DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF HYDROCORTISONE SODIUM SUCCINATE

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ABSTRACT:
A simple, precise and sensitive stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the analysis of Hydrocortisone sodium succinate both in bulk and in powder dosage form. The seperation was performed on pre-coated silica gel 60 GF_{254} plates using ethyl acetate: methanol (8.5:1.5 v/v) as mobile phase. The retention factor (R_f) was found to be 0.45 ± 0.05. The detection of band was carried out at 242 nm. The drug was subjected to different stress conditions like acid, base hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH Q_2 (R1) guidelines. The linear regression analysis data for the calibration plot showed good linear relationship with $R^2 = 0.9922$ in the range 500-3000 ng/band. The method found to be accurate as results of the recovery studies are close to the 100%. The developed method was found to be simple, sensitive, selective, accurate and repeatable for analysis of Hydrocortisone sodium succinate and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

KEYWORDS: High performance thin layer chromatography (HPTLC), Hydrocortisone sodium succinate, method validation, stability indicating.

INTRODUCTION:
Hydrocortisone sodium succinate (HSS) is chemically sodium;4-[2-[(8~{S},9~{S},10~{R},11~{S}, 13~{S},14~{S},17~{R})-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1~{H}-cyclopenta[a]phenanthren-17-yl]-2-oxoethoxy]-4-oxobutanoate. Hydrocortisone Sodium Succinate is the sodium salt of hydrocortisone succinate with glucocorticoid property. Hydrocortisone sodium succinate is chemically similar to the endogenous hormone that stimulates anti-inflammatory and immunosuppressive activities, in addition to exhibiting minor mineralocorticoid effects. [1] The literature survey reveals that all the HPTLC methods are on hydrocortisone, hydrocortisone acetate [2-3] and stability testing method of hydrocortisone succinate with multiple regression analysis versus dynamic neural network. [4]
To the best of our knowledge no stability indicating HPTLC method has been reported for estimation of Hydrocortisone sodium succinate. The present work describes a simple, stability indicating HPTLC method for the determination of Hydrocortisone sodium succinate in bulk and powder dosage form (Hydrocort-100) according to ICH guidelines. [5-6]

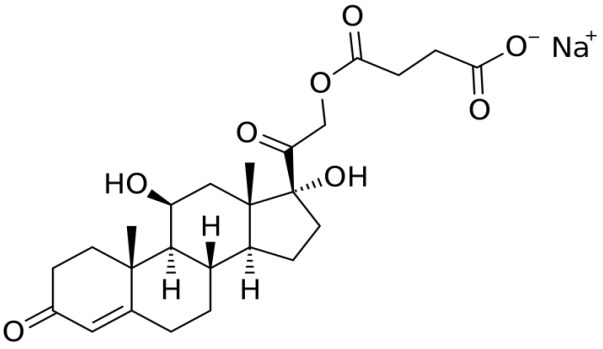

Fig.1 Chemical structure of Hydrocortisone Sodium Succinate

MATERIALS AND METHODS

Reagents and chemicals

The formulation Hydrocort-100 labeled to contain Hydrocortisone sodium succinate was procured from local market. Methanol (AR grade), Ethyl acetate (AR grade) were purchased from S.D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH); all AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Chromatographic conditions:

Chromatographic seperation of drug was performed on aluminum plates precoated with silica gel 60 $F_{254,}$ (10cm × 10cm with 250 µm layer thickness). Sample was applied on the plate as a band of 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Ethyl acetate: methanol (8.5:1.5 v/v). 10cm × 10cm Camag twin trough glass chamber was used for linear ascending development of TLC plate under 12 min saturation conditions and 10 ml of mobile phase was used per run. Migration distance was 80 mm. Densitometric scanning was performed was performed at 242 nm using Camag TLC scanner 3, operated by winCATS software (version 1.4.3), slit dimensions were 4.00×0.45 mm and Deuterium lamp was used as a radiation source.

Selection of detection wavelength

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 242 nm. Representative UV spectrum of Hydrocortisone sodium succinate is shown in Fig 2.

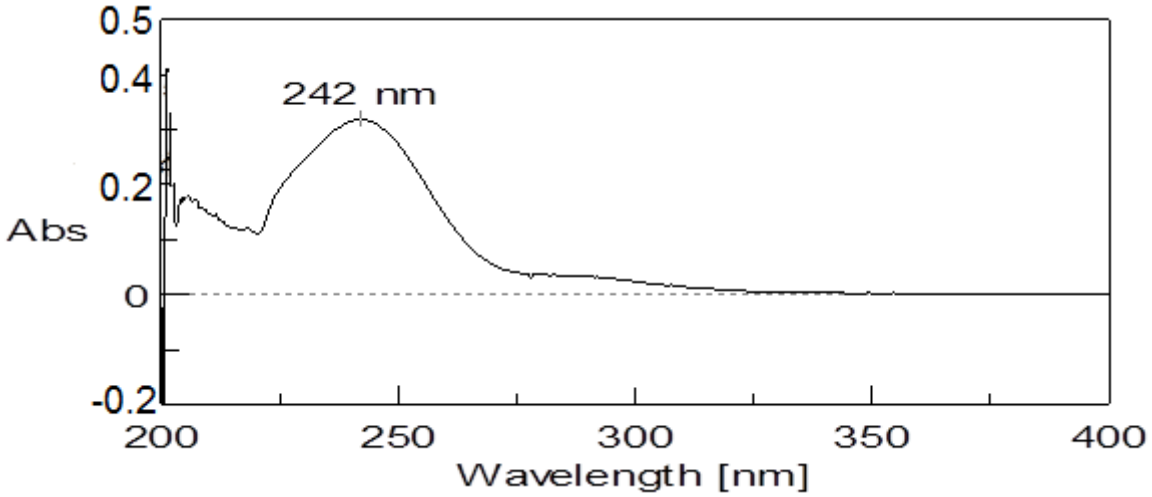

Fig.2: UV spectrum of Hydrocortisone sodium succinate

Preparation of Standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard solution was prepared containing 250 µg/ml of Hydrocortisone sodium succinate. Representative densitogram of Hydrocortisone sodium succinate (1000 ng/band) is shown in Fig 3.

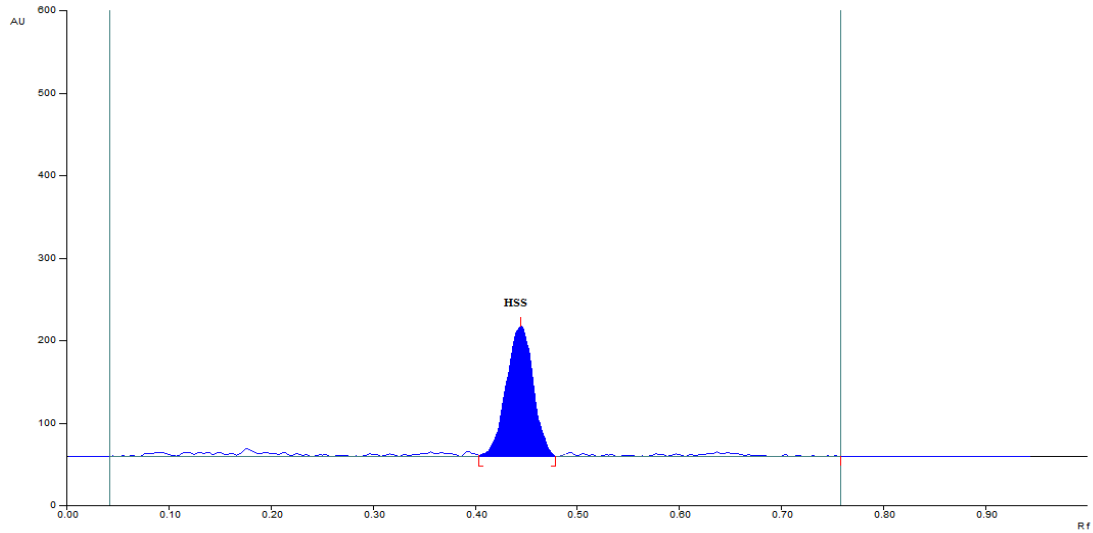

Fig 3: Densitogram of standard solution of Hydrocortisone sodium succinate (1000 ng/band)

Preparation of sample solution:
For determination of the content of Hydrocortisone sodium succinate in Hydrocort-100 (label claim: each vial contain Hydrocortisone sodium succinate USP equivalent to Hydrocortisone 100 mg). A quantity of powder equivalent to 10 mg of Hydrocortisone sodium succinate was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The sample was ultra sonicated for 1 min and volume was made up to 10 ml with the methanol. 2.5 ml of this solution was diluted to 10 ml with the methanol to prepare a final sample stock solution of 250 µg/ml.

Stress degradation studies of bulk drug
Stability studies were carried out to provide evidence on how quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline hydrolysis, oxidation, dry heat and photolytic degradation. Dry heat and photolytic degradation were carried out in the solid state. All studies are carried out at concentration level 1500 ng/band.

Acid degradation:
2.5 ml of working standard solution of Hydrocortisone sodium succinate (1000 µg/ml) was mixed with 1ml of 0.5 N HCl. Sample aliquot was kept for half hour degradation and volume was made up with methanol upto 10 ml. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Average 89.75% Hydrocortisone sodium succinate was recovered with two peaks of degradant. Representative densitogram is shown in Fig 4.

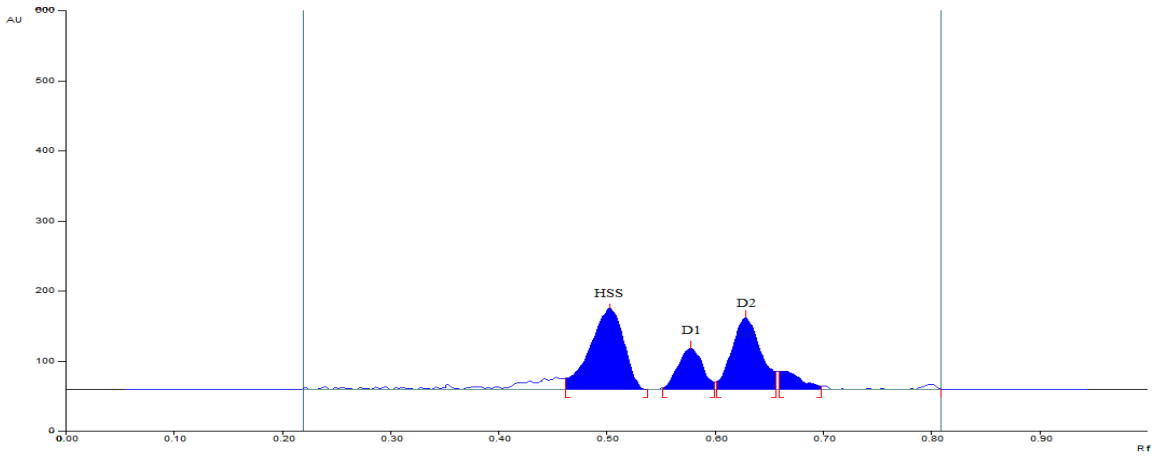

Fig.4: Representative densitogram of acid induced degradation of Hydrocortisone sodium succinate (1500 ng/band)

Alkaline degradation:
2.5 ml of working standard solution of Hydrocortisone sodium succinate (1000 µg/ml) was mixed with 1 ml of 0.5N NaOH. Sample aliquots was kept for half an hour degradation & volume was made up with methanol upto 10 ml. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Average 80.13 % Hydrocortisone sodium succinate was recovered with one peak of degradant. Representative densitogram is shown in Fig 5.

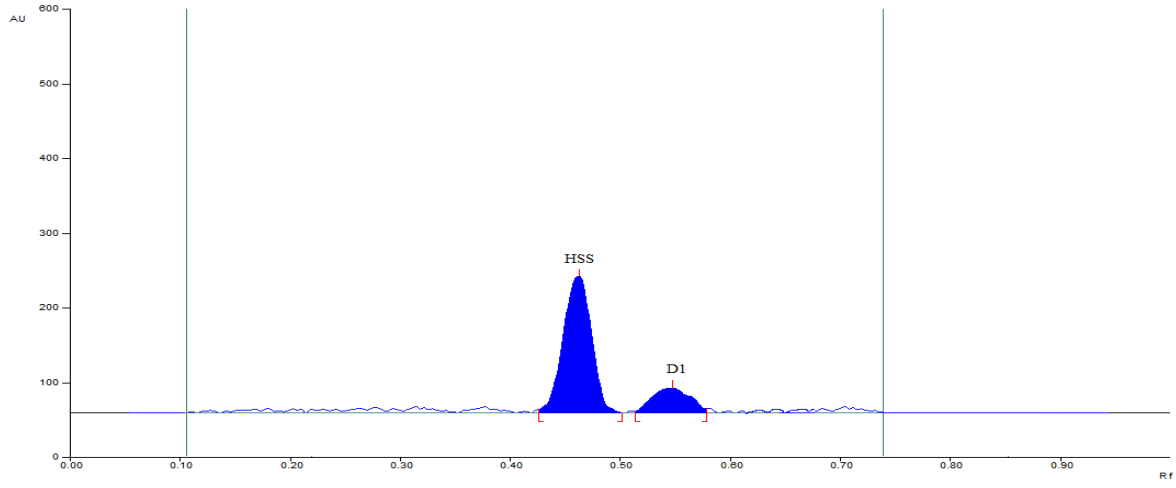

Fig. 5: Representative densitogram of alkali induced degradation of Hydrocortisone sodium succinate (1500 ng/band)

Degradation under oxidative conditions

2.5 ml of working standard solution of Hydrocortisone sodium succinate (1000 µg/ml) was mixed with 1 ml of 6% H_2O_2. Sample aliquots was kept for half hour degradation & volume was made up with methanol upto 10 ml. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Average 98.78% Hydrocortisone sodium succinate was recovered with one peak of degradant. Representative densitogram is shown in Fig 6.

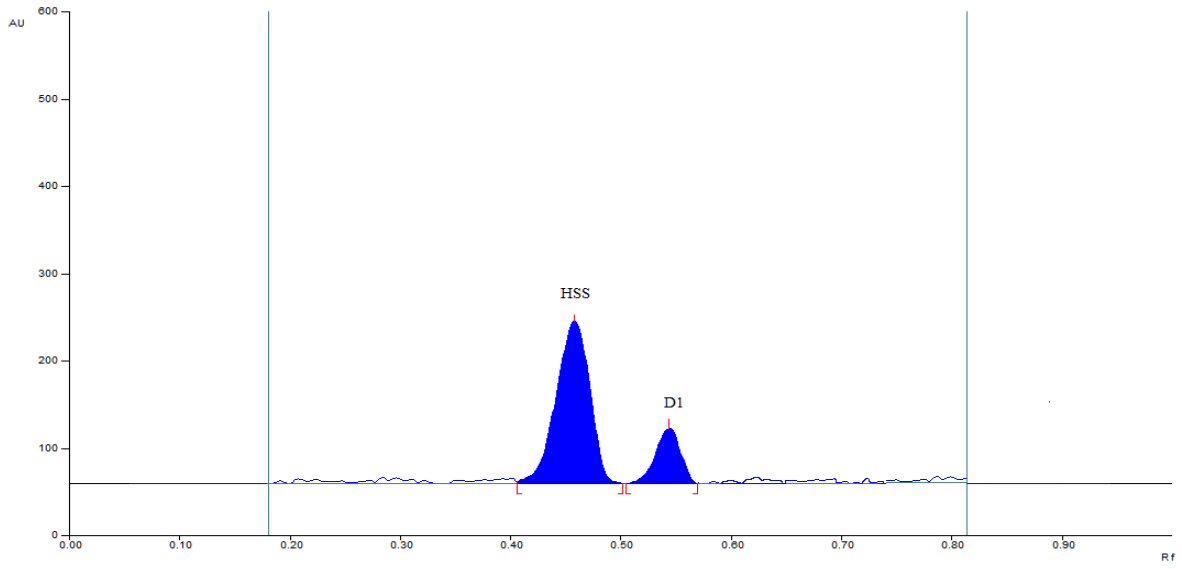

Fig.6: Representative densitogram of peroxide induced degradation of Hydrocortisone sodium succinate (1500 ng/band)

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80℃) for a period of 24 hours. Sample was withdrawn, dissolved in methanol and diluted to get 250 µg/ml. 6 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 98.87% of Hydrocortisone sodium succinate was recovered with one peak of degradant. Representative densitogram obtained for sample subjected to dry heat is shown in Fig 7.

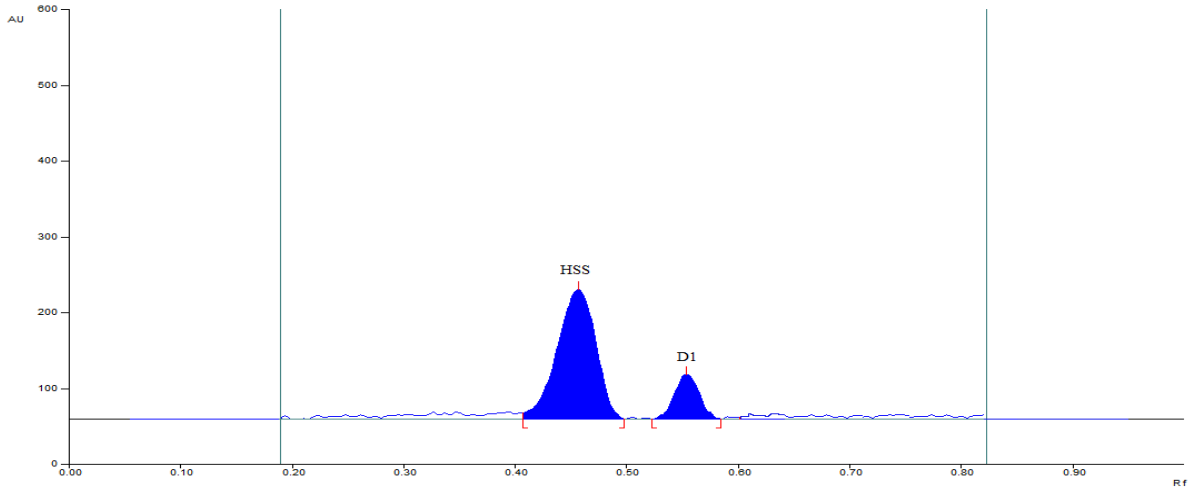

Fig. 7: Representative densitogram of dry heat degradation of Hydrocortisone sodium succinate (1500 ng/band)

Photodegradation studies:

1. **UV Illumination:**

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask. The volume was made up with methanol to obtain 1000 µg/ml. 2.5 ml of the resultant solution was then diluted with methanol to get the concentration of 250 µg/ml. 6 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 77.39 % of Hydrocortisone sodium succinate was recovered with one peak of degradant. Representative densitogram obtained for sample subjected to dry heat is shown in Fig 8.

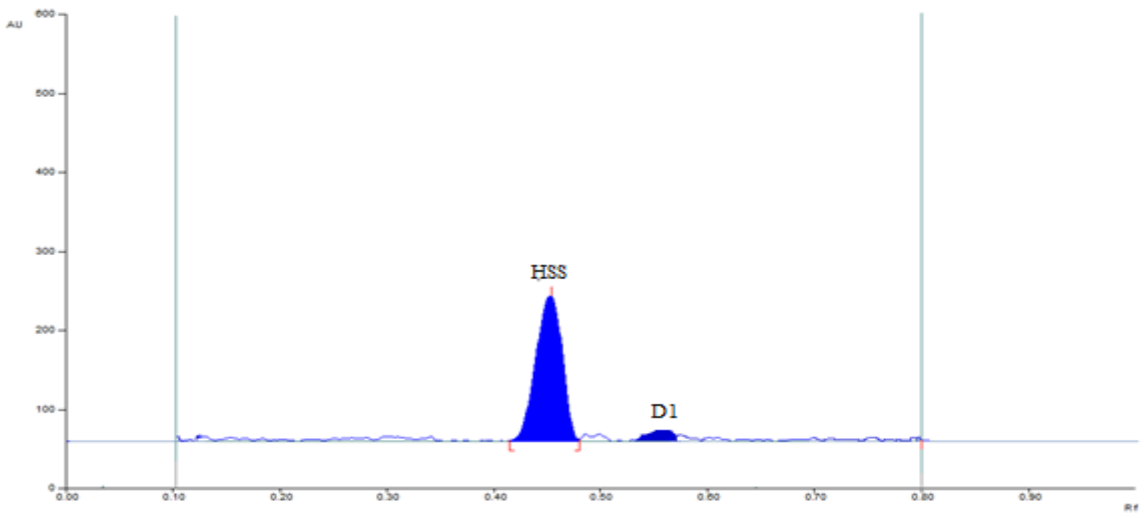

Fig.8: Representative densitogram of photolytic uv degradation of Hydrocortisone sodium succinate (1500 ng/band)

2. Fluroscent light:

The photo degradation study of the drug was studied by exposing the drug to fluroscent light providing illumination of NLT 1.2×10^6 Lux hr of fluroscent light. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask. The volume was made up with methanol to obtain 1000 µg/ml. 2.5 ml of the resultant solution was then diluted with methanol to get the concentration of 250 µg/ml. 6 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 98.76% of Hydrocortisone sodium succinate was recovered with two peaks of degradant. Representative densitogram obtained for sample subjected to dry heat is shown in Fig 9.

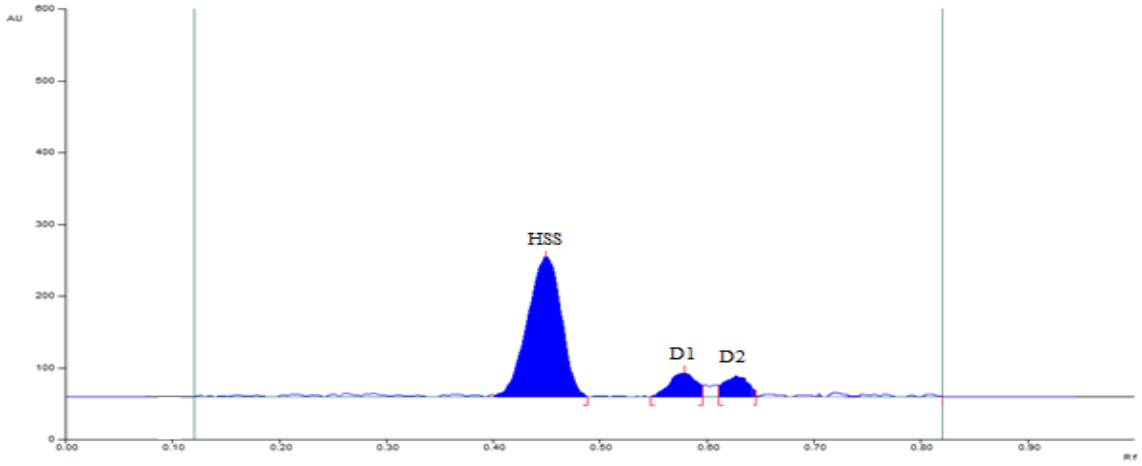

Fig.9: Representative densitogram of photolytic fluroscent light degradation of Hydrocortisone sodium succinate (1500 ng/band)

VALIDATION OF ANALYTICAL METHOD [6]

Specificity:

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity and Range

From the standard stock solution (1000 µg/ml) of Hydrocortisone sodium succinate, solution was prepared containing 250 µg/ml of Hydrocortisone sodium succinate. This solution was further used for spotting. Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analysing six concentrations over the concentration range 500-3000 ng/band for Hydrocortisone sodium succinate to obtain calibration curve. The results found to be linear with regression equation of $y = 3.2793x + 940.11$ and $R^2 = 0.9922$. The calibration curve is shown in Fig. 10.

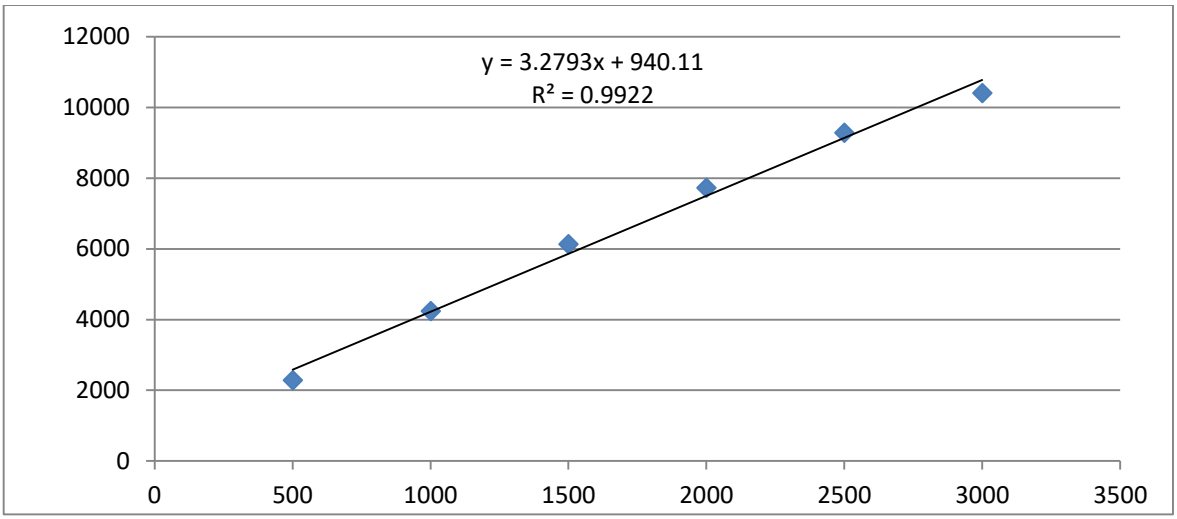

Fig 10: Calibration curve of Hydrocortisone sodium succinate (500-3000 ng/band) reference standard

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inta-day studies 3 replicates of 3 concentrations were analysed on the same day, and % RSD was calculated. For the interday variation studies, 3 concentraions were analysed on 3 consecutive days and % RSD was calculated. For intraday precision and interday precision results obtained are shown in Table 1.

Table 1: Intraday and interday variation studies data for Hydrocortisone sodium succinate

Concentration (ng/band)	Intra-day precision			Inter-day precision		
	Area	% recovery	% RSD	Area	% recovery	% RSD
1500	4245	100.78	0.50	4201.5	99.45	1.07
	4222	100.07		4268.2	101.48	
	4212.7	99.79		4256.7	101.13	
2000	7630.2	102.00	1.31	7609.4	101.68	1.26
	7595.3	101.47		7502.1	100.05	
	7465.4	99.49		7445.9	99.19	
2500	9152.3	100.17	0.58	9304.1	102.02	1.06
	9247.1	101.32		9254.8	99.87	
	9216.9	100.95		9199.5	101.05	

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

From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ / S and LOQ = 10 σ / S where, σ = standard deviation of the response at lowest concentration in range and S = slope of the calibration curve. The LOD and LOQ were found to be 74.32 ng/band and 225.22 ng/band, respectively.

Assay:

Hydrocort-100 powder formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. 4 µl volume of sample solution was applied and area was recorded. Basic concentration of sample chosen was 1000 ng/band from powder solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 2.

Table 2: Assay of marketed formulation

Drug	Peak area	Amount recovered (ng/band)	% recovery	% RSD
Hydrocortisone sodium succinate	4191.5	991.489	99.149	0.764
	4245	1007.803	100.780	

	4222	1000.790	100.079
	4212.7	997.954	99.795
	4198.3	993.563	99.35
	4173.9	986.122	98.612

Accuracy:
To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 1000 ng/band. % recovery was determined from linear equation. Accuracy results obtained are shown in Table 3

Table 3: Accuracy studies of Hydrocortisone sodium succinate

Level	Amount of sample taken (ng/band)	Amount of standard spiked (ng/band)	Area	% recovery	% RSD
50%	1000	500	5909.1	101.01	1.14
			5864.2	100.10	
			5797.2	98.74	
100%	1000	1000	7633.4	102.05	1.01
			7504.6	100.08	
			7603.5	101.59	
150%	1000	1500	9335.2	102.40	0.47
			9257.2	101.44	
			9309.1	102.08	

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Robustness
Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength, chamber saturation time, mobile phase composition, time from spotting to development, time from development to scanning was changed and the effect on the area was noted. It was found that method is robust.

Results and discussion:
Hydrocortisone sodium succinate has absorbance maxima at 242 nm. The calibration curve data show good linear relationship in concentration range 500-3000 ng/band. Recovery study is carried out at three different level 50%, 100% and 150% by adding pure drug to the previously analysed test sample. Percentage recovery for drug was determined by linearity equation method and found to be within acceptance criteria. The precision and accuracy was found to be good, which is evident by low standard deviation values. The summary of validation parameters is presented in Table 4.

Table 4: Summary of Validation Parameters

Sr. No.	Validation parameters	Hydrocortisone sodium succinate
1.	Linearity equation R^2 Range	y = 3.2793x + 940.11 R^2= 0.9922 500 – 3000 ng/band
2.	Precision	(%RSD)
	Intraday	0.80
	Interday	1.13
3.	Assay	99.62 %
4.	Accuracy	
	50	99.95 %
	100	101.24 %
	150	101.97 %
5.	Limit of detection	74.32 ng/band
6.	Limit of quantitation	225.22 ng/band
7.	Specificity	Specific
8.	Robustness	Robust

REFERENCES

1. https://pubchem.ncbi.nlm.nih.gov/
2. Alina Pyka, Magdalena Babuska-Roczniak , Paulina Bochenska. 2011. Determination Of Hydrocortisone in Pharmaceutical Drug by TLC with Densitometric Detection In UV, Journal of Liquid Chromatography & Related Technologies, 34(9), 753–769.
3. Ariaya Hymete, Adnan A Bekhit. 2012. Development and validation of HPTLC assay method for simultaneous quantification of hydrocortisone and clotrimazole in cream and applying for stability indicating test. Journal of the Chilean Chemical Society , 57, Nº 3,1199-1203 .
4. Ljiljana N. Solomun, Svetlana R. Ibric, Vjera M. Pejanovic, Jelena D. duris, Jelena M. Jockovic, Predrag D. Stankovic, Zorica B. Vujic. 2012. In silico methods in stability testing of Hydrocortisone, powder for injections: Multiple regression analysis versus dynamic neural network. Hem. Ind. 66 (5), 647–657.
5. ICH guidelines for stability testing of new drug substances and products Q1A (R2), Geneva, Switzerland, 2004, 1-24.
6. ICH guidelines for validation of analytical procedures: text and methodology Q2 (R1), Geneva, Switzerland, 2005, 1-17.