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"SIMULTANEOUS ESTIMATION OF SALICYLIC ACID AND LACTIC ACID IN PHARMACEUTICAL DOSAGE FORM BY USING HPTLC"

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Abstract

A simple, accurate and precise high-performance thin layer chromatographic method for simultaneous estimation of Salicylic acid (SA) and Lactic acid (LA) in their combined liquid dosage form has been developed. The method employed thin layer chromatographic aluminium plates pre-coated with silica gel 60 F_{254} as the stationary phase and chloroform: formic acid: methanol (8.5: 1:0.5 v/v/v) as mobile phase. Chromatographic analysis was carried out in the reflectance/absorbance mode at 229 nm. The method was validated with respect to linearity, specificity, accuracy, precision, limit of detection and limit of quantitation and applied for analysis of SA and LA in combined dosage form. The Rf values were found to be 0.285 ± 1.922 and 0.688 ± 1.094 for SA and LA, respectively. The linear regression analysis data for the calibration plots showed a linear relationship in the concentration range 200-1200 ng/band with correlation coefficient 0.9983 for SA and 200-1200 ng/band with correlation coefficient 0.998 for LA. The proposed method can be applied for routine analysis of dosage form containing SA and LA in combination.

Keywords: HPTLC analysis, Salicylic acid, Lactic acid

Introduction

Salicylic acid (SA), chemically 2-Hydroxybenzoic acid, [1,2,3], it is the most generally consumed analgesic, antipyretic, and anti-inflammatory agent within the world [4,5]. It's a natural product found within the bark of a willow and has been used for hundreds of years to alleviate fever and pain [6,7]. SA is employed topically for its keratolytic, bacteriostatic, fungicidal, and photoprotective properties [8]. Lactic acid (LA), chemically 2-Hydroxypropanoic acid, a compound belonging to the family of carboxylic acids, present in certain plant juices, within the blood and muscles of animals, and within the soil. it's the most typical acidic constituent of fermented milk products like sour milk, cheese, and buttermilk. LA is employed in tanning leather and dyeing wool; as a flavouring agent and preservative in process cheese, salad dressings, pickles, and carbonated beverages; and as a material or a catalyst in numerous chemical processes. [9,10] In addition to these applications, it is utilized in solutions to exfoliate thicker skin in hyperkeratotic situations.[11] Salicylic acid is thought to diminish intercellular cohesiveness between corneocytes by dissolving the intercellular cement material and lowering the pH of the stratum corneum, hence enhancing hydration and softening. [12,13] LA penetrates the epidermis, causing stratum corneum turnover and desquamation of the outermost layer

while maintaining barrier function. [14] They lessen intercorneocyte connections via increasing the distance between corneocytes owing to increased stratum corneum water content, decreasing cell surface charges, suppressing enzymes involved in corneocyte cohesion, and splitting desmosomes when the pH of the medium decreases. This lowering can also directly destroy keratinocytes, increasing cell growth.[11] Combined dosage form of SA and LA is available in the Indian market for treatment of various skin related issues.

Various HPLC [15], HPTLC [16, 17], fluorimetric [18], and LC-MS [19] techniques for estimating SA individually and in combination with other medicines have been documented in the literature. Techniques such as UPLC [20], HPLC [21], LC-MS [22], fluorimetry [23], TLC [24, 25, 26] and UV Visible spectrophotometry [27], have been reported in literature for estimation of LA. There is no HPTLC method documented in the literature for simultaneous measurement of SA and LA. HPTLC is a straightforward, less time-intensive, and solvent-intensive method. The development and validation of an HPTLC method for the simultaneous estimation of SA and LA in their combined dose form are described in this study.

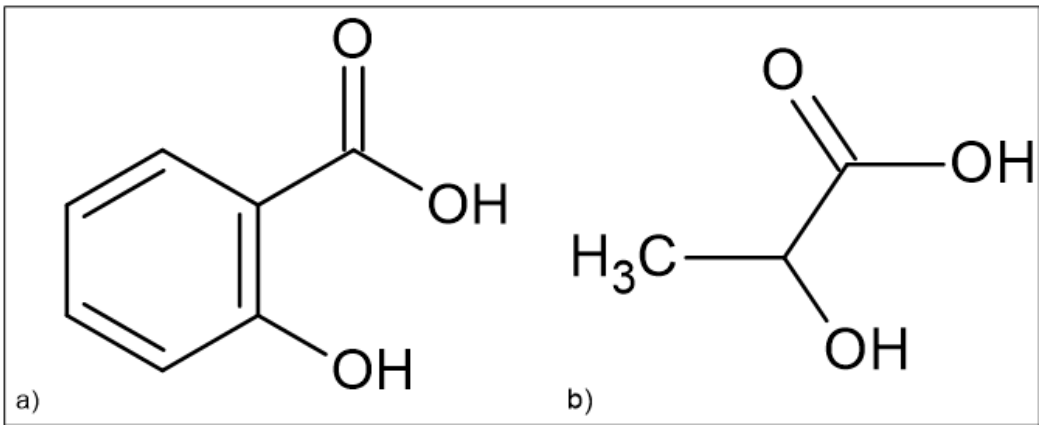

Fig.1. Structure of a) Salicylic acid and b) Lactic acid

Materials and Methods

Chemicals

Salicylic acid and Lactic acid were obtained as a gift samples Nulife Pharmaceuticals pvt. Ltd, Pune. Drug formulation Salactin Paint was obtained from the market. Whereas HPLC-grade chemicals such as Formic acid, Acetonitrile, Chloroform, Methanol were procured from Merk Life Sciences.

Instrumentation

Chromatographic separation of drugs was performed on Merck TLC plates pre-coated with silica gel 60 F254 (10 cm ×10 cm with 250 mm layer thickness) from E. Merck, Germany. The HPTLC system (Camag, Switzerland) consisting of Linomat- V applicator, a TLC Scanner III (Camag, Switzerland), twin-trough developing chamber (10 x10 cm), win CATS software V- 1.4.2, microlitre syringe (Hamilton) were used for chromatographic study. UV-Visible Double beam spectrophotometer with single Monochromator (Jasco Model V-730), and electronic analytical balance (Shimadzu Model AY-120) were used during the study.

Preparation of Standard stock solution

Standard stock solution of Lactic Acid and Salicylic Acid were prepared separately by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml of Lactic Acid and Salicylic Acid, separately in methanol

Preparation of sample solution

Salactin Paint each containing 16.7 % w/v of Lactic Acid and 16.7 % w/v Salicylic Acid was taken and volume equivalent to 100 mg of Lactic Acid (100 mg of Salicylic Acid) was transferred to 100 ml volumetric flask and was diluted with methanol, sonicated for 10 min and volume made to 100 ml (1000 µg/ml of Lactic Acid and 1000 µg/ml of Salicylic Acid) with methanol. Solution was filtered and further dilutions were made to get the final concentration of 100 µg/ml of Lactic Acid and 100 µg/ml of Salicylic Acid.

Selection of detection wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 229 nm.

Development of the optimum mobile phase

The TLC plates were used to detect the functioning standards of both medications, which were then developed using various solvent systems. For SA and LA, various mobile phases were tried. The mobile phase made up of chloroform, formic acid, and methanol (8.5: 1: 0.5 v/v/v) produced the best results. At room temperature, the mobile phase had filled the compartment to saturation. Resolution for two medicines with developed mobile phase was 0.285 ± 1.922 and 0.688 ±1.094 for SA and LA, respectively. Figure 2 shows the example densitogram.

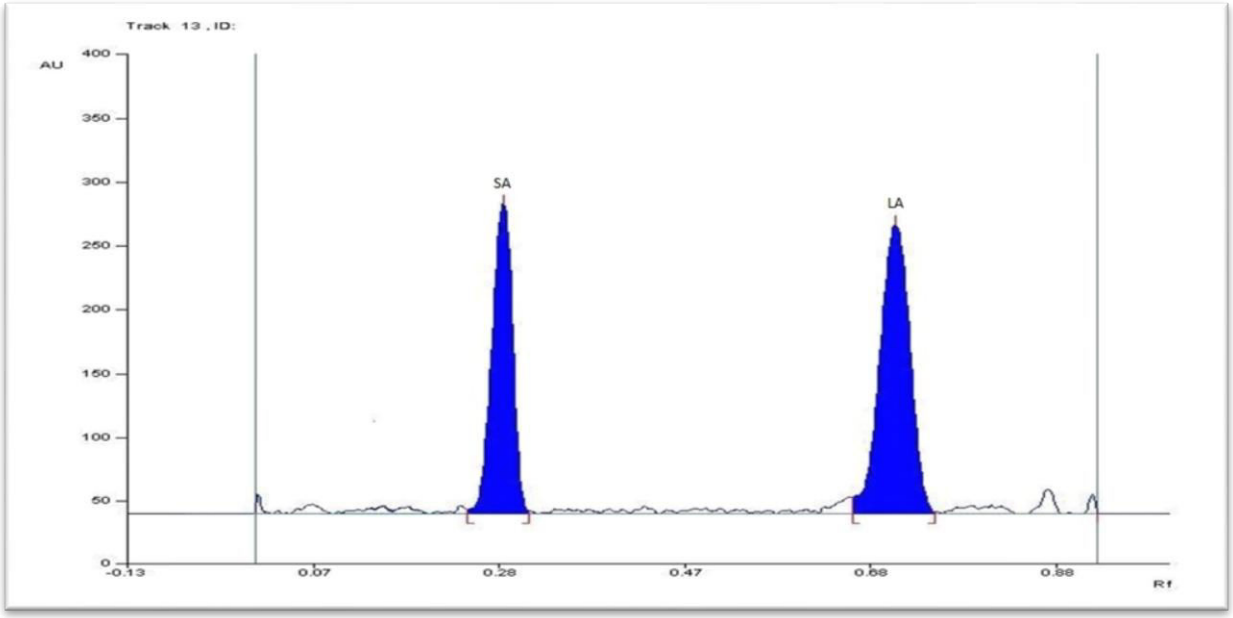

Fig.2.Densitogram of blank and mixed standard solution of Salicylic Acid

Method validation

The developed method was method validated for the simultaneous estimation of SA and LA using following parameters.

Specificity:

Specificity of a method is ability to measure accurately and specifically the analyte whether there is any presence of any other component such as impurity or excipient. The specificity study of revealed that no interference of methanol and mobile phase was observed.

Linearity and Range

From the standard stock solution (1000 µg/ml) of Lactic Acid and Salicylic Acid, solution was prepared containing 100 µg/ml of Lactic Acid and 100 µg/ml of Salicylic Acid, separately. Different volumes were applied on TLC plate to obtain linear range. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range 200-1200 ng/band for Lactic Acid as well as for Salicylic Acid. The results obtained are shown in Table 1 for Lactic Acid and in Table 2 for Salicylic Acid.

Table 1. Linearity study of Lactic Acid

Replicates	Concentrations of Lactic Acid (ng/ spot)					
	200	400	600	800	1000	1200
	Peak Area					
1	1420.2	2727.4	4456.6	6035.1	7619.8	8976.9
2	1398.6	2739	4280.5	5825.5	7401.3	8905.3
3	1418.4	2767	4295.6	6070.7	7805.1	9101.6
4	1370.2	2773	4342.3	6079.9	7550.5	8923.8
5	1373	2703.5	4284.3	6098	7645	9284.9
6	1381.9	2678.9	4277.3	6091.4	7549.2	9014.1
Mean	1450.43	2731.467	4322.77	6033.433	7595.150	9034.43
Std.dev.	22.41	36.349	69.81	104.227	133.308	141.34
%RSD	1.54	1.331	1.61	1.727	1.755	1.56

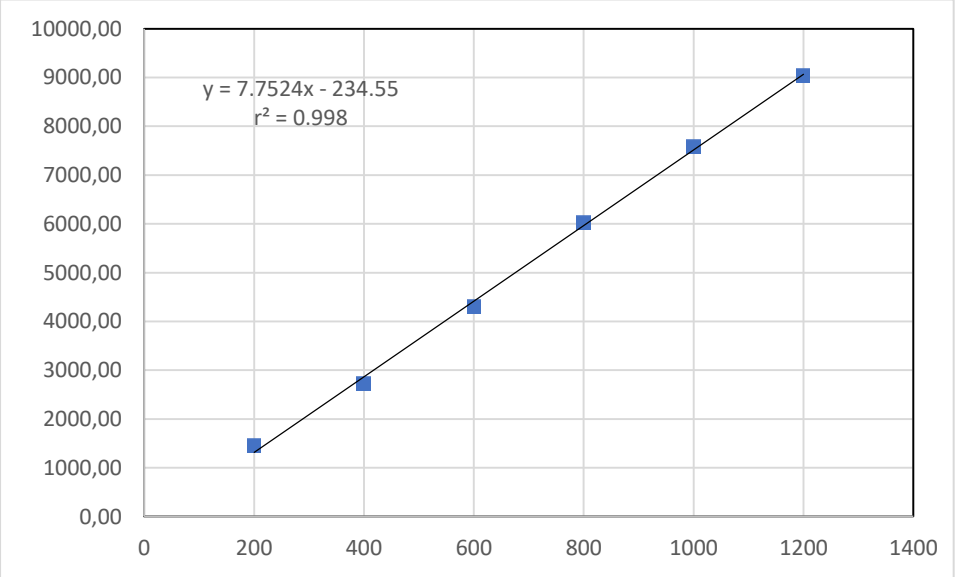

Fig 3. Calibration curve of Lactic acid

Table 2. Linearity study of Salicylic Acid

Replicates	Concentrations of Salicylic Acid (ng/band)					
	200	400	600	800	1000	1200
	Peak Area					
1	2352	3989.6	5527	7680.7	9521.11	11595.3
2	2383.6	4041.6	5622	7681.11	9539.6	11510.6

3	2379.4	4192	5697	7802.9	9601.8	11353.9
4	2419.5	4157.8	5689	7643.4	9627	11718.3
5	2355.1	4059	5691.4	7749	9618	11714
6	2412.7	4243.3	5679.9	7650.5	9623	11229.8
Mean	2383.717	4113.883	5651.050	7701.268	9588.418	11520.317
Std.dev.	28.157	88.451	66.700	62.230	46.157	197.242
%RSD	1.181	1.393	1.180	0.808	0.481	1.712

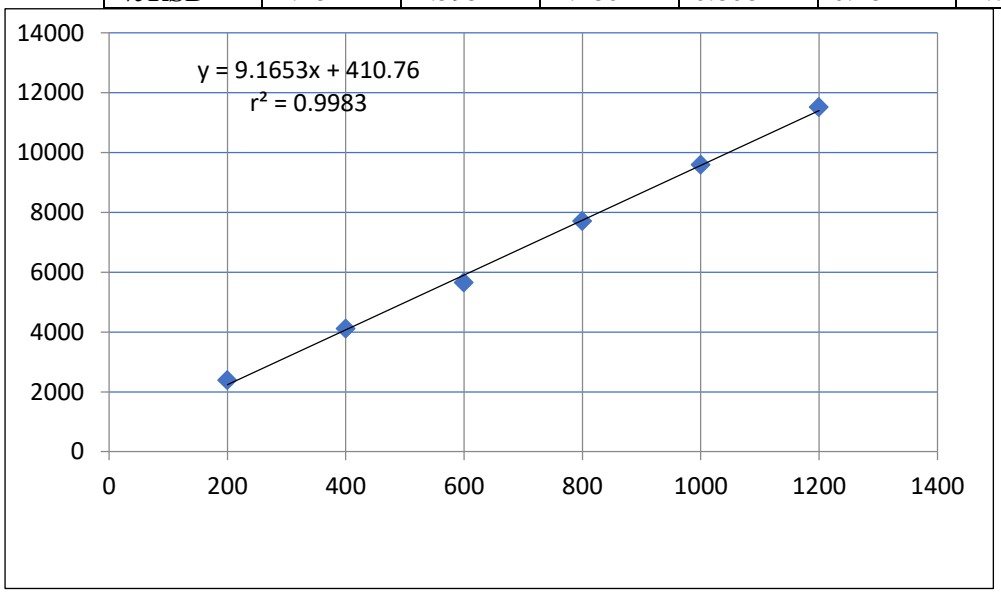

Fig.4. Calibration curve of Salicylic acid

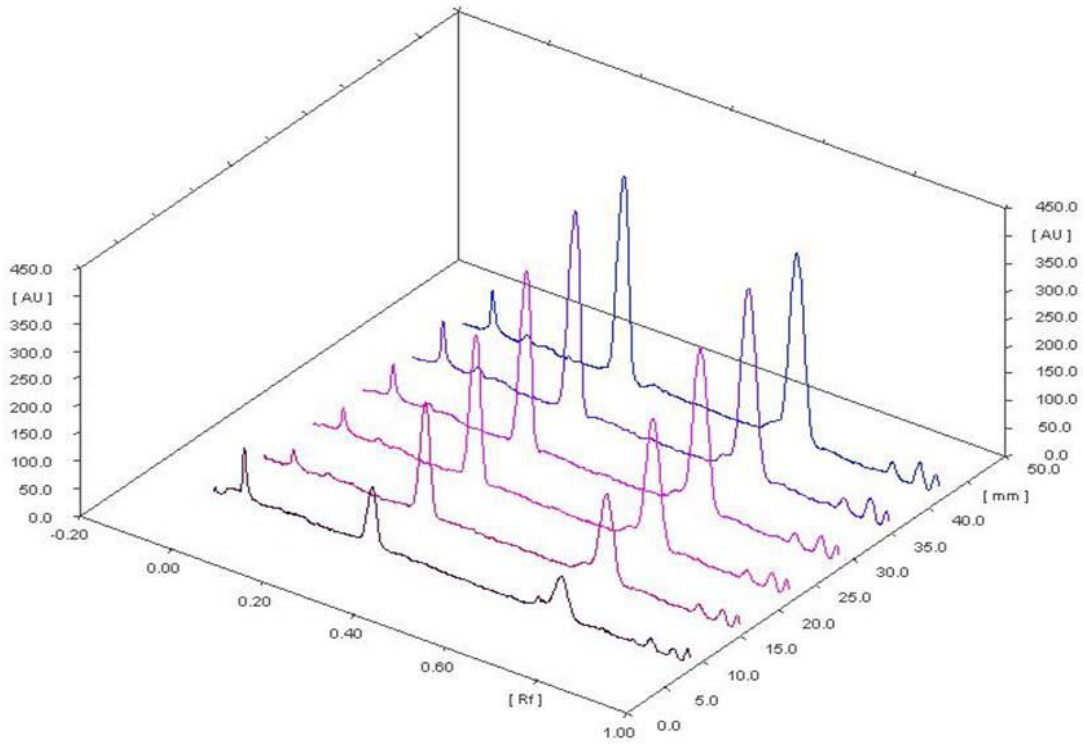

Fig.5. 3D Densitogram of linearity of Lactic Acid and Salicylic Acid

Precision

The precision of the method was demonstrated by intra-day and inter-day 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 concentrations were analysed on 3 consecutive days and % RSD was calculated. The results obtain for intra-day variations are shown in Table 3 and Table 4. The result obtained for inter day variations are shown in Table 5 and Table 6.

Table 3. Intra-day precision study of Lactic Acid

Concentration (ng/spot)	Area	Amount recovered	% Recovery	Mean % Recovery	Std. Dev.	% RSD
400	2866.29	402.191	100.5477	100.006	0.5408	0.542
	2832.6	397.8643	99.46606			
	2849.4	400.0218	100.0055			
600	4372.4	595.6168	99.26946	99.897	0.667	0.676
	4398.31	598.9443	99.82405			
	4434.5	603.5921	100.5987			
600	5915.3	793.7674	99.22093	100.055	0.742	0.742
	6003.9	805.1461	100.6433			
	5982.6	802.4106	100.3013			

Table 4. Intra-day precision study of Salicylic Acid

Concentration (ng/spot)	Area	Amount recovered	% Recovery	Mean % Recovery	Std. Dev.	% RSD
400	4117	404.391	101.098	100.230	0.76	0.758
	4073.5	399.644	99.911			
	4065.1	398.728	99.682			
600	5885.2	597.320	99.553	99.986	1.009	1.010
	5869.3	595.585	99.264			
	5972.4	606.835	101.139			
600	7708.1	796.218	99.527	99.375	0.132	0.134
	7692.5	794.516	99.315			
	7690.2	794.265	99.283			

Table 5. Inter-day precision of Lactic Acid

Concentration (ng/spot)	Area	Amount recovered	% Recovery	Mean % Recovery	Std. Dev.	% RSD
400	2866.29	402.191	100.5477	100.006	0.5408	0.542
	2832.6	397.8643	99.46606			
	2849.4	400.0218	100.0055			
600	4372.4	595.6168	99.26946	99.897	0.667	0.676
	4398.31	598.9443	99.82405			
	4434.5	603.5921	100.5987			
600	5915.3	793.7674	99.22093	100.055	0.742	0.742
	6003.9	805.1461	100.6433			
	5982.6	802.4106	100.3013			

Table 6. Inter-day precision of Salicylic Acid

Concentration (ng/spot)	Area	Amount recovered	% Recovery	Mean % Recovery	Std. Dev.	% RSD
400	4121.43	404.874	101.218	99.89	1.82	1.828
	3989.2	390.446	97.612			
	4108.38	403.450	100.863			
600	5883.5	597.135	99.522	99.58	0.733	0.737
	5926.7	601.848	100.308			
	5846.1	593.054	98.842			
600	7724.45	798.002	99.750	100.01	0.844	0.844
	7694.46	794.730	99.341			
	7813.48	807.716	100.965			

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150 %. Basic concentrations of sample chosen were 4 µl of 100 µg/ml of Lactic Acid as well as of Salicylic Acid sample solution. These solutions were applied on TLC plates in triplicate to obtain the densitogram. The drug concentrations of Lactic Acid and Salicylic Acid were calculated by using linearity equations of Lactic Acid and Salicylic Acid. The results obtained are shown in Table 7 and Table 8.

Table 7. Recovery studies of Lactic Acid

Level	Conc. (ng/band)		Area	Amount recovered	% Recovery	Mean	Std. Dev.	% RSD
	Sample	Std.						
50 %	400	200	4348.4	592.5345	98.75	99.214	0.397	0.400
			4379.9	596.58	99.43			
			4381.2	596.74	99.45			
100 %	400	400	5898.1	791.55	98.94	99.721	0.684	0.686
			5978.5	801.884	100.23			
			5962.9	799.88	99.98			
150 %	400	600	7552.7	1004.05	100.40	100.979	0.372	0.372
			7498.7	997.11	99.71			
			7507.2	998.21	99.82			

Table 8. Recovery studies of Salicylic Acid

Level	Conc. (ng/band)		Area	Amount recovered	% Recovery	Mean	Std. Dev.	% RSD
	Sample	Std.						
50 %	400	200	5851	593.589	98.931	99.470	0.466	0.471
			5897.6	598.673	99.779			
			5893.3	598.204	99.701			
100 %	400	400	7781	804.172	100.522	99.674	0.738	0.739
			7683	793.480	99.185			
			7692.6	794.527	99.316			
150 %	400	600	9632	1006.136	100.614	100.339	0.251	0.249
			9587.3	1001.259	100.126			
			9601.3	1002.787	100.279			

Assay

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 400 ng/band of sample solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 9 and Table 10.

Table 9. Assay of marketed formulation (% of LA)

Sr. No.	Peak area	Amount recovered (ng/band)	% Recovery
1	2871.3	403.039	100.760
2	2845.55	399.722	99.930
3	2837.8	398.723	99.681
4	2848.1	400.050	100.013
5	2869.8	402.846	100.712
6	2825.1	397.087	99.272
Mean	2849.608	400.244	100.061
SD	18.095	2.331	0.583
%RSD	0.635	0.583	0.583

Table 10. Assay of marketed formulation (% SA)

Sr. No.	Peak area	Amount recovered (ng/band)	% Recovery
1	4117.3	404.423	101.106
2	4041.6	396.164	99.041
3	4096.8	402.187	100.547
4	4089.5	401.390	100.348
5	4069.75	399.235	99.809
6	4091.3	401.586	100.397
Mean	4084.375	400.831	100.208
SD	25.908	2.827	0.707
%RSD	0.634	0.705	0.705

Limit of Detection (LOD)

According to the ICH [28] criteria, the LOD for SA and LA was determined to be 36.630 ng/band and 42.766 ng/band, respectively.

Limit of Quantification (LOQ)

According to the ICH criteria, the LOQ for SA and LA was determined to be 111 ng/band and 129.594 ng/band, respectively.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which the chamber saturation time, wavelength and time form application to development are altered and the effect area were noted. The results obtained are shown in the Table 11, 12 and 13.

Table 11. Robustness Study (Saturation Time)

Sr.no	Salicylic Acid			Lactic Acid		
Time(min)	13	15	17	13	15	17
1	4047.5	4156.2	4102.2	2751.45	2723.175	2752.815
2	3998.2	4131.7	4115.4	2828.28	2747.095	2711.15
3	4103.5	4146.2	4099.5	2803.905	2769.975	2763.41
Avg	4049.733	4144.700	4105.700	2794.545	2746.748	2742.458
STD DEV	52.686	12.319	8.508	39.261	23.402	27.626
% RSD	1.301	0.297	0.207	1.405	0.852	1.007
RSD. Avg.	0.602			1.088		

Table 12. Robustness Study (Wavelength)

Sr.no	Salicylic Acid			Lactic Acid		
Wavelength(nm)	228	229	230	228	229	230
1	4215.2	4155.7	4112.3	2636.595	2720.38	2666.17
2	4218	4128.1	4099.7	2646.865	2666.82	2668.445
3	4188.4	4159.6	4056.2	2616.705	2671.695	2662.205
Avg	4207.200	4147.800	4089.400	2633.388	2686.298	2665.607
STD DEV	16.341	17.172	29.434	15.334	29.616	3.158
% RSD	0.388	0.414	0.720	0.582	1.102	0.118
RSD. Avg.	0.507			0.601		

Table 13. Robustness Study (Application to development time)

Sr.no	Salicylic Acid			Lactic Acid		
Time(min)	0	30	60	0	30	60
1	4156.3	4085.2	4091.8	2699.645	2713.88	2666.17
2	4172.1	4202.8	4045.3	2711.865	2699.32	2668.445
3	4125.7	4040.3	4069.7	2681.705	2704.195	2703.162
Avg	4151.367	4109.433	4068.933	2697.738	2705.798	2679.259
STD DEV	23.590	83.917	23.259	15.170	7.411	20.732
% RSD	0.568	1.91	0.572	0.562	0.274	0.774
RSD. Avg.	1.016			0.537		

Table 14. Summary

r. lo.	alidation Parameter	esults	
		actic Acid	alicylic Acid
1.	Linearity	y = 7.7524x - 234.55 r^2 = 0.998	y = 9.165 x + 410.7 r^2 = 0.998
2.	Range	200-1200 ng/band	200-1200 ng/band
3.	Precision	%RSD	%RSD
	A) Intraday precision	0.542 – 0.742	0.134 – 1.010
	B) Interday precision	0.197 – 0.705	0.737 – 1.990
4.	Assay (Mean ± % RSD)	100.061 ± 0.583	100.208 ± 0.705
5.	Accuracy	% recovery ± % RSD	% recovery ± % RSD
	50%	99.214 ± 0.400	99.470 ± 0.471
	100%	99.712 ± 0.686	99.674 ± 0.739
	150%	100.979 ± 0.372	100.339 ± 0.249
6.	LOD	36.630 ng/ band	42.766 ng/band
7.	LOQ	111.000 ng/band	129.594 ng/band
8.	Specificity	Specific	Specific
9.	Robustness	Robust	Robust

Result and Discussion

High performance thin layer chromatography method had developed and validated for the estimation of SA and LA in combined dosage formulation. The method uses pre-coated silica gel plate 60 F254 TLC plates as stationary phase with Chloroform: Formic acid: Methanol (8.5: 1:0.5 v/v/v) as mobile phase. Densitometric analysis was carried out in the absorbance mode at 229 nm. The linearity range lies between 200 and 1200 ng/band for both the compound with correlation coefficients of 0.994 and 0.995 respectively. The Rf value for SA is 0.285 ± 1.922 and for LA is 0.688 ± 1.094. Percentage Recoveries of SA and LA was close to 100%. Limit of detection value for SA was 36.63 ng/band and for LA was 42.76 ng/band. Limit of quantitation value for SA was 111 ng/band and for LA was 129.54 ng/band. The results of formulation analysis were validated as per ICH guidelines indicating high degree of accuracy. The robustness study suggested that the developed HPTLC methods were unaffected by small changes in process parameters. The HPTLC method showed good separation and sharp peak of salicylic acid and lactic acid. Chromatography method thus been validated for the salicylic acid and lactic acid respectively.

Conclusion

A specific, accurate and precise HPTLC method was developed for simultaneous estimation of SA and LA in a dosage form. The developed method was applied for assay of formulation and results were found to be in agreement with the label claim. The proposed method can be used in routine analysis of dosage form containing salicylic acid and lactic acid in combination.

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