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Determination of Thiomersal, Lidocaine and Phenylepherine in their Ternary Mixture

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Abstract

This work is concerned with the development and validation of two accurate, precise and specific spectrophotometric and chromatographic methods for analysis of Phenylepherine (PE), Lidocaine (LC) and Thiomersal (TM) in their ternary mixture and in Chromoderm® solution. The developed spectrophotometric method is the mean centering of ratio spectra (MCR) spectrophotometric method that depends on using the mean centered ratio spectra in two successive steps which eliminates the derivative steps and therefore the signal to noise ratio is enhanced. On the other hand, the developed chromatographic method isocratic RP-HPLC method that depends chromatographic separation on Zorbax C_{18} column using a mobile phase consisting phosphate buffer: acetonitrile: triethylamine (40:60:0.1, by volume pH=6 with orthophosphoric acid). The flow rate was 0.6 mL min^{-1} and the eluent was monitored at 245 nm.

Factors affecting the developed methods were studied and optimized. Moreover, they have been validated as per ICH guidelines. The developed methods have been successfully applied for determination of PE, LC and TM in different laboratory mixtures and in solution dosage form. Also, Statistical analysis of the results of the two developed methods with each other using F and student's t tests showed no significant difference.

Keywords: Phenylepherine; Lidocaine; Thiomersal; Mean centering of ratio spectra; RP-HPLC

Introduction

Phenylepherine (PE), chemically known as 3-[(1R)-1-hydroxy-2-(methylamino) ethyl] phenol [1]. It is an official drug in BP [2] and USP [3]. PE is a selective α1-adrenergic receptor agonist used primarily as a decongestant [4]. It is commonly used as a vasopressor to counteract the vasodilating effect of bacterial toxins and the inflammatory response in sepsis and systemic inflammatory syndrome [4,5]. Therefore, PE vasoconstricts arteries reducing bleeding and also delays resorption of Lidocaine, almost doubling the duration of anesthesia [6-8].

Lidocaine (LC), is chemically designated as (2-(diethyl amino)-N-(2,6-dimethyl phenyl)acetamide [1]. It is an official drug in BP [2] and USP [3]. LC is a common local anesthetic and antiarrhythmic drug [4]. It is used topically to relieve itching, burning, pain from skin inflammations [9] and is used as a local anesthetic for minor surgery [4,9-11]. It is characterized by rapid onset of action and intermediate duration of efficacy [12-14].

Thiomersal (TM) is (Ethyl(2-mercaptobenzoato-(2-)-O,S) mercurate(1-)sodium or mercury((O-carboxy phenyl)thio)ethyl sodium salt [1]. It is an official drug in BP [2] and USP [3]. TM acts as antiseptic and antifungal agent [4,15]. In multidose drug delivery systems, it prevents serious adverse effect such as the staphylococcus infection [16]. It has been also used as a preservative in many other topical products [17-20].

After meticulous research, it is found that none of the recognized pharmacopoeias reported an analytical method for determination of the studied drugs in their ternary mixtures. Also, there is no reports have been published for their determination together. On the other hand, all the reported methods have been developed only for determination of each of the proposed drugs either alone or in combination with other drugs. Therefore, the proposed methods are the first in the determination of the proposed mixture and, so there is no comparison with other techniques because till now there are no methods available.

Phenylepherine was determined by several techniques including colorimetric [5], spectrophotometric [21,22], RP-HPLC [8,21-24], and electro chemical [25] methods. Whereas, LC was determined by RP-HPLC [26-29] and electrochemical [11,13] methods. TM was determined by several methods such as spectrophotometric [16], HPLC [15,18,30-32], and electrochemical [19,32] methods. On the other hand, PE and LC were determined together by HPTLC [7] method while TM and PE was determined together by a spectrophotometric [33] method.

Due to the pharmaceutical importance of the studied drugs and the lacking in the methods developed for their determination in ternary mixture, this work aims to develop and validate accurate and precise spectrophotometric and chromatographic methods for determination of PE, LC and TM in their ternary mixture with the application to Chromoderm® solution which is used as analgesic, astringent, anaesthetic and antiseptic in minor surgery and wound infection.

Materials

Instruments

For spectrophotometric method, a double beam UV-Visible spectrophotometer with quartz cell of 1 cm path length, model UV-1601 PC (SHIMADZU, Japan) connected to IBM compatible computer. UVPC personal spectroscopy software version 3.7 was used.

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For HPLC method, the HPLC system (Agilent Chem Station HPLC B.04.03) consisted of a quaternary system with automatic injection facility, loop capacity 20 µL, UV-visible detector and LC solution version 1.25 software. The column used was Zorbax C_{18} (250×4.6 mm, 5 µm i.d). The detector was adjusted at 245 nm.

Samples

Pure standard samples: Phenylepherine (PE, 99.8%), Lidocaine (LC, 98.8%) and Thiomersal (TM, 99.2%) were kindly supplied by OCTOBERPHARMA (6th October, Cairo, Egypt).

Commercial product: Chromoderm® solution (Batch No.0380209) labeled to contain 0.25 gm% Phenylepherine, 1 gm% Lidocaine and 0.1 gm% Thiomersal, was kindly supplied by OCTOBERPHARMA(6th October, Cairo, Egypt).

Reagents

All solvents and chemicals used through this work were of analytical grade and were used without purification.

Hydrochloric acid and sodium hydroxide were purchased from EL-NASR Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt.

Deionized water purchased from (SEDICO, Pharmaceuticals co., Cairo, Egypt).

Acetonitrile, methanol, orthophosphoric acid, triethylamine and potassium dihydrogen phosphate were of HPLC grade (Sigma-Aldrich® Chemie GmbH, Germany).

Solutions

Stock standard solutions of PE, LC and TM in the concentration of 1 mgmL^{-1} were prepared in methanol. Working standard solutions of PE, LC and TM in the concentration of 0.1 mgmL^{-1}, were prepared in methanol (for spectrophotometric method) and in the used mobile phase (for HPLC method).

Procedures

Mean centering of ratio spectra method (MCR)

Spectral characteristics: Zero order absorption spectra of 20 µgmL^{-1} each of PE, LC and TM were recorded in the range of 200-400 nm using methanol as a solvent.

Linearity and range: The developed spectrophotometric method was validated according to ICH guidelines [34]. Accurate aliquots equivalent to 5-50 µgmL^{-1} of PE, 5-70 µgmL^{-1} of LC and 5-40 µgmL^{-1} of TM were transferred from their respective working standard solutions (0.1 mgmL^{-1}) into three series of 10-mL volumetric flasks, the volume was completed to the mark with methanol. The absorption spectra of the prepared solutions were scanned and the spectra in the range of 200-270 nm were used for measuring the studied drugs.

For PE, the recorded spectra were divided by standard spectrum of 10 µgmL^{-1} LC to obtain the first ratio spectra which is then mean centered. These vectors (mean centered ratio spectra) were then divided by the mean centered (MC) ratio of (αTM/αLC) corresponding to 10 µgmL^{-1} each, then mean centering of the second ratio spectra were then obtained. By the same way, the recorded spectra of LC were divided by the standard spectrum of 10 µgmL^{-1} PE and the obtained ratio spectra were mean centered, these vectors were divided by the mean centered ratio of (αTM/αPE) corresponding to 10 µgmL^{-1} each to obtain the second ratio spectra which were then mean centered. For TM, the scanned spectra of its prepared solutions were divided by the standard spectrum of 10 µgmL^{-1} LC and the obtained ratio spectra were mean centered. These vectors were divided by the mean centered ratio of (αTM/αLC) corresponding to10 µgmL^{-1} each to obtain the second ratio spectra which were then mean centered. The mean centered values of the second ratio spectra at 229.2-229.4 (peak to peak), 215.8-216 (peak to peak) and 216.8-217 (peak to peak) for PE, LC and TM, respectively were measured and plotted against the corresponding concentration of each drug to construct their respective calibration graphs.

Laboratory prepared mixtures: Different laboratory prepared mixtures containing different ratios each of PE, LC and TM within their calibration ranges were prepared. The spectra of these mixtures were then recorded and the procedure under construction of calibration curves was then followed but using the recorded spectra of the prepared mixtures.

RP-HPLC method

Chromatographic conditions: Chromatographic analysis was performed in isocratic mode with acetonitrile: buffer: triethylamine (60:40:0.1, by volume PH=6 with orthophosphoric acid) as a mobile phase delivered at 0.6 mL min^{-1}, injection volume 20 µL and scanning at 245 nm at room temperature.

Linearity and range: Linearity of the developed RP-HPLC method was evaluated by preparing standard solutions of each drug at different concentration levels ranging from 5-55 µgmL^{-1} for PE, 5-70 µgmL^{-1} for LC and 5-60 µgmL^{-1} for TM. Injections in triplicates were made for each concentration then the calibration curves were obtained by plotting the area under the curve against the corresponding concentration of each drug.

Laboratory prepared mixtures: Specificity of the method was ascertained by analyzing different mixtures containing the three components in different ratios following the procedure under linearity. By applying in the computed regression equations, concentrations of the studied drugs in the prepared mixtures could be obtained.

Application to commercial product

5 ml of Chromoderm® solution was accurately transferred (after vigorous shaking) into 50 mL measuring flask, sonicated in 50 mL methanol for 5 min to prepare sample stock solution containing 0.25 mg mL^{-1} PE, 1 mg mL^{-1} LD and 0.1 mg mL^{-1} TM. Appropriate dilutions of the prepared solution were made to prepare solutions within the linearity ranges of PE, LC and TM.

Results and Discussion

Methods development and optimization

Mean centering of ratio spectra spectrophotometric method: The UV absorption spectra of PE, LC and TM (Figure 1), displays considerable overlap, where the application of conventional spectrophotometry, its derivative and derivative ratio techniques failed to resolve these overlapping.

The developed MCR method depends on the mean centering of ratio spectra, it eliminates the derivative steps and therefore signal- to-noise ratio is enhanced [35]. It has been applied for resolving binary and ternary mixtures in complex samples with unknown matrices [35]. The mathematical explanation of the method was illustrated by Afkhami and Bahram [36-38].

In order to optimize the developed MCR method, effect of divisor concentration on the selectivity of the method has been tested. Different

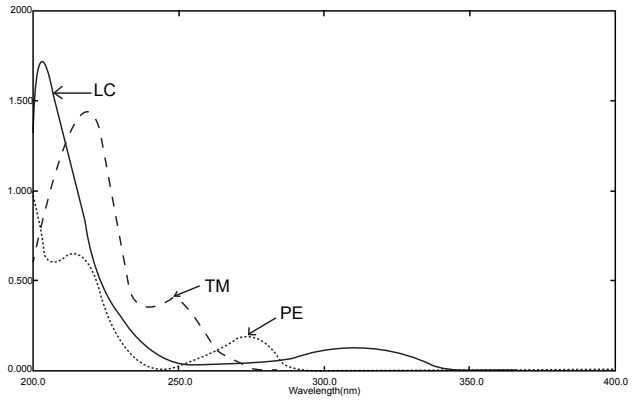

Figure 1: Zero order absorption spectra of 20 μgmL^{-1} each of LC (____), PE (…), TM (------) using methanol as a solvent.

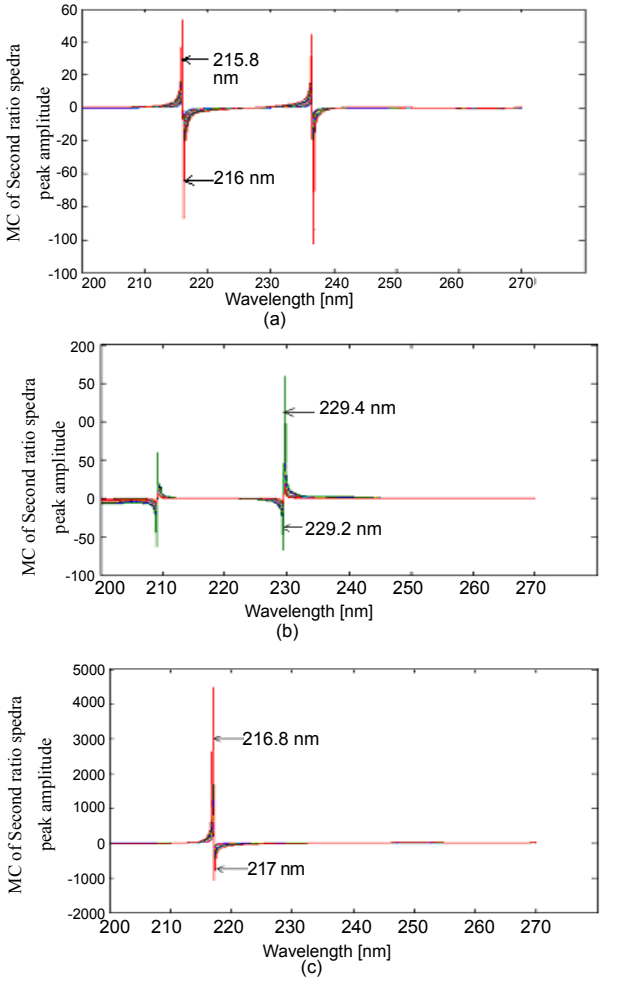

Figure 2: The mean centered second ratio absorption spectra of (a) LD, (b) PE, and (c) TM in methanol.

concentrations each of PE, LC and TM (5, 10, 20 and 30 μgmL^{-1}) were tested. It was found that the divisor had a great effect on the selectivity of determination of PE, LC and TM where reproducible and good results have been obtained upon using concentration of 10 μgmL^{-1} each of LC and TM (for PE), 10 μgmL^{-1} each of PE and TM (for LC) and 10 μgmL^{-1} each of PE and LC (for TM) as divisors.

After method optimization the studied drugs have been successfully determined at 229.2 & 229.4, 215.8 & 216 and 216.8 & 217 nm for PE, LC and TM, respectively as shown in Figure 2.

RP-HPLC method: An isocratic HPLC method was developed to provide a suitable procedure for rapid and reliable quality control analysis of PE, LC and TM in their combined pharmaceutical preparation. The most important aspect in LC method development is the achievement of sufficient resolution with acceptable peak symmetry in reasonable analysis time.

The parameters affecting the chromatographic separation such as stationary phase, mobile phase (composition and pH), flow rate and detection wavelength, had been studied and optimized.

For optimization of the stationary phase, several columns were tested (C_8 and C_{18}) where Zorbax C_{18} column gave acceptable, sharp and symmetrical peaks for all the separated components.

Concerning the mobile phase, the chosen mobile phase was one that gave stable baseline, adequate separation and sharp peaks in a suitable analysis time. Different mobile phases were tried to achieve best chromatographic separation, firstly type of organic modifier used is tested, methanol: water combination in different ratios (40-60% methanol) were tested, unluckily all the three components eluted together as a one peak. Another trial was the replacement of methanol with acetonitrile in different ratios (40-60% acetonitrile), it was observed that for all the tested ratios PE, LC eluted together as one peak even as TM eluted after very long time as a tailed peak except when using mobile phase containing 60% acetonitrile somewhere a little resolution among the studied drugs was obtained.

Effect of mobile phase pH on the separation was tested by changing water with 0.05 M potassium dihydrogen phosphate buffer (pH=3-8): acetonitrile (40:60, v/v,). On using acidic buffer (pH<6) PE and LC eluted as frocked peaks and hence bad resolution was obtained, on the other hand basic buffer (pH>7) brought about strong retention of TM on the stationary phase. Adjusting the buffer pH at pH 6 gave considerably good separation for the three components but with tailed broad peaks.

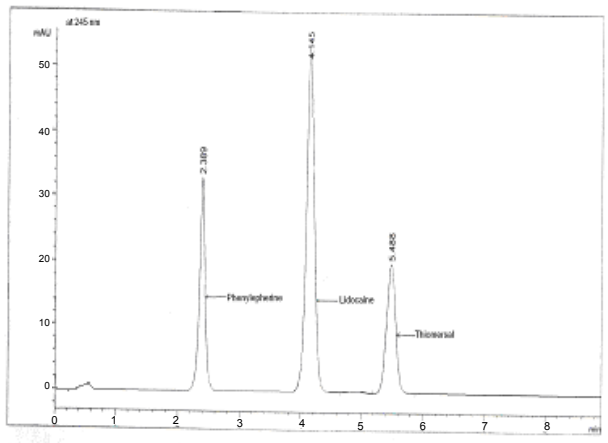

Figure 3: HPLC chromatogram of PE, LC and TM using phosphate buffer: acetonitrile: triethylamine (40:60:0.1, by volume at pH=6).

So as to improve the peaks a symmetry, triethylamine was added to the mobile phase in different ratios (0.05-0.1%) where acceptable peaks with minimum tailing were obtained upon using triethylamine (0.1%) in the mobile phase, thus improving performance and system suitability parameters of the chromatographic separation.

Different scanning wave lengths (230, 245, 260 and 280 nm) were tested in a trial to enhance the sensitivity of the method. Wavelength of 245 nm was the chosen scanning wavelength regarding signal to noise ratio. Different mobile phase flow rates were tried (0.5, 0. 6, 0.75 and 1 mL min^{-1}) to obtain maximum separation within realistic analysis time, delivering the mobile phase at 0.6 mL min^{-1} was found to be suitable for complete separation and good resolution.

Respectable chromatographic separation of the three components was achieved by using Zorbax C_{18} column, with mobile phase consisting of 0.05 M $KHPO_4$ (pH=6): acetonitrile: triethylamine (40:60:0.1, by volume) delivered at 0.6 mL min^{-1} followed by UV scanning at 245 nm. The respective compounds were well separated at reasonable retention times, where PE was eluted at 2.38 min, LC after 4.15 min and TM after 5.48 min (Figure 3).

Methods application

The developed spectrophotometric and RP-HPLC methods have been successfully applied for determination of the ternary mixture in Chromoderm® solution, where acceptable percentage recoveries were obtained (Table 1). Moreover, validity of the methods was established by applying standard addition technique where no interference from excepients was found (Table 1). The results obtained from the suggested methods for analysis of three studied drugs in their pure forms were statistically compared to those obtained by the reported methods [26,31] (using F and student's t-tests) and there was no significant difference among them (Table 2), regarding both accuracy and precision.

Methods validation

Validation has been performed according to ICH guidelines [34].

Linearity and range: Beer's Lambert's law was obeyed in the concentration ranges of 5-50, 5-70 and 5-40 μgmL^{-1} for PE, LC and TM, respectively (for spectrophotometric method) and in the range of 5-55, 5-70 and 5-60 μgmL^{-1} for PE, LC and TM, respectively (for RP-HPLC method). The evaluation parameters like correlation coefficients, intercept and slope values were calculated and presented in Table 2.

Accuracy: Accuracy of the spectrophotometric and RP-HPLC methods was judged by their application for determination of the drugs in different blind samples of pure drugs and calculating the percentage recoveries. Good results have been obtained and presented in Table 2. Accuracy was further evaluated by application of standard addition technique at different levels (80,100 and 120%), by addition of known amounts of pure drugs to known concentrations of Chromoderm® solution and then analyzing the prepared mixtures. Acceptable results were given in Table 1.

Precision: It was studied with respect to repeatability and intermediate precision. Repeatability was evaluated by repeating the assay of three different concentrations (10, 20 and 30 μg mL^{-1} for each drug) three times in the same day while intermediate precision was evaluated by assaying the same samples in triplicates on three successive days, using the developed chromatographic methods and calculating the percentage recoveries and RSD values. Results in Table 2 confirmed the satisfactory precision of the proposed methods.

Specificity

Spectrophotometric method: When the suggested spectrophotometric methods were applied for analysis of laboratory prepared mixtures containing PE, LC and TM in different ratios, good percentage recoveries and low SD values were produced (Table 1) confirming the high specificity of the developed methods.

RP-HPLC method: The specificity of the method was confirmed by good resolution of the proposed drugs as shown in the chromatogram (Figure 2). Also good percentage recoveries obtained upon applying the method to pharmaceutical preparation proved the high selectivity of the method. Values of resolution, selectivity and peak a symmetry factors were within the acceptable limits.

Robustness of RP-HPLC method

Robustness of the method was established by making small deliberate changes in the conditions of the chromatographic separation, e.g. changing in flow rate by ± 0.1 mL min^{-1}, changing in pH by ± 0.1 pH unit, changing the organic composition of the mobile phase by ± 1% and changing triethylamine percent by ± 0.02%, then calculating the resolution among the studied drugs. It was found that the changes in the studied parameters have no significant effect on the chromatographic resolution or peaks areas.

Parameters	Spectrophotometric method			RP-HPLC method		
	PE	LC	TM	PE	LC	TM
L.P Mixtures[a]	98.71 ± 0.940	98.55 ± 1.030	99.19 ± 0.938	98.68 ± 1.196	99.33 ± 1.497	100.39 ± 1.285
Chromoderm® solutionb (B.No.A0380209)	97.82 ± 1.104	101.41 ± 0.660	99.65 ± 0.578	97.87 ± 0.573	101.05 ± 1.102	100.28 ± 0.983
Standard addition[a]	99.72 ± 0.569	99.96 ± 0.724	98.97 ± 0.519	97.96 ± 1.107	98.82 ± 0.997	98.93 ± 0.543
Degree of freedom[c] F-test	10 (3.178) 2.363	10 (4.657) 3.178	7 (4.283) 1.588	8 (4.387) 4.202	7 (3.153) 2.418	7 (3.971) 3.605
Degree of freedom[c] Student'st- test	10 (2.100) 0.826	10 (2.100) 0.143	7 (2.179) 0.848	8 (2.200) 0.227	7 (2.231) 0.599	7 (2.178) 0.627

[a]Average of 3 determinations.
[b]Average of 6 determinations
[c]The values in the parenthesis are the corresponding theoretical values at p=0.05.
[c]Reported RP-HPLC method for PE and LC using Nucleosil5 C_{18} and methanol-0.01 M sodium dihydrogen phosphate (7:3) containing 0.005 M sodium dodecane sulphate as the mobile phase.
[c]Reported RP-HPLC for TM using anion exchange resin column and aqueous perchlorate solution as a mobile phase.

Table 1: Determination of the studied drugs in the laboratory prepared mixtures (L.P.) and pharmaceutical preparation by the proposed methods and statistical comparison with the reported RP-HPLC methods.

Parameters	Spectrophotometric method			HPLC method		
	PE	LC	TM	PE	LC	TM
Calibration range	5-50 μgmL^{-1}	5-70 μgmL^{-1}	5-40 μgmL^{-1}	5-55 μgmL^{-1}	5-70 μgmL^{-1}	5-60 μgmL^{-1}
Slope	5.0306	2.0664	1.4087	0.5861	1.1924	0.7678
Intercept	-0.0862	3.5372	-1.2562	-0.4193	0.3133	-0.2720
Correlation coefficient(r)	0.9999	0.9999	0.9998	0.9999	0.9999	0.9998
Accuracy	100.25	99.67	100.53	99.85	99.78	99.53
Precision						
Repeatability	1.635	0.340	1.027	1.037	1.382	1.188
Intermediate precision	0.582	0.548	0.348	0.680	0.627	0.870

Table 2: Regression and analytical parameters of the proposed methods for determination of Lidocaine, Phenylepherine and Thiomersal.

Parameters	Obtained value			Reference value
	PE	LC	TM	
Resolution(Rs)	2.5		1.7	>1.5
Relative retention(α)	1.9		1.32	>1
Tailing factor(T)	1	1	1	T=1, for a typical symmetrical peak
Capacity factor(Ḱ)	3.8	7.3	9.7	1-10 acceptable
Number of theoretical plates(n)	1825	2360	1685	Increase with the efficiency of the separation
HETP[b]	0.018	0.009	0.025	The smaller the value the higher the column efficiency

b=height equivalent to theoretical plate, ($cm.plate^{-1}$).

Table 3: Statistical analysis of parameters required for system suitability testing of RP-HPLC.

System suitability test for RP-HPLC method

The system suitability test confirms that the analytical procedure is valid as well as ensures the resolution between different peaks of interest. The system suitability parameters were calculated according to ICH guidelines and all parameters tested met the accepted criteria (Table 3).

Conclusion

The developed methods are the first developed methods for determination of the studied mixture with successful application to different laboratory prepared mixtures and marketed sample. The developed MCR method is simple, rapid and data processing steps do not need the application of complex algorithms. Also, it does not need derivative steps and so signal to noise ratio has been enhanced.

On the other hand, the developed RP-HPLC method is more specific but it needs high cost equipment and materials. Moreover, all the obtained results confirmed the applicability, accuracy and precision of these methods.

References

1. Budavari S (2002) The Merk Index, An Encyclopedia of Chemicals, Drugs and Biologicals, (13th edn), Merck and Co. Inc., Whitehouse Station NJ, USA.
2. The British Pharmacopoeia (2007) Her Majestys. The Stationary Office, London, UK.
3. The United States Pharmacopoeia (2007) National Formulary 25, United States Pharmacopoeia Convention Inc. (30th edn), 713: 1427.
4. Martindale-Extra Pharmacopoeia, (34th edn) The Complete Drug References. The Pharmaceutical Press, London, UK.
5. Auerbach ME (2006) Colorimetric determination of Phenylephrine (neo-synephrine) in pharmaceutical products. J Pharm Sci 39: 50-52.
6. Vaida GT, Moss P, Capan LM, Turndorf H (1986) Prolongation of lidocaine spinal anesthesia with phenylephrine. AnesthAnalg 65: 781-785.
7. Devarajan PV, Adani MH, Gandhi AS (2000) Simultaneous determination of lignocaine hydrochloride and phenylephrine hydrochloride by HPTLC. J Pharm Biomed Anal 22: 685-690.
8. Sheikh S, Asghar S, Patni SA (2012) A validated specific stability indicating reverse phase liquid chromatographic method for the simultaneous estimation of PhenylepherineHCl in pharmaceutical ointment. Int J Sci R Pub 2.
9. Sporkert F, Pragst F (2000) Determination of lidocaine in hair of drug fatalities by headspace solid-phase microextraction. J Anal Toxicol 24: 316-322.
10. Cox SK, Hamner T, Bartges J (2005) Monoethylglycinexylidide and lidocaine determination in porcine microsomal preparations. J Pharm Biomed Anal 37: 801-804.
11. Gamze T, Gulcin B, Mehmet OA, Serdar A (2012) Determination of Lidocaine based on electrocatalysis of a chemically modified electrode. Turkian J Chem 36: 593-600.
12. de Jong GJ, Koster EHM (2000) Solid-Phase micro-extraction in bioanalysis, exemplified by Lidocaine determination. Chromatographia 52: S12-S13.
13. Oliveria RTS, Banda GRS, Ferreia VS, Oliveria SC, Avaca LA (2007) Electroanalytical determination of Lidocaine in pharmaceutical preparations using Boron-Doped Diamond Electrodes, Electroanalysis 19: 1189-1194.
14. Deglin SM, Deglin JM, Wurtzbacher J, Litton M, Rolfe C, et al. (1980) Rapid serum lidocaine determination in the coronary care unit. JAMA 244: 571-573.
15. Lam SW, Meyer RC, Takahashi LT (1981) Determination of thimerosal in ophthalmic solutions by radial compression separation HPLC. J Parenter Sci Technol 35: 262-265.
16. Shrivastaw KP, Singh S (1995) A new method for spectrophotometric determination of thiomersal in biologicals.Biologicals 23: 65-69.
17. Santos EJ, Herrmann AB, Santos AB, Baik LM, Sato CS, et al. (2010) Determination of Thiomersal in human and veterinarian vaccines by photochemical vapor generation coupled to ICP OES, J. Anal. At.Spectrom 25: 1627-1632.
18. Reader MJ, Lines CB (1983) Decomposition of thimerosal in aqueous solution and its determination by high-performance liquid chromatography. J Pharm Sci 72: 1406-1409.
19. Silva MP, Rodriguez J, Hemandez L (1994) Electrochemical oxidative determination of Thiomersal in soft contact lens care solutions by cyclic voltammetry. Analyst 119: 1971-1974.
20. HE Peng, QIU Shaoli, Yufen GUO (2010) Determination of Thiomersal content in biological products by various methods Chinese J Biologic 2.
21. Nora H Al-Shaalan (2010) Determination of Phenylepherine hydrochloride and chloropheniramine maleate in binary mixture using chemometric assisted spectrophotometric and high-performance liquid chromatographic-UV methods. J Pharm Sci 14: 15-21.
22. Khoshay RM, Abdollahi H, Ghaffari A, Shariatpanahi M, Farzanegan H, Simultaneous spectrophotometric determination of Paracetamol, phenylepherine and chloropheniramine in pharmaceuticals using chemometricapproachs. Daru 18: 292-297.
23. Amer SM, Abbas SS, Shehata MA, Ali NM (2008) Simultaneous determination of phenylephrine hydrochloride, guaifenesin, and chlorpheniramine maleate in cough syrup by gradient liquid chromatography. J AOAC Int 91: 276-284.
24. Palabiyik IM,Onur F (2007) The simultaneous determination of Phenylepherine hydrochloride, Paracetamol, Chlorpheniramine maleate and dextromethorphan hydrobromidein pharmaceutical preparations. Chromatographia, 66: 93-96.
25. Kang L, Mingfang Z, Jie Z (2013) Electrochemical determination of phenylepherine hydrochloride based on Garphene-Tio2 Modified Glassy Carbon Electrode. Int J ElectrochemSci 8: 4047-4054.
26. Helboe P, Thomsen M (1979) Single dose determination of suppositories containing phenylepherine, Lidocaine and beta methasonevalerate by reversed-phase ion pair liquid chromatography, Int J Pharm 2: 317-324.
27. Kang L, Jun HW, McCall JW (1999) HPLC assay of Lidocaine in plasma with solid phase extraction and UV detection. J Pharm Biomed Anal 19: 737-745.
28. Junior ER, Bentley MVLB,Marchetti JM (2002) HPLC assay of Lidocaine in vitro dissolution test of thepoloxamer 407 gels. RBCF 38.

29. O'Neal CL, Poklis A (1996) Sensitive HPLC for simultaneous quantification of lidocaine and its metabolites monoethylglycinexylidide and glycinexylidide in serum. Clin Chem 42: 330-331.

30. Meyer RC, Cohn LB (1978) Thimerosal determination by high-pressure liquid chromatography. J Pharm Sci 67: 1636-1638.

31. Fu CC, Sibley MJ (1978) Quantitative high-pressure liquid chromatographic determination of thimerosal in pharmaceutical formulation of Thimerosal in pharmaceutical formulations. J Pharm Sci 67: 1636-1638.

32. Kang SH, Kim H (1997) Simultaneous determination of methylparaben, propylparaben and thimerosal by high-performance liquid chromatography and electrochemical detection. J Pharm Biomed Anal 15: 1359-1364.

33. Collado MS, Mantovani VE, Goicoechea HC, Olivieri AC (2000) Simultaneous spectrophotometric multivariate calibration determination of several components of ophthalmic solutions: phenylepherine, chloramphenicol, antipyrine, methylparaben and Thimerosal. Talanta 52: 909-920.

34. ICH Q2 (R1) (2005) Validation of analytical procedures. Proceedings of the International Conference on Harmonization, Geneva

35. Abdelaleem EA, Abdelwahab NS (2012) Simultaneous determination of some antiprotozoal drugs in different combined dosage forms by mean centering of ratio spectra and multivariate calibration with model updating methods. Chem Centr J 6: 27.

36. Afkhami A, Bahram M (2004) Mean centering of ratio kinetic profiles as a novel spectrophotometric method for the simultaneous kinetic analysis of binary mixtures. Anal ChimActa 526: 211-218.

37. Afkhami A, Bahram M (2005) Mean centering of ratio spectra as a new spectrophotometric method for the analysis of binary and ternary mixtures. Talanta 66: 712-720.

38. Afkhami A, Bahram M (2006) A novel spectrophotometric method for the simultaneous kinetic analysis of ternary mixtures by mean centering of ratio kinetic profiles. Talanta 68: 1148-1155.