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Original Article

RP-HPLC Method Development and Validation of Choline Salicylate and Lignocaine HCl in Mouth Ulcer Gel

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ABSTRACT

Objectives: The prime intent of the current study is to develop a rapid, reliable, robust and cost-effective reversed-phase HPLC method for simultaneous estimation of Choline Salicylate and Lignocaine HCl in mouth Ulcer Gel. **Materials and Methods:** Buffer was prepared with 3.5 g of disodium hydrogen orthophosphate in 1000 mL of water and adjusted to pH 4.5 with dilute orthophosphoric acid. A mixture of Buffer and Methanol (55:45) containing 0.01M (2.062 g) 1-Hexane Sulphonic acid comprised the mobile phase. The separation was carried out with a Hypersil C_{18} column (250x4.6 mm, 5 µ) maintained at a temperature of 30ºC at a flow rate of 1.0 mL/min with detection at 220 nm. The Injection volume was 20 µL and the run time was 20 min. **Results:** Retention time for choline salicylate and Lignocaine HCl was 3.163 and 9.629 min respectively. Linearity for Choline Salicylate and Lignocaine Hydrochloride was found to be in the range of 12.5-50 µg/mL. The correlation coefficient for the linear curve obtained between concentration vs. area for standard preparations of Choline Salicylate and Lignocaine HCl was 0.9992 and 0.9993 respectively. The projected procedure successfully satisfied the specificity and robustness parameters. **Conclusion:** The analytical method was successfully validated according to ICH guidelines (ICH, Q2 (R1)). The proposed novel method is reliable and cost-effective and is well-suited for use in the pharmaceutical industry.

Keywords: Choline Salicylate, Lignocaine HCl, Sensitivity, Isocratic elution, Specificity.

INTRODUCTION

Choline Salicylate (CS) can be described as a compound composed of 2-carboxy phenolate and 2-hydroxy ethyl (trimethyl) azanium. It is used to reduce swelling and cure mild to severe discomfort.[1,2] Choline salicylate works to relieve pain by preventing the production of prostaglandins, while it reduces temperature by having an impact on the hypothalamus's heat-regulating area. In addition, it prevents nerve impulse production by blocking the cyclooxygenase enzyme. Salicylic acid is the source of Choline Salicylate (CS), a type of Non-Steroidal Anti-Inflammatory Medication (NSAID).[3,4] Lignocaine Hydrochloride (LH) is known as 2-(diethyl amino)-N-(2,6-dimethyl phenyl) acetamide hydrochloride. This drug is used to reduce pain during some medical operations.[5-7] Raising the threshold for electrical excitation in nerves causes numbness in the mouth, throat, or nose before these procedures.[5-7] By decreasing the spread of nerve impulses and lowering the pace at which action potentials rise, it successfully prevents the creation and conduction of nerve impulses. It is a local anesthetic drug.[8-13]

MATERIALS AND METHODS

Equipment

A Shimadzu HPLC system was used for the analysis and a PDA detector was used to develop and validate the method. The data processing and collecting were done using the LC solution chromatographic program. Hypersil C_{18} column (250x4.6 mm, 5 µ) was the analytical column employed for the separation.

Chemical and Reagents

Rankem supplied HPLC-grade methanol and orthophosphoric acid. Rankem and Thermo Fisher Scientific India Pvt. Ltd., supplied the AR-grade sodium hydrogen orthophosphate. The water utilized in the experiment was purified HPLC-grade water.

Optimized Chromatographic conditions

Buffer was prepared with 3.5 g of disodium hydrogen orthophosphate in 1000 mL of water and adjusted to pH 4.5 with dilute orthophosphoric acid. A mixture of Buffer and Methanol (55:45) containing 0.01 M (2.062 g) 1-Hexane Sulphonic acid

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served as the mobile phase. The separation was carried out with a Hypersil C_{18} column (250x4.6 mm, 5 µ) maintained at a temperature of 30°C at a flow rate of 1.0 mL/min with detection at 220 nm. The Injection volume was 20 µL and the run time was 20 min.

Diluent: Mobile Phase is employed as a diluent.

Preparation of buffer solution

Dissolve about 3.5 g of Di-Sodium hydrogen orthophosphate in 1000 mL of Milli-Q water.

Use orthophosphoric acid to adjust the pH level to 4.5.

Mobile phase preparation

The mixture of Buffer and Methanol (55:45) containing 0.01 M (2.062 g)1-Hexane Sulphonic acid was prepared.

Standard Solution Preparation

Stock Solution (A)

In a clean, dry 100 mL volumetric flask, accurately weigh about 435 mg of Choline Salicylate WS of known purity and add 50 mL of diluent. After sonicating for 5 min, dilute using the same diluent. Transfer 5 mL of this solution to a 50 mL volumetric flask and dilute with the same diluent.

Stock Solution (B)

In a clean, dry 100 mL volumetric flask, accurately weigh around 50 mg of Lignocaine HCl of known purity before adding 50 mL of diluent. After sonicating for 5 min, dilute using the same diluent. Transfer 5 mL of this solution to a 50 mL volumetric flask and dilute with the same diluent.

Standard Preparation: Transferred 5 mL of stock solution (A) and 5 mL of stock solution (B) in a 50 mL volumetric flask and dilute with the same diluent.

Sample Solution Preparation

Weighed approximately 2.5 g of material in a clean, dry 100 mL volumetric flask, add 50 mL of diluent, sonicated for 20 min, then diluted with the same solvent. Transfer 5 mL of this solution to a 50 mL volumetric flask and dilute with the same diluent.

RESULTS AND DISCUSSION

To optimize the chromatographic for the concurrent assessment of Choline Salicylate and Lignocaine HCl, the isocratic elution method was used. This study aimed to develop a rapid and accurate method for the estimation of CS and LH by RP-HPLC. Different combinations of mobile phase were tried but better results were obtained using a mobile phase of phosphate buffer with a pH of 4.5 (adjusted by OPA) and Methanol (55:45) containing 0.001 M (2.062 g) 1-Hexane Sulphonic acid which exhibited sharp peaks for CS and LH at a retention time of 3.163 and 9.629 min respectively (Figure 1). By using the Hypersil C_{18} column (250x4.6 mm, 5 µ) maintained at 30°C at a flow rate of 1 mL/min, both the drugs showed good absorbance at 220 nm.

Method validation

The proposed technique was validated by the International Conference of Harmonization requirements for several aspects such as accuracy, precision, linearity, robustness, system applicability and solution stability.[14-17] To assess the system's appropriateness, five replicate injections of the medication were performed. It was analysed for peaks, tailing factors, theoretical plates and relative retention time.[18]

Specificity: Prepared Blank, placebo, standard and sample all had been injected. The blank and placebo did not interfere with the retention time of the Choline salicylate and Lignocaine HCl peak.

System suitability: 5 replicates were injected and %RSD, tailing factor and Theoretical plates were found to be within limits.

Linearity: Linearity was assessed by plotting a calibration curve correlating peak response with their corresponding concentrations. A concentration range of 12.5-50 µg/mL was used (Tables 1 and 2). The linear regression equations were y=76177x+76693 (r^2=0.9992) for CS and y=71642x-158781 (r^2=0.9993) for LH. (Figures 2 and 3).

Accuracy

Prepared three independent sample preparations at each level of 50%, 100% and 150% using the same batch of API of the target analytical weight (100%) [Total of 9 samples]. (50%, 100%, 150%). The Test results for Choline Salicylate and Lignocaine HCl indicate that the %RSD of results are within limits (Tables 3 and 4).

Precision

Test results for Choline Salicylate and Lignocaine HCl indicate that the %RSD results are within limits (Tables 5-8).

Intermediate precision

The Test results for Choline Salicylate and Lignocaine HCl indicate that the %RSD results are within limits. (Tables 9-12).

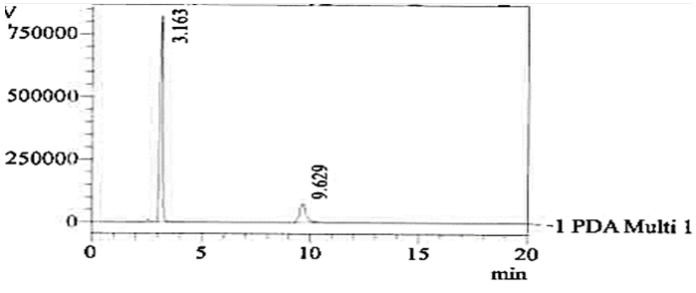

Figure 1: Chromatogram obtained with an optimized method.

Table 1: Results of Choline Salicylate 5 standard preparations

% of Sample	Sample Concentration µg/mL	Peak area
50	12.5	3901416
75	18.75	5791148
100	25	7684586
150	37.5	11472375
200	50	15335787

Table 2: Results of Lignocaine HCl 5 standard preparations.

% of Sample	Sample Concentration µg/mL	Peak area
50	12.5	740812
75	18.75	1195630
100	25	1594222
150	37.5	2563854
200	50	3410096

Table 3: Accuracy Results of Choline Salicylate.

Concentration Level	Preparation	Retention Time	Area	Mean	%RSD
50%	1	3.112	3837968	98.37	0.4
	2	3.140	3861445	98.98	
	3	3.140	3861783	98.98	
100%	1	3.175	7712533	100.36	0.2
	2	3.168	7700163	100.20	
	3	3.163	7684165	99.99	
150%	1	3.191	11450334	99.81	0.6
	2	3.162	11370270	99.11	
		3.172	11315438	98.63	

Table 4: Accuracy Results of Lignocaine HCl.

Concentration Level	Preparation	Retention Time	Area	Mean	%RSD
50%	1	9.610	761771	102.83	1.5
	2	9.638	784337	105.88	
	3	9.641	769059	103.81	
100%	1	9.639	1663309	104.33	0.5
	2	9.606	1679317	105.34	
	3	9.629	1675937	104.13	
150%	1	9.609	2610607	101.82	1.4
	2	9.572	2566444	100.10	
	3	9.605	2637401	102.87	

Table 5: Precision Results of Standard Choline Salicylate.

Sl. No.	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Choline Salicylate	Standard_01	3.125	6938576	0.976	3551
2		Standard_02	3.124	6914358	0.976	3546
3		Standard_03	3.127	6949147	0.979	3531
4		Standard_04	3.125	6938280	0.978	3537
5		Standard_05	3.127	6945061	0.988	3532
		Average	3.126	6937084	0.979	3539
		SD	0.002	13503	0.005	8.781
		%RSD	0.043	0.195	0.501	0.248

Table 6: Precision Results of Standard Lignocaine HCl.

Sl. No.	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Lignocaine HCl	Standard_01	9.448	1680402	1.194	5550.4
2		Standard_02	9.444	1677592	1.197	5540
3		Standard_03	9.447	1679073	1.195	5534
4		Standard_04	9.442	1677696	1.195	5530.7
5		Standard_05	9.446	1668568	1.197	5511.7
		Average	9.445	1676666	1.195	5533.3
		SD	0.002	4670	0.001	14.262
		%RSD	0.026	0.279	0.113	0.258

Table 7: Precision Results of Sample Choline Salicylate.

Sl. No	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Choline Salicylate	Sample_01	3.120	6674576	0.978	3589
2		Sample_02	3.119	6663255	0.971	3577
3		Sample_03	3.12	6664959	0.974	3573
4		Sample_04	3.121	6669702	0.975	3589
5		Sample_05	3.123	6662529	0.98	3594
6		Sample 06	3.123	6680665	0.982	3596
		Average	3.121	6669281	0.977	3586
		SD	0.002	7189	0.004	9.147
		%RSD	0.056	0.108	0.411	0.255

Table 8: Precision Results of Sample Lignocaine HCl.

Sl. No.	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Lignocaine HCl	Sample_01	9.445	1688602	1.208	5659.5
2		Sample_02	9.443	1689201	1.194	5573.1
3		Sample_03	9.446	1682769	1.194	5588.3
4		Sample_04	9.445	1685401	1.195	5563.8
5		Sample_05	9.449	1683583	1.193	5563.1
6		Sample 06	9.448	1679203	1.193	5568.1
		Average	9.446	1684793	1.196	5586
		SD	0.002	3771	0.006	37.184
		%RSD	0.023	0.224	0.5	0.666

Table 9: Intermediate precision Results of Standard Choline Salicylate.

Sl. No.	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Choline salicylate	Standard 01	3.17	7705496	0.987	3548
2		Standard 02	3.17	7720461	0.985	3549
3		Standard 03	3.172	7714818	0.968	3537
4		Standard 04	3.171	7692844	0.989	3532
5		Standard 05	3.166	7659653	0.98	3539
		Average	3.17	7698655	0.9818	3541
		SD	0.002	24179	0.008	6.542
		% RSD	0.074	0.314	0.766	0.185

Table 10: Intermediate Precision Results of Standard Lignocaine HCl.

Sl. No.	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Lignocaine HCl	Standard 01	9.638	1666528	1.197	5540
2		Standard 02	9.641	1673880	1.195	5534
3		Standard 03	9.663	1701429	1.195	5530.7
4		Standard 04	9.646	1697322	1.197	5511.7
5		Standard 05	9.615	1689354	1.195	5533.3
		Average	9.641	1685702	1.1958	5529.94
		SD	0.017	15026	0.001	9.615
		% RSD	0.179	0.891	0.082	0.174

Table 11: Intermediate precision Results of Sample Choline Salicylate.

Sl. No.	Sample Name	Sample ID	Ret. Time	Arca	Tailing Factor	Theoretical Plate
1	Choline Salicylate	Sample 01	3.17	7439894	0.862	2479
2		Sample 02	3.165	7443991	0.86	2409
3		Sample 03	3.158	7427003	0.857	2458
4		Sample 04	3.162	7336934	0.853	2435
5		Sample 05	3.144	7424475	0.86	2406
6		Sample 06	3.147	7438911	0.859	2458
		Average	3.158	7418535	0.861	2436
		SD	0.01	40706	0.008	28.18
		%RSD	0.317	0.549	0.911	1.157

Table 12: Intermediate precision Results of Sample Lignocaine HCl.

Sl. No.	Sample Name	Sample ID	Ret. Time	Area	Tailing Factor	Theoretical Plate
1	Lignocaine HCl	Sample 01	9.668	1744070	1.313	5488
2		Sample 02	9.659	1754109	1.314	5521
3		Sample 03	9.654	1732357	1.313	5433
4		Sample 04	9.652	1730202	1.287	5405
5		Sample 05	9.642	1754361	1.308	5547
6		Sample 06	9.64	1742539	1.269	5329
		Average	9.653	1742940	1.301	5454
		SD	0.011	10304	0.017	73.893
		%RSD	0.114	0.591	1.304	1.355

Table 13: **Results of Robustness.**

Parameter	Choline salicylate				Lignocaine HCl			
	RT (min)		TF		RT (min)		TF	
	Std	Spl	Std	Spl	Std	Spl	Std	Spl
Column Temperature								
27°	3.209	3.196	0.86	0.852	10.13	10.11	1.227	1.244
30°	3.165	3.164	0.826	0.819	9.654	9.651	1.243	1.281
33°	3.146	3.133	0.852	0.861	9.492	9.47	1.23	1.298
Wave Length								
218 nm	3.163	3.163	0.827	0.824	9.683	9.674	1.273	1.332
220 nm	3.165	3.164	0.826	0.819	9.654	9.651	1.243	1.281
222 nm	3.167	3.158	0.817	0.824	9.632	9.633	1.267	1.288
Flow Rate								
0.9 mL	3.495	3.488	0.832	0.83	10.74	10.72	1.317	1.311
1.0 mL	3.165	3.164	0.826	0.819	9.654	9.651	1.243	1.281
1.1 mL	2.892	2.893	0.827	0.833	8.847	8.851	1.246	1.26

Table 14: **Results of solution stability.**

Sl. No.	Hr	Choline salicylate				Lignocaine HCl			
		Avg Std Area	%RSD	Tailing Factor	Theoretical Plate	Avg Std Area	%RSD	Tailing Factor	Theoretical Plate
1	0 Hr	6937084	0.195	0.979	3539	1676666	0.279	1.195	5533
2	8 Hr	7650489	0.198	0.820	2448	1956936	1.573	1.224	5437
3	16 Hr	7631446	0.792	0.832	2415	1834623	0.334	1.298	5335
4	24 Hr	7636704	0.56	0.849	2359	1838665	0.597	1.31	5078

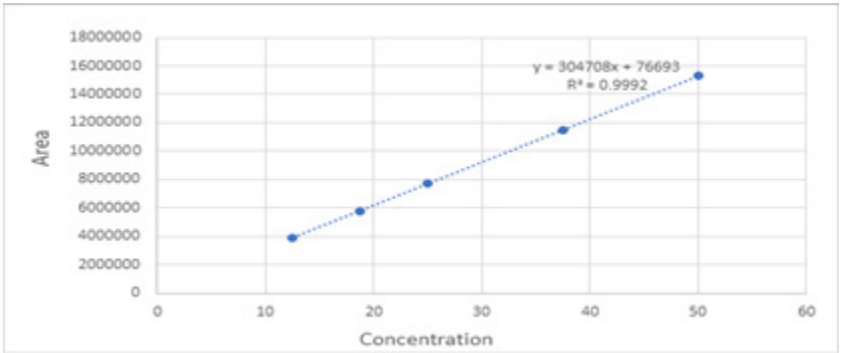

Figure 2: Linearity graph of Choline salicylate.

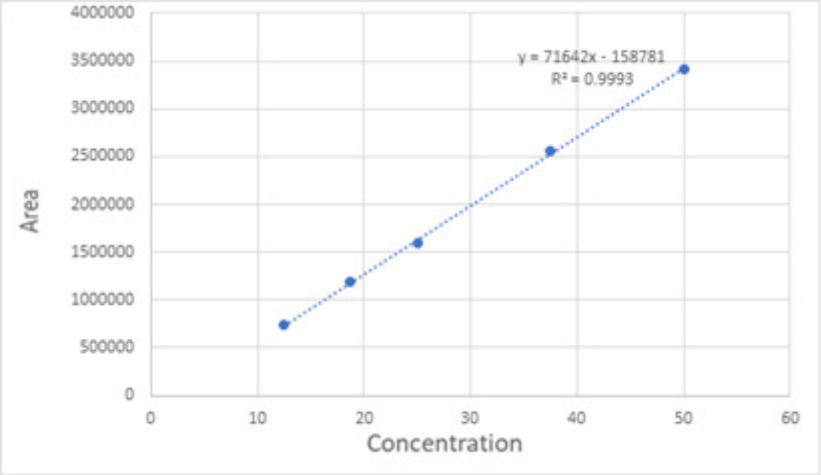

Figure 3: Linearity graph of Lignocaine HCl.

Robustness

The robustness results were obtained by varying the flow rate, wavelength and column temperature. The tailing factor did not vary significantly with deliberate adjustments in flow rate, wavelength, or column temperature. The tailing factor was determined to be within the limits for choline salicylate and lignocaine HCl (Table 13).

Solution stability

From the standard solution at 0, 8, 16 and 24 hr 5 replicate injections were injected and % RSD, Theoretical plate and Tailing factor were found to be within the limits (Table 14).

CONCLUSION

This study developed a unique, quick, cost-effective and sensitive HPLC technique for estimating choline salicylate and Lignocaine HCl simultaneously. The main advantages of this approach are its shorter run time, low cost, accessibility, sensitivity, dependability and reproducibility. These characteristics are important when it comes to evaluating a large number of samples. All characteristics, including linearity, accuracy, specificity, robustness and precision, were validated and determined to be within acceptable limits. For all parameters, the RSD values were less than 2%, indicating that the method's validity and the outcomes obtained by this methodology are in good accord. As a result, the proposed method might be used in quality control laboratories to investigate pharmaceutical formulations of choline salicylate and Lignocaine HCl without the need for any preparatory separation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatographic; **CS:** Choline Salicylate; **LH:** Lignocaine HCl; **NSAID:** Non-Steroidal Anti-Inflammatory Drug; **HCl:** Hydrochloride; **OPA:** Orthophosphoric Acid; **RSD:** Relative Standard Deviation; **RT:** Retention Time.

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