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Research Article

**DEVELOPMENT AND VALIDATION OF TLC
DENSITOMETRIC METHOD FOR THE SIMULTANEOUS
ESTIMATION OF ASPIRIN AND TICLOPIDINE
HYDROCHLORIDE IN TABLET DOSAGE FORM****Bhusal Ramesh Dattatraya*, Kolhe Mahesh Hari, Laware Ravindra Bhimraj, Hajare
Pranit Pandurang.**Department of Pharmaceutics, Pravara Rural College of Pharmacy, Loni, Pravaranagar, Tal-
Rahata, Dist- Ahmednagar, Maharashtra**Abstract:**

Accurate, specific, precise and robust TLC densitometric method has been developed for simultaneous estimation of Aspirin (ASP) and Ticlopidine hydrochloride (TIC) in tablet dosage form. The chromatographic separation was performed on precoated silica gel TLC 60 F254 plates using n-Hexane: Ethyl acetate: Methanol (6.5:1.5:2 v/v/v) as mobile phase. This system was found to give compact bands for Aspirin and Ticlopidine hydrochloride (RF values 0.52 ± 0.05 and 0.75 ± 0.05 respectively). Densitometric analysis of Aspirin and Ticlopidine hydrochloride were performed at 239 nm. Regression analysis data for the calibration plots were indicative of good linear relationships between response and concentration over the range 1000-5000 ng/band for Aspirin and 500-2500 ng/band for Ticlopidine hydrochloride. The method was validated as per ICH guidelines for accuracy, precision, LOD, LOQ and robustness

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INTRODUCTION:**Aspirin (ASP)****Description:**

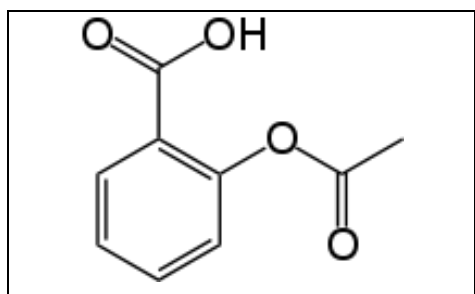
The prototypical analgesic used in the treatment of mild to moderate pain. It has anti-inflammatory and antipyretic properties and acts as an inhibitor of cyclooxygenase which result in the inhibition of the biosynthesis of prostaglandins. Acetylsalicylic acid also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis.

Colour: off white to white

Appearance: Fine crystalline powder slightly hygroscopic

Odor: Odorless

The structural formula of Aspirin is

**Chemistry:**

IUPAC name : 2-acetoxybenzoic acid

Molecular Formula : $C_9H_8O_4$

Molecular weight : 180.2 g/mol

Solubility: Freely soluble in Ethanol, methanol, Dimethyl formamide. Also soluble in DMSO.

Category: Antiplatelet agent, Anti-inflammatory; analgesic.

Ticlopidine Hydrochloride (TIC)**Description:**

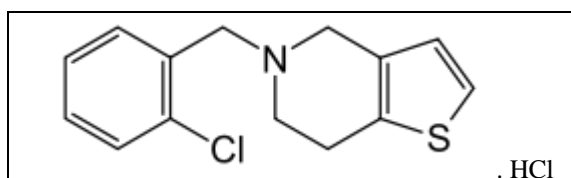
Ticlopidine is an effective inhibitor of platelet aggregation. The drug has been found to significantly reduce infarction size in acute myocardial infarcts and is an effective antithrombotic agent in arteriovenous fistulas, ortho-coronary bypass grafts, ischemic heart disease, and venous thrombosis.

Colour: off white to white

Appearance: Fine crystalline powder

Odor: Odorless

The structural formula of Ticlopidine Hydrochloride is

**Chemistry:**

IUPAC name : 5-(2-chlorobenzyl)-4, 5, 6, 7-tetrahydrothieno[3, 2-c]pyridine

Molecular Formula : $C_{14}H_{14}ClNS.HCl$

Molecular weight : 263.786 g/mol.

Solubility: Freely soluble in ethanol (95%), in chloroform and in ether; practically insoluble in water.

Category: Anti-inflammatory; analgesic.

Detailed survey of literature for ASP alone or in combination with other drugs is reported to be estimated by several methods based on different techniques such as UV spectrophotometry, HPLC and HPTLC for its determination from pharmaceuticals. Similarly literature survey for TIC alone or in combination with other drugs is reported to be estimated by UV spectrophotometry, HPLC and HPTLC method. But no methods have been reported for simultaneous determination of ASP and TIC. Hence in the present work a successful attempt has been made to estimate both these drugs simultaneously by TLC densitometric method. Currently TLC densitometric is becoming a routine technique for analysis of drug.

MATERIALS AND METHODS:**Chemicals and Reagents**➤ **Preparation of Standard Stock Solutions**

- **Standard stock solution A:** Accurately weighed quantity of ASP (10.0 mg) was transferred to 25.0 ml volumetric flask, dissolved and diluted to the mark with methanol. (concentration 400 μ g/ml)
- **Standard stock solution B:** Accurately weighed quantity of TIC (25.0 mg) was transferred to 25.0 ml volumetric flask, dissolved and diluted to the mark with methanol. (Concentration 1000 μ g/ml).
- **Standard stock solution C:** An accurately weighed quantity of ASP (10.0 mg) and TIC (25.0 mg) was transferred to 25.0 ml volumetric flasks, dissolved and diluted up to mark with methanol. (Concentration: 400 μ g/ml of ASP and 1000 μ g/ml of TIC).

➤ **Selection of Mobile Phase**

Aliquot portions of standard stock solutions A and B (5 μ l each) were applied on TLC plates in the form of band (band size: 6 mm) and the plates were run in different solvent systems. In an attempt to achieve the desired R_f value range (0.2-0.8) with a compact band, several trials

were made by using different solvent systems containing non-polar and relatively polar solvents. Among the different mobile phase combinations tested, mixture of n-Hexane, Ethyl acetate and Methanol (6.5:1.5:2, v/v/v) gave compact bands which showed symmetrical peak on chromatogram and desired R_f value. The R_f values were found to be 0.52 and 0.75 for Aspirin and Ticlopidine hydrochloride, respectively.

➤ **Selection of Wavelength for Densitometric**

Evaluation of Separated Bands:

Standard stock solution A and B were individually applied on TLC plate with the help of CAMAG LINOMAT-V automatic sample applicator, the plate was chromatographed in twin-through glass chamber saturated with mobile phase for 20 min. After chromatographic development, the plate was removed and air dried. The bands on the TLC plate were scanned over the wavelength range of 200-700 nm. The wavelength 239 nm was selected for densitometric evaluation of ASP and TIC bands as both the drugs showed significant absorbance at this wavelength. The spectrum obtained for ASP and TIC are depicted in Fig.No.1.

➤ **Optimum Chromatographic Conditions**

The following chromatographic conditions were optimized by trial and error for densitometric analysis of ASP and TIC

Stationary phase	: Aluminium plates precoated with silica gel 60 F ₂₅₄ (Merck)
Mobile phase	: n-Hexane: Ethyl acetate: Methanol (6.5:1.5:2 v/ v/v)
Plate size	: 10 cm x 10 cm
Mode of application	: Band
Band size	: 6 mm (Distance between two bands: 5.6 mm)
Sample volume	: 5 μ l
Development chamber:	Twin-through glass chamber, 10 cm x 10 cm with stainless steel lid.
Saturation time	: 20 min.
Separation technique	: Ascending
Migration distance	: \approx 80 mm
Temperature	: $25 \pm 5^\circ\text{c}$
Scanning mode	: Absorbance/Reflectance
Slit dimensions	: 5 x 0.45 mm
Scanning wavelength	: 239 nm
The retention factors of ASP and TIC were	
ASP	: 0.52 ± 0.05
TIC	: 0.75 ± 0.05

The typical densitogram obtained for ASP and TIC is shown in Fig.No.2

➤ **Study of Linearity Range:**

The standard stock solution C containing ASP (400 μ g/ml) and TIC (1000 μ g/ml) was applied on the TLC plates in the range 2-12 μ l with the help of micro syringe using LINOMAT-V automatic sample applicator. The plate was then developed and scanned under the above mentioned chromatographic conditions.

Peak area was recorded for each drug concentration and the calibration curves of Concentration Vs. Peak area were constructed for both the drugs. The calibration curve for TIC and ASP are depicted in Fig.No.3 and 4, respectively.

➤ **Analysis of Standard Laboratory mixture:**

Six sample solutions were prepared and analyzed in following manner:

An accurately weighed quantity of 10 mg ASP and 25 mg TIC was transferred to 25.0 ml volumetric flasks, dissolved and diluted up to the mark with methanol. (Concentration 400 μ g/ml ASP and 1000 μ g/ml TIC).

On TLC plate two bands of standard stock solution C and four bands of sample solution, 5 μ l each, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the peaks obtained for standard and sample were integrated and the peak area obtained for standard and sample bands was recorded.

Amount of the drugs present in sample (in mg) was obtained by comparing the mean peak area of sample bands with that of standard bands. Results of analysis of standard laboratory mixture and its statistical evaluation are shown in Table No. 1 and 2, respectively.

➤ **Analysis of the Tablet Formulation**

Six samples were prepared and analyzed in following manner:

Twenty tablets were weighed, average weight was calculated and the tablets were then crushed to obtain fine powder. Accurately weighed quantity of tablet powder equivalent to about 50 mg of ASP and 125 mg of TIC was transferred to 25.0 ml volumetric flask, added 15 ml methanol and ultrasonicated for 20 min; volume was then made upto mark with methanol. The solution was mixed and filtered through Whatman filter paper No. 42. From the filtrate, 2.0 ml solution was diluted to 10.0 ml with methanol.

On the TLC plate two bands of standard stock solution C and four bands of sample solution, 5.0 μ l each, were applied and the plate was

developed and scanned under the optimum chromatographic conditions. After scanning, the peak obtained for standard and sample bands were integrated and the peak area obtained for standard and sample bands was recorded.

The amount of ASP/TIC (in mg/tablet) was calculated by comparing mean peak area of sample bands with that of standard bands.

Result of analysis of tablet formulation and its statistical evaluation are shown in Table No.3 & 4, respectively.

➤ **Method Validation**

The proposed method was validated by studying several parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

• **Accuracy:**

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

An accurately weighed quantity of pre-analyzed tablet powder equivalent to about 50 mg ASP /125 mg TIC was transferred individually in nine different 50.0 ml volumetric flasks. To each of the flask following quantities of ASP /TIC were added:

- Flask No.1: 40.1 mg ASP + 100.3 mg TIC,
- Flask No.2: 40.0 mg ASP + 100.2 mg TIC,
- Flask No.3: 40.2 mg ASP + 100.0 mg TIC,
- Flask No.4: 50.0 mg ASP + 125.1 mg TIC,
- Flask No.5: 50.2 mg ASP + 125.4 mg TIC,
- Flask No.6: 50.4 mg ASP + 125.1 mg TIC,
- Flask No.7: 60.2 mg ASP + 150.0 mg TIC,
- Flask No.8: 60.0 mg ASP + 150.4 mg TIC,
- Flask No.9: 60.2 mg ASP + 150.1 mg TIC.

Then, 30.0 ml methanol was added to each flask and contents of the flask were ultrasonicated for 20 min, volume was made up to the mark with methanol. The solution was individually mixed and filtered through Whatman filter paper No. 42. From the filtrate, 2.0 ml solution was diluted to 10.0 ml with methanol.

On TLC plate two bands of standard stock solution C and four bands of sample solution, 5 µl each, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the chromatograms obtained for standard and sample were integrated and peak area for standard and

sample bands was recorded. Total amount of drug estimated in sample was obtained by comparing the peak area of sample bands with that of the standard bands.

Results of recovery studies and its statistical validation are shown in Table 5 and 6, respectively.

• **Precision :**

Intra-day and inter-day precision was determined by analyzing tablet sample solutions at different time intervals on the same day and on three different days, respectively. Tablet sample solution was prepared and analyzed in the similar manner as described under analysis of the tablet formulation.

Results of intra-day and inter-day precision are shown in Table No.7 and 8, respectively.

• **Limit of Detection and Limit of Quantification:**

The LOD and LOQ were separately determined which is based on the standard deviation of response of the calibration curve. The standard deviation of y- intercept and slope of the calibration curves were used to calculate the LOD and LOQ. Results are shown in Table No.9

• **Robustness:**

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters such as composition of the mobile phase, change in amount of mobile phase, duration for chamber saturation, time from spotting to development, and time from development to scanning were done. The composition of the mobile phase, amount of mobile phase and chamber saturation time were varied in the range of ± 0.1 ml, ± 1.0 ml and ± 5 min, respectively, of the used optimized conditions. In variation for time from spotting to development, after applying the sample bands the TLC plate was developed at 5 min, 20 min and 1h time interval. In variation for time from development to scanning, after developing the TLC plated in optimized chromatographic conditions the TLC plate was scanned 5 min, 20 min and 1h time interval. The effect of these changes on both the R_f value and peak shape of the drug peak was studied.

Result of robustness studies are shown in Table No.10.

RESULTS:

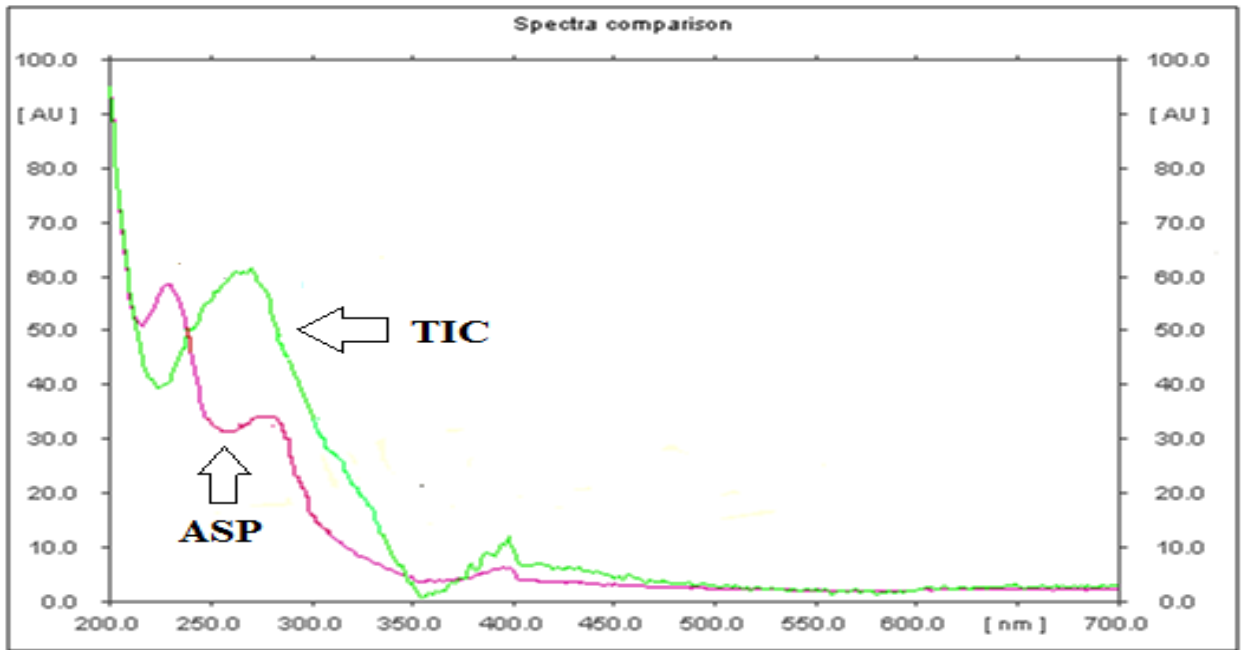


Fig 1: Spectrum for Aspirin and Ticlopidine HCl

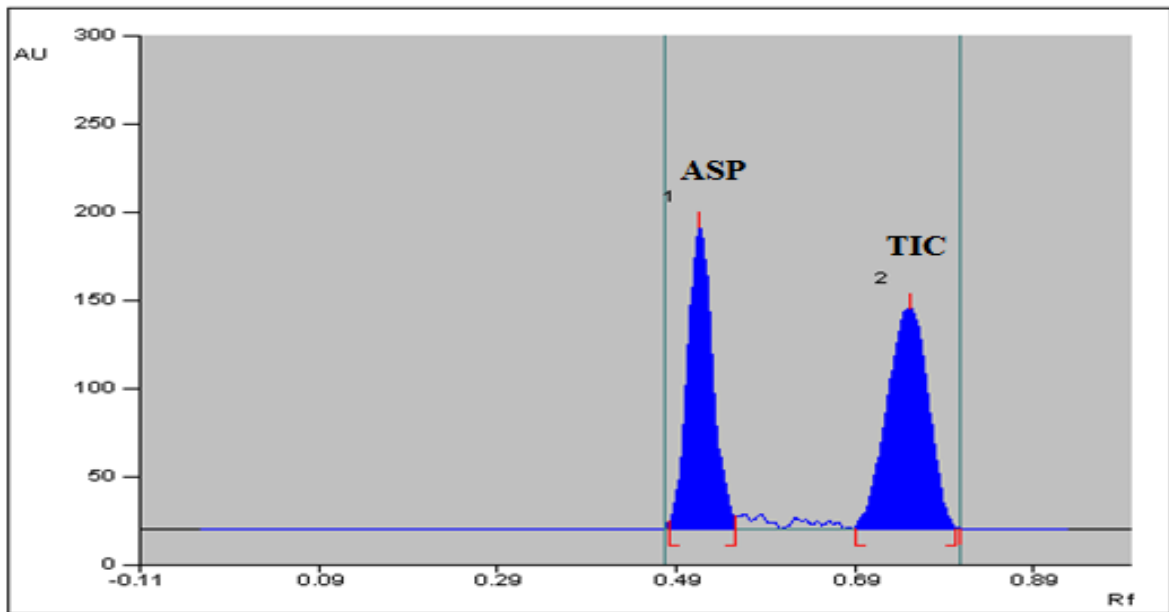


Fig 2: Typical Densitogram of Aspirin and Ticlopidine HCl

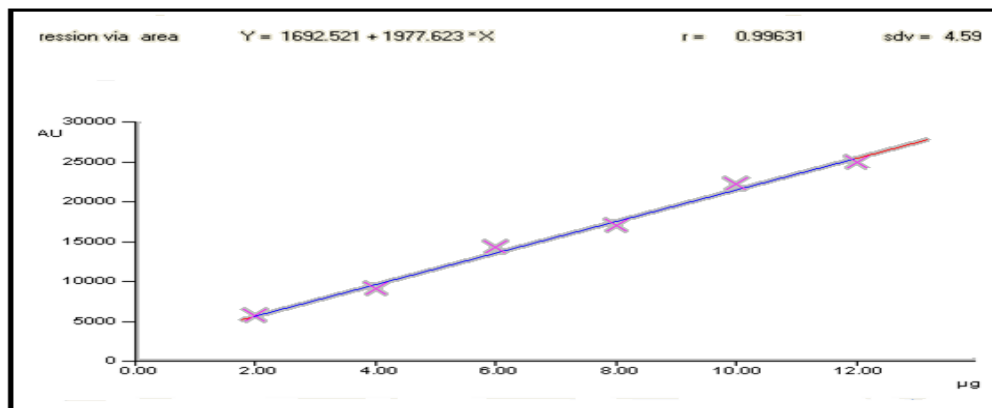


Fig 3: Standard Calibration Curve for Ticlopidine HCl

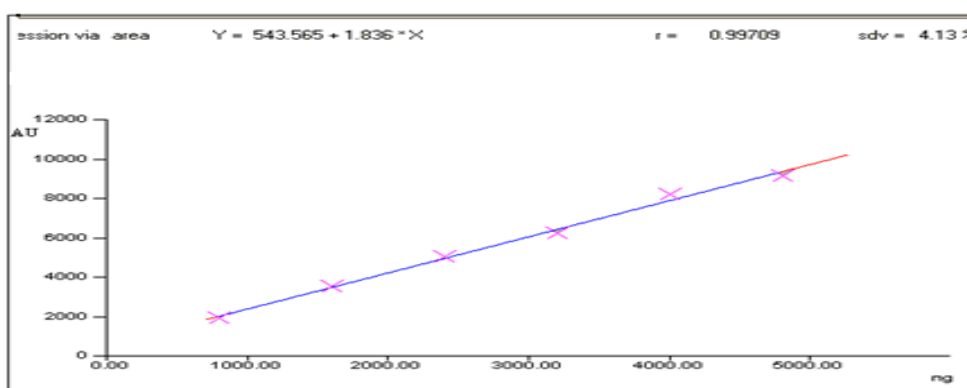


Fig 4: Standard Calibration Curve for Aspirin

Table 1: Results of Analysis of Standard Laboratory Mixture

Sr. No.	Amount of pure drug taken (mg)		Sample Peak area*		Standard Peak area*		Amount of pure drug estimated (mg)		% Estimation	
	ASP	TIC	ASP	TIC	ASP	TIC	ASP	TIC	ASP	TIC
1.	10.1	25.1	7127.46	14395.15	7163.55	14420.16	9.95	24.96	98.51	99.43
2.	10.1	25.0	6036.2	14595.85	6077.45	14434.7	9.93	25.28	98.34	101.12
3.	10.0	25.2	5855.9	15407.85	5885.30	15250.10	9.95	25.26	99.50	100.23
4.	10.0	25.1	6650.17	13968.67	6634.15	13848.55	10.02	25.22	100.24	100.47
5.	10.0	25.1	5774.20	14341.60	5824.30	13942.90	9.91	25.71	99.14	102.45
6.	10.0	25.0	5854.50	13196.43	5753.90	13309.60	10.17	24.79	101.75	99.15

*denotes average of four determinations

Table 2: Statistical Validation for Standard Laboratory Mixture Analysis

Sr. No	Drug	% Estimation*	S.D	C.V
1.	ASP	99.58	± 0.778	0.781
2.	TIC	100.16	±0.418	0.417

*denotes average of six determinations

Table 3: Result of Analysis of Tablet Formulation

DOPRIN PLUS		Avg.wt.: 595.42mg				ASP-100 mg,TIC-250mg			
Sr. No.	Weight of tablet powder taken (mg)	Sample Peak area*		Standard Peak area*		Amount of drug estimated (mg/tablet)		% Label claim	
		ASP	TIC	ASP	TIC	ASP	TIC	ASP	TIC
1	297.7	10539.22	6112.02	10554.10	6098.40	99.86	250.57	99.86	100.23
2	297.7	11156.72	4843.70	11257.20	4846.65	99.11	249.86	99.11	99.94
3	297.7	9903.30	6687.40	9936.20	6603.90	99.67	253.17	99.67	101.27
4	297.7	12361.45	3826.21	12298.23	3824.11	100.52	250.15	100.52	100.06
5	298.5	8861.00	3731.80	8680.60	3683.70	101.81	252.59	101.81	101.04
6	298.1	10503.40	7827.10	10314.00	7828.80	101.70	249.62	101.70	99.85

*denotes average of four determinations

Table 4: Statistical Evaluation for Analysis of Tablet Formulation

Sr. No.	Drug	Amount of drug estimated (mg/tablet)*	% Label claim*	S.D	C.V
1	ASP	100.45	100.45	1.110	1.105
2	TIC	250.99	100.40	0.603	0.600

*denotes average of six determinations

Table 5: Result for Recovery Studies.

Sr. No.	Level of recovery	Weight of tablet powder taken (mg)	Amount of drug added(mg)		Amount of drug recovered(mg)		% Recovery	
			ASP	TIC	ASP	TIC	ASP	TIC
1	80 %	297.7	40.0	100.3	100.47	100.47	100.17	100.17
		297.7	40.0	100.2	100.05	100.05	99.85	99.85
		297.7	40.2	100.0	99.90	99.90	99.90	99.90
2	100 %	297.7	50.0	125.1	123.69	123.69	98.87	98.87
		297.7	50.2	125.4	125.65	125.65	100.20	100.20
		297.7	50.4	125.1	123.65	123.65	98.84	98.84
3	120 %	297.7	60.2	150.0	150.69	150.69	100.46	100.46
		297.7	60.0	150.4	150.93	150.93	100.35	100.35
		297.7	60.2	150.1	146.79	146.79	98.13	98.13

Table 6: Statistical Validation for Recovery Study.

Level of recovery	% Recovery *		S.D		C.V	
	ASP	TIC	ASP	TIC	ASP	TIC
80 %	98.77	99.87	0.130	0.023	0.131	0.023
100 %	98.81	99.30	0.292	0.776	0.295	0.781
120 %	100.19	99.65	1.112	1.315	1.109	1.319

* denotes average of three determinations

Table 7: Intra-Day Precision Data.

Drug	Label claim*	S.D	C.V
ASP	98.24	± 0.246	0.250
TIC	100.16	± 0.832	0.831

*denotes average of three determinations.

Table 8: Inter-Day Precision Data.

Drug	Label claim*	S.D	C.V
ASP	99.34	± 1.293	1.301
TIC	100.33	± 1.616	1.610

*denotes average of three determinations.

Table 9: Results of LOD and LOQ

Parameter	ASP	TIC
LOD (ng/band)	2.602	1.437
LOQ (ng/ band)	7.886	4.357

Table No. 10: Result of Robustness Study

Chromatographic Changes			
Factors	Level	R _f Value	
Mobile phase composition (± 0.1 ml)		ASP	TIC
6.4:1.4:1.9	- 0.1	0.57	0.74
6.5:1.5:2	0	0.56	0.75
6.6:1.7:2.1	+ 0.1	0.57	0.75
	S.D	0.006	0.006
Amount of Mobile Phase (v/v)(± 1ml)		ASP	TIC
9	- 1.0	0.56	0.74
10	0	0.57	0.74
11	+1.0	0.55	0.76
	S.D	0.010	0.012
Duration for chamber saturation (± 5min)		ASP	TIC
15 min	- 5	0.54	0.75
20 min	0	0.55	0.74
25 min	+ 5	0.57	0.75
	S.D	0.015	0.006
Time from spotting to development		ASP	TIC
5min	-	0.53	0.73
20min	-	0.55	0.74
1h	-	0.54	0.73
	S.D	0.010	0.015
Time from development to scanning		ASP	TIC
5min	-	0.52	0.76
20min	-	0.53	0.74
1h	-	0.52	0.74
	S.D	0.006	0.012

CONCLUSION:

The TLC densitometric method was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. Hence, it can be concluded that the developed HPTLC method was accurate, specific, precise and robust can be employed successfully for the estimation of ASP and TIC in tablet dosage form.

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