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HPLC/MS Analysis of Lidocaine, Benzocaine, and Tetracaine in Anaesthetic Ointment

Stanislav V. Yefimov[1]*

[1] Pharmetric Laboratory LLC, 7265 Ulmerton Road Largo, FL USA

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***Corresponding author:** Stanislav V. Yefimov
Pharmetric Laboratory LLC, 7265 Ulmerton Road Largo, FL USA

Abstract **Original Research Article**

Benzocaine, Lidocaine, Tetracaine (BLT) cream is a potent, prescription-only compounded topical anaesthetic used to numb skin before dermatologic procedures like laser resurfacing, fillers, and microneedling. It combines three agents for rapid-acting (Benzocaine) and deep, prolonged (Lidocaine/Tetracaine) numbness. It is typically applied to intact skin. The composition of the cream included the BLT, and non-active components. HPLC/MS is used for qualitative and quantitative analysis and thoroughly validated for specificity, linearity, limit of detection, accuracy, precision, and recovery. Detailed experimental data are presented to assist chemists in analyzing BLT in creams using HPLC.

Keywords: Lidocaine, Benzocaine, Tetracaine, HPLC/MS, method validation.

INTRODUCTION

BLT numbing cream is a topical anesthetic cream it broken into 3 main components, Benzocaine, Lidocaine and Tetracaine. Benzocaine (20% concentration) - rapid onset, short duration (fast acting) frequently used anesthetic in many med spas, dermatology offices and dental settings. Benzocaine is generally well tolerated by most patients. Lidocaine (4% concentration) - medium onset, medium duration, another commonly used anesthetic that has an onset of approx 4 minutes. Tetracaine (2% concentration) - rapid onset, extended duration (long acting). Among the scientific publications, one can find several articles covering various aspects of BLT analysis, a brief overview of these publications is provided below. The stability study of BLT (Mihaela, *et al.,* 2026) indicates that combined Benzocaine (20%), Lidocaine (6%), and Tetracaine (4%) compounded in VersaPro™ Cream Base was stable for the duration (180 days) of the study under the examined conditions. Gas chromatography–time-of-flight mass spectrometry and gas chromatography–triple quadrupole mass spectrometry methods are used simultaneously to determine 18 local anesthetics including BLT (Kyeong *et al.,* 2025). HPLC method Determination of Local Anesthetic Drugs in Human Plasma Using Magnetic Solid-Phase Extraction described in the publication (Liang *et al.,* 2022). HPLC Method to quantify lidocaine from Polyethylene-co-Vinyl Acetate matrices and biological fluids described in (Bhusal *et al.,* 2017). HPLC and mass spectrometry analysis of few substances including local anesthetics in cosmetic creams is interesting because a lot of components are analyzed simultaneously (Orsi *et al.,* 2009). Waters Corporation validated a fast UPLC Method for the analysis of Benzocaine, and Tetracaine Hydrochloride in solutions (Aubin *et al.,* 2005).

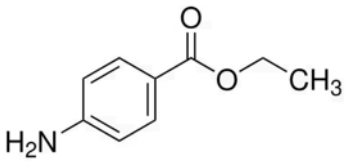
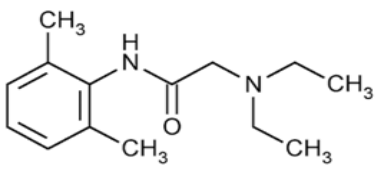
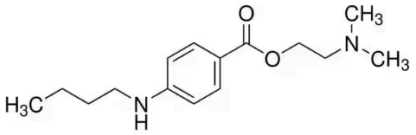
The objectives of the study are to develop a simple HPLC-MS method for the routine quantitative determination of BLT in ointments or creams that allows for the simultaneous determination of components, and to validate this method for accuracy, precision, repeatability, selectivity, specificity, and robustness in accordance with the requirements of the ICH Q2 (R2) 2023 guideline. The molecular structures and basic chemical properties of BLT are presented in Table 1.

MATERIALS AND METHODS

Chemicals. Water HPLC grade purchased from Agilent. HPLC-grade solvents were used. Lidocaine, Tetracaine, and Benzocaine USP reference standards was used.

Mobile phase: ACN/H2O 50/50 + 0.1% formic acid (FA) analytical grade (Sigma). Mobile phase was used as a diluent.

Table 1: BLT

Benzocaine. $C_9H_{11}NO_2$, 165.192 g·mol−1, pKa = 2.5 (25°C). Benzocaine is highly soluble in acetonitrile, m/z+=166.	**Lidocaine.** $C_{14}H_{22}N_2O$, 234.343g·mol^{-1}, pKa=7.7; pKb=(5.8 -7.9), (25°C). Lidocaine base is soluble in methanol, ethanol, and other common organic solvents, practically insoluble in water. m/z+= 235, 236.	**Tetracaine.** $C_{15}H_{24}N_2O_2$, 264.369 g·mol^{-1}, pKa = 8.5 (25°C). Tetracaine is highly soluble in methanol, ethanol, and almost insoluble in water, m/z+=265.
		

Cream containing Lidocaine, Tetracaine, Benzocaine 6%, 4%, 20%. Also containing propylene glycol (5%), polysorbate 80 (2%), and Salt Stable Advanced - silicone-based transdermal cream base.

Standard solution preparation: Weight 20 mg USP standard dissolve in 10ml ACN, filtrate 0.45µm pore syringe filter.

Samples preparation: Weight 50, 300, 1000, and 1200 mg cream, and dissolve it in 45ml acetonitrile vigorously stirring on a magnetic stirrer for 30 minutes, then filter through a filter with 0.45 µm pores. The samples were diluted with a diluent to a concentration approximately corresponding to the midpoint of the calibration curve.

Instrument. Agilent HPLC/DAD/MS instrument consists of the following components: Diode Array Detector (DAD), quaternary pump, single quadrupole mass selective detector (MSD) with electrospray ionization and 150 V fragmentor, the gas temperature is 300°C, the capillary voltage is 4000 V, and the nebulizer is 15psi.

Column - Poroshell 120 EC-C18 250x4.6mm with particles size 2.7 µm.

HPLC/MS conditions: column temperature 40°C, flow rate 1ml/min, flow time 5 min, DAD 266 and 313nm, samples injection volume – 0.5 µL; m/z+: 235, 236 for Lidocaine; 265, 266 for Tetracaine; 166,167 for Benzocaine. RT≈ 2.0 min Lidocaine, 2.3min Tetracaine, and 4.2min Benzocaine.

Qualitative analysis of the components was carried out using UV and MS spectra specific for each of the components (Fig. 1, 2. and 3).

Quantitative analysis was done using a calibration curve built for each of the components (Fig. 1, 2. and 3).

System suitability has been validated according to the Center for Drug Evaluation and Research (CDER, 1994) and the System Suitability Assessment Guidelines (Evaluating System Suitability, 2019; Dr. Deepak, 2013; Bose, 2014). The parameters were peak area, retention time, number of theoretical plates (N), and tailing factor (T).

Calibration curve and coefficient of correlation (r). The range was chosen so that, the calibration curve should be strictly linear (r≥0.999).

The precision/accuracy of the method was determined by the RSD value from the analysis of five samples of the same concentration under the same experimental conditions. The intraday and interday analysis was compared by RSD and recovery.

Limits of detection (LOD). LOD characterizes the sensitivity of a method; it is the minimum amount of a substance that can be measured by a given method, whereas the LOQ is the lowest concentration with acceptable linearity, accuracy, and precision. If the equation of the calibration curve is an equation of the first degree (straight line) then LOD is calculated by formula [1] (Yefimov, 2021):

$$LOD = \frac{3.3 \times \sigma}{a} [1]$$

where (σ) is the residual standard deviation of the regression line, and (a) is the slope of the line (ICH Q2(R2), 2023; FDA, 2015). LOQ is 3 times LOD.

A measure of repeatability is the RSD of the mean of five independent tests of the samples of the same concentration.

To prove the specificity of the method, the peak areas of the component in the drug sample and the standard solution of the same concentration were compared. At the same time, the retention time of the component in both chromatograms was almost the same (RSD<1.2%). A minor discrepancy in the magnitude of the peak area indicated the specificity of the method for this component.

To demonstrate the robustness of the method the column temperature, and mobile phase composition

were varied. The tailing factor (T) and the number of theoretical plates (N) were calculated. The results were compared with the acceptable limits.

The forced degradation study involved incubating samples with formic acid, ammonium hydroxide, and hydrogen peroxide, as well as exposing them to UV radiation (20 W/cm) and high temperature (80 °C) for 19 hours. The % degradation was determined.

The resolution factor was considered satisfactory if it was at least 2.0, based on the separation between the main peak of the active substance of interest and the nearest peak of another component.

Statistical analysis included calculating mean, standard deviation (S.D.), relative standard deviation (RSD), and correlation coefficient (r). Results $p < 0.05$ were considered statistically significant. The Least-squares regression analysis was used. In most cases, the calculation was performed automatically by the Open LAB CDS software of the instrument.

RESULTS AND DISCUSSION

System Suitability Test (Table 2, 3, 4) was used to verify that chromatographic system is adequate for the intended analysis. The standard solutions were prepared and injected 5 times at the middle concentration levels (5 in total). The following parameters were determined: peak area, retention time, number of theoretical plates, tailing factor, and Relative Standard Deviation was calculated (%). The data in the following table demonstrated the specification for system suitability was met the acceptance criteria: % RSD of Retention Time should be ≤ 2.0, Tailing factor should be ≤ 2.0, Average of Theoretical Plates number should be > 2000, Resolution ≥ 2.0. The results were averaged, and the RSD was calculated automatically using the OpenLAB CDS software. The acceptable limit is in line with the recommendations (Dr. Deepak, 2013; Bose, 2014).

Table 2: Lidocaine system suitability

Resolution* >2				
Lidocaine, sample #	**RT**	**Tailing factor**	**Theoretical plates N.**	**Peak area**
1	2.217	1.200	36192.000	1917.800
2	2.218	1.180	35728.000	1956.000
3	2.217	1.180	35693.000	1978.000
AVE	2.217	1.187	35871.000	1950.600
SD	0.001	0.012		
% RSD	0.026	0.973		

Table 3: Benzocaine system suitability

Resolution*	**14.5**			
Benzocaine sample #	**RT**	**Tailing factor**	**Theoretical plates N**	**Peak area**
1	4.272	1.055	116563.000	2495.370
2	4.272	1.052	116501.000	2451.130
3	4.271	1.043	116501.000	2517.940
AVE	4.272	1.050	116521.667	2488.147
SD	0.001	0.006		
% RSD	0.014	0.595		

Table 4: Tetracaine system suitability

Resolution*	**>2**			
Tetracaine sample #	**RT**	**Tailing factor**	**Theoretical plates N.**	**Peak area**
1	2.704	1.204	64720.000	199.190
2	2.704	1.204	64802.000	198.020
3	2.704	1.111	63470.000	197.060
AVE	2.704	1.173	64330.667	198.090
SD	0.000	0.054		
% RSD	0.000	4.577		

(*) resolution between two adjacent peaks on the chromatogram.

Qualitative analysis

Qualitative determination of BLT in cream was carried out using three parameters, namely, retention time, UV spectrum, and MS spectrum (Figure 1, 2, 3). It has been proven that the tested cream contains benzocaine, lidocaine, and tetracaine.

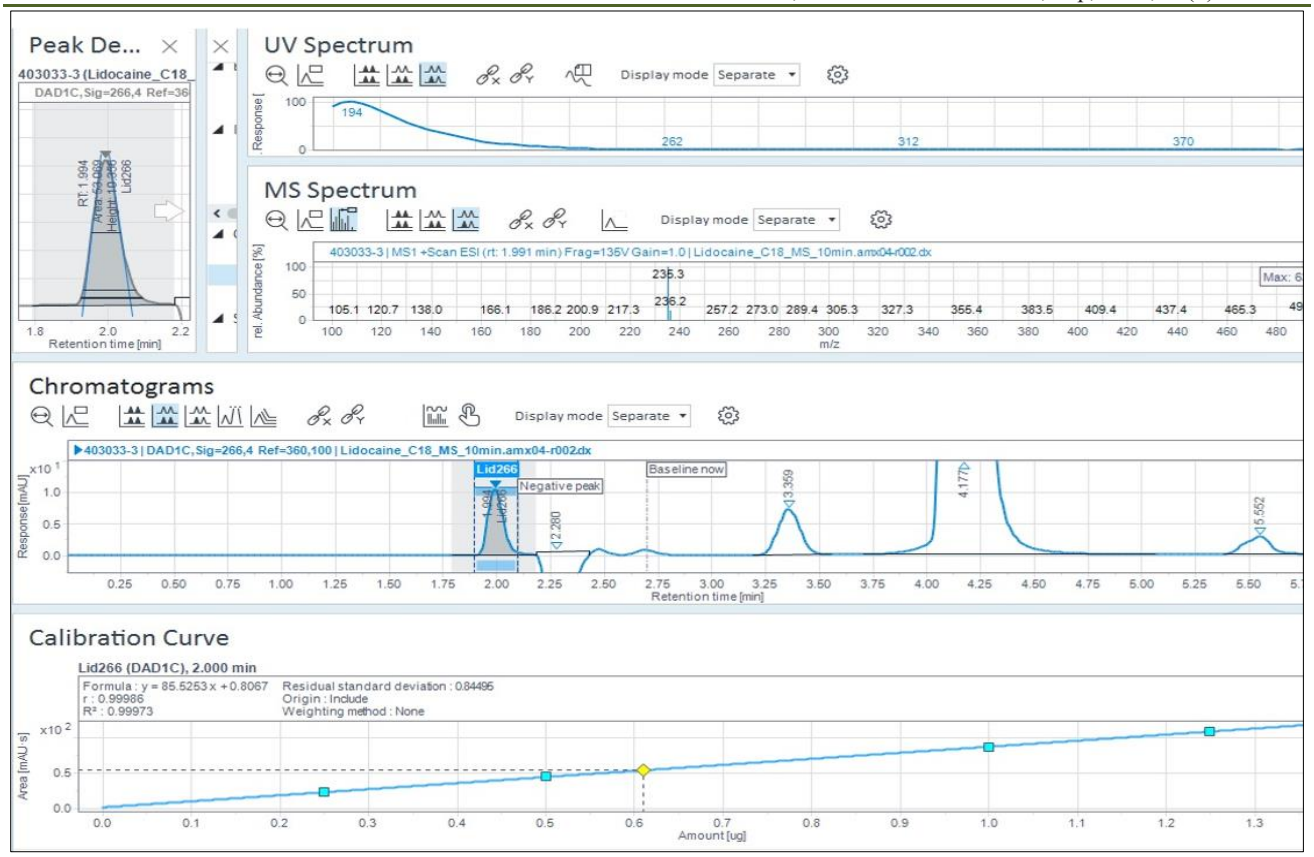


Figure 1: Lidocaine. Chromatograms, spectres, and calibration curves

Figure 2: Benzocaine. Chromatograms, spectres, and calibration curves

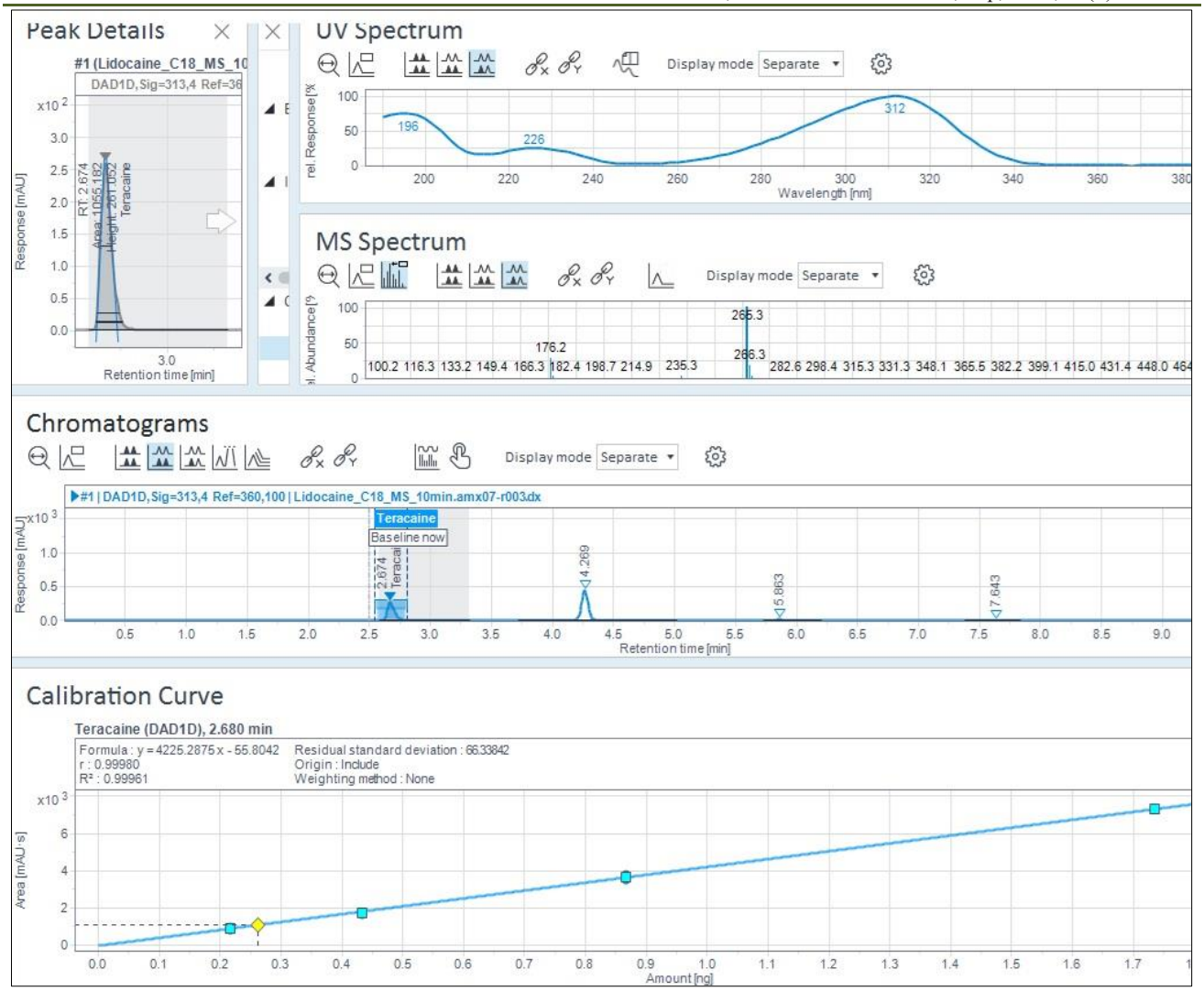


Figure 3: Tetracaine. Chromatograms, spectres, and calibration curves

Linearity, Range, and Limit of Detection (Tables 5, 6, and 7). The LOD for BLT is ranged from 0.013µg (lidocaine) to 0.048 µg (benzocaine) per injection which is quite enough for API testing.

Table 5: Lidocaine, Range, and LOD

Y = ax+b			
X (µg)	Mean Y (n=5)		Y calc.
0.224	864	870	6
0.448	1575	1592	17
0.896	3073	3037	36
1.792	5913	5926	13
a	3224.18		
b	147.97		
c	N/A		
r	1.000		
Mean DY (n=xx)	18		
S.D. DY (n=x)	13		
LOD (µg)	**0.013**		
"X"- the content of lidocaine in the sample; "Y" - the peak area; "Y calc."- the calculated peak area; "DY" - the residues; "a" - the slope of the regression line; "a", "b", "c" - the parameters of regression curve; "r" - the correlation coefficient; "S.D. DY" - the residual standard deviation of the regression curve.			

Table 6: Benzocaine, Range, and LOD

Y = ax+b			
X (µg)	Mean Y (n=5)	Y calc.	DY
0.226	366	334	32
0.452	569	604	36
0.904	1141	1144	2
1.808	2229	2223	6
a	1193.56		
b	64.63		
c	N/A		
r	0.999		
Mean DY	19		
S.D. DY	17		
LOD (µg)	**0.048**		
"X"- the content of benzocaine in the sample; "Y" - the peak area; "Y calc."- the calculated peak area; "DY" - the residues; "a" - the slope of the regression line; "a", "b", "c" - the parameters of regression curve; "r" - the correlation coefficient; "S.D. DY" - the residual standard deviation of the regression curve.			

Table 7: Tetracaine, Range, and LOD

Y= ax + b			
X (µg)	Mean Y (n=5)	Y calc.	DY
0.217	874.61	827	47
0.434	1680.55	1751	71
0.868	3622.60	3600	23
1.736	7296.53	7296	0
a	4258.83		
b	-97.05		
c	N/A		
r	1.000		
Mean DY	35		
S.D. DY	30		
LOD (µg)	**0.024**		
"X"- the content of tetracaine in the sample; "Y" - the peak area; "Y calc."- the calculated peak area; "DY" - the residues; "a" - the slope of the regression line; "a", "b", "c" - the parameters of regression curve; "r" - the correlation coefficient; "S.D. DY" - the residual standard deviation of the regression curve.			

Accuracy/recovery and precision (Tables 8, 9, 10).

The accuracy (based on the recovery of known amounts of analyte) was carried out by spiking the tested solution with API. Spiked solutions were prepared in three (3) levels of concentration: 80%, 100% and 120% of the nominal target of 1.0 - 1.5 µg. Samples containing three different concentrations of the component of interest were measured five times, and the mean values were calculated. The recovery was determined based on the calibration curve. The data confirm the accuracy, reproducibility, and precision of the method. Intraday analysis (check the next day) shows no significant degradation.

Table 8: Lidocaine Accuracy/recovery and precision. The total amount of API represents the sum of the API content in the sample and in the spike

µg API in sample	API Total (µg)	Tested (µg)	Recovery %
0.22	1.16	1.20	103.45
0.21	1.21	1.25	103.00
0.17	1.45	1.43	98.26

Table 9: Benzocaine Accuracy/recovery and precision. The total amount of API represents the sum of the API content in the sample and in the spike

µg API in sample	API Total (µg)	Tested (µg)	Recovery %
0.69	1.08	1.09	100.09
0.66	1.13	1.13	99.98
0.53	1.36	1.34	101.08

Table 10: Tetracaine Accuracy/recovery and precision. The total amount of API represents the sum of the API content in the sample and in the spike

µg API in sample	API Total (µg)	Tested (µg)	Recovery %
0.13	1.04	1.04	100.03
0.13	1.09	1.09	99.19
0.10	1.30	1.31	99.31

Repeatability and **Precision**:

(Tables 11, 12, and 13). In the present study, 4 samples were prepared and tested. The injection peak areas of the samples were recorded. The mean, standard deviation and RSD were calculated for obtained results. The method demonstrates the satisfactory repeatability and precision.

Table 11: Lidocaine 0.396 g/l. Repeatability and Precision

Sample preparation	peak area	result g/l	Recovery %
1	1382.80	0.40	100.1
2	1371.32	0.39	99.2
3	1390.03	0.40	100.7
4	1373.55	0.39	99.4
mean	1379.42	0.40	99.8
SD	8.64	0.001	
RSD %	**0.63**	**0.67**	

Table 12: Benzocaine 1.36g/l. Repeatability and Precision

Sample preparation	peak area	result g/l	Recovery %
1	1700.80	1.37	100.7
2	1679.40	1.35	99.4
3	1697.46	1.37	100.5
4	1700.48	1.37	100.7
mean	1694.54	1.36	100.3
SD	10.20	0.01	
RSD %	**0.60**	**0.62**	

Table 13: Tetracaine 0.266 g/l. Repeatability and Precision

Sample preparation	peak area	result g/l	Recovery %
1	1065.50	0.27	99.8
2	1059.28	0.26	99.2
3	1073.89	0.27	100.5
4	1078.11	0.27	100.9
mean	1069.19	0.27	100.1
SD	8.44	0.001	
RSD %	0.79	0.74	

Intermediate precision (Tables 14, 15, and 16). The % RSD of all compounded preparation sample analysis was ≤ 5.0. But these data indicate that freshly prepared samples should be analyzed.

Table 14: Lidocaine Intermediate precision

nominal (g/l)	Day1 (g/l) mean 3	Day2 (g/l) mean 3	mean	SD	RSD
0.387	0.38	0.41	0.39	0.01	3.40
0.264	0.26	0.28	0.27	0.01	3.84
0.638	0.58	0.63	0.60	0.03	4.52
1.221	1.14	1.19	1.17	0.03	2.72

Table 15: Benzocaine Intermediate precision

nominal (g/l)	Day1 (g/l) mean 3	Day2 (g/l) mean 3	mean	SD	RSD
1.29	1.3239	1.3827	1.35	0.03	2.39
0.88	0.9222	0.9492	0.94	0.02	2.25
0.25	0.2368	0.2432	0.24	0.00	1.55

2.13	2.1554	2.2086	2.18	0.05	2.44

Table 16: Tetracaine Intermediate precision

nominal (g/l)	Day1 (g/l) mean 3	Day2 (g/l) mean 3	mean	SD	RSD
0.25825	0.2625	0.2742	0.27	0.01	2.41
0.1762	0.1836	0.1926	0.19	0.01	2.71
0.42552	0.419	0.4448	0.43	0.02	3.62
0.81395	0.7948	0.8168	0.81	0.01	1.62

Standard solution stability (Tables 17, 18, and 19).

Standard solutions were prepared according to the procedure described here and stored at room temperature for 7 days. Based on the testing of standard solutions at the end of the 7-day period, we recommend using freshly prepared standard solutions.

Table 17: Lidocaine standard solution 2.43 g/l

Day#	RT (min)	Area
1	2.18	8070.31
1	2.18	7762.94
Aver	**2.18**	7916.62
7	2.18	7670.53
7	2.18	7630.54
Aver	**2.18**	7650.53
RSD (%)	0.001	2.56

Table 18: Benzocaine standard solution 2.6 g/l

Day#	RT (min)	Area
1	4.38	1367.96
1	4.38	1417.04
Aver	**4.38**	1392.5
7	4.38	1414.59
7	4.38	1405.02
Aver	**4.38**	1409.8
RSD (%)	0.001	1.62

Table 19: Tetracaine standard solution 2.17 g/l

Day#	RT (min)	Area
1	2.62	4530.74
1	2.62	4762.63
Aver	**2.62**	4646.68
7	2.62	4621.11
7	2.62	4565.46
Aver	**2.62**	4593.29
RSD (%)	0.001	2.21

Robustness (Tables 20, 21, and 22).

As part of establishing the robustness of the method, the chromatographic parameters, tailing factor (T) and RT (min) of each of the components were determined with a change in column temperature (38°C), and composition of the mobile phase (48/52). These parameters changed insignificantly and were within acceptable limits. Thus, the method is robust.

Table 20: Benzocaine Robustness

Benzocaine	Control, 40°C, MP 50/50	Control, 40°C, MP 50/50	38°C	38°C	MP 48/52	MP 48/52
	RT	Tailing factor	RT	Tailing factor	RT	Tailing factor
#1	4.272	1.055	4.271	1.061	4.372	1.052
#2	4.272	1.052	4.292	0.992	4.368	1.048
#3	4.271	1.043	4.285	1.030	4.369	0.989
AVE	4.272	1.050	4.283	1.028	4.370	1.030
% RSD	0.014	T<2	0.250	T<2	0.048	T<2

Table 21: Lidocaine Robustness.

Lidocaine	control 40°C, MP 50/50	control 40°C, MP 50/50	38°C	38°C	MP 48/52	MP 48/52
	RT	Tailing factor	RT	Tailing factor	RT	Tailing factor
#1	2.217	1.200	2.19	1.21	2.183	1.477
#2	2.218	1.180	2.20	1.17	2.207	1.126
#3	2.217	1.180	2.20	0.938	2.207	1.112
AVE	2.217	1.187	2.197	1.106	2.199	1.238
% RSD	0.026	T<2	0.263	T<2	0.630	T<2

Table 21: Tetracaine Robustness

Tetracaine	Control, 40°C, MP 50/50	Control, 40°C, MP 50/50	38°C	38°C	MP 48/52	MP 48/52
	RT	Tailing factor	RT	Tailing factor	RT	Tailing factor
#1	2.704	1.204	2.649	1.243	2.687	1.323
#2	2.704	1.204	2.662	1.289	2.695	1.386
#3	2.704	1.111	2.673	1.162	2.695	1.289
AVE	2.704	1.173	2.661	1.231	2.692	1.333
% RSD	0.000	T<2	0.451	T<2	0.172	T<2

Specificity

The results of forced degradation studies (acid, alkali, an oxidizing agent, UV radiation, and high temperature stress) conducted over a period of 19 hours are presented in Table 22. The weight of the cream and the amount of the stressing agent added are indicated in the tables. Chromatograms and spectra of the degraded Lidocaine, Benzocaine, and Tetracaine compared to control are presented in Figures 5, 6, 7, 8, 9, and 10. BLT is resistant to bases (NH3). Furthermore, lidocaine is resistant to formic acid, while benzocaine is resistant to UV radiation.

Table 22: Forced Degradation study results

FD agent	Cream (mg)	FD agent (ml)	Lidocaine degradation (%)	Benzocaine degradation (%)	Tetracaine degradation (%)
UV (20W/m^2)	1021.2	NA	5.7	0.0	4.7
Formic acid 98%	1495.9	1.0	0.0	98.0	28.6
80°C	1559.9	NA	10.1	7.9	8.5
NH_3 30%	1148.6	1.0	0.0	0.0	0.0
H_2O_2 30%	1036.3	1.0	77.8	3.6	100.0

Figure 5: Lidocaine control

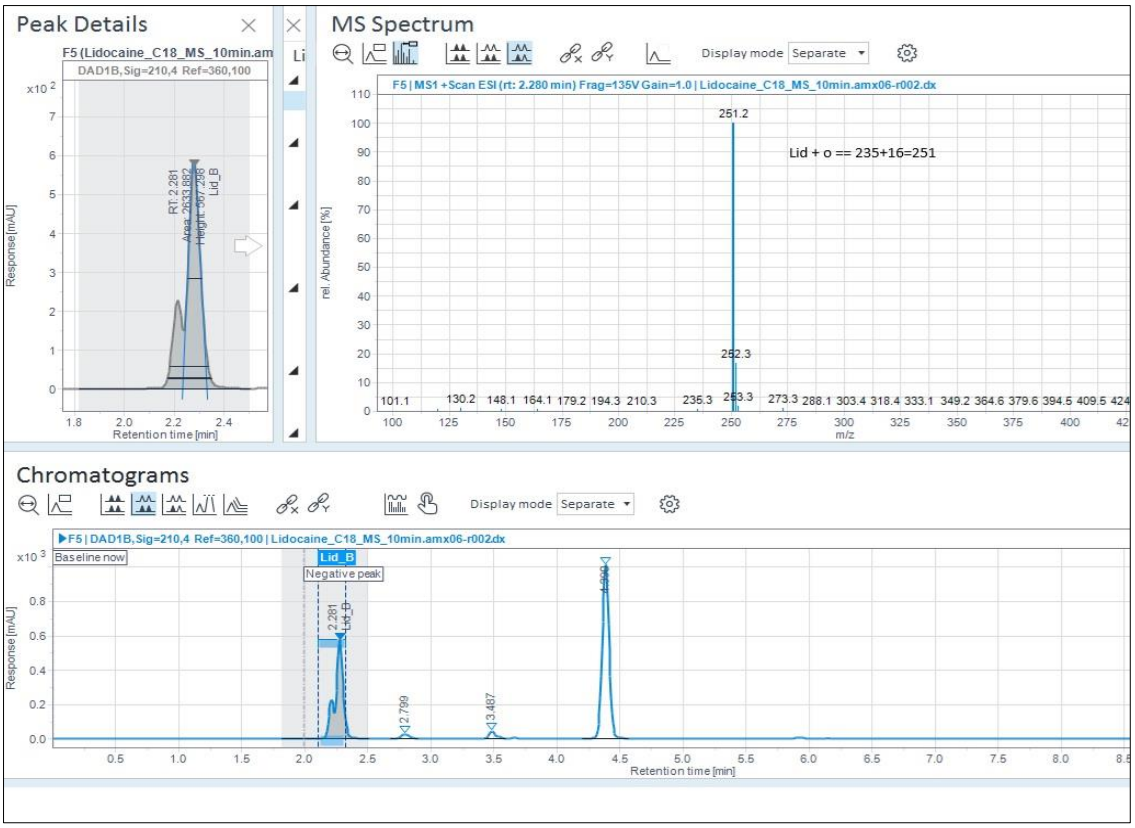


Figure 6: Lidocaine. H_2O_2 oxidative stress

The effect of the oxidizing agent (H_2O_2) on lidocaine is evidenced by a double peak in the chromatogram and a change in the MS spectrum, specifically, an increase of 16 in the m/z+ value, corresponding to the mass of a single oxygen atom.

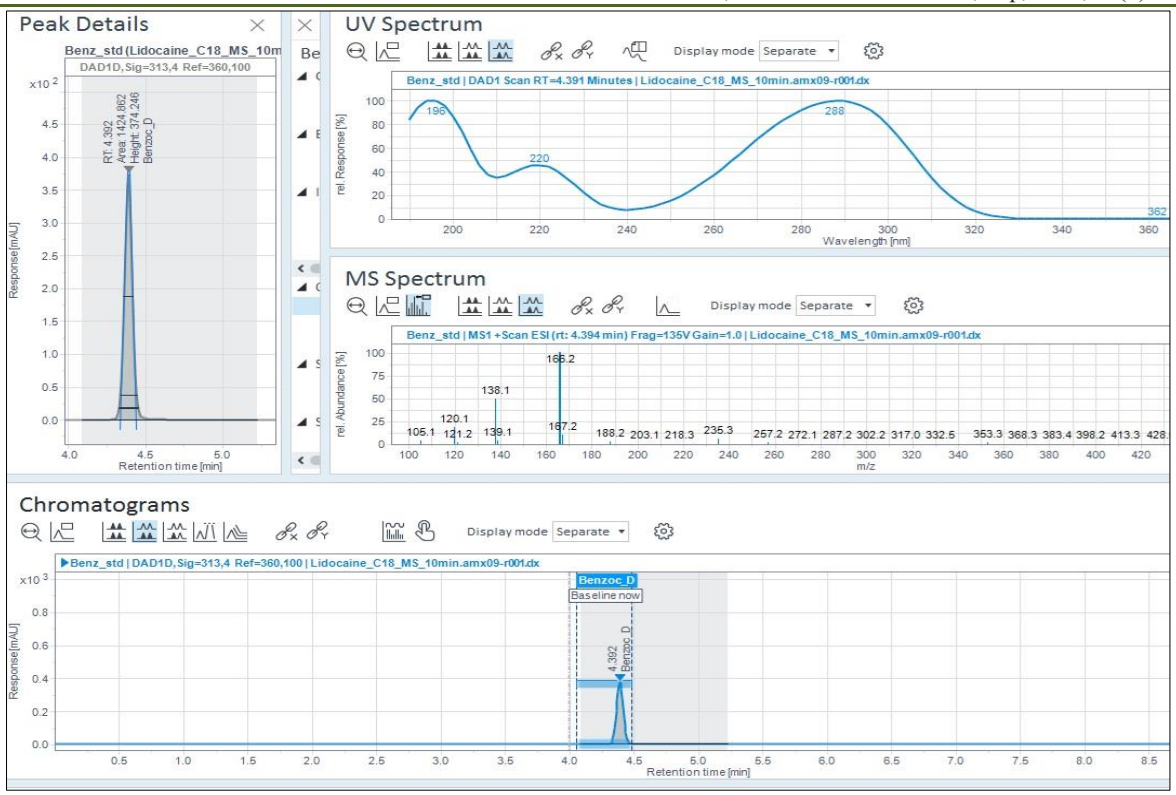


Figure 7: Benzocaine control

Figure 8: Benzocaine. Formic acid stress

The destructive effect of formic acid on benzocaine is evidenced by a decrease in peak height (from 400 to 10 mAU) and the appearance of an m/z+ = 235 signal in the mass spectrum.

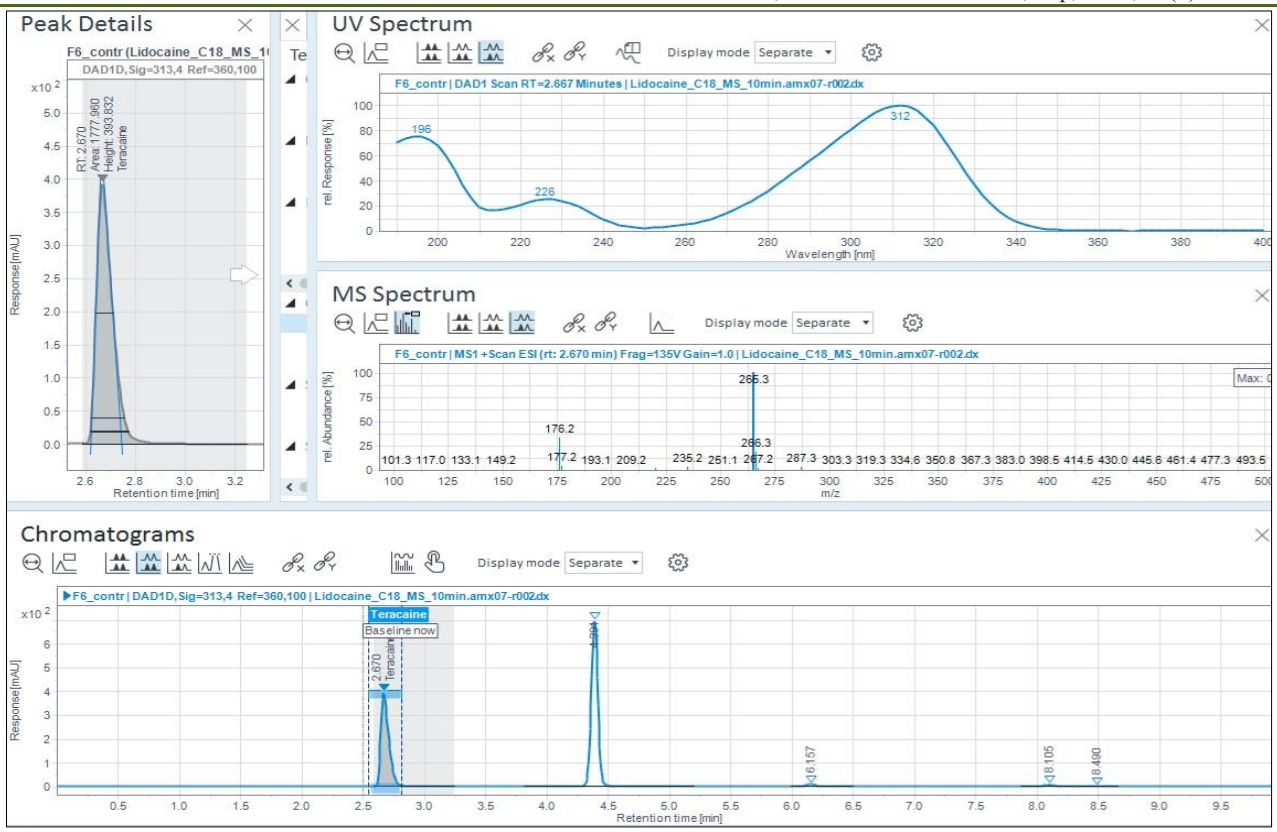


Figure 9: Tetracaine control

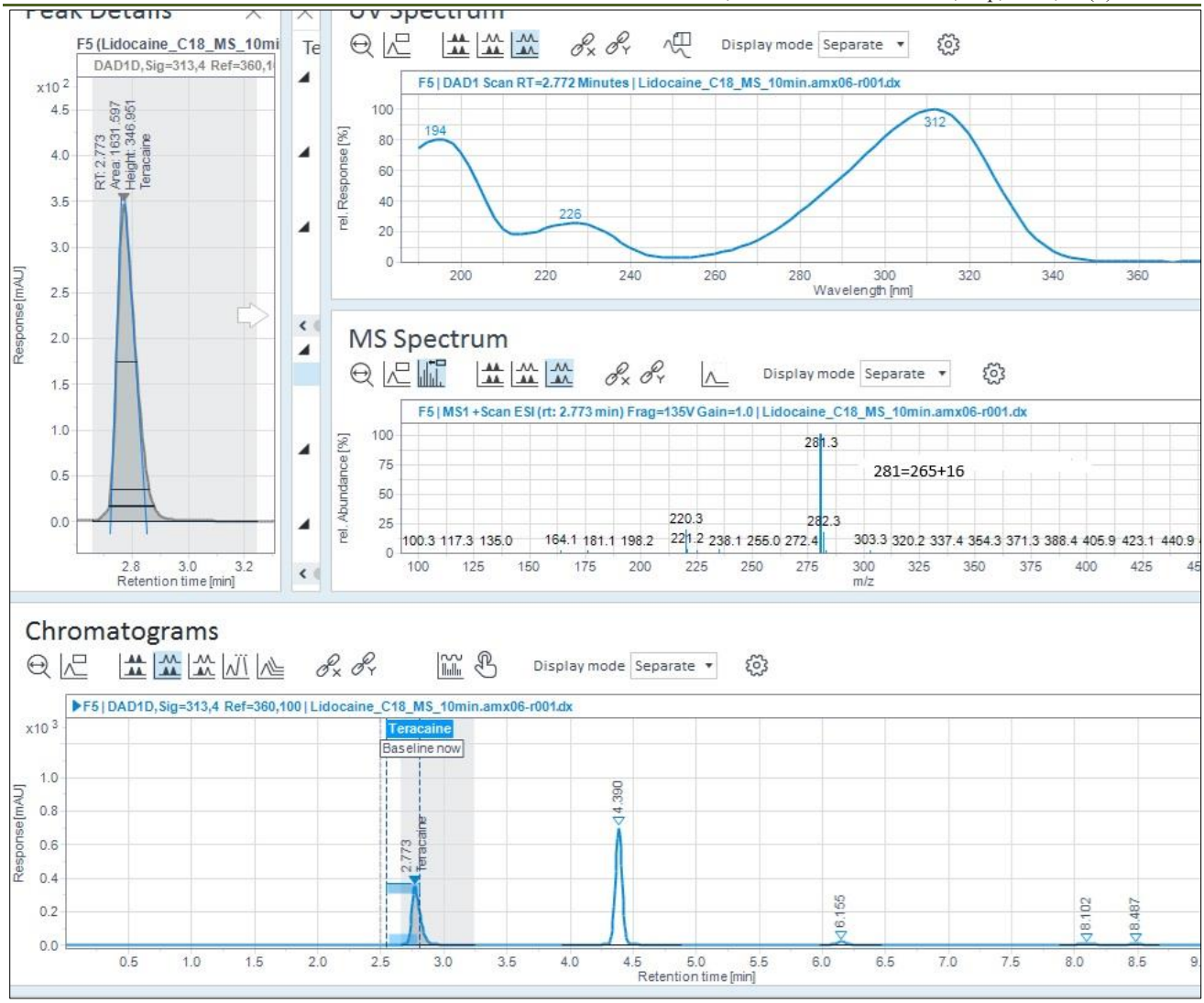


Figure 10: Tetracaine. H_2O_2 oxidative stress

The effect of the oxidizing agent (H_2O_2) on tetracaine is evidenced by a shift in retention time of 0.103 min and a change in the mass spectrum, specifically, an increase in the m/z+ value by 16 units, corresponding to the mass of a single oxygen atom.

CONCLUSION

The method presented here was developed and proved in our laboratory and is used for the routine determination of the potency of creams containing BLT. Analysis using this method can be performed via either HPLC/MS or HPLC/UV instruments. We recommend this method for use by analytical laboratories.

The authors claim that there is **no conflict of interest**.

Abbreviations:
DAD -Diode Array Detector
LOD – limit of detection
MSD - mass selective detector
MP-mobile phase
MW- Molecular weight
N – number of theoretical plates
RP- reversed-phase
RSD – relative standard deviation
SQ - single quadrupole
T – tailing factor
API - Active Pharmaceutical Ingredients

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