	0.3235	8.24	32.35
6	12	0.102	0.3718	8.5	30.98
7	14	0.1229	0.4309	8.7	30.77
8	16	0.1421	0.4789	8.8	29.93
9	18	0.1636	0.5262	9.08	29.23
10	20	0.1829	0.6443	9.145	32.21

The mean absorptivity of SM at 261 nm ( $\text{ay}_1$ ) = 7.814

at 222 nm ( $\text{ay}_2$ ) = 1.913

#### Development of Simultaneous Equations

The absorbance and absorptivity coefficients of standard dilutions of both drugs, within the linearity range (2-20  $\mu\text{g/ml}$ ) were determined at both the selected wavelengths (261 nm and 222 nm). The method employed for the development of simultaneous equations using Cramer's rule and matrices, employing the mean absorptivity values. According to the Beer-Lambert law, The absorbance (A) is directly proportional to the concentration (C) of the solution of the

sample.

$$A \propto C \dots\dots\dots (1)$$

And the absorbance (A) is directly proportional to the length of the light path (l), which is equal to the width of the cuvette.

$$A \propto l \dots\dots\dots (2)$$

Combining Equations (i) and (ii):

$$A \propto c * l \text{ or}$$

$$\epsilon = A / C * l$$

The constant 'ε' is called **molar absorptivity** or **molar extinction coefficient** and is a measure of the probability of the electronic transition. The unit of 'ε' is "Lit.mol<sup>-1</sup>.cm<sup>-1</sup>," where the unit of C is mol. Lit<sup>-1</sup>, l is in cm.

But due to complexity of the calculation, in our study we convert the molar absorptivity into absorptivity, the unit of which is Lit.gm<sup>-1</sup>.cm<sup>-1</sup> because all the calculations were in gm/Lit or μg/Lit.

**Molar absorptivity = Absorptivity \*Molecular Weight**

Therefore,

Absorptivity = A/conc (gm/lit) x l (cm)

Here, l is 1 cm (the width of quartz cell was 1 cm).

If,

- The absorptivity of drug X at λ<sub>max1</sub> and λ<sub>max2</sub> are a<sub>x1</sub> and a<sub>x2</sub> respectively.
- The absorptivity of drug Y at λ<sub>max1</sub> and λ<sub>max2</sub> are a<sub>y1</sub> and a<sub>y2</sub> respectively and
- The absorbances of the diluted sample at λ<sub>max1</sub> and λ<sub>max2</sub>, A<sub>1</sub> and A<sub>2</sub> respectively.
  - C<sub>X</sub> and C<sub>Y</sub> are the concentrations of X and Y respectively in the diluted sample. A set of two simultaneous equations were framed:

At λ<sub>max1</sub>, A<sub>1</sub> = (a<sub>x1</sub>\*C<sub>X</sub>\*1) + (a<sub>y1</sub>\*C<sub>Y</sub>\*1).....(3)

At λ<sub>max2</sub>, A<sub>2</sub> = ( a<sub>x2</sub>\*C<sub>X</sub>\*1) + (a<sub>y2</sub>\*C<sub>Y</sub>\*1)..... (4) Substituting for C<sub>Y</sub> in eq. (iii) and rearranging gives

C<sub>Y</sub>= A<sub>1</sub> - a<sub>x1</sub>\*C<sub>X</sub> a<sub>y1</sub>.....(5)

Substituting value of C<sub>Y</sub> in eq. no. (iv)

$$C_X = \frac{A_2 \cdot a_{y1} - A_1 \cdot a_{y2}}{a_{y1} \cdot a_{x2} - a_{y2} \cdot a_{x1}} \dots\dots\dots (6)$$

In this study,

- The drug X was INH
- The drug Y was Silymarin
- λ<sub>max1</sub> was 261nm and λ<sub>max2</sub> was 222 nm
- Absorptivity (mean) at a<sub>x1</sub> and a<sub>x2</sub> were 22.46 and 15.48 respectively
- Absorptivity (mean) at a<sub>y1</sub> and a<sub>y2</sub> were 7.814 and 31.913 respectively

**RESULT AND DISCUSSION:**

The proposed simultaneous equation method has been found to be very simple that can be applied for the estimation of the two drugs in a mixed solution simultaneously by use of any spectrophotometer and does not require any costly instrument equipped with special package. It also shows good linearity values and sensitivity. The result of recovery study demonstrated that simultaneous equation method by UV/visible spectrophotometer could be successfully used for the determination of INH and SM when they are given in a same dosage form or present in same solution etc. The absorbance was measured at different wavelengths and then concentration of both the drugs can be determined using derived equations.

A study of overlain spectra of INH and SM in methanol shows that at 261 INH shows maximum absorbance whereas SM shows almost zero absorbance and at 222 nm SM shows maximum absorbance where INH shows almost zero absorbance. This indicates that there is considerable difference in absorbance peaks of both the drugs and hence there is no interference of one drug in the estimation of both drugs simultaneously. Recovery study for simultaneous estimation was performed using standard addition method.

The proposed method for simultaneous estimation of INH and SM in combined sample solutions was found to be simple, reproducible and accurate. Once the equations are determined then only value substitution is required in order to calculate the amount of drug released. It is a new novel method and can be used for routine analysis in quality control laboratories.

**REFERENCES:**

1. Quinonez CG, Lee JJ, Lim J, Odell M, Lawson CP, Anyogu A, Raheem S, Eoh H. The role of fatty acid metabolism in drug tolerance of Mycobacterium tuberculosis. *Mbio*. 2022 Jan 11;13(1):e03559-21.
2. Tabriz AG, Nandi U, Hurt AP, Hui HW, Karki S, Gong Y, Kumar S, Douroumis D. 3D printed bilayer tablet with dual controlled drug release for tuberculosis treatment. *International Journal of Pharmaceutics*. 2021 Jan 25; 593:120147.
3. Chakaya J, Khan M, Ntumi F, Aklillu E, Fatima R, Mwaba P, Kapata N, Mfinanga S, Hasnain SE, Katoto PD, Bulabula AN. Global Tuberculosis Report 2020—Reflections on the Global TB burden, treatment and prevention efforts. *International Journal of Infectious Diseases*. 2021 Dec 1; 113:S7-12.
4. Court R, Chirehwa MT, Wiesner L, de Vries N, Harding J, Gumbo T, Maartens G, McIlleron H.

Effect of tablet crushing on drug exposure in the treatment of multidrug-resistant tuberculosis. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease*. 2019 Oct 1;23(10):1068.

5. Pepping J. Milk thistle: *Silybum marianum*. *Am J Health Syst Pharm* 1999;56:1195-7.
6. Genina N, Boetker JP, Colombo S, Harmankaya N, Rantanen J, Bohr A. Anti-tuberculosis drug combination for controlled oral delivery using 3D printed compartmental dosage forms: From drug product design to in vivo testing. *Journal of controlled Release*. 2017 Dec 28;268:40-8.
7. Terracciano E, Amadori F, Zaratti L, Franco E, ‘Tuberculosis: an ever present disease but difficult to prevent’. *Igiene e sanita pubblica*. 2020 Jan-Feb
8. Perrier S.; Lunn A.; Unnikrishnan M.,” Dual pH-Responsive Macrophage-Targeted Isoniazid Glycoparticles for Intracellular Tuberculosis Therapy” *Biomacromolecules* 2021, 2022,
9. A. Matteelli, A. Roggi, A.C. Carvalho, Extensively drug-resistant tuberculosis: epidemiology and management, *Clin. Epidemiol.* 6 (2014) 111–118.
10. J. Deacon, S.M. Abdelghany, D.J. Quinn, D. Schmid, J. Megaw, R.F. Donnelly, et al., Antimicrobial efficacy of tobramycin polymeric nanoparticles for *Pseudomonas aeruginosa* infections in cystic fibrosis: formulation, characterization and functionalization with dornase alfa (DNase), *J. Control. Release: official journal of the Controlled Release Society* 198 (2015) 55–61.
11. K.Dua, R. Awasthi, J.R. Madan, D.K. Chellappan, B.N. Nalluri, G. Gupta, M. Bebawy, P.M. Hansbro, Novel drug delivery approaches in treating pulmonary fibrosis, *Panminerva Med.* (2018)
12. S.G. Mpagama, N. Ndusilo, S. Stroup, H. Kumburu, C.A. Peloquin, J. Gratz, et al., Plasma drug activity in patients on treatment for multidrug-resistant tuberculosis, *Antimicrob. Agents Chemother.* 58 (2014) 782–788.
13. Bhardwaj A., Mehta S., Yadav S., Singh S., Anne Grobler, Goyal A. & Mehta A. (2016) Pulmonary delivery of antitubercular drugs using spray-dried lipid-polymer hybrid nanoparticles, *Artificial Cells, Nanomedicine, and Biotechnology*, 44:6, 1544-1555.
14. World Health Organization Global Tuberculosis Report 2020. <https://www.who.int/publications/i/item/9789240013131>