Rapid, Simple, and Cost-Effective Analytical Method Validation of the Mixture of Lidocaine and Methylparaben Combination for Assay Determination

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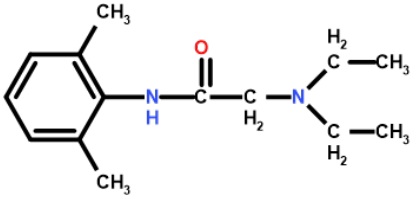
Summary: Lidocaine, a versatile analgesic, serves as a local anesthetic for topical dermal applications and as an agent to mitigate discomfort associated with chemotherapy in cancer patients. It is also utilized for postoperative pain management. Given its diverse applications, it is essential to investigate analytical and detection methods for lidocaine and its associated compounds, such as methylparaben. The present study introduces a simple and validated procedure for performing such analyses. The chromatographic system employed in this study comprised a GL Science Inc. Intersil ODS-3 column (150 mm length, 4.6 mm internal diameter, 5 µm particle size), with a mobile phase consisting of methanol:buffer (1:1), a detection wavelength of 220 nm, and operation at room temperature. A comprehensive validation study was conducted to confirm the accuracy, reproducibility, and precision of the results through the application of system suitability criteria. The UV-HPLC analytical method developed and validated in this research was evaluated for its capacity to detect low levels of lidocaine (Lido) and methylparaben (MP) within an efficient 8-minute run time. The limits of detection (LOD) were statistically determined as 6.636 µg/mL for Lido and 0.713 µg/mL for MP. The method demonstrated high recovery rates for both compounds, with an accuracy range of 98.7% to 101.1%. Furthermore, excellent linearity was observed, with coefficients of determination (R^2) of 0.99992 for Lido and 0.99991 for MP.

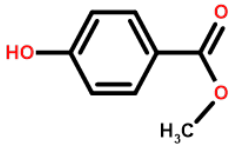
Keywords: HPLC, Validation, Lidocaine, Methylparaben, LOD, LOQ.

Introduction

Lidocaine (Lido) serves as a pain reliever, functioning as a local anesthetic that can be applied topically to the skin [1]. It is also utilized as an aesthetic to ease the discomfort of chemotherapy in cancer patients [2]. Moreover, it finds use as a painkiller for post-surgical pain management [3]. The necessity arises to study the analysis and detection methods of lidocaine and its accompaniments, such as methylparaben, given its diverse applications [4].



(A)



(B)

Fig. 1: A) Lidocaine (Lido), B) Methylparaben (MB).

The Lidocaine scientific name is (2-(diethylamino) -N- (2,6-dimethyl phenyl) acetamide); it is a molecule with molecular mass ($C_{14}H_{22}N_2O$) 234.34 g/mol, which is composed of two parts: one is hydrophilic (lipophobic), and the other is hydrophobic (lipophilic), connected through a single carbon chain **(Fig. 1A)**. The hydrophilic portion consists of a tertiary amine (methyl-diethylamine), while the lipophilic part forms an aromatic, unsaturated 2,6-dimethyl aniline. Lidocaine is a weak base with a pKa of 8 [5]. Methylparaben is a member of the Paraben family widely used as a preservative. It is the methyl derivative of parahydroxybenzoic acid. MP is an aromatic compound with molecular mass ($C_8H_8O_3$) 152.15 g/mol **(Fig. 1B)**. MP is employed as an antifungal agent and finds use as a preservative in food, cosmetics, personal care products, and pharmaceutical formulations. MP is a weak base with a pKa of 8.4 [6, 7].

Nalkiashary *et al.* reported their paper analyzing Lido, Hydrocortisone, and MP using the mobile phase at pH 8.0. The reported analysis method revealed the retention times as 1.57, 4.38, and 6.3 min for Hydrocortisone, Lido, and MP, respectively, using a high percentage of methanol.

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The peak shapes did not undergo the ideal Gaussian peak shape for all three items with high tailing. Also, the method did not implement most of the method validation parameters [8]. Additionally, the high pH of the mobile phase in the reversed-phase HPLC leads to dissociations of the packaging material for the column, resulting in distortion of its separation performance [9, 10].

Although Maslii *et al.* introduced their analysis method according to the ideal chromatographic method guidelines, the method was restricted to a specific pH of 3.0 with high retained Lido and MP on the column at about 5.2 and 10.1 min, respectively, which makes the method time-consuming [11]. Also, they used the gradient mode for separation via acetonitrile in a ratio of 50% as mobile phase B at column temperature 30°C, which makes this method cost-ineffective.

Furthermore, the United States Pharmacopeia (USP-47) submitted its analysis method of Lido and MP using water and glacial acetic acid in ratio of (930:50) after pH adjusting to 3.4 then mixed with acetonitrile in ratio (80:20), which makes the same previously issue in the analysis method as Maslii *et al.* reported. The USP analysis method was also recommended for separating the column with specs, 3.9-mm × 30-cm; packing L1, and flow rate 1.5 mL/ min. So this method is time-consuming and primarily costly. On the contrary, the British Pharmacopeia (BP-2024) recommended determining the lidocaine using the old-fashioned technique (extraction and non-aqueous titration) using hazardous materials such as chloroform for the extraction process of lidocaine. Comparing the BP analysis method with the HPLC method, the analysis method using titration in this path lacks many of the required separation competencies according to modern analytical techniques.

It is undeniable that earlier analytical techniques possessed significant limitations, including high costs, lengthy procedures, potential hazards, and the requirement for specialized conditions or reagents during derivatization. These methods frequently lacked precision and selectivity, particularly when adjusting the pH of the mobile phase or employing titration techniques. Nevertheless, the contributions of scientists who diligently endeavored to analyze lidocaine (Lido) and methylparaben (MP) using these approaches merit recognition. Presently, many analytical methods in use do not meet comprehensive validation standards. Only a limited number of studies have underscored the critical importance of accurately quantifying pharmaceutical preservatives, such as parabens (e.g., MP), when co-present with other compounds [12].

This study focuses on creating a rapid, simple, and effective method for the simultaneous quantification of Lidocaine (Lido) and Methylparaben (MP) through reversed-phase HPLC.

Experimental

Analytical standards, materials, and reagents

Lidocaine HCl (Lido) working standard batch# API/LIH/212040; Methylparaben (MP) batch# PE 2731 were kindly provided from (UP Pharma Assuit; Egypt); methanol HPLC grade (Scharlau); sodium 1-pentane sulfonic acid (Merck); phosphoric acid 85% (Adwic), hydrochloric acid 35-37% (Advent); sodium hydroxide (Scharlau); hydrogen peroxide 33% (Scharlau); potassium permanganate (Loba). The buffer solution was prepared as a mixture of 0.2 % v/v Phosphoric acid 85% and 0.2% sodium 1- pentane sulfonic acid w/v in deionized water.

Chromatographic conditions

The chromatographic parameters were as follows: The study was conducted using HPLC model (HP 1100) connected with the variable wavelength detector Agilent 1100; the mobile phase comprised of buffer and methanol (1:1), flow rate of 1.0 mL/min, detection wavelength established at 220 nm, injection volume of 10 µL, a stainless steel GL Science Inc. ODS3 Intersil column measuring 150 x 4.6 mm with a particle size of 5 µm, and the column compartment temperature sustained at ambient conditions.

Method validation

System Suitability

The mobile phase was used to dissolve the Lido standard equivalent to about 50 mg/100 mL and 5 mg/ 100 mL of MP standard in a 100 mL volumetric flask. Then the solution was injected six times in concentration of 0.5 mg/mL and 0.05 mg/mL of Lido and MP, respectively.

Precision and Repeatability

To assess repeatability, six separate preparations of the 100% test concentration were injected as the system suitability solution's in

concentration of 0.5 mg/mL and 0.05 mg/mL of Lido and MP, respectively[9]. This test was carried out by a single analyst over three days to evaluate inter-precision.

Linearity and Range

The concept of linearity is characterized by the correlation coefficient, which should be determined to be greater than 0.999 [13, 14]. This coefficient is derived from the relationship between peak area responses and their corresponding concentrations. Linearity is a crucial factor in single-point standardization, and it should encompass at least two concentrations preceding and succeeding the 100% specification target concentration. The linearity equation can be expressed as follows:

$$Y = a + bX \quad (1)$$

The mean peak area denoted as Y, and the concentration in mg/mL, represented as X, is used to calculate the linearity, with 'a' representing the intercept and 'b' representing the slope. To assess linearity, five distinct concentrations were prepared at 50%, 70%, 100%, 120%, and 150%. The stock solution was meticulously crafted by dissolving an amount of Lido equivalent to 1000.45 mg/200 mL and 99.4 mg/200 mL of MP standard in a volumetric flask, which was then filled to volume with the mobile phase. Subsequent serial dilutions were performed to achieve the desired concentrations. Specifically, 5 mL, 7 mL, 10 mL, 12 mL, and 15 mL were taken to obtain 50%, 70%, 100%, 120%, and 150% concentrations, respectively. The final volume was adjusted to 200 mL using the mobile phase, and duplicate replicates of each concentration were analyzed using HPLC.

Limit of Detection

The detection limit, often referred to as the point at which a peak becomes discernible [15], can be statistically determined using the parameters of the linearity equation. This calculation is performed using the following formula:

$$LOD = 3.3\sigma / S \quad (2)$$

Where: σ: is the standard error, S: is the slope of the linearity curve.

Limit of Quantitation

The quantification limit, which signifies the point at which a peak can be accurately and precisely quantified, can be statistically derived using the parameters of the linearity equation. This determination is made through the application of the following formula [16]:

$$LOQ = 10\sigma / S \quad (3)$$

Accuracy and Recovery

The accuracy assessment involved analyzing three distinct solutions, each representing 70%, 100%, and 120% of the mixture of Lido and MP standards. These concentrations were meticulously prepared by directly weighing the two drug substances and combining them in the same volumetric flask. Specifically, for the 70% Lido standard solution, approximately 36.1 mg/100 mL of Lido and 3.51 mg/100 mL of MP standard were weighed and placed in a 100 mL volumetric flask, using the mobile phase as a solvent. At the optimum standard concentration 100% of the mixture (at concentration about 0.5 mg/mL and 0.05 mg/mL of Lido and MP, respectively) Lido standard solution was prepared by weighing approximately 50.2 mg/100 mL of Lido and 5.06 mg/100 mL of MP standard in a 100 mL volumetric flask using the mobile phase as a solvent. Similarly, the 120% Lido standard solution was crafted by weighing approximately 59.3 mg/100 mL of Lido and 6.01 mg/100 mL of MP standard in a 100 mL volumetric flask, again completing the volume with the mobile phase. Subsequently, three replicates of each concentration were injected for analysis.

Actual conc. (mg/mL) = (Mean peak area (P. A). of the actual analysis - intercept of the linearity calibration curve) / slope of the linearity calibration curve (4)

Recovery (%) = Actual conc. (mg/mL)/ experimental conc. (mg/mL) x 100 (5)

Selectivity/ specificity

Selectivity was confirmed by individually injecting the following solutions: mobile phase, Lido standard, and MP standard. Forced degradation studies were conducted on each drug substance separately to evaluate the stability-indicating properties, selectivity, and specificity of the procedure. Accelerated degradation was performed using acid hydrolysis, base hydrolysis, and H_2O_2 oxidative degradation [10]. Acid, base, and H_2O_2 hydrolysis were carried out as system suitability tests at 100% of the standard concentration. For acid hydrolysis, 10 mL of 0.1 M HCl was added to the drug substance, followed by dilution with the mobile

phase to a final volume of 100 mL. The solution was allowed to react for 30 minutes, neutralized, and then injected into the HPLC system. A similar procedure was followed for base hydrolysis using 10 mL of 0.1 M NaOH and for H_2O_2 hydrolysis using 10 mL of 3% (w/v) H_2O_2. Neutralization and HPLC injection were common steps across all three hydrolysis methods.

Robustness and Ruggedness

* *Robustness*

Robustness, which assesses a method's resilience to minor variations in normal operating parameters [17], was evaluated for HPLC by introducing slight changes in mobile phase composition and flow rate. Specifically, the analytical method was conducted with varying organic modifier ratios, maintaining a 50%±5% methanol content while keeping the flow rate and column parameters constant. Additionally, the technique was applied with different flow rates, ranging from 1.0 mL/min±0.1 mL/min, while maintaining a constant mobile phase composition and column to gauge their impact on analytical performance.

* *Ruggedness*

The assessment of ruggedness, which involves evaluating a method's performance under significant variations from normal operating parameters [18], was conducted through several experiments. To gauge inter-precision, three distinct preparations of standard and test solutions at a 100% concentration were analyzed by both the same analyst and different analysts. Additionally, two different preparations of standard and test solutions at approximately 100% concentration, prepared by two different analysts, were compared. Furthermore, column-to-column inter-precision was investigated by performing the analytical method at the claimed sample concentration for the standard on three HPLC columns with the same packing material but different serial numbers.

Actual analysis of fished product sterile ampoule of Lidocaine 1% 3.5mL and 5.0 mL (UP Pharma ampoule)

After performing the full analytical method validation of Lido and MP in combination, the actual analysis of the finished products was tested. The assay of Lido and MP in sterile ampoules of Lidocaine 1% 3.5 mL and 5.0 mL (UP Pharma ampoule) was determined. The acceptance assay limits are 95-105% and 80-120% from the stated amount of Lido and MP, respectively.

The assay test was performed as follows: After mixing 10 ampoules, 5 mL from each finished product batch was separately transferred using a volumetric pipette into a 100 mL volumetric flask and completed to the mark using the mobile phase. Then, it was injected into HPLC under the optimum conditions of the analysis method, as previously introduced using the standard system suitability solution.

The assay of Lido and MP was calculated using the following equations:

Lido assay (%) =

Test P.A/std. P.A × (std. conc. in mg/mL) × 100/ 50 × Lido std. assay (%) (6)

Where,

Test P.A and std. P. A is the test and standard peak areas of Lido, respectively, std. Conc. In mg/mL is the standard concentration in mg/mL, 100 is the test volume, 50 is the theoretical content in mg of Lido HCl in 5 mL and Lido std. Assay (%) is the Lido HCl standard assay as is (%).

MP assay (%) = Test P.A/std. P.A × (std. conc. in mg/mL) × 100/ 5 × MP std. assay (%) (7)

Where, Test P.A and std. P. A is the test and standard peak areas of MP, respectively, std. Conc. In mg/mL is the standard concentration in mg/mL, 100 is the test volume, and 5 is the theoretical content in mg of MP in 5 mL and MP std. Assay (%) is the MP standard assay as is (%).

Results and discussions

System suitability

In the first, each of the mobile phases, Lido and MP were injected to establish their specific retention times and confirm the absence of any overlap. The retention time for Lido was approximately 3.8±0.2 minutes, while for MP, it was around 6.4±0.2 minutes **(Fig. 2)**.

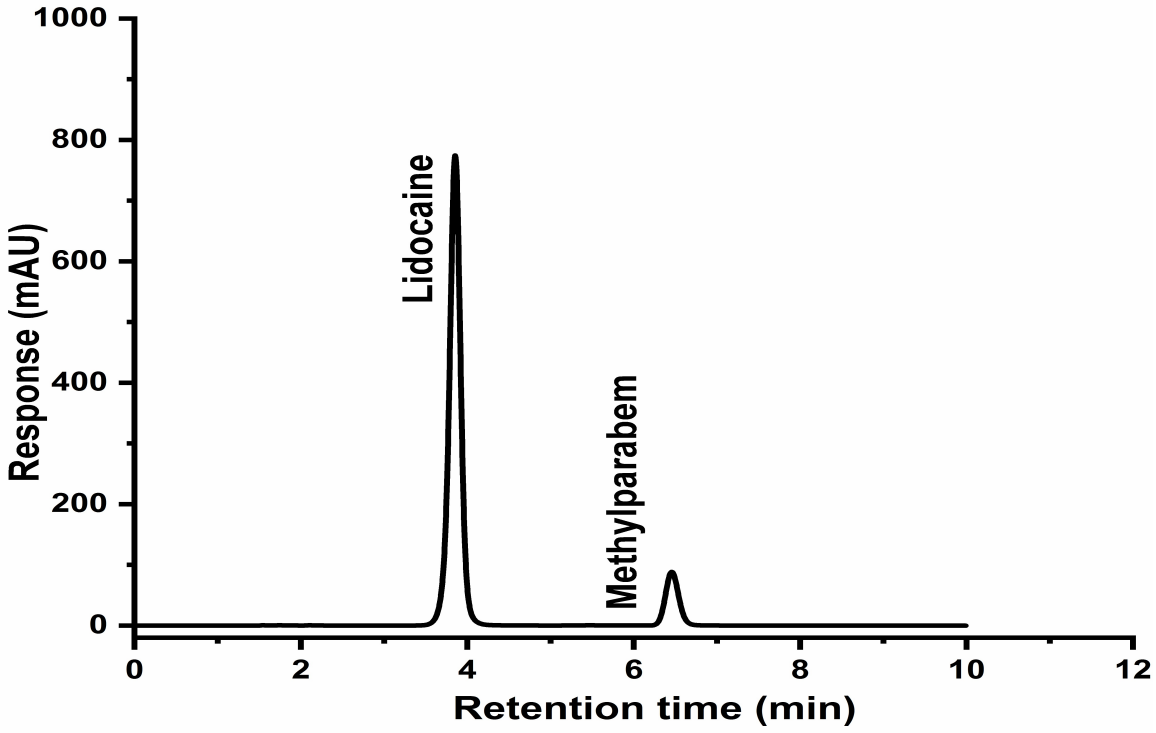

Fig. 2: Lido and MP chromatogram with concentrations of 0.5 mg/mL and 0.05 mg/mL, respectively, at optimum conditions.

Table-1: System suitability parameters.

Item	Day--1		Day--2		Day--3	
	Lido	MP	Lido	MP	Lido	MP
Wt in mg	49.01	5.02	48.6	5.0	47.9	4.75
1	7547.1	994.3	7473.3	988.0	7490.5	936.3
2	7543.5	994.4	7470.1	987.9	7351.1	936.1
3	7545.3	994.7	7481.5	988.9	7356.8	935.9
4	7547.1	994.6	7478.2	988.6	7364.4	936.3
5	7550.1	994.8	7484.9	989.2	7362.7	936.4
6	7554.8	994.7	7497.2	990.6	7367.4	937.0
Mean	7548.0	994.6	7480.9	988.9	7382.2	936.3
STDEV	4.00	0.19	9.63	0.99	53.40	0.37
RSD (%)	0.05	0.02	0.13	0.10	0.72	0.04
USP tailing	0.94562	1.09644	0.95205	1.07729	0.94908	1.08512
Plates	3967	7627	3808	7133	3857	7289
Resolution	9.62		9.162		9.242	

The system suitability test is a vital component that requires careful evaluation to confirm that all elements of the instrument system and analytical method procedures function cohesively to meet the intended analytical goals [19]. This assessment requires careful monitoring of several parameters, including column performance and theoretical plates, peak asymmetry, resolution, relative standard deviation percentage (RSD%), stable retention times, and peak area responses, **(Fig. 3)**.

The validation of system suitability parameters confirmed that all specifications were satisfied according to established acceptance criteria. A complete overview of the system performance metrics obtained during the two-day evaluation period is detailed in Table 1, demonstrating that each measurement remained within the predefined acceptable limits. The assessed parameters included chromatographic retention time stability, reproducibility of peak areas and heights with relative standard deviation percentages at or below 2.0%, column efficiency and theoretical plate numbers exceeding 1500, tailing factors per USP guidelines remaining under 2.0, and peak resolution maintaining values of 2.0 or above. [20].

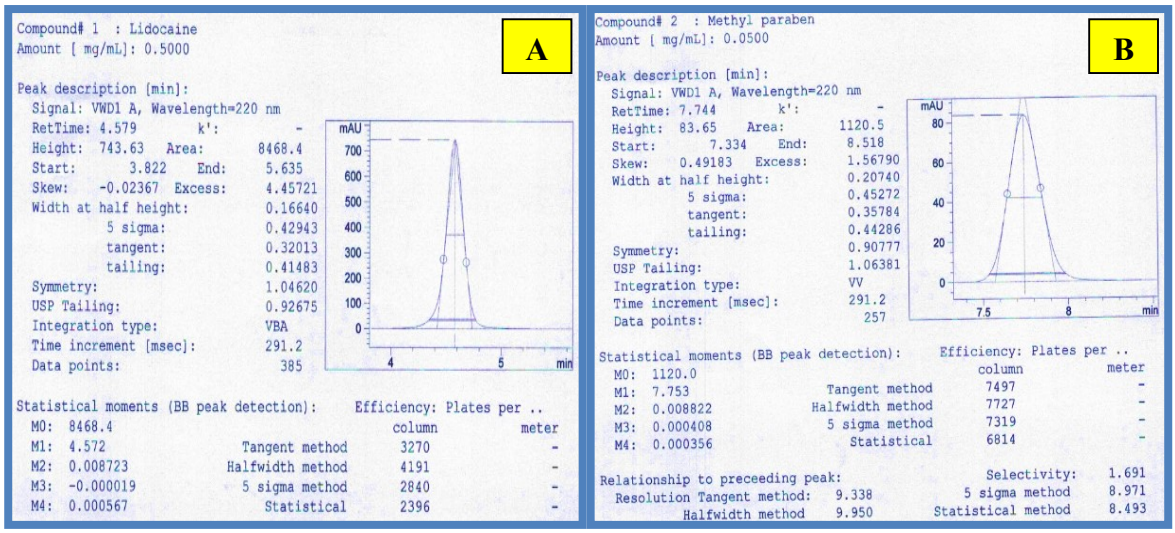

Fig. 3: Parameters of the system suitability in the extended performance chromatogram of A) Lido at 0.5 mg/mL concentration, B) MP at 0.05 mg/mL concentration.

Table-2: Repeatability assessment results.

Item	Day--1				Day--2				Day--3			
	Lido		MP		Lido		MP		Lido		MP	
	Weight (mg)	P.A	Weight (mg)	P.A	Weight (mg)	P.A	Weight (mg)	P.A	Weight (mg)	P.A	Weight (mg)	P.A
1	50.3	7557.9	5.03	995.3	49.7	7471.2	4.98	983.6	48.4	7238.6	4.75	938.5
2	50.1	7532.8	5.01	992.1	50.0	7512.2	5.00	990.3	49.9	7495.6	4.79	947.4
3	50.1	7555.8	5.02	994.4	50.1	7534.6	5.00	987.7	48.7	7288.5	4.75	942.8
4	50.2	7555.2	5.03	995.1	49.8	7474.3	4.97	986.5	48.4	7226.7	4.75	938.6
5	50.3	7545.4	5.02	993.5	49.7	7481.6	5.00	987.9	48.4	7289.9	4.79	945.4
6	50.6	7605.2	5.06	1001.8	49.4	7417.1	4.96	979.2	48.2	7242.2	4.77	940.0
Mean		7558.72		995.4		7481.8		985.9		7296.9		942.1
STDEV		24.62		3.36		40.17		3.93		100.92		3.72
RSD %		0.33		0.34		0.54		0.40		1.38		0.39

Precision and repeatability results

Repeatability assessment served as a fundamental measure for evaluating analytical instrument functionality, balance accuracy, and procedural handling techniques. This criterion was employed to determine intra-assay precision through the execution of six replicate analyses using distinct sample preparations performed within a single day from one uniform sample source. Furthermore, inter-assay precision was investigated by conducting analyses with the same operator over three separate days. Table 2 presents the data generated from these intra-precision and inter-precision tests, which spanned three days. The output data confirms the robustness of the analysis method, as the relative standard deviation (RSD%) remained below 2.0% in all cases, ranging from 0.33% to 1.38%.

Linearity and range

Linear response evaluation was conducted using eight different concentration levels for both Lido [ranging from 0.25 to 0.75 mg/mL] and MP [spanning 0.025 to 0.075 mg/mL], analyzed simultaneously within individual chromatograms. The analytical approach demonstrated proportional response characteristics following linear equation principles, whereby mean peak area responses correlated directly with respective active pharmaceutical ingredient concentrations throughout the examined range **(Fig. 4)**. Outstanding linearity performance was confirmed through correlation coefficient values exceeding 0.999 [21]. The established criteria for analytical method linearity encompass the concentration interval where correlation coefficient requirements are fulfilled, extending from the lowest acceptable concentration level to the highest permissible limit.

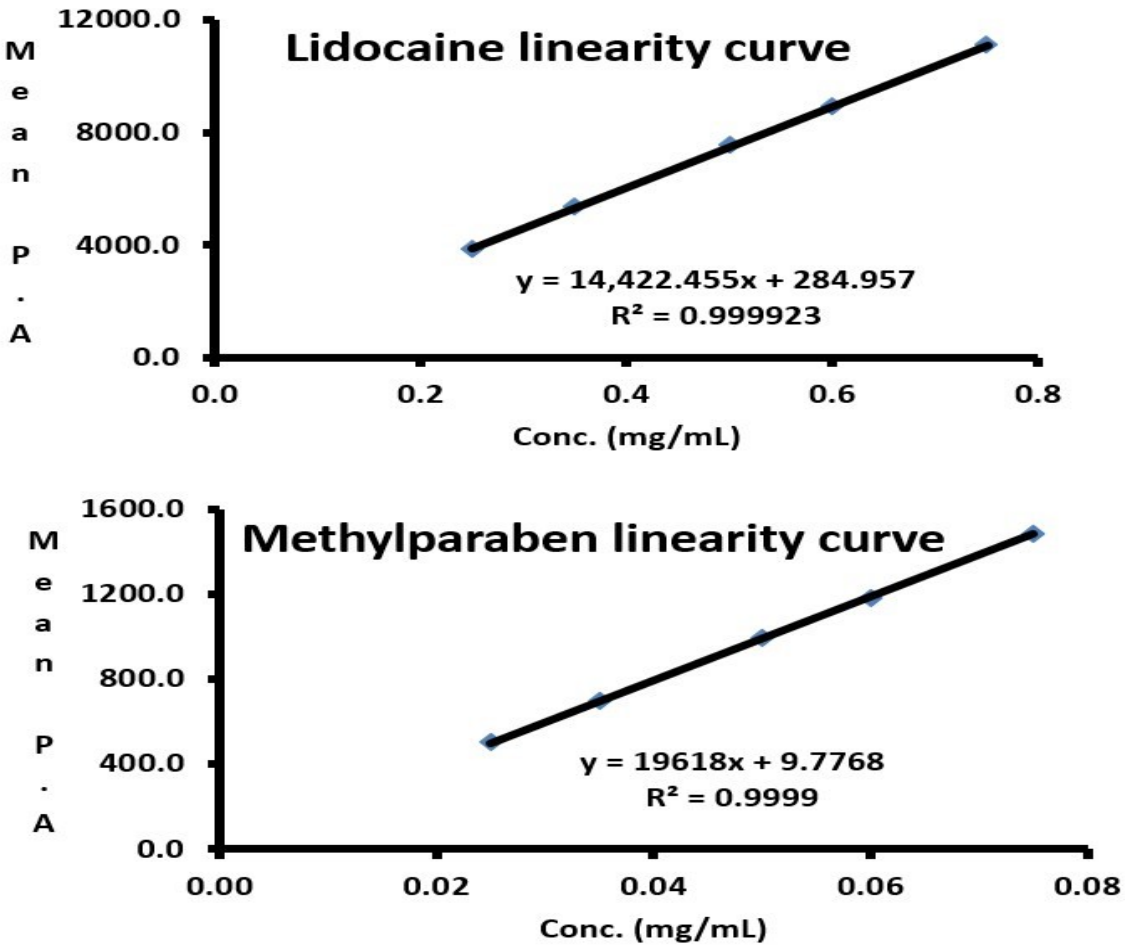

Fig. 4: Lido and MP linearity assessment.

Table-3: Lido recovery results.

Claimed concentration		Experimental Concentration (mg/mL)	P. A.	Mean P. A.	Actual Concentration (mg/mL)	Recovery (%)
(%)	(mg/mL)					
70	0.35	0.361	5394.1 5441.2 5435.3	5424	0.3563	98.7
100	0.50	0.502	7554.4 7549.8 7548.6	7551	0.5038	100.4
120	0.60	0.593	8926.2 8927.3 8931.2	8928	0.5993	101.1

Table-4: MP recovery results.

Claimed concentration		Experimental Concentration (mg/mL)	P. A.	Mean P. A.	Actual concentration(mg/mL)	Recovery (%)
(%)	(mg/mL)					
70	0.0350	0.0351	703.0 705.0 705.0	704.6	0.0354	100.9
100	0.0500	0.0506	994.9 993.7 994.0	994.2	0.0502	99.2
120	0.0600	0.0601	1182.0 1182.3 1182.7	1182.3	0.0598	99.5

Accuracy and recovery

Accuracy, often assessed as percent recovery, is a critical parameter when applying an analytical method to quantify an active pharmaceutical ingredient (API) in the presence of an inactive matrix (placebo) or another API. It is essential to ensure that these matrix components do not interfere with the API assay [22]. The acceptable limit for interference or deviation between the theoretically prepared concentration and the experimentally determined concentration of the API within the matrix is established at 100% ± 2% of the intended concentration. Recovery was evaluated using a minimum of three concentration levels, including the target concentration. The developed method exhibited excellent accuracy, with recoveries determined for three replicates each of Lido and MP at concentration levels ranging from 70% to 120% of the intended concentrations. The data presented in Tables 3 and 4 demonstrate that the recovery percentages remained within the acceptable range of 98.7% to 101.34%.

Selectivity/specificity

Selectivity and stability are essential characteristics of the method, ensuring that each API peak is well-resolved and distinct from any matrix-related peaks that may arise due to the degradation process. The resolution should be equal to or greater than 2.0 [23], and the results obtained were within the range of 9.21-9.69 Table 5 The selectivity test plays a crucial role in confirming the absence of interference between potential degradants and the principal peak under investigation, providing insight into the impurity profile that could be generated under aggressive conditions.

Table-5: Resolution of specificity item.

Item	Resolution
Mobile phase Blank	-
Lido	-
MP	-
HCl hydrolysis	**9.69**
NaOH hydrolysis	**9.21**
H_2O_2 hydrolysis	**9.67**

Detection and quantitation limits

The detection limit (LOD) constitutes an essential analytical parameter that defines the minimum measurable concentration of an API achievable through the designated analytical technique. This concentration must fall beneath the maximum acceptable carryover (MACO) threshold, which proves fundamental for cleaning validation procedures following pharmaceutical production operations. The LOD additionally demonstrates both instrumental sensitivity and methodological appropriateness for specific applications. Tables 6 and 7 present statistical LOD calculations for the three investigated APIs, validating the technique's capability to identify minimal quantities of these substances. Methylparaben (MP) specifically shows remarkable detection efficiency at extremely dilute levels. Moreover, the quantitation threshold (LOQ) maintains substantial importance, particularly within related compound analysis, functioning as the primary criterion for establishing detection limits of both known and unknown contaminants that may arise during product storage duration [24].

Table-6: Limit of detection results.

Item	σ value	S value	Equation	Result
Lido	**29.003**	**14422.455**	**3.3*σ/S**	**6.636 µg/mL**
MP	**4.241**	**19618.025**		**0.713 µg/mL**

Table-7: Limit of quantitation results.

Item	σ value	S value	Equation	Result
Lido	**29.003**	**14422.455**	**10*σ/S**	**20.109 µg/mL**
MP	**4.241**	**19618.025**		**2.162 µg/mL**

Robustness and Ruggedness

The established criteria for a reliable and stable analytical method stipulate that the relative standard deviation (RSD%), calculated from replicate measurements, should not exceed 2.0% [25, 26].

The primary objective of conducting robustness and ruggedness tests is to evaluate the analytical method's ability to remain unaffected by deliberate variations in experimental parameters, whether minor or major, during the analysis [27]. These variations include factors such as different analysts, flow rates, testing days, columns, mobile phase compositions, and ratios of buffer to organic components **(Figs. 5-7)**.

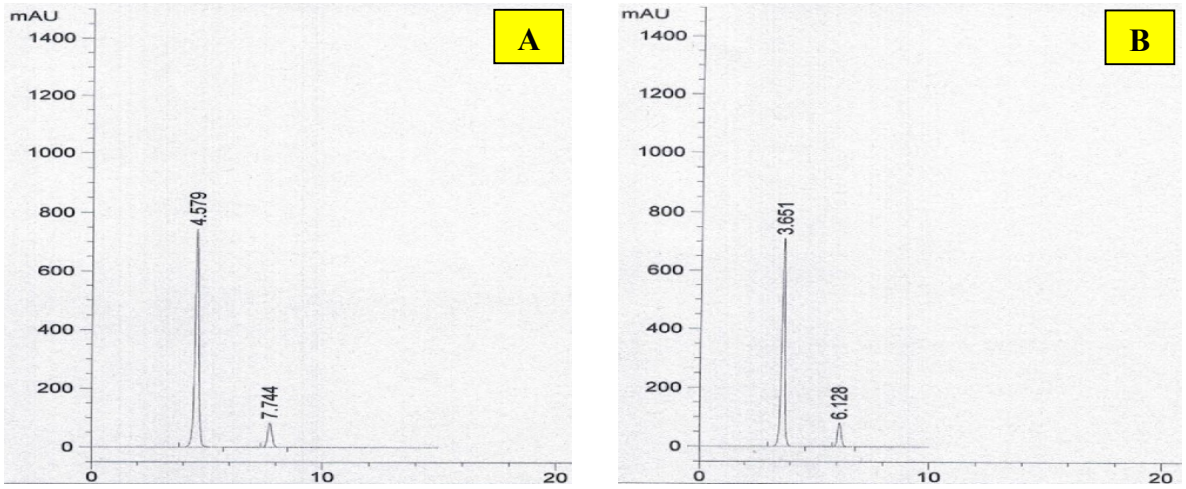

Fig. 5: HPLC chromatogram of working standard of Lido and MP at 0.5 mg/mL and 0.05 mg/ mL, respectively at different flow rates; A) flow rate 0.9 mL/min, B) flow rate 1.1mL/min.

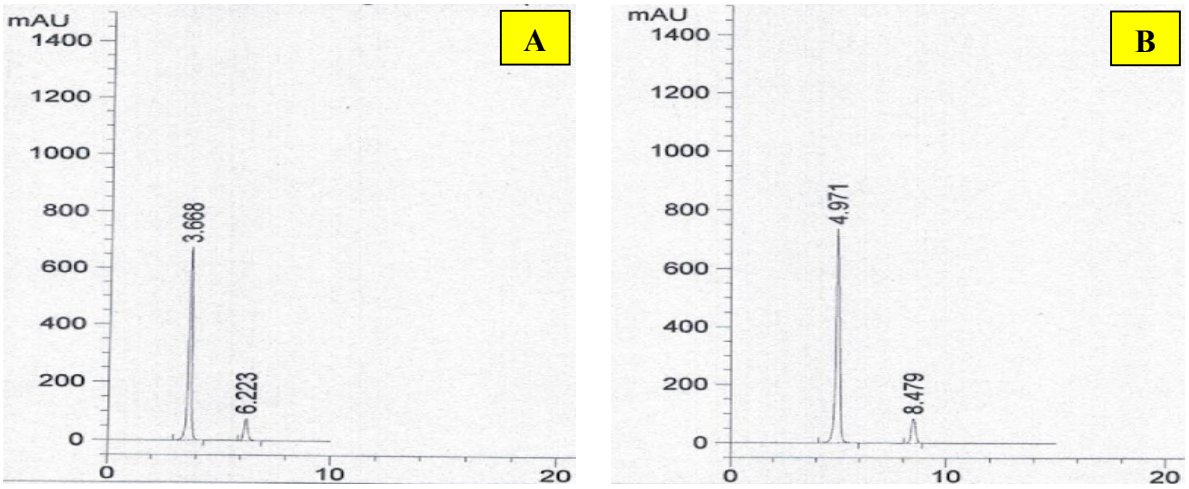

Fig. 6: HPLC chromatogram of working standard of Lido and MP at 0.5 mg/mL and 0.05 mg/ mL, respectively at different organic ratios ±5%; A) At 525 mL methanol, B) At 475 mL methanol.

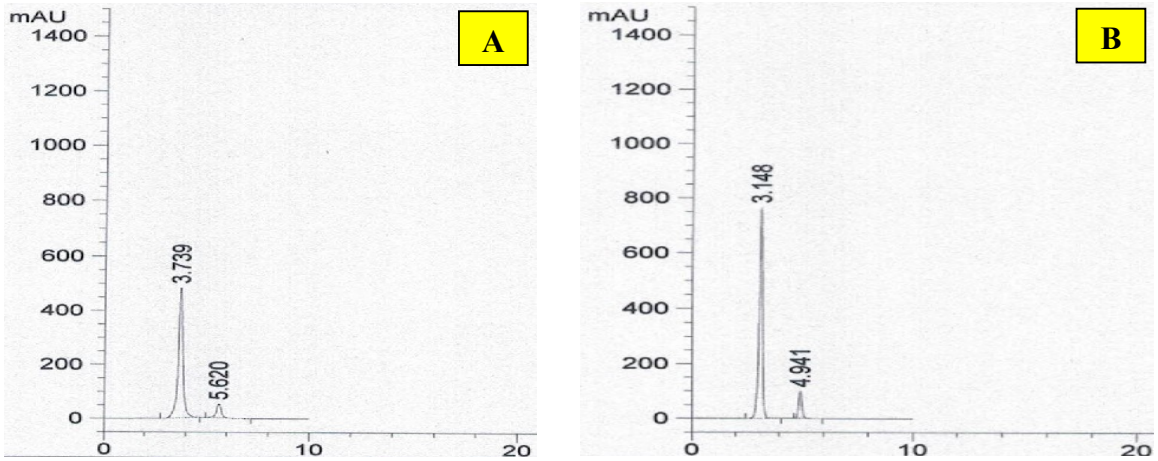

Fig. 7: HPLC chromatogram of working standard of Lido and MP at 0.5 mg/mL and 0.05 mg/ mL, respectively at different columns; A) At column #2, B) At column #3.

The current method demonstrated exceptional stability across all variations that were applied. In every scenario, the relative standard deviation (RSD%) remained within the allowable acceptance criteria, as evidenced by the results presented in Tables 8-11, which fell within the range of 0.02%-1.74%. Additionally, all system suitability parameters were closely monitored and found to be within their accepted limits, even under non-optimal conditions.

Table-8: Analyst-to-Analyst precision results.

Item	Analyst--1		Analyst--2	
	Lido	MP	Lido	MP
Weight (mg)	49.01	5.02	50.3	5.0
1	7547.1	994.3	7779.0	1000.7
2	7543.5	994.4	7630.9	1002.9
3	7545.3	994.7	7625.2	1003.1
4	7547.1	994.6		
5	7550.1	994.8		
6	7554.8	994.7		
Mean	7548.0	994.6	7678.4	1002.2
STDEV	4.00	0.19	87.20	1.33
RSD %	0.05	0.02	1.14	0.13

Table-9: Change in mobile phase precision results.

Item	500 mL methanol		525 mL methanol		475 mL methanol	
	Lido	MP	Lido	MP	Lido	MP
Weight (mg)	49.01	5.02	49.01	5.02	49.01	5.02
1	7547.1	994.3	6667.5	876.0	9056.3	1185.3
2	7543.5	994.4	6650.2	876.2	9001.5	1177.9
3	7545.3	994.7				
4	7547.1	994.6	6665.1	875.5	9014.3	1176.9
5	7550.1	994.8				
6	7554.8	994.7				
Mean	7548.0	994.6	6660.9	875.9	9024.0	1180.0
STDEV	4.00	0.19	9.37	0.36	28.67	4.59
RSD %	0.05	0.02	0.14	0.04	0.32	0.39
USP tailing	0.94562	1.09644	0.91806	1.08735	0.90972	1.06522
Plates	3967	7627	3590	6458	4273	7849
Resolution	9.62		9.2		10.2	

Table-10: Change in flow rate precision results.

Item	Flow (1.0 mL/min)		Flow (1.1 mL/min)		Flow (0.9 mL/min)	
	Lido	MP	Lido	MP	Lido	MP
Weight (mg)	49.01	5.02	49.01	5.02	49.01	5.02
1	7547.1	994.3	6900.4	908.2	8469.7	1120.5
2	7543.5	994.4	6883.6	906.7	8482.9	1120.3
3	7545.3	994.7				
4	7547.1	994.6	6887.3	908.5	8528.6	1123.2
5	7550.1	994.8				
6	7554.8	994.7				
Mean	7548.0	994.6	6890.4	907.8	8493.7	1121.3
STDEV	4.00	0.19	8.83	0.96	30.91	1.62
RSD %	0.05	0.02	0.13	0.11	0.36	0.14
USP tailing	0.94562	1.09644	0.94444	1.07139	0.92675	1.06381
Plates	3967	7627	3640	6753	4191	7727
Resolution	9.62		9.153		9.95	

Table 11: Column-to-Column precision results

Item	Column--1		Column--2		Column--3	
	Lido	MP	Lido	MP	Lido	MP
Weight (mg)	49.01	5.02	47.9	4.75	47.9	4.75
1	7547.1	994.3	7778.2	856.4	8043.0	1018.1
2	7543.5	994.4	7764.2	862.5	8057.4	1020.3
3	7545.3	994.7	7757.7	861.1	8039.0	1018.7
4	7547.1	994.6	7762.4	862.0	8051.4	1020.3
5	7550.1	994.8	7772.8	870.4	8047.1	1020.4
6	7554.8	994.7	7761.8	897.8	8052.5	1020.7
Mean	7548.0	994.6	7766.2	868.4	8048.4	1019.8
STDEV	4.00	0.19	7.72	15.11	6.72	1.07
RSD %	0.05	0.02	0.10	1.74	0.08	0.11
USP tailing	0.94562	1.09644	0.94118	0.79798	0.86371	1.07576
Plates	3967	7627	2671	2856	2514	5308
Resolution	9.62		4.778		6.853	

Table-12: Standard solution stability performance results.

Day	Theoretical plates		Mean P.A		P.A RSD (%)		USP Tailing		Resolution	
	Lido	MP	Lido	MP	Lido	MP	MP	MP	Lido	MP
Starting	3967	7627	7548.0	994.6	0.05	0.02	0.94562	1.09644	---	9.62
24 hours	3823	7168	7497.8	990.2	0.06	0.05	0.95567	1.1	---	9.16

Solution stability

The standard solution exhibited excellent stability and utility over 24 hours following its preparation. This was confirmed by assessing the system suitability parameters for Lido and MP, as presented in Table 12. Consequently, the standard solution can be utilized within 24 hours of its preparation without compromising the performance of the method in terms of separation and analysis.

Actual analysis of fished product sterile ampoule of Lidocaine 1% 3.5mL and 5.0 mL (UP Pharma ampoule)

The method was successfully employed to determine the assay of Lidocaine 1% w/v ampoules for two different volumes available in the Egyptian local market. The results indicated an assay of 98.5% and 96.7% for Lidocaine HCl in the 3.5 mL and 5.0 mL ampoules, respectively. Additionally, the assay of the MP was determined, revealing values of 92.6% and 101.6% for the 3.5 mL and 5.0 mL ampoules, respectively.

Conclusion

The developed method showcased remarkable sensitivity, detecting low levels of Lidocaine (Lido) and Methylparaben (MP) with limits of detection (LOD) at 6.636 µg/mL and 0.713 µg/mL, respectively, and limits of quantitation (LOQ) at 20.109 µg/mL and 2.162 µg/mL, respectively. It demonstrated high accuracy, with recovery rates of 98.7%–101.1% for Lido and 99.2%–100.9% for MP, within a 70%–120% range. Precision was confirmed over three days, with intra-day precision values of 0.05%–0.72% for Lido and 0.02%–0.10% for MP. The method exhibited excellent linearity across 50%–150% of the target concentrations, with regression coefficients (R^2) of 0.99992 for Lido and 0.99991 for MP. Robustness was verified through intentional variations in flow rates, mobile phase compositions, testing days, and analysts, consistently meeting chromatographic system suitability criteria, including theoretical plates and column efficiency ≥ 1500 and USP tailing ≤ 2.0. Selectivity and specificity were confirmed with a minimum resolution of 9.21 between Lido and MP peaks. The method effectively separated Lido and MP principal peaks from forced degradation peaks, with retention times of 3.1–4.9 minutes for Lido and 4.9–8.5 minutes for MP. Its applicability for cleaning validation was supported, as LOD values were below the maximum allowable carry-over limit. The method was successfully applied to quantify Lido and MP in a finished product.

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