Indian J. Applied & Pure Bio. Vol. 41(1), 451-459 (2026). **ISSN: 0970-2091**

Method development of validated HPTLC method for simultaneous estimation of Sumatriptan and Naproxen in Pharmaceutical Dosage Forms

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Abstract

A simple, precise, accurate and stability-indicating High Performance Thin Layer Chromatographic (HPTLC) method was developed and validated for the simultaneous estimation of sumatriptan succinate and naproxen sodium in pharmaceutical dosage forms[1]. Chromatographic separation was achieved on pre-coated silica gel 60 GF_{254} plates using chloroform: methanol (8:2, v/v) as mobile phase. Densitometric scanning was performed at 250 nm using a deuterium lamp[2]. Well-resolved bands were obtained with Rf values of 0.59 for sumatriptan and 0.21 for naproxen. The method was validated in accordance with ICH Q2(R1) guidelines[3]. Linearity was established over the concentration range of 0.5–5.0 µg/mL for sumatriptan and 5–500 µg/mL for naproxen, with correlation coefficients of 0.9995 and 0.9991, respectively[4]. The limits of detection and quantitation were found to be 100 ng/mL and 300 ng/mL for sumatriptan and 10 ng/mL and 30 ng/mL for naproxen. Intra-day and inter-day precision studies showed %RSD values less t han 1%, indicating excellent precision. Accuracy studies demonstrated recoveries in the range of 101.66–103.21% for sumatriptan and 97.86–99.01% for naproxen[5]. The method was found to be robust with no significant variation upon deliberate changes in chromatographic conditions. Stability studies indicated minimal degradation of both drugs over 72 hours at 4 °C and 25 °C.[6]

Key words HPTLC; Sumatriptan; Naproxen; Method validation; Stability-indicating method.

Migraine is a chronic neurological disorder characterized by recurrent episodes of severe headache often associated with nausea, photophobia, and phonophobia[7]. Combination therapy using sumatriptan succinate and naproxen sodium has proven to be more effective than monotherapy, as it provides rapid pain relief and reduces recurrence

by targeting both vascular and inflammatory components of migraine.[8] Owing to their widespread clinical use in combined pharmaceutical dosage forms, reliable analytical methods are essential to ensure the quality, safety, and efficacy of these formulations.[9]

Sumatriptan succinate is a selective serotonin (5-HT_1 B/1D) receptor agonist used for the acute treatment of migraine attacks, while naproxen sodium is a non-steroidal anti-inflammatory drug (NSAID) that exerts analgesic and anti-inflammatory effects through inhibition of cyclooxygenase enzymes[10]. The combination of these two drugs has gained significant importance in migraine management due to improved therapeutic outcomes. Consequently, accurate and simultaneous estimation of sumatriptan and naproxen in pharmaceutical dosage forms is required for routine quality control and regulatory compliance[11].

In recent years, High Performance Thin Layer Chromatography (HPTLC) has emerged as a versatile and powerful analytical technique for qualitative, semi-quantitative, and quantitative analysis of drugs and pharmaceutical formulations. Compared to other chromatographic techniques, HPTLC offers several advantages such as simplicity, low solvent consumption, rapid analysis, and the ability to analyze multiple samples simultaneously.[12] These advantages make HPTLC particularly suitable for routine quality control analysis in pharmaceutical industries.[13]

Chromatographic conditions :

The different parameters of HPTLC such as mobile phase, band width, and detection wavelength were tried and then were optimized.

Instrumentation :

The chromatographic analysis was performed using a **CAMAG HPTLC system** (Muttenz, Switzerland) equipped with a **Linomat V sample applicator** and a **TLC Scanner III**. Data acquisition and processing were carried out using **Win CATS software (version 1.3.0)**. Sample preparation was facilitated by an **ultrasonicator** (Enertech Electronics Pvt. Ltd., India), and an **analytical balance (SHIMADZU AUX-120)** was used for accurate weighing of all reagents and standards. The instrumentation was operated according to the respective manufacturer's instructions to ensure precision and reproducibility of the chromatographic results.[14]

Reagents and Chemicals :

All chemicals and reagents used during the study were of analytical reagent (AR) grade and procured from Merck Chemicals Ltd., Mumbai. Methanol was selected as the primary solvent for preparation of standard and sample solutions due to its good solubility for both drugs and compatibility with the chromatographic system. Distilled water was used wherever required[15].

Selection of Stationary Phase :

Pre-coated aluminum TLC plates of **Silica Gel 60 GF_{254}** (Merck) were employed as the stationary phase. Prior to use, the plates were pre-washed with methanol to remove any impurities and subsequently activated at **60 °C for 5 minutes** to enhance reproducibility

and ensure proper adsorption characteristics[16].

Optimization of Mobile Phase :

The selection of the mobile phase was performed to achieve **adequate separation of both analytes**, ensuring **sharp and symmetrical peaks** and **Rf values within the acceptable range of 0.2–0.8**. Optimization of the mobile phase composition was carried out to obtain reproducible results and clear resolution of sumatriptan and naproxen on the HPTLC plates, following standard guidelines for method development and validation.

Several solvent systems with different polarity combinations were evaluated on a trial-and-error basis to achieve optimal separation of naproxen and sumatriptan. Among the tested systems, **chloroform:methanol (8:2, v/v)** provided the best resolution, producing well-defined peaks with reproducible Rf values. Under the optimized conditions, the **Rf value of naproxen** was 0.21 and the **Rf value of sumatriptan** was 0.59. Based on these results, this solvent system was selected as the **mobile phase for all subsequent HPTLC studies**.

Optimization of Band Width :

Different band widths of 6 mm, 7 mm, and 8 mm were evaluated to determine the optimal sample application conditions. A **band width of 7 mm** was found to be optimal as it provided **better peak shape**, **adequate separation without spot diffusion**, and the **ability to accommodate 12 tracks per 20 × 10 cm TLC plate**. Based on these observations, a band width of 7 mm was selected for all subsequent analyses[17].

Detection Wavelength Selection :

The UV absorption spectra of sumatriptan and naproxen were recorded individually to determine their respective λmax values, which were found to be **229 nm for sumatriptan** and **272 nm for naproxen**. For simultaneous estimation, a **compromise wavelength of 250 nm** was selected, at which both drugs exhibited adequate absorption with maximum area under the curve (AUC) and minimal baseline noise. Based on these observations, **densitometric scanning** of the HPTLC plates using a **deuterium lamp**.[18]

Optimized Chromatographic Conditions :

The simultaneous estimation of sumatriptan and naproxen was carried out using **pre-coated Silica Gel 60 GF_{254} plates (Merck)** as the stationary phase, and **chloroform: methanol (8:2, v/v)** as the mobile phase. Chromatographic development was performed in a **20 × 10 cm twin-trough glass chamber** saturated with the mobile phase for **15 minutes**. Linear ascending development was carried out for a migration distance of **8 cm** over **15 minutes**, and a **band width of 7 mm** was used for sample application. After development, the plates were air-dried and scanned densitometrically in reflectance-absorbance mode using CAMAG TLC Scanner III operated through WinCATS software. Quantification was based on the measurement of diffusely reflected light intensity.[19]

Preparation of Standard Solutions

Standard Stock Solution of Naproxen Sodium:

An accurately weighed **10 mg** of

naproxen sodium reference standard was transferred into a **10 mL volumetric flask**, dissolved in distilled water, and diluted up to the mark with distilled water to obtain a stock solution of **1000 µg/mL**.

Standard Stock Solution of Sumatriptan Succinate :

Similarly, **10 mg** of sumatriptan succinate reference standard was accurately weighed and transferred into a **10 mL volumetric flask**, dissolved in distilled water, and diluted to volume to obtain a stock solution of **1000 µg/mL**.

Preparation of Sample Solution :

Twenty tablets of the marketed formulation (**Treximet®**) were accurately weighed, and the average tablet weight was calculated. The tablets were then finely powdered to obtain a homogeneous mixture. An accurately weighed quantity of the powder equivalent to one tablet was transferred into a **100 mL volumetric flask**. The contents were dissolved in distilled water and sonicated for **30 minutes** to ensure complete extraction of sumatriptan succinate and naproxen sodium from the tablet matrix. The solution was allowed to cool to room temperature and then diluted up to the mark with distilled water. The resulting solution was filtered through **Whatman No. 41 filter paper** to remove insoluble excipients. The clear filtrate obtained was used as the sample solution for HPTLC analysis.[20]

Method validation :
Linearity :

Linearity was established by least-squares regression of peak area versus concentration over the range **5–100 µg/mL for Naproxen** and **0.5–10.0 µg/mL for Sumatriptan**. Good linearity was observed with correlation coefficients of **0.9991 (Naproxen)** and **0.9995 (Sumatriptan)**.[21]

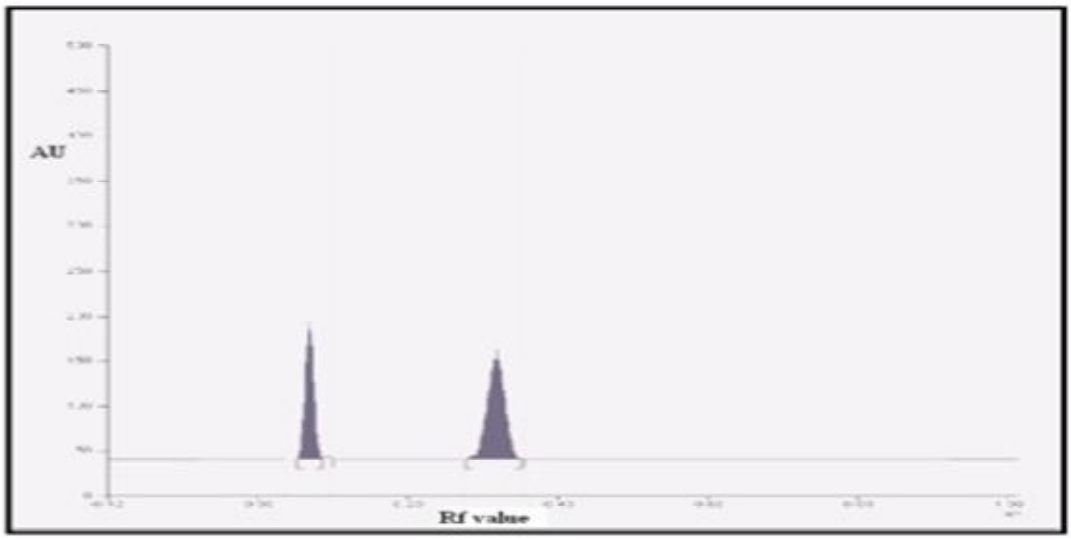

Fig. 1. HPTLC Chromatogram of Naproxen and Sumatriptan

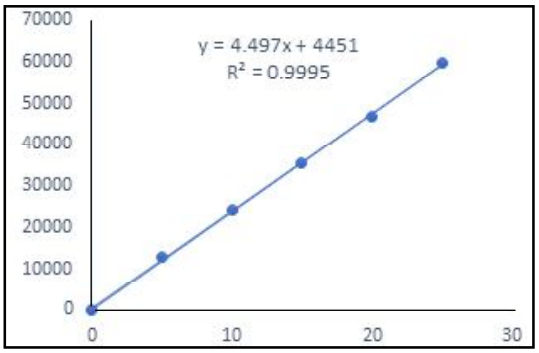

Fig. 2. Calibration curve of Sumatriptan

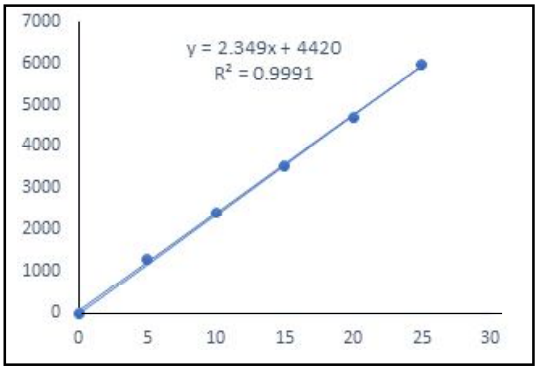

Fig. 3. Calibration curve of Naproxen

Limit of detection (LOD) and quantitation (LOQ) :

LOD and LOQ were calculated from the calibration curve using the equations **LOD = 3σ/S** and **LOQ = 10σ/S**, where σ is the standard deviation and S is the slope. The LOD and LOQ were found to be **100 and 300 ng/mL for Sumatriptan** and **10 and 30 ng/mL for Naproxen**, respectively.

Precision was evaluated by intra-day and inter-day studies at three concentration levels. The %RSD values were found to be **less than 1%**, indicating good precision of the method.

Both precision studies showed R.S.D. less than 1%, indicating a sufficient precision.[22]

Results of Intra-day Precision Studies of Sumatriptan and and Naproxen

Accuracy was assessed by recovery studies at **80%, 100%, and 120%** levels using the standard addition method. The percentage recoveries were within **97.86–103.21%**, indicating good accuracy of the method.[23]

Robustness

Robustness was evaluated by small deliberate variations in mobile phase composition (±2%) and detection wavelength (±2 nm), with no significant effect on

Table-1. Results of Intra-day Precision Studies of Sumatriptan & Naproxen

Sr.No	AUC for concentration of Sumatriptan (µg/ml)			AUC for concentration of Naproxen (µg/ml)		
	2.0	3.0	4.0	20.0	30.0	40.0
1.	2105.22	1631.61	1169.43	978.1	1354.2	1761.25
2.	2120.41	1624.3	1183.14	974.25	1359.4	1754.4
3.	2128.12	1635.01	1178.3	980.47	1360.9	1769.29
Mean	2117.91	1630.30	1176.95	977.60	1358.16	1761.64
% RSD	0.55	0.33	0.59	0.32	0.26	0.42

Table-2. Results of Inter-day Precision Studies of Sumatriptan and Naproxen

Sr.No	AUC for concentration of Sumatriptan (µg/ml)			AUC for concentration of Naproxen (µg/ml)		
	2.0	3.0	4.0	20.0	30.0	40.0
1.	2170.17	1642.3	1199.34	967.9	1366.16	1774.02
2.	2159.12	1653.61	1201.72	961.94	1354.09	1770.45
3.	2166.48	1660.02	1214.51	972.04	1371.15	1787.19
Mean	2165.25	1651.97	1205.19	967.29	1363.8	1777.22
% RSD	0.23	0.54	0.67	0.52	0.64	0.50

Accuracy studies

chromatographic results. **Stability studies** confirmed that sample solutions were stable up to **72 h** at **4°C and 25°C**, with non-degraded content above **96%**.

Robustness was evaluated by deliberate variation of the mobile phase composition (chloroform:methanol, 8:2 v/v ±2%) and detection wavelength (250 and 252 nm). No significant effect on chromatographic performance was observed.

Stability studies showed that sample solutions were stable up to **72 h** at **4°C and 25°C**, with percentage non-degraded content ranging from **96.11–99.45%**, indicating acceptable stability.[24]

Table-3. Robustness (Mobile phase variation) studies of Sumatriptan and Naproxen

Sr. No	Mobile phase composition (v/v)		R_f		AUC	
	Chloroform	Methanol	SUT	NAP	SUT	NAP
1.	8	2	0.59	0.21	2139.01±0.90	1757.11±0.21
2.	8.1	1.9	0.59	0.21	2141.11±0.22	1755.40±0.09
3.	7.9	2.1	0.59	0.21	2139.17±0.14	1755.21±0.15
4.	7.6	2.4	0.59	0.21	2139.89±0.05	1756.06±0.11
5.	8.2	1.8	0.59	0.21	2140.81±0.04	1756.01±0.18
S.D	-	-	-	-	0.94	0.74

SUT= Sumatriptan NAP= Naproxen

Table-4. Robustness (Detection wavelength variation) studies of Sumatriptan and Naproxen

Sr.No	Detection Wavelength(nm)	R_f		AUC	
		SUT	NAP	SUT	NAP
1.	248	0.59	0.21	2139.01±0.90	1757.11±0.21
2.	250	0.59	0.21	2138.78±0.01	1757.75±0.14
3.	252	0.59	0.21	2139.89±0.10	1756.48±0.20
S.D	-	-	-	0.59	0.64

Table-5. Results of Stability Studies of Sumatriptan in formulations

Formulations	Temperature					
	4°C			25°C		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Brand 1	99.02	98.79	98.07	98.87	98.15	97.54
Brand 2	98.22	97.83	97.46	98.01	97.87	97.01
Brand 3	98.01	97.54	96.91	97.89	97.07	96.50

Table-6. Results of Stability Studies of Naproxen in formulations

Formulations	Temperature					
	4°C			25°C		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Brand 1	99.45	98.99	97.07	97.91	97.09	96.11
Brand 2	98.22	97.99	96.37	97.33	96.82	96.09
Brand 3	97.89	97.01	95.99.	97.11	96.06	95.74

Quantitative analysis for simultaneous estimation of Naproxen & Sumatriptan content by HPTLC

The validated method was successfully applied to various dosage forms, showing no interference from excipients, with acceptable assay results for Sumatriptan and Naproxen.

Table-7. Percent Content of Sumatriptan and Naproxen in various formulations

Formu-lations	Percent Content ± S.D. (%)		Weight of unit Dose	Content per unit dosage form of SUT	Content per unit dosage form of NAP
	SUT	NAP			
Brand 1	0.698±0.09	0.646±0.15	250 mg	1.745mg	16.15mg
Brand 2	0.737±0.21	0.293±0.07	200 mg	1.474mg	0.586mg
Brand 3	0.758±0.19	0.704±0.16	3 g(1tbsp)	22.74mg/tbsp	21.12mg/tbsp.
Brand 4	0.137±0.13	0.250±0.11	2 g	27.4mg/10ml	5.0 mg/10ml

A simple, precise, accurate, and stability-indicating HPTLC method was successfully developed and validated for the simultaneous estimation of sumatriptan succinate and naproxen sodium in combined pharmaceutical dosage forms.[25] The optimized chromatographic conditions using precoated silica gel 60 GF_{254} plates and chloroform:methanol as the mobile phase provided well-resolved, compact, and reproducible bands with satisfactory separation

of both drugs.[26]

The method demonstrated excellent linearity over the selected concentration ranges for sumatriptan and naproxen with high correlation coefficients, confirming its suitability for quantitative analysis.[27] Validation studies in accordance with ICH Q2(R1) guidelines established the method's precision, accuracy, sensitivity, and robustness, with low %RSD values, acceptable recovery results, and consistent performance under deliberate variations in experimental conditions.[28]

Overall, the proposed HPTLC method is reliable, cost-effective, and suitable for routine quality control, assay determination, and stability testing of sumatriptan succinate and naproxen sodium in combined pharmaceutical dosage forms.[29]

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