ISSN: 2455-8109

International Journal of Farmacia (IJF)

IJF |Vol.11 | Issue 2 | Apr - Jun -2025

www.ijfjournal.com

DOI : https://doi.org/10.61096/ijf.v11.iss2.2025.87--92

Research

Stability Indicating Method Development And Validation Of Indomethacin And Sulphamethoxazole By Rp-Hplc

G. Harshitha*, Priyanka. K[1], Sameena Begum[2], M. Bhaskar[3], Yakub pasha[4], K. Sreevani[5]

*Author for Correspondence: G. Harshitha
Email: teelavath@gmail.com

Published on: 08 May 2025

Published by:
DrSriram Publications

Abstract

A simple, accurate, precise approach was devised to estimate Indomethacin (IDM) and Sulphamethaxazole (SMZ) in tablets. A Phenomenex Luna C18 250 x 4.6 mm, 5mm chromatogram was conducted. Mobile phase with Buffer 10Mm Ammonium Acetate: Acetonitrile 50:50v/v was injected through column at 0.7ml/min. Optimal wavelength was 285nm. IDM and SMZ retention times were 2.861min and 7.273, respectively, and their %RSDs were 0.9 and 0.9. % Recovery was 100.46% for IDM and 100.20% for SMZ. IDM and SMZ regression equations yielded LOD, LOQ values of 0.08, 0.25, and 0.04, 0.12. IDM regression equation is y = 15895x + 41.3 and SMZ y = 3720x + 244.1. The method devised was straightforward and economical for routine quality control tests in industries because retention and run times were reduced.

Keywords: Indomethacin, Sulphamethaxazole, RP-HPLC, Validation

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for separating, identifying, and quantifying components in a mixture. It operates on the principle of liquid-phase chromatography, where a liquid mobile phase carries the analytes through a stationary phase (column), leading to differential retention and separation based on chemical properties such as polarity, molecular weight, and interaction with the stationary phase.[1]

Indomethacin (IDM) is a nonsteroidal anti-inflammatory drug (NSAID) with a molecular formula of $C_{28}H_{27}N_3O_3$ and a molecular weight of 357.788 g/mol. It appears as a solid powder with low solubility (0.937 mg/L at 25°C) and should be stored at 25°C. Its IUPAC name is *2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid*.

IDM is a reversible, nonspecific inhibitor of cyclooxygenase (COX) enzymes, primarily COX-1 and COX-2. By inhibiting prostaglandin G/H synthase, it reduces PGE_2 levels, leading to decreased pain, inflammation, and fever. Though it inhibits both COX isoforms, IDM is more selective for COX-1, contributing to gastrointestinal side effects. Additionally, it blocks phospholipase A_2, preventing arachidonic acid release. Its

antipyretic action affects the hypothalamus, causing vasodilation and heat dissipation. IDM is marketed under the brand names Indocin and Tivorbex.[2],[3]

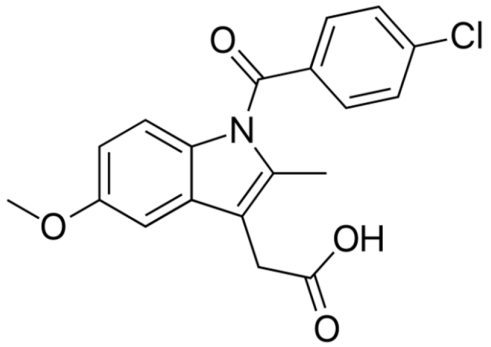

Fig 1: Structure of IDM

Sulfamethoxazole (SMZ) is a sulfonamide antibiotic belonging to the isoxazole class, with a molecular formula of $C_{10}H_{11}N_3O_3S$ and a molecular weight of 253.28 g/mol. It appears as a solid powder, with an aqueous solubility of 3942 mg/mL at 25°C and should be stored at 25°C. Its IUPAC name is *4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide*. SMZ inhibits dihydrofolic acid synthesis by mimicking para-aminobenzoic acid (PABA), disrupting bacterial growth. It functions as an antibacterial, anti-infective, P450 inhibitor, and dihydropteroate synthase inhibitor, and is also classified as an environmental contaminant and xenobiotic. [4],[5]

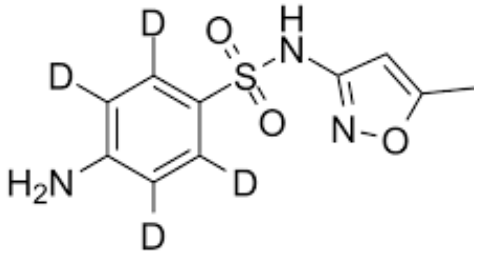

Fig. 2: SMZ structure

Several studies have explored analytical methods and clinical efficacy of IDM and SMZ. Tsvetkova et al. developed an RP-HPLC method for separating indomethacin and impurities in tablets.[6] Assali et al. created an indomethacin-paracetamol codrug, validated via RP-HPLC. [7] Kwong et al. analyzed indomethacin potency in suppositories and capsules, [8] while Johnson et al. measured plasma concentrations in infants using HPLC with high accuracy. [9] Sayar et al. (2010) developed an HPLC method to simultaneously detect trimethoprim (TMP) and SMZ in human plasma, proving sensitive, specific, accurate, and reliable for pharmacokinetic analysis. [10]
While there is no existing literature on the combination of IDM and SMZ, studies confirm no drug-drug interactions. Since they have distinct mechanisms of action, their combination reduces antibiotic resistance, supporting the development of a novel antibiotic formulation and an RP-HPLC method for its analysis.

MATERIALS AND METHODS

Chemicals and Solvents

HPLC grade Water and Methanol, Formic Acid AR Grade, IDM and SMZ API Standards and combined formulation. Tablets with 500mg of CFT and 400mg of SMZ were punched in our laboratory. SMZ (working standard) was obtained as a gift sample from Emcure Pharmaceuticals, India and CFT from GMT Pharma International.

Instrumentation

HPLC analysis was performed using Shimadzu LC 2030C 3D Plus HPLC (Prominence-i series) with Empower-2 software. The Agilent Zobrax C18 column (250 × 4.6 mm, 5µm) was used for separation.

Preparation of Standard Solution

To prepare the primary standard solution, 5 mg of IDM and 40 mg of SMZ were weighed and dissolved in 100 mL volumetric flasks, degassed for 10 minutes, and adjusted to obtain 50 µg/mL IDM and 400 µg/mL SMZ. Aliquots of 1–5 mL were diluted to 10 mL, yielding final concentrations of 2-10 µg/mL. 10 µL of each sample was injected thrice, and a calibration graph was plotted, showing a linear relationship between peak area and drug strength.

Assay

Tablets labelled 50mg IDM and 400mg SMZ were manufactured in our laboratory. The punched formulation was utilised for the experiment. After weighing 10 tablets, an amount equivalent to 50 mg IDM and 400 mg SMZ was transferred into a 10 mL volumetric flask. 5 mL of diluent was added, followed by 10 minutes of degassing. After adjusting the final volume, the solution was filtered using HPLC filters, obtaining a 50 µg/mL IDM and 400 µg/mL SMZ solution.

Method Validation

System suitability was assessed by injecting standard solutions of IDM (5µg/ml) and SMZ (40µg/ml) five times to evaluate the tailing factor, area, and USP plate count, with an RSD of less than 2%. Specificity was confirmed as no interfering peaks were observed at the drug retention times. Accuracy and linearity were evaluated by analyzing IDM and SMZ at concentrations ranging from 10–50 µg/ml, with a calibration curve confirming linearity and a recovery rate of 98–102%. Precision was assessed using spiked solutions at 50%, 100%, and 150% levels. Robustness was tested by varying the flow rate and mobile phase composition, with %RSD remaining within acceptable limits. Sensitivity was confirmed through LOD and LOQ studies, with sample dilutions of 0.25 ml and 0.3 ml demonstrating reliable detection.

RESULTS AND DISCUSSIONS

The optimized chromatographic conditions included a mobile phase of 10mM ammonium acetate buffer, acetonitrile, and methanol (50:25:25 v/v/v) with a flow rate of 8 mL/min. Separation was performed using a Phenomenex Luna C18 column (4.6 × 250 mm, 5 µm) at a column temperature of 10°C. The detector operated at a wavelength of 285 nm, with an injection volume of 10 mL and a run time of 8 minutes. A 50:50 v/v mixture of methanol and acetonitrile was used as the diluent. The experiment was considered optimized as the eluted peaks demonstrated good resolution, tailing factor, and theoretical plate count, ensuring the method's accuracy and reliability.

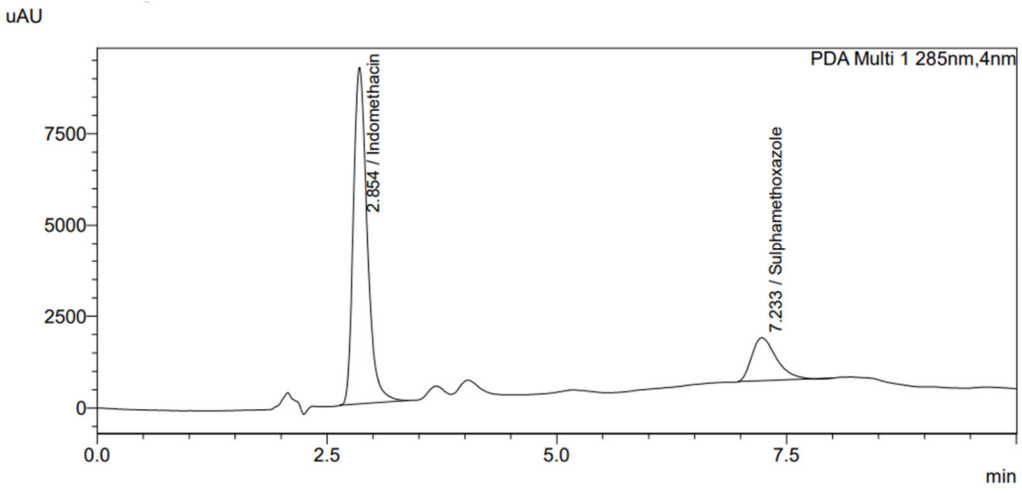

Fig 3: Chromatogram (Optimised)

The following table 1 and Figs. 4 and 5 shows the calibration data of IDM and SMZ. Correlation coefficients obtained were 0.994 for IDM and 0.996 for SMZ.

Table 1: Linearity data of CFT and SMZ

	CFT	SMZ
Conc (µg/ml)	**Area**	**Area**
2	29307	7507
4	66111	14875
6	96132	21791
8	127580	28122
10	157527	38083

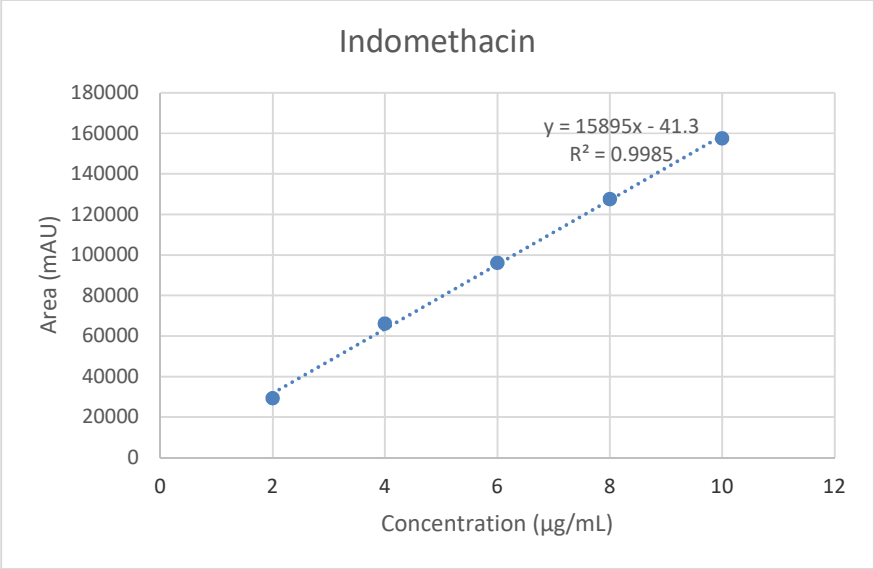

Fig. 4: Calibration graph for IDM

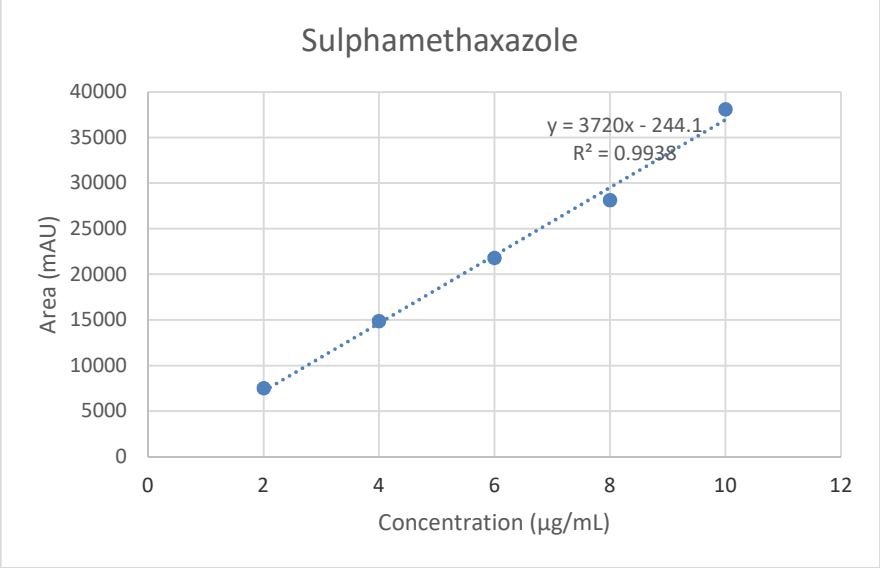

Fig. 5: Calibration graph for SMZ

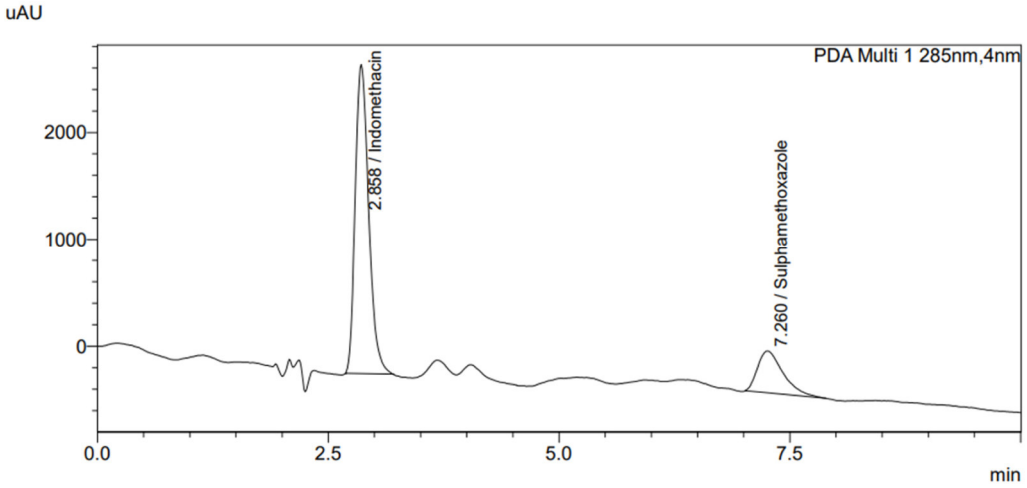

Fig 6: Chromatogram of Assay

IDM and SMZ were analyzed at five linear doses (2–10 µg/mL), with correlation coefficients of 0.9985 for IDM and 0.9938 for SMZ, confirming strong linearity. System precision was validated with %RSD values of 0.8% (IDM) and 0.6% (SMZ), remaining within acceptable limits. Accuracy testing using the conventional addition approach showed recovery rates of 100.46% for IDM and 100.20% for SMZ, demonstrating method reliability. The system suitability parameters met the required standards with minimal deviation.

Degradation Results

The degradation studies of IDM and SMZ were conducted under acidic, basic, oxidative, heat, UV, and neutral conditions to evaluate their stability. In acidic conditions, IDM showed 96.20% recovery with 3.80% degradation, while SMZ had 95.21% recovery and 4.79% degradation. Under basic treatment, IDM and SMZ exhibited 94.99% and 96.89% recovery, respectively, with degradation percentages of 5.01% and 3.11%. Oxidative conditions resulted in 96.52% recovery for IDM and 95.74% for SMZ, with 3.48% and 4.26% degradation, respectively. Heat exposure led to 97.98% recovery for IDM and 98.53% for SMZ, with minimal degradation of 2.02% and 1.47%. Under UV exposure, IDM and SMZ showed 99.17% and 98.76% recovery, with degradation of 0.83% and 1.24%, respectively. Neutral conditions provided the highest stability, with IDM and SMZ recovering 99.19% and 99.25%, and degradation of 0.71% and 0.75%, respectively. These results indicate that IDM and SMZ are most stable under neutral and UV conditions, while acidic and basic conditions cause greater degradation.

CONCLUSION

The method proved to be accurate, precise, robust, and cost-effective for the simultaneous estimation of IDM and SMZ in tablets. IDM and SMZ were retained at 2.854 and 7.233 minutes, respectively. LOD and LOQ values were 0.08 µg/mL and 0.25 µg/mL (IDM), and 0.04 µg/mL and 0.12 µg/mL (SMZ). With a regression coefficient of 0.999, the method was efficient, economical, and well-suited for drug testing laboratories.

REFERENCES

1 Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken, NJ: Wiley-Blackwell; 2009.
2 https://go.drugbank.com/drugs/DB00328
3 https://pubchem.ncbi.nlm.nih.gov/compound/3715
4 https://go.drugbank.com/drugs/DB01015
5 https://www.ncbi.nlm.nih.gov/books/NBK513232/
6 Tsvetkova B, Ivanka Pencheva, Alex Zlatkov, Pl. Peikov. High performance liquid chromatographic assay of indomethacin and its related substances in tablet dosage forms International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(3):549-552
7 Assali M, Abualhasan M, Zohud N, Ghazal N. RP-HPLC method development and validation of synthesized codrug in combination with Indomethacin, Paracetamol, and Famotidine. Int J Anal Chem

[Internet]. 2020;2020:1894907. Available from: http://dx.doi.org/10.1155/2020/1894907

[8] Kwong E, Pillai GK, McErlane KM. HPLC analysis of indomethacin and its impurities in capsule and suppository formulations. J Pharm Sci [Internet]. 1982;71(7):828–30. Available from: http://dx.doi.org/10.1002/jps.2600710730

[9] Johnson, A. G.; Ray, J. E.. Improved High-Performance Liquid Chromatographic Method for the Determination of Indomethacin in Plasma. Therapeutic Drug Monitoring 14(1):p 61-65, February 1992.

[10] Sayar E, Sahin S, Cevheroglu S, Hincal AA. Development and validation of an HPLC method for simultaneous determination of trimethoprim and sulfamethoxazole in human plasma. Eur J Drug Metab Pharmacokinet [Internet]. 2010;35(1–2):41–6. Available from: http://dx.doi.org/10.1007/s13318-010-0006-9