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Liquid Chromatographic Technique for Stability Indicating Analytical Method Development and Validation of Salicylic Acid and Tolnaftate in Pharmaceutical Ointment by High Performance

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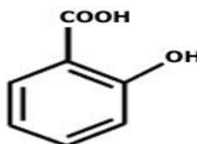
Abstract- A stability indicating high performance liquid chromatography (HPLC) method is developed for the quantification of salicylic acid (SA) and Tolnaftate (TF) in combined pharmaceutical ointment base formulations. The separation was performed on a Merck" C-18 column with the mobile phase consisting of Acetonitrile**:** Methanol: Water (50:20:30v/v) at flow rate 1.5ml/min. Both the drugs were resolved successfully with retention time 1.318 and 8.805minute when detection was carried out at UV 245nm.The overall retention time of analytes were 10.0minutes.The method was validated with respect to linearity, precision, accuracy and recovery. The relative standard deviation for six replicate measurements of SA and TF were 0.259% and 0.240% respectively. Total recoveries of analytes were 100.56, 100.63, 100.58% and 100.23, 100.73, 100.22% of SA and TF respectively when examine over the range of 80, 100, and 120% of added drugs in placebo. No chromatographic interference from the formulation excipient was found. The linearity of SA and TF were found in the range of 256-384µg/ml and 32.0 to 48.0µg/ml respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operator proven that the method is robust and rugged

Index Terms- Stability, Salicylic Acid, Tolnaftate, pharmaceutical ointment, HPLC-UV development, Assay, Method Validation and liquid chromatography.

I. INTRODUCTION

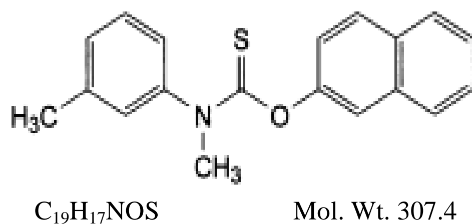
The ointment contains tolnaftate, a highly active synthetic antifungal agent effective in the treatment of superficial fungal infections of the skin. The ointment contains tolnaftate with the classical keratolytic agent, salicylic acid. The ointment is indicated in superficial dermatomycoses where a keratolytic effect is required. An extensive literature survey revealed HPLC determination for salicylic acid (SA) with betamethasone dipropionate and Tolnaftate (TF) with betamethasone dipropionate. But there is no method which describes the simultaneous determination of salicylic acid and Tolnaftate from ointment base formulation. The objective of this investigation was to develop simple, precise, accurate and economical procedure for simultaneous estimation of SA and TF from an ointment by HPLC.

Salicylic acid is 2-hydroxy benzoic acid. White or colorless, acicular crystals or white, crystalline powder. Freely soluble in ethanol (95%) and in ether; sparingly soluble in chloroform; slightly soluble in water.



$C_7H_6O_3$ Mol. Wt. 138.12

Figure.1: Chemical Structure of Salicylic Acid



$C_{19}H_{17}NOS$ Mol. Wt. 307.4

Figure.2: Chemical Structure of Tolnaftate

O-Naphthalen-2-yl methyl (3-methylphenyl) carbamothioate. It is a White or yellowish-white powder. Practically insoluble in water, freely soluble in acetone and in methylene chloride, very slightly soluble in ethanol (96 per cent).

II. Experimental

Materials and Reagents

Salicylic acid (SA) and Tolnaftate (TF) were of USP. Acetonitrile and Methanol was procured from Merck and company. Water was deionised and double distilled. Pharmaceutical formulation containing SA and TF was of our in house formulation.

Apparatus and Chromatographic Conditions

Chromatographic separation was performed on a Shimadzu HPLC system consisting of Shimadzu LC2010 CHT with a PDA detector, and window based LC solutions software. An ODS Merck" C18 RP-Column (250mm X 4.6mm, 5µm) was used for separation. The chromatographic separation was carried isocratically at room temperature with a flow rate of 1.5ml/min using Acetonitrile: Methanol: Water (50:20:30v/v/v) as mobile phase. The injection volumes for samples and standards were 20µl.

III. Preparation of Standard Solutions

Salicylic acid

Weigh about 80.0mg of standard (i.e. salicylic acid) and transfer it to 50ml volumetric flask. Dissolve the drug in sufficient amount of acetonitrile with shaking and make the volume up to the mark with Acetonitrile to obtain the concentration of 1600µg/ml (Stock-1).Dilute 10ml of the stock-1 further to 50 ml to obtain the concentration of 320µg/ml. Filter it with 0.2µ filter paper and use the filtrate for further analysis.

Tolnaftate

Weigh about 50.0mg of standard (i.e. tolnaftate) and transfer it to 50ml volumetric flask. Dissolve the drug in sufficient amount of acetonitrile with shaking and make the volume up to the mark with acetonitrile to obtain the concentration of 1000µg/ml (Stock-2) Dilute 1ml of the stock-2 further to 25 ml to obtain the concentration of 40µg/ml. Filter it with 0.2µ filter paper and use the filtrate for further analysis.

Sample Solution

Weigh accurately 1gm of sample in a beaker. Add sufficient amount of acetonitrile and heat the sample on water bath until it gets converted into liquid. Thereafter keep the hot beaker on the magnetic stirrer until the beaker gets cooled and the liquefied wax gets converted into solid and gets separated. Transfer the solution obtained into 50 ml volumetric flask Repeat the heating and chilling method till the drug is completely extracted out of the waxy material. Make up the volume to 50 ml with Acetonitrile. Filter the stock sample with filter paper (Stock). Dilute 5ml of the stock further to 25 ml to obtain the concentration of 320µg/ml for salicylic acid and 40µg/ml for tolnaftate. Filter it with 0.2µ filter paper and use the filtrate for further analysis.

IV. Method Validation

The proposed method was subjected for various parameters like system suitability, specificity range and linearity, accuracy, precision and robustness in accordance with International Conference on Harmonization guidelines. The replicates drugs were carried out to assess the system suitability. It was further evaluated by analyzing the repeatability, peak symmetry (Symmetry factor), theoretical plate of the column, resolution between the peaks, tailing factor and relative retention time. The specificity of the chromatographic method was determined to ensure separation of SA and TF. Specificity was also determined in the presence of excipient used in formulation, SA and TF was spiked in placebo and chromatograms was observed and compared with that of reference standard. PDA detector was used to check the peak purity.

The linearity is important to demonstrate that the response of the measurement of detector system is linear over the range of interest of the method. This was determined by means of calibration graph using increasing amounts of a standard solution (80, 90, 100, 110, and 120%) of both the drugs. Six replicates of the standard were tested according to ICH guidelines. A calibration curve was constructed and the proposed method was evaluated by its correlation coefficient and intercept value. The correlation coefficient found within limit. The method was validated for accuracy and precision. The accuracy of the method was studied as mean % recovery. Accuracy was determined by means of recovery experiments, by spiked addition of active drugs to placebo formulations. It was shown that the recoveries were independent of the concentration of the active drug over reasonable concentration range normally 80 to 120% of the nominal concentration. The accuracy of the assay was measured by analyzing samples of SA and TF by spiking known amount of said drugs in the placebo, at different concentration levels (80%, 100% and 120%).

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ICH guidelines recommend that, precision must be considered at two levels, i.e. repeatability and intermediate precision, repeatability of the method was performed by injecting 100% concentration of both analyte of regular analytical working value consecutively for six times and the effects on the results were examined. The intermediate precision is with respect to laboratory variations, i.e. by using different equipments, different analyst and days. The relative standard deviation (% RSD) was determined to assess the precision of the assay and it was not be more than 2.0%.

Robustness studies was done by making slight variations in flow rate, amount of acetonitrile, and detection wavelength changes once at a time.

V. Result and Discussion

Quality control of drugs/ drug products is of prime importance. HPLC methods are used for the quantitation of drug substances. The goal of this study was to develop a rapid, sensitive, accurate, precise and reliable HPLC method for the analysis of SA and TF from ointment formulations using the most commonly employed C18 column with PDA detector.

Method Development and Optimization

This isocratic-mode method with PDA detection was developed for the determination of the active ingredients, SA and TF at 100% level. Firstly, the reversed-phase column was tested. The column gives very efficient and reproducible separation of the components while minimizing usage. Consequently, it was selected for the method development. The system suitability studies were carried out as specified in ICH guidelines.

The mobile phase consisted of Acetonitrile: methanol and water at various ratios (75: 10:15, 85: 5: 10, 80: 5:15, 30 and 50:20:30 v/v/v) was tested as starting solvent. The variations at the mobile phase lead to considerable changes in the chromatographic parameters. However, the proportion acetonitrile: methanol: water (50:20:30v/v) yielded the best results.

For studying the effect of excipient on quantification of SA and TF, a placebo was analysed. The result obtained indicates that excipient have no interference. Based on the highest UV absorbance for SA and TF, 245nm was chosen for the detection of this new HPLC method at which the best detector response for both the substances were obtained.

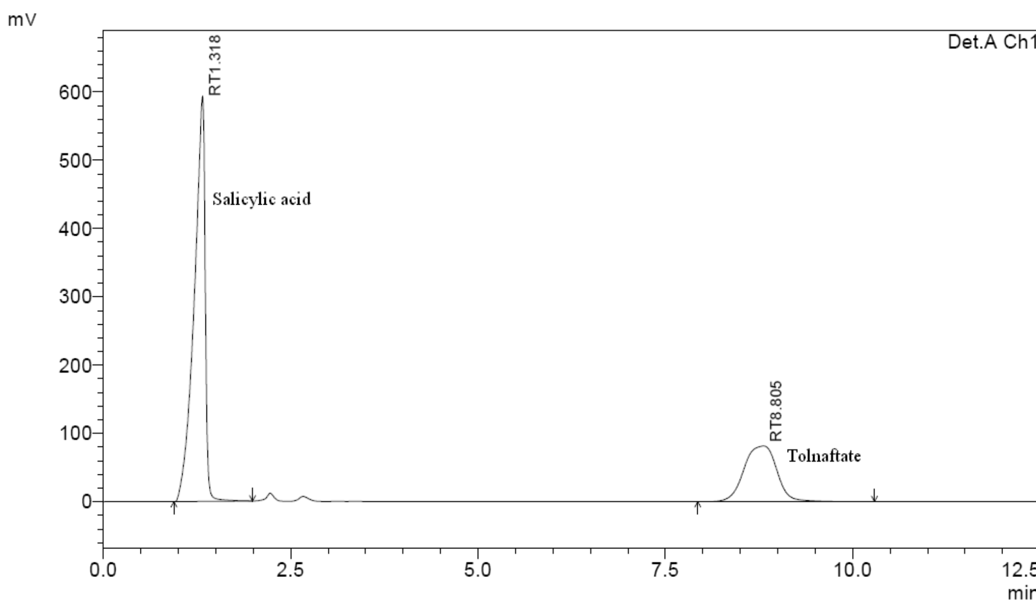

Figure 3:- Typical chromatogram of sample shows Salicylic acid and Tolnaftate.

VI. Method validation

System suitability testing

System suitability testing is the integral part of chromatographic methods and is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The results obtained for system suitability are summarized in Table I.

Table I: - System Suitability Parameter

Parameter	SA	TF
Precision of the method (n = 6)	0.259 %	0.240%
Theoretical Plates	2442	5176
Tailing Factor	1.000	1.000
Retention time	1.73	8.32

Range and linearity

The range of an analytical method is the interval between analytical concentration for upper and lower limit of a sample where the method has shown to demonstrate acceptable accuracy, precision and linearity. The linearity of the method was observed in the expected concentration range demonstrating its suitability for analysis. The calibration curves were constructed with five concentrations. The regression static's are shown in Table II.

Table II: - Calibration characteristics

Parameters	SA	TF
Linearity Range	256 to 384µg/ml	32 to48µg/ml
Regression Equation	Y =20129x + 18475	Y = 60878x + 6099.1
Correlation coefficient (r^2)	0.999	0.998

Precision

The precision of the method was determined by inter-day and intra-day precision. It was expressed as %RSD of a series of six measurements. The experimental values obtained for the repeatability of SA and TF in samples in terms of area was listed below in table III. All the data obtained were in within the acceptance criteria.

TF Table III: Precision for SA and

Number of injections	Area's of Active ingredients		Percent RSD NMT 2.0%	
	Salicylic Acid	Tolnaftate		
Injection 1	6449600	2469529	Salicylic Acid	Tolnaftate
Injection 2	6426366	2468797		
Injection 3	6477173	2469062	**0.259%**	**0.240%**
Injection 4	6456189	2457615		
Injection 5	6450709	2456915		
Injection 6	6461898	2466990		
Mean Area	**6453655**	**2464818**		
±SD	16716.91	5917.7		

*Average of six determinations, S.D. is standard deviation; RSD is the Relative Standard Deviation.

Accuracy

The accuracy was evaluated by the recovery of SA and TF at three different concentration levels (80, 100, and 120%). The recoveries of SA and TF were within limit respectively which is evident that the method is accurate in the desired range.

Table IV: - Results of Accuracy Experiment

DRUGS	Amount of drug added (%)	Amount of drug added (mg)	Amount recovery (mg)	Amount recovery (%)	Mean recovery (%)	±SD	%RSD
Salicylic Acid	80	256	256.51	100.19	100.56	0.557	0.554
	80	256	256.80	100.31			
	80	256	259.10	101.21			
	100	320	320.28	100.08	100.63	0.694	0.689
	100	320	321.30	100.40			
	100	320	324.50	101.41			
	120	384	384.73	100.19	100.58	0.652	0.684
	120	384	389.13	101.34			
	120	384	384.90	100.23			
%RSD NMT 2.0%							
Tolnaftate	80	32.0	32.13	100.41	100.23	0.150	0.149
	80	32.0	32.05	100.15			
	80	32.0	32.05	100.15			
	100	40.0	40.09	100.23	100.73	0.805	0.800
	100	40.0	40.11	100.28			
	100	40.0	41.66	101.65			
	120	48.0	47.95	99.89	100.22	0.729	0.727
	120	48.0	47.87	99.72			
	120	48.0	48.51	101.06			

SD is Standard deviation and; RSD is the Relative Standard Deviation.

Specificity

Specificity is the ability of the method to asses unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradation-products and matrix materials etc. did not interfere with the drug peak and thus the method was found to be specific. The placebo was not interfering with the actives.

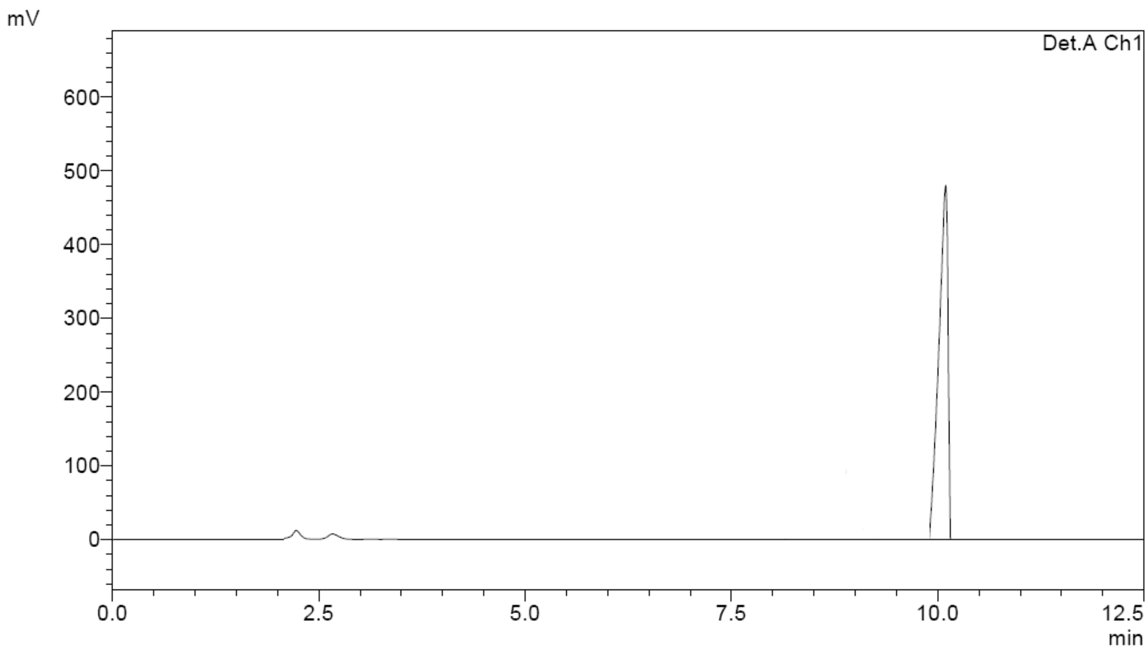

Figure 4: Chromatogram of Placebo solution showing no interference.

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Robustness: Various parameters were deliberately varied. The parameters include variation of flow rate, percentage of acetonitrile and methanol, detection wavelength, column etc.

Table V: - Results of Robustness.

Parameter	Variation	Retention Time	
		SA	**TF**
Flow rate	1.4	1.318	8.805
	1.6	1.311	8.800
Wavelength	235	1.318	8.805
	255	1.318	8.805
Mobile phase composition	(55:15:30)	1.306	8.802
	(60:20:30)	1.320	8.809

VII. Conclusions

A reverse-phase HPLC method was developed and validated for the simultaneous determination of SA and TF proven to be more convenient and effective for the quality control of these drugs in pharmaceutical formulations. The method is found to be simple, precise, accurate, specific, selective and linear over the concentration range tested (80-120%) with correlation coefficient of 0.999. The good percentage recovery in formulation suggests that the excipient present in the dosage forms have no interference in the determination. The percent RSD was also less than 2.0% showing high degree of precision of the proposed method. So, the method is suitable for the determination of the drugs in tables without interference from commonly used excipient, and could be used in a quality control laboratory for routine sample analysis.

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