A Study of Method Development, Validation and Forced Degradation for Simultaneous Quantification of Choline Salicylate and Lignocaine in Bulk and Pharmaceutical Dosage Form

B. Urukunda[1], G Srinivasa Rao[2], Guduru Rajeswari[3], Kolavali Yalla Reddy[4]

[1]*Department of pharmaceutical analysis, Saastra college of Pharmaceutical Education and Research, Varigonda (v) T.P Gudur (m) SPSR Nellore Dt -524311*

[2]*Principal and Professor, Saastra college of Pharmaceutical Education and Research, Varigonda (v) T.P Gudur (m) SPSR Nellore Dt -524311*

[3]*Principal and Professor, Department of Pharmacology, Saastra College of Pharmaceutical Sciences for Women, Varigonda (v) T.P. Gudur (m) SPSR Nellore Dt -524311*

[4]*Associate Professor, Saastra College of Pharmaceutical Sciences for Women, Varigonda (v) T.P. Gudur (m) SPSR Nellore Dt -524311*

***Abstract*—A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Choline Salicylate and Lignocaine in pharmaceutical dosage form. Chromatographic separation of Choline Salicylate and Lignocaine was achieved on Waters Alliance-e2695, by using Waters X-Bridge C18, 150x4.6mm, 3.5µm column and the mobile phase containing 1ml Triethyl amine is dissolved in 1lt water adjust pH-7.0 with OPA & ACNin the ratio of 60:40% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 249nmusing a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Choline Salicylate and Lignocaine were NLT 2000 and should not more than 2respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Choline Salicylate and Lignocaine and study of its stability.**

***Index Terms*—HPLC, Choline Salicylate, Lignocaine, ICH guidelines**

I. INTRODUCTION

The concurrent administration of multiple active pharmaceutical ingredients (APIs) in fixed-dose combination therapies has become an established approach in clinical practice, offering enhanced therapeutic efficacy, improved patient compliance, and synergistic pharmacological effects.[1] Among these combinations, the formulation containing Choline Salicylate and Lignocaine (also known as Lidocaine) is widely utilized for its dual action in managing painful inflammatory conditions of the oral cavity,[2] ear, or localized topical regions. Choline Salicylate the choline salt of salicylic acid acts as an effective non-steroidal anti-inflammatory drug (NSAID)[3] and analgesic by inhibiting prostaglandin synthesis to alleviate localized pain and swelling.[4] Concurrently, Lignocaine functions as a potent local anesthetic of the amide type, providing rapid and reversible block of nerve conduction to deliver immediate surface pain relief.[5,6]

In modern pharmaceutical manufacturing and regulatory compliance, ensuring the safety, efficacy, and consistency of such combinations demands highly reliable analytical methodologies.[7] The pharmaceutical industry heavily relies on High-Performance Liquid Chromatography (HPLC) as the

gold standard for quality control due to its high resolution, automated capabilities, and accuracy. For fixed-dose formulations, the challenge lies in achieving an analytical method capable of simultaneous quantification meaning it must cleanly separate and measure both Choline Salicylate and Lignocaine in a single chromatographic run without interferences from excipients or matrix elements.[8]

Furthermore, dynamic regulatory expectations established by the International Council for Harmonisation (ICH) mandate that analytical methods not only be validated for routine testing but also be inherently stability-indicating.[9] Forced degradation studies (stress testing) are an integral component of this process. By intentionally exposing the drug substance and product to severe conditions—such as acid, base, oxidation, thermal, and photolytic stress researchers can evaluate how the molecules degrade over time.[10] A robust method must successfully resolve the active analytes from any potential degradation products, proving that the assay can accurately assess shelf-life and drug stability.[11]

While separate analytical profiles for these drugs may exist, there is a continuous need for optimization to yield methods that are faster, more cost-effective, and less chemically complex. This study addresses that need by establishing a simple, rapid, precise, and reproducible Reverse-Phase HPLC (RP-HPLC) method utilizing a Waters X-Bridge C18 column and a budget-friendly isocratic mobile phase.[12] This paper details the systematic development of the method, its comprehensive validation according to current ICH guidelines, and its successful application to forced degradation studies for the simultaneous estimation of Choline Salicylate and Lignocaine in bulk and pharmaceutical dosage forms.[13]

1.1 Aim

To develop and validate a robust, stability-indicating Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation and quantification of Choline Salicylate and Lignocaine in bulk drug forms as well as commercially available multi-branded pharmaceutical formulations.

Objectives

Method Development:

To establish a simple, rapid, cost-effective, and highly specific RP-HPLC chromatographic system optimized for the clean separation and simultaneous resolution of Choline Salicylate and Lignocaine without interference from formulation excipients.

Method Validation (ICH Compliance):

To rigorously validate the developed analytical method in accordance with the International Council for Harmonization (ICH) guidelines by evaluating critical validation parameters, including:

- System Suitability: Confirming system performance metrics (e.g., theoretical plates N \ge 2000, tailing factor T \le 2.0, and peak area repeatability).
- Specificity: Ensuring no cross-interference between the active drugs, excipients, or degradation matrices.
- Linearity & Range: Establishing a proportional relationship between peak response and analyte concentration across a specified operational range.
- Accuracy (Recovery Studies): Verifying the closeness of the test results to the true value via standard addition techniques.
- Precision: Assessing both system repeatability (intraday precision) and intermediate precision (interday precision), targeting a relative standard deviation (%RSD) of less than 2.0%.
- Sensitivity (LOD & LOQ): Determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ) to quantify the method's lowest threshold of visibility and measurement.
- Robustness: Evaluating the method's capacity to remain unaffected by small, deliberate variations in operational parameters (e.g., small shifts in mobile phase pH, flow rate, or organic modifier ratio).

Forced Degradation (Stability-Indicating Studies):

To subject Choline Salicylate and Lignocaine to standard stress testing conditions to evaluate the stability-indicating capability of the method.

1.3 Drug Profile

Drug profile of choline salicylate

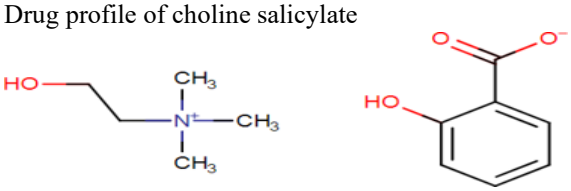

Fig No. 1: Molecular structure of Choline Salicylate

Table No.1: Drug profile of Choline Salicylate

IUPAC name	(2-hydroxyethyl) trimethyl azanium 2-hydroxybenzoate
Molecular Formula	C12H19NO4
Molecular Weight	241.287 g/mol
Description	Choline salicylate is an anti-inflammatory pain reliever agent that is related to aspirin. It is used to decrease swelling and to treat mild-moderate pain
Solubility	water-soluble
Therapeutic category	Anti-inflammatory
Storage	Store in tightly sealed containers in cool place.
Melting point	36 - 38°C

Uses of Choline Salicylate

Choline salicylate is an anti-inflammatory pain reliever agent that is related to aspirin. It is used to decrease swelling and to treat mild-moderate pain. It is used to treat arthritis in both children and adults. This medicine can also be used for fever.

Mechanism of action: Choline salicylate relieves pain by inhibition of prostaglandin synthesis and reduces fever by acting on the hypothalamus heat-regulating center. It also inhibits the generation of impulses through the inhibition of cyclooxygenase enzyme (COX) Cyclo oxygenase is involved in the production of prostaglandins, in response to injury and after various other stimuli. The prostaglandins promote pain, swelling, and inflammation. The choline salicylate decreases inflammation and pain by reducing the production of these prostaglandins in the area of the mouth it is applied.

Absorption: Onset: 1-2 hr after ingestion

In the oral form, choline salicylate is absorbed across the buccal mucosa. There is a need for caution not to exceed the stated dose and monitor for any signs of suggested salicylism, especially when this drug is used for infants

Adverse reactions:

- trouble breathing;
- ringing in your ears, hearing loss;
- behavior changes with nausea and vomiting in a child using this medicine;
- worsening fever or pain; or.
- signs of stomach bleeding--feeling light-headed, ongoing stomach pain, bloody or tarry stools, coughing up blood or vomit that looks like coffee grounds.

Drug Profile of Lignocaine

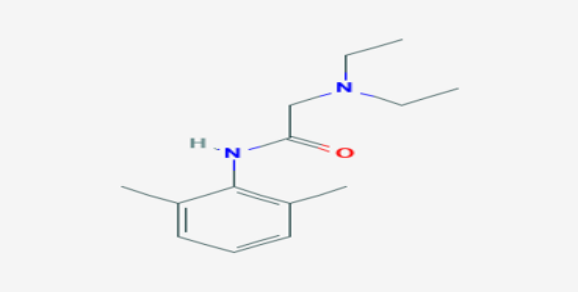

Fig No. 2: Molecular structure of Lignocaine

Table No. 2: Drug profile of Lignocaine[14]

Absorption	Information derived from diverse formulations, concentrations and usages reveals that lidocaine is completely absorbed following parenteral administration.
Melting Point	68.5 °C
pKa	8.01
Therapeutic category	Anti-Arrhythmia Agents

IUPAC name	2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide.[15]
Molecular Formula	C14H22N2O
Molecular Weight	234.343 g/mol
Description	White or slightly yellow, crystalline powder
Solubility	Water soluble

Mechanism of Action:[16]

Lidocaine stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses thereby effecting local anesthetic action. Lidocaine alters signal conduction in neurons by blocking the fast voltage gated sodium (Na+) channels in the neuronal cell membrane that are responsible for signal propagation.[17] With sufficient blockage the membrane of the postsynaptic neuron will not depolarize and will thus fail to transmit an action potential. This creates the anaesthetic effect by not merely preventing pain signals from propagating to the brain but by aborting their birth in the firstplace.[18]

Use:
Lignocaine Injection belongs to two groups of medicines known as local anaesthetics and antiarrhythmic drugs.[19] Local anaesthetics stop pain and feeling in the area around where it is injected; and antiarrhythmic drugs work by restoring irregular and/or rapid heartbeats to normal.[20]
Adverse reactions:

- nausea,
- dizziness,
- numbness in places where the medicine is accidentally applied,or.
- bruising, redness, itching, or swelling where the medication wasinjected.[21]

II. METHODOLOGY

RP-HPLC Simultaneous Method Development for Choline Salicylate and Lignocaine.

2.1 Equipment and Reagents

- System: Waters Alliance e2695 HPLC with PDA Detector & Empower 2.0 software.
- Solvents & Chemicals: HPLC Grade Acetonitrile, Methanol, Water (Milli-Q), Triethylamine (TEA), and Orthophosphoric Acid (OPA).

2.2 Optimized Chromatographic Conditions
The method was optimized over 10 trials. The final validated conditions are:

- Column: Waters X-Bridge C18 ($150 \times 4.6mm, 3.5\mu\m)
- Mobile Phase: Acetonitrile: 0.1\% TEA Buffer (pH 7.0 adjusted with OPA) in a 40:60\% v/v ratio
- Flow Rate: 1.0\text {mL/min
- Wavelength: 249\nm (Isobestic point determined via UV scan from 200-400nm)
- Injection Volume / Run Time: 10mu\L / 7.0\tmin
- Retention Times: Choline Salicylate approx 2.52\min; Lignocaine approx 5.21 min (Resolution = 13.21)

2.3 Preparation of Solutions & Assay[22]

- Buffer: Dissolve 1\ mL of TEA in 1\ L of water; adjust pH to 7.0 with OPA. Filter (0.45\ \mu\tm).
- Standard Stock Solution: Weigh 220\ mg} Choline Salicylate and 50\mg Lignocaine into a 100 mL volumetric flask, dissolve, and dilute to volume with mobile phase (diluent).
- Working Standard & Sample Solution (220\ppm / 50\ ppm: Dilute 5\text{mL} of the stock solution to 50mL with diluent. (For samples: sonicate, centrifuge, and filter sample powder equivalent to the standard weight before final dilution).
- Assay Evaluation: Inject standard and sample solutions ($10\ \mu\text{L}$). Calculate % Assay using the peak area ratio:

2.4. Method Validation Protocol (ICH Guidelines)[23]

- Specificity: Verified by injecting a blank; no interfering peaks occurred at the drug retention times.
- Linearity & Range: Prepared 6 calibration levels (10\% to 150%). Acceptance Criteria: Correlation coefficient R^2 \ge 0.999.
- Accuracy: Evaluated via standard recovery at 50\%, 100\%, and 150\% spiking levels. Acceptance Criteria: Mean recovery must be 98.0\%-102.0\%.
- Precision: Confirmed via System, Method, and Intermediate precision protocols ($n=6$ injections). Acceptance Criteria: Peak area \% RSDle 2.0\%.
- Sensitivity: Derived via calibration slope metrics:
  - Choline Salicylate: LOD = 2.2\ \mu\g/mL; LOQ = 22.0\ \mu\g/mL
  - Lignocaine: LOD = 0.5\ \mu\g/mL; textLOQ = 5.0\ \mu\g/mL
- Robustness: Evaluated by making deliberate variations in flow rate pm 0.2\mL/min) and minor shifts in mobile phase ratio.

2.5. Forced Degradation Studies[24]
Stock solutions were subjected to the following stress conditions to confirm the method's stability-indicating capability:

- Acid Hydrolysis
- Alkali Hydrolysis
- Thermal Stress
- Oxidative Degradation

III. RESULTS AND DISCUSSION

3.1 RP-HPLC METHOD
Determination of Working Wavelength (λ_{max}):

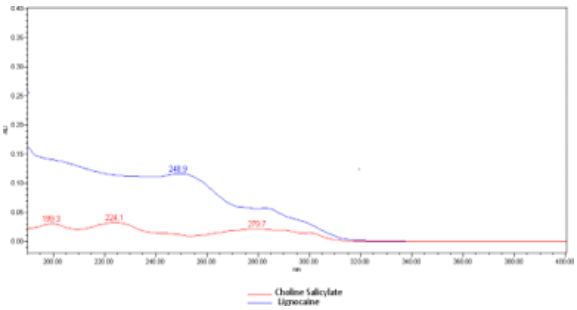

Fig No.:3.1 PDA - Spectrum of Choline Salicylate and Lignocaine

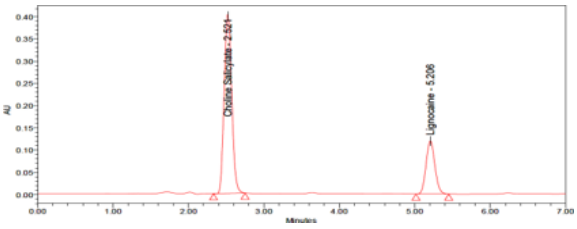

Fig No-3.2: Optimized chromatogram -Trial-10

Table No.3.1: Results for (Optimized trail)

Name	Area	% Area	USP Tailing	USP Plate Count
Choline Salicylate	2068122	72.36	1.12	4852
Lignocaine	845623	27.64	1.10	5542

Table 3.2: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument used	Waters HPLC with auto sampler and UV detector.
Injection volume	10µl
Mobile Phase	Acetonitrile and 0.1%triethylamine Ph 7.0 with OPA 40:60
Column	Waters X-Bridge C_{18} 150x4.6mm, 3.5µ
Detection Wave Length	249 nm
Flow Rate	1 mL/min
Runtime	7min
Temperature	Ambient (25°C)
Mode of separation	Isocratic mode

Analytical Method Validation (Hplc)

The method was validated for its linearity range, accuracy, precision, and specificity. Method validation was carried out as per ICH guidelines.[25]

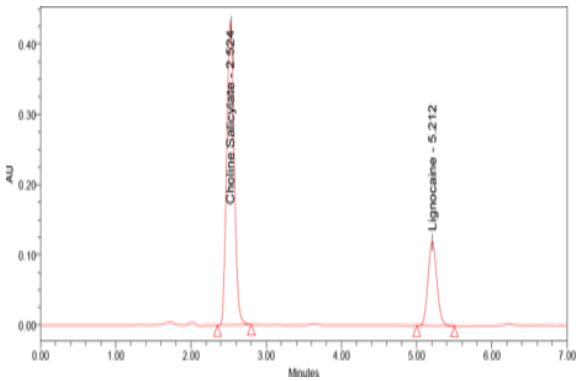

Fig. No. 3.3: Optimized chromatogram

Linearity:

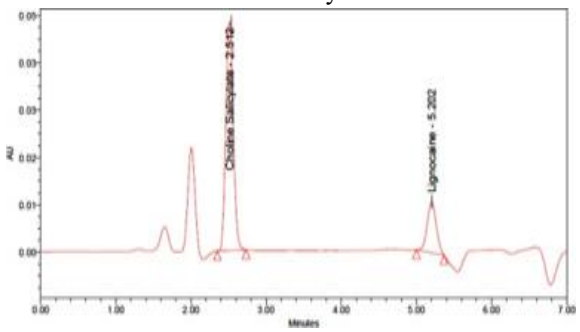

Fig No.3.4: Chromatogram of Linearity-10%

Precision

System Precision:

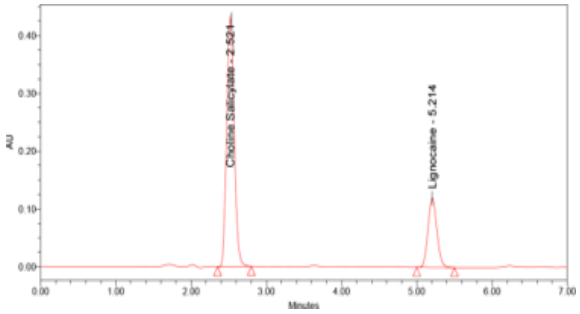

Fig. 3.5: System precision chromatogram-1

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.34% and 0.08% respectively for Choline salicylate and Lignocaine. As the limit of Precision was less than "2" the system precision was passed in this method.[26]

Accuracy:

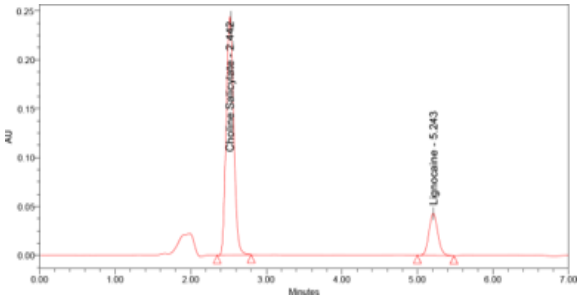

Fig. 3.6: Chromatogram of Accuracy 50%

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 100.25% and 100.86% for Choline salicylate and Lignocaine respectively.[27]

LOD and LOQ (µg/ml):

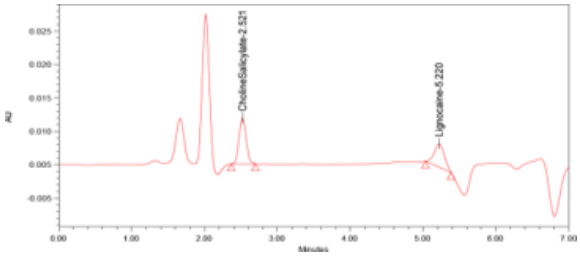

Fig No.3.7: chromatogram for LOD

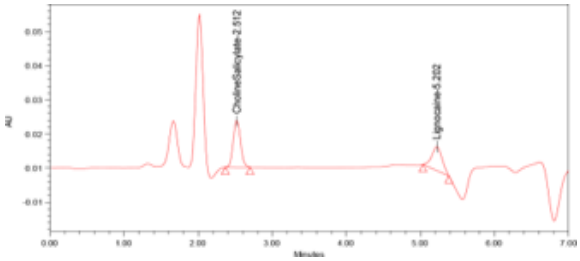

Fig No.3.8: chromatogram for LOQ

Table No.3.3: Sensitivity parameters (LOD & LOQ) by RP-HPLC

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Choline Salicylate	2.2	22
Lignocaine	0.5	5

IV. SUMMARY AND CONCLUSION

This study details the development, validation, and stability-indicating profile of a novel Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous quantification of Choline Salicylate and Lignocaine.[28] While these active ingredients are widely co-formulated in topicals to provide combined anti-inflammatory and local anesthetic relief, previous analytical literature lacked a unified method for their concurrent determination. This research successfully bridges that gap, offering a single, optimized chromatographic run that eliminates the need for separate assays, thereby reducing processing time and solvent consumption.[29]

The experimental setup was optimized using a Waters Alliance e2695 system coupled with a photodiode array (PDA)/UV detector. Effective baseline separation was achieved on a Waters X-Bridge C18 (150 \times 4.6mm, 3.5\ \mu\m) stationary phase. The mobile phase consisted of an isocratic mixture of 0.1\% v/v} Triethylamine buffer (adjusted to pH 7.0 with orthophosphoric acid) and Acetonitrile in a 60:40\%\ v/v ratio. Operating at a flow rate of 1.0\mL/min and monitored at an isobestic wavelength of 249 nm} $, Choline Salicylate and Lignocaine resolved cleanly into sharp, symmetrical peaks with retention times of 2.521min and 5.206 min, respectively.[30]

The analytical method was rigorously validated in compliance with standard ICH guidelines, proving to be highly linear (R^2 \ge 0.999), sensitive, and precise, with all repeatability metrics falling well below the strict le 2.0\% relative standard deviation (%RSD) threshold.[31] Accuracy was verified through standard addition recovery studies, which yielded excellent results within the acceptable $]98.0% to 102.0\% range, demonstrating zero interference from common pharmaceutical excipients. Furthermore, forced degradation studies under acidic, basic, oxidative, and thermal stress confirmed that the method is truly stability-indicating, as all generated degradation artifacts were successfully resolved from the active parent drug peaks.[32]

Conclusion

The developed RP-HPLC method provides a rapid, accurate, and highly reproducible analytical tool for the simultaneous estimation of Choline Salicylate and Lignocaine. Featuring a short runtime of just 7 minutes, the method offers a reliable approach for high-throughput testing in industrial environments. Because it satisfies all ICH validation criteria and exhibits robust stability-indicating properties during stress testing, this method can be confidently

implemented for routine quality control, manufacturing batch release, and long-term shelf-life stability monitoring of these combined drugs in both bulk substances and commercial dosage forms.

Disclosure statement

The authors declare no potential conflicts of interest.

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