Reverse Phase-HPLC Method for Simultaneous Estimation of Tetracaine and Oxymetazoline in Bulk Samples

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Abstract
A simple, precise, reliable, rapid and reproducible reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Tetracaine HCl and Oxymetazoline HCl chromatography is carried out isocratically on Xbridge C18 (250 × 4.6 mm, 5μm) with a mobile phase composed of 0.1% OrthoPhosphoric Acid and Acetonitrile (90:10 (v/v) at a flow rate of 0.9 ml/min. Detection was carried out using a PDA detector at 290 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH-Q2 (R1) guidelines. The retention times of Tetracaine HCl and Oxymetazoline HC are 2.97, 6.97min respectively. The linearity range is 30-225 μg/ml for Tetracaine HCl and 150-1125 μg/ml for Oxymetazoline HCl respectively. The correlation coefficients of Tetracaine HCl and Oxymetazoline HCl are 0.9975 and 0.9782. The proposed method can be useful in quality control of bulk Manufacturing and Pharmaceutical dosage forms.
Key words: Tetracaine HCl and Oxymetazoline HCl , HPLC, Validation, Retention time.

INTRODUCTION

Tetracaine is a local anesthetic of the ester type and exerts its activity by blocking sodium ion channels required for the initiation and conduction of neuronal impulses. Oxymetazoline is an imidazoline derivative with sympathomimetic activity. It is believed to be a mixed α1/α2 adrenoceptor agonist and, by stimulating adrenergic receptors, it elicits vasoconstriction of dilated arterioles and reduces nasal blood flow [1].

Tetracaine HCl (Fig.1.0.) Tetracaine hydrochloride is an ester local anesthetic. Chemically it is 2-(dimethylamino) ethyl 4 (butylamino)benzoate hydrochloride. Its molecular weight is 300.8 for the hydrochloride salt and 264.4 for the free base. It is freely soluble in water and soluble in ethanol [2].
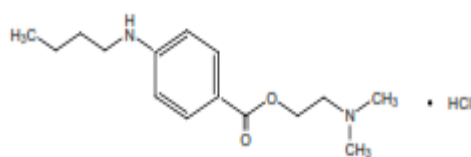

Fig. 1.0: Chemical structure of Tetracaine HCl

Oxymetazoline hydrochloride (Fig:2.0) is a vasoconstrictor. Chemically it is 3-[(4,5-dihydro-1*H*-imidazol-2-yl) methyl]-6-(1,1,-dimethylethyl)-2,4-dimethylphenol mono-hydrochloride. Its molecular weight is 296.8 for the hydrochloride salt and 260.4 for the free base. It is freely soluble in water and ethanol [1].
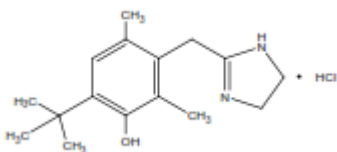

Fig. 2.0: Chemical structure of Oxymetazoline HCl

Various analytical techniques have been used for the determination of Tetracaine HCl which include high-performance liquid chromatography [2,5,6,16-19], Spectrometry [3,9-11,23], Fluorescene [4,7,8], capillary electrophoresis [12, 14], voltametry [13], gas chromatography [15,20-22] and for the determination of Oxymetazoline hydrochloride which include HPLC [25,26,31], spectroscopy [27, 28-30] methods have been used in the determination of concentration of Tetracaine HCl and Oxymetazoline hydrochloride in pharmaceutical preparations.

The literature review revealed that up today, no method has been published for simultaneous determination of Tetracaine HCl and Oxymetazoline HCl by RP-HPLC method. Hence, the present work was aimed to develop Simple, sensitive and reproducible RP-HPLC method for the determination of oxymetazoline hydrochloride in bulk (API) samples by RP-HPLC method. The developed method has been validated as per ICH guidelines [32].

MATERIALS AND METHODS

Instrumentation

Chromatographic separation was performed on a Agilent chromatographic system equipped with 1200 series isocratic pump; Rheodyne injector with 20μl fixed volume loop, variable wavelength programmable UV detector and the output signal was monitored and integrated by EZICHROME ELITE Chromatographic Software. Double beam UV-Visible spectrophotometer (Labindia-3120) was used to carry out spectral analysis and the data was recorded by UVWIN-5 software. Ultrasonicator (1.5L) was used for degasification of mobile phase and samples. Standard and sample drugs were weighed by using shimadzu electronic analytical balance (AX-220) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Chemicals and solvents

All chemicals and reagents used were of HPLC grade. Pure standards of Tetracaine HCl and Oxymetazoline HCl employed in the study were obtained as gift sample from MICRO LABS, Bangalore. The other reagents used were Methanol and Acetonitrile from Qualigens ltd. Mumbai, India, OrthoPhosphoric Acid from Hi-media, Mumbai, India, and HPLC grade water from Merck chemicals, Mumbai, India.

Chromatographic conditions

Separation and estimation was carried out using HPLC (waters-2469 with PDA detector), column used in experiment was Xbridge C18 (250 × 4.6 mm, 5µm). The mobile phase was prepared by mixing 0.1% OrthoPhosphoric Acid- pH adjusted to 2.5 buffer: acetonitrilein the ratio of (90:10) was filtered and degassed. Injection volume is 20µL and the detection was at 290nm.

Preparation of standard stock solution

Standard stock solution of Tetracaine (TC) and Oxymetazoline (OZ) API (pure drug) (1mg/ml) was prepared by accurately weighing about 100 mg drug and transferring in to 100 ml volumetric flask and dissolved in diluent.

Preparation of Buffer (0.1% OrthoPhosphoric Acid- pH adjusted to 2.5)

Accurately weighed and transferred 1ml of Concentrated Ortho phosphoric acid in a 1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degas in ultrasonic water bath for 10 minutes and finally make up the volume with water, then pH adjusted to 2.5 with dil. Ortho phosphoric acid solution. Filter through 0.45 µ filtered under vacuum filtration.

Preparation of mobile phase

The mobile phase used was freshly prepared 0.1% OrthoPhosphoric Acid buffer solution (P^H 2.5) and Acetonitrile in the ratio of 90:10 (v/v) and the mobile phase was filtered through 0.45 µ membrane filter and sonicated before use.

Preparation of Diluent

Transfer measured volume of 50ml methanol in 100ml volumetric flask and add 50ml of milli-Q water. Filtered through 0.45 µ membrane filter and sonicated before use.

METHOD VALIDATION

Specificity

A solution containing a mixture of tablet was prepared using sample preparation procedure and injected in to the system , to evaluate possible interfering peaks.

System suitability

System suitability tests were carried out on freshly prepared standard stock solution of Tetracaine HCl and Oxymetazoline HCl. Equal volume of Standard concentration was mixed well. From the prepared solution 20µl of the sample was injected into HPLC system and the results obtained were used to express the system suitability of the developed method. The results were depicted in Table.1.0.

**Table. 1.0: System precision of Tetracaine HCl and Oxymetazoline HCl**

|		
Retention Time	Tetracaine	2.83 min
	Oxymetazoline	7.42 min
Peak Area	Tetracaine	1963213
	Oxymetazoline	8824977
Theoretical plates	Tetracaine	3699
	Oxymetazoline	5430
Tailing Factor	Tetracaine	1.4
	Oxymetazoline	1.32
Resolution	Tetracaine	-
	Oxymetazoline	5.9

Linearity & Range

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of Tetracaine and Oxymetazoline. Linearity was assessed by performing single measurement at several analytes concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 30, 75, 105, 150, 180, 225 µg/ml of Tetracaine and 50, 75 525, 750, 900, 1125 µg/ml of Oxymetazoline. Injection was made at intervals of 10.0 min. Linearity of Tetracaine was found to be exist between 30-225 µg/ml and for Oxymetazoline was 50-1125 µg/ml. The chromatograms were recorded and linearity graph was plotted by using peak area of drug against respective concentrations to obtain the linearity range. The results were depicted in Table.2.0, Fig.3.0. and Fig.4.0.

Table. 2.0: Linearity and range of Tetracaine (TC) and Oxymetazoline (OZ)

S.NO	Concentration µg/ml	Area of TC	Concentration µg/ml	Area of OZ
1	30	386185	50	956891
2	75	960922	75	1105678
3	105	1355242	525	6040731
4	150	1963213	750	8824977
5	180	2357969	900	10595121
6	225	2942729	1125	13242269
Concentration range	30-225µg/ml		150-1125ml	
Slope (m)	150036		18956	
Correlation coefficient	0.9975		0.9782	
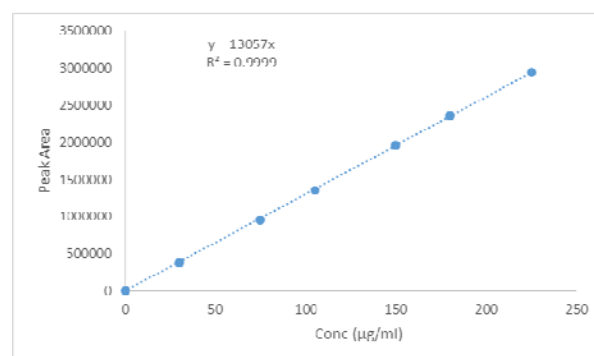
Fig:3: Linearity of Tetracaine (TC)
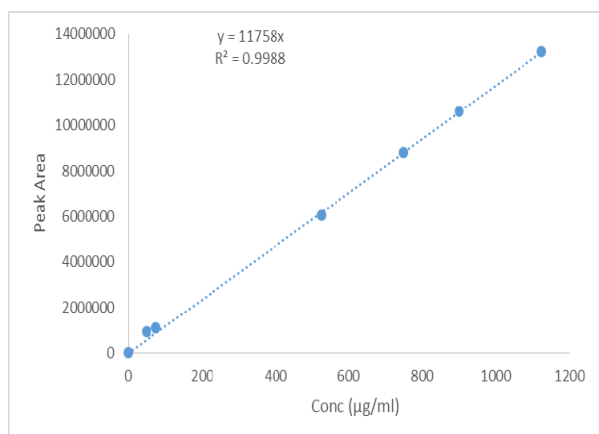
Fig:4: Linearity of Oxymetazoline (OZ)

Precision

The intra-day and inter-day precision studies were carried out with six replicates on the same day and different days. The results were depicted in Table. No- 3.0 to 4.0.

Table. 3.0: Intraday precision data for Tetracaine (TC) and Oxymetazoline (OZ)

Sample. No	Area of TC	Area of OZ
1.	1440866	6484965
2.	1428777	6594345
3.	1430407	6560373
4.	1423047	6576012
5.	1421048	6524590
6.	1442223	6501156
Mean	1431061	6540240
SD	8841.45	43451.43
%RSD	0.617	0.664

Table. 4.0: Interday precision data for Tetracaine (TC) and Oxymetazoline (OZ)

Sample. No	Area of TC	Area of OZ
1.	1416000	6987622
2.	1463054	6906570
3.	1494373	6729675
4.	1437579	6762279
5.	1459677	6873276
6.	1458394	6729367
Mean	1454846	6814798
SD	26354.6	107067.2
%RSD	1.80	1.50

Ruggedness

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from system to system and from analyst to analyst. It was carried out by using a test sample assay method with six replicates using different analyst, column and system. The results were depicted in Table. No- 5.0.

Table.5.0: Ruggedness data for Tetracaine (TC) and Oxymetazoline (OZ)

Sr. No.	TC (%Purity)			OZ (%Purity)		
	SET I	SET II	SET III	SET I	SET II	SET III
1	99.43	99.83	99.43	99.03	99.67	99.67
2	100.91	100.01	100.91	100.41	100.01	100.61
3	98.64	98.24	98.64	98.74	98.34	98.88
4	103.56	103.66	103.56	103.06	103.76	103.03
5	100.58	100.58	100.58	100.28	100.54	100.43
6	102.67	102.07	102.67	102.07	102.12	102.67
Average	100.80	100.10	100.80	100.40	100.23	100.37
SD	1.21	1.45	1.914	1.344	1.614	1.34
% RSD	1.4	1.2	1.6	1.56	1.53	1.33
Overall Average	101.9			100.71		
Overall % RSD	1.05			1.05		

SET – I   : Variability due to HPLC system
SET – II   : Variability due to HPLC column
SET – III   : Variability due to analyst

Robustness

Robustness was performed by change in mobile phase ratio, mobile phase flow rate and wavelength of the detector. The test was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentrations 100 µg/ml for TC and 100 µg/ml for OZ and %change was calculated. %change in the results was calculated. The results were depicted in Table. No- 6.0.

Table. 6.0. Robustness of Tetracaine (TC) and Oxymetazoline (OZ)

S. No	Parameter	Condition	TC		OZ	
			Area (n=3)	% change	Area (n=3)	% change
1	Standard	Standard conditions	1438318	0	6529991	0
2	Mobile phase	0.1%OrthoPhosphoric acid (pH-2.5): Acetonitrile (80:20%v/v)	1414243	0.1	6527790	0.14
		0.1%OrthoPhosphoric acid (pH-2.5): Acetonitrile (95:5%v/v)	1446992	1.33	6536602	0.55
3	Mobile phase pH	2.7	1432336	0.83	6526608	0.11
		2.3	1423680	0.52	6553068	0.56
4	Wavelength	288nm	1446791	0.19	6490488	0.07
		292 nm	1438318	0.23	6527425	1.51
5	Flow rate	1.2	1414243	0.25	6490465	0.07
		0.8	1446992	0.18	6527234	1.51

Limit of detection and Limit of quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines. LOD = 3 .3 × σ / S; L OQ = 1 0 × σ / S; Where σ is the standard deviation of y-intercepts of

regression lines and S is the slope of the calibration curve. The results were depicted in Table. No- 7.0.

Solution Stability

Solution stability was assed using standard and test stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 hrs and % differences was calculated. The results were depicted in Table. No.8.0 to 11.0.

Table.7.0: LOD and LOQ of Tetracaine (TC) and Oxymetazoline (OZ)

Parameter	TC	OZ
LOD(µg/ml)	2.50034	7.5767
LOQ(µg/ml)	2.94827	8.9341

Table.8.0. Solution Stability of Tetracaine (TC) at room temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	1438318	1438543	NA	1438324	1438312	NA
6hrs	1414243	1414240	0.1	1414268	1414245	0.7
12hrs	1446992	1446981	0.0	1446911	1446993	0.9
20hrs	1432336	1432333	0.3	1432362	1432339	1.2
26hrs	1423680	1423626	0.8	1423682	1423681	0.1
30hrs	1446791	1446791	0.2	1446797	1446795	0.4
36hrs	1433727	1433761	0.5	1433729	1433721	0.8

Table. 9.0: Solution Stability of Oxymetazoline (OZ) at room temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	6529991	6529992	NA	6529993	6529990	NA
6hrs	6527790	6527793	0.1	6527795	6527793	0.7
12hrs	6536602	6536645	0.0	6536601	6536605	0.9
20hrs	6526608	6526662	0.3	6526607	6526601	1.2
26hrs	6553068	6553061	0.8	6553068	6553067	0.1
30hrs	6490488	6490482	0.2	6490481	6490489	0.4
36hrs	6527425	6527421	0.5	6527424	6527421	0.8

Table. 10.0: Solution Stability of Tetracaine (TC) at refrigerated temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	1438312	1438318	NA	1438318	1438311	NA
6hrs	1414241	1414243	0.1	1414243	1414247	0.7
12hrs	1446993	1446990	0.0	1446991	1446996	0.9
20hrs	1432335	1432336	0.3	1432356	1432332	1.2
26hrs	1423686	1423680	0.8	1423620	1423685	0.1
30hrs	1446792	1446791	0.2	1446701	1446790	0.4
36hrs	1433723	1433727	0.5	1433722	1433722	0.8

Table. 11.0: Solution Stability of Oxymetazoline (OZ) at refrigerated temperature

| Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	6529991	6529993	NA	6529909	6529993	NA
6hrs	6527790	6527795	0.1	6527792	6527791	0.7
12hrs	6536602	6536602	0.0	6536601	6536600	0.9
20hrs	6526608	6526609	0.3	6526608	6526604	1.2
26hrs	6553068	6553060	0.8	6553062	6553012	0.1
30hrs	6490488	6490484	0.2	6490486	6490432	0.4
36hrs	6527425	6527422	0.5	6527423	6527421	0.8

Filter validation

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Test solution was prepared as per the test method. Some portion of the above solution was filtered through three different filters namely 0.45µm PVDF filter, 0.45µm PTFE and 0.45µm Nylon filter and some portion was centrifuged and injected into the HPLC system. The% difference values between centrifuged and filtered sample were calculated. The results were depicted in Table. No.12.0.

Table. 12.0: Filter Interference Results for Tetracaine (TC) and Oxymetazoline (OZ)

TC				
Filtration Method	Centrifuged	Nylon	PTFE	PVDF
Area (Inj. 1)	1438318	1438313	1438312	1438319
Area (Inj. 2)	1414243	1414244	1414241	1414242
Avg. Area	1446992	1446995	1446996	1446992
% Difference		-0.2	0.2	0.5
OZ				
Filtration Method	Centrifuged	Nylon	PTFE	PVDF
Area (Inj. 1)	6529990	6529990	6529994	6529991
Area (Inj. 2)	6527792	6527791	6527795	6527790
Avg. Area	6536603	6536602	6536602	6536602
% Difference		-0.4	0.3	0.5
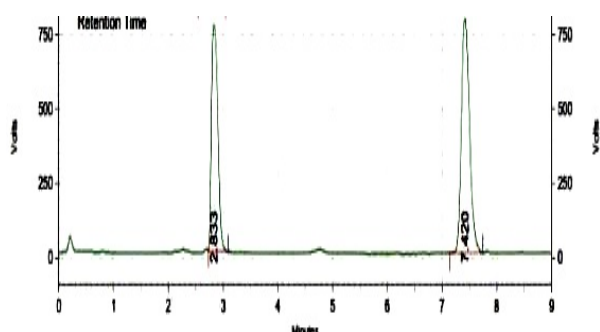
Fig.5.0: Standard chromatogram of Tetracaine HCl and Oxymetazoline HCl

RESULTS & DISCUSSION

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with 0.1%ortho Phosphoric Acid buffer: Acetonitrile (90:10 v/v) at flow rate of 1.0 ml/min was found to be robust method. The developed method was validated as per the ICH guidelines for the quantification of Tetracaine (TC) and Oxymetazoline (OZ) in bulk samples.

A suitability test was applied to various system suitability parameters and the results obtained were within acceptable limits of tailing factor ≤2.0 and theoretical plates >2000. The calibration curve was constructed with series of concentration in the range of 30-225 μg/ml and 50-1125 μg/ml for Tetracaine (TC) and Oxymetazoline (OZ). This concluded that the method was linear throughout the range selected. Specificity was studied for the quantification of impurities in the API of Tetracaine (TC) and Oxymetazoline (OZ). From the results it was indicated that none of excipients were interfere at analytes retention time. Hence the developed method was specific.

The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated and results were the acceptable limits. The low values of RSD indicate

that the method was precise. The % recovery for each case was calculated and was found to be 99.98 to 100.18% for TC and 99.51 to 100.09 for OZ and found to be results were within acceptance limits. Hence the developed method is accurate throughout the selected range.

Robustness test was carried out by small variation in the chromatographic conditions and %change was calculated. The %change in the results was calculated and it was found robust as % change was below 2.0%.

A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 2.50034μg/ml for TC and 7.5767g/ml for OZ and LOQ is found to be 2.94827μg/ml for TC and 8.934μg/ml for OZ.

Sample and standard solution are stable at 5°C for 36 hrs as the % difference in the area was found to be less than 2.0%. Filter interference was done on three types of 0.45μ filters (Nylon, PVDF) and the % difference was found to be below 2.0% for sample solutions and standard solutions calculated against centrifuged samples and standard.

CONCLUSION

Thus the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common impurities/excipients used in the manufacturing of pure drugs of Tetracaine (TC) and Oxymetazoline (OZ).

REFERENCES

1) British Pharmacopoeia, version 4, CD-ROM, Her Majesty's Stationery Office, (2005), pp.1185.
2) G. N. Menon, B. J. Norris. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography. 70 (5), 1981, 569–570.
3) Robert I. Ellin, Albert A. Kondritzer. The Spectrophotometric Determination of Tetracaine and Phenylephrine Hydrochloride, JPS, 1952, 41(2), 71–74.
4) Gan X, Liu S, Liu Z, Hu X. Determination of Tetracaine Hydrochloride by Fluorescence Quenching Method with Some Aromatic Amino Acids as Probes. Journal of Fluorescence, 2012, 22 (1), 129–135.
5) JiansongWang, JinrongLu.LingxiaZhang, YuzhuHu. Determination of cetylpyridinium chloride and tetracaine hydrochloride in buccal tablets by RP-HPLC. Journal of Pharmaceutical and Biomedical Analysis. 32(2), 2003, 381-386.
6) Lories I. Bebawy. Stability-Indicating Method for the Determination of Meloxicam and Tetracaine Hydrochloride in the Presence of their Degradation Products. An International Journal for Rapid Communication. 1998, 31(4), 797-820.
7) Qin MY, Liu SP, Liu ZF, Hu XL. Fluorescence quenching reaction of tetracaine hydrochloride–erythrosin system and its analytical application. Spectrosc Spect Anal. 2008, 28(2), 389–393.
8) Nevado BJJ, Pulgarin MJA, Escudero ROI. Determination of procaine and tetracaine in cocaine samples by variable-angle synchronous fluorimetry. Appl. Spectrosc. 2000, 54(11), 1678–1683.
9) Zhang R, Baeyens WRG, Weken GV, Calokerinos AC, Imai K. Chemiluminescence determination of some local anaesthetics. Anal Chim Acta. 1995, 303, 137–142.
10) Pasekova H, Polasek M. Determination of procaine, benzocaine and tetracaine by sequential injection analysis with permanganate-induced chemiluminescence detection. Talanta. 2000, 52, 67–75.
11) Fan J, Wang AJ, Feng SL, Wang JJ. Non-equilibrium determination of metoclopramide and tetracaine hydrochloride by

sequential injection spectrophotometry. Talanta. 2005, 66, 236–243.

12) Sun HW, Wen HF, Su M, Li LQ. Simultaneous determination Tetracaine and Procaine in blood plasma by Capillary electrophoresis—pyridine ruthenium electrochemical luminescence method. J. Instrum. Anal. 2000, 28(2):168–172.

13) Yang YF. Tetracaine in glassy carbon electrode's voltammetric behavior and determination. Chin J Anal Chem. 1999, 27(10):1156–1159.

14) Sun HW, Wen HF, Su M, Li LQ. Simultaneous determination Tetracaine and Procaine in blood plasma by Capillary electrophoresis—pyridine ruthenium electrochemical luminescence method. J Instrum Anal. 2009, 28(2):168–172

15) Masaru T, Mohammed NI, Zaw T, Katsuya H, Choei W, Tatsuo S, Jun-ichi Y, Toshinori Y, Yukio K. Determination of ester-type local anesthetic drugs (Procaine, Tetracaine, and T-caine) in human serum by wide-bore capillary gas chromatography with nitrogen-phosphorus detection. J Anal Toxicol. 1996, 20(5):318–322.

16) Grouls RJE, Ackerman EW, Korsten HHM, Hellebrekers LJ, Breimer DD. Partition coefficients(n-octanol/water)of N-butyl-p-aminobenzoate and other local anesthetics measured by reversed-phase high-performance liquid chromatography. J Chromatogr B. 1997, 694:421–425.

17) Zhang CH, Zhao Q, Kang SY, Chen B, Ma M, Yao SZ. Determination of local anaesthetics in human plasma by liquid-liquid-liquid microextraction coupled with high performance liquid chromatography. Chin J Anal Chem. 2006, 34(2):165–168.

18) Wang JS, Lu JR, Zhang LX, Hu YZ. Determination of cetylpyridinium chloride and tetracaine hydrochloride in buccal tablets by RP-HPLC. J Pharm Biomed Anal. 2003, 32:381–386.

19) Murtaza R, Jackman HL, Alexander B, Lleshi-Tali A, Winnie AP, Lgic R. Simultaneous determination of mepivacaine, tetracaine, and p-butylaminobenzoic acid by high-performance liquid chromatography. J Pharmacol Toxicol Meth. 2001, 46(3):131–136.

20) Shshima T, Takayasu T. Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography–mass spectrometry with solid-phase extraction. J Chromatogr B. 1999, 726:185–194.

21) Kudo K, Hino Y, Ikeda N, Inoue H, Takahashi S. Blood concentrations of tetracaine and its metabolite following spinal anesthesia. Forensic Sci Int. 2001, 116:9–14.

22) Hino Y, Ikeda N, Kudo K, Tsuji A. Sensitive and selective determination of tetracaine and its metabolite in human samples by gas chromatography-mass spectrometry. J Anal Toxicol. 2000, 24:165–169.

23) Qin MY, Liu SP, Liu ZF, Hu XL. Resonance Rayleigh scattering spectra, non-linear scattering spectra of tetracaine hydrochloride–erythrosin system and its analytical application. Spectrochim Acta Part A. 2009, 71:2063–2068.

24) Qin MY, Liu SP, Liu ZF, Hu XL. Resonance Rayleigh scattering spectra, of tetracaine hydrochloride–erythrosin system and its analytical application. J Southwest Univ (Nat Sci). 2008, 30(5):48–51.

25) Stanisz B, Nowiński W. Determination of oxymetazoline hydrochloride and decomposition products by high-performance liquid chromatography. Acta Pol Pharm. 2000, 57(6), 399-401.

26) Sirimas Sudsakorn. Leonard Kaplan. David A.Williams. Simultaneous determination of triamcinolone acetonide and oxymetazoline hydrochloride in nasal spray formulations by HPLC. Journal of Pharmaceutical and Biomedical Analysis. 2006, 40 (5), 1273-1280.

27) Nan-Nan Wang, Yan-Qing Shao, Yu-Hai Tang, He-Ping Yin, Xiao-Zhong Wu. Flow-injection chemiluminescence method for the determination of naphazoline hydrochloride and oxymetazoline hydrochloride. luminescence , Volume 24, Issue 3, May/June 2009, Pages 178–182.

28) Theia'a N. Al-Sabha and Bassam A. Rasheed. Spectrophotometric Determination of Oxymetazoline Hydrochloride Based on the Oxidation Reactions. Jordan Journal of Chemistry. 2011, 6(4), 403-411.

29) Theia'a N. Al-Sabha, Nidhal M. S. Mohammed, Payman A. Abdul-Jaba. Spectrophotometric determination of oxymetazoline hydrochloride via charge transfer reaction using 2,3-dichloro-5,6-dicyano1,4-benzoquinone (ddq) reagent. Eur. Chem. Bull., 2015, 4(8), 372-377

30) Safaa A. Zakaria. Spectrophotometric Determination of Oxymetazoline Hydrochloride Via Oxidative Coupling Reaction with 4-Aminoantipyrine in the Presence of Potassium Periodate. Raf. J. Sci., Vol. 22, No.4 pp 97-108, 2011.

31) Kabeer Ahmed Shaikh, and Ashish Tanaji Patil. Stability-Indicating HPLC Method for the Determination of Mometasone Furoate, Oxymetazoline, Phenyl Ethanol and Benzalkonium Chloride in Nasal Spray Solution. Journal of Trace Analysis in Food and Drugs. (2013) 1: 14-21.

32) ICH, Q2 (R1): Validation of Analytical Procedures: Text and Methodology. 2005 International Conference on Harmonization, Geneva, Switzerland.