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Stability-indicating HPLC assays for the determination of prilocaine and procaine drug combinations

Meredith L. Storms, James T. Stewart *

Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA 30602-2352, USA

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Abstract

Stability-indicating, reversed phase high-performance liquid chromatographic (HPLC) methods have been developed for the determination of several procaine hydrochloride and prilocaine hydrochloride combinations. The separation and quantitation of epinephrine–prilocaine and epinephrine–procaine drug combinations were achieved on a phenyl column using a mobile phase of 80:20% v/v 25 mM phosphate buffer (pH 3.0) containing 50 mM heptanesulfonic acid sodium salt–acetonitrile at a flow rate of 1 ml min^{-1} and UV detection at 254 nm. The method showed linearity for the epinephrine and prilocaine hydrochloride mixture in the 0.25–2.5 and 8–200 μg ml^{-1} ranges, respectively. The intra- and inter-day relative standard deviations (RSDs) ranged from 0.26 to 2.05% and 0.04 to 0.61% for epinephrine and prilocaine hydrochloride, respectively. The epinephrine and procaine hydrochloride mixture yielded linear ranges of 0.25–2.0 and 5–100 μg ml^{-1} and intra- and inter-day RSDs ranged from 0.23 to 1.88% and 0.07 to 0.26% for epinephrine and procaine hydrochloride, respectively. The assays were shown to be suitable for measuring epinephrine–prilocaine and epinephrine–procaine combinations in their respective injection dosage forms. Stability-indicating HPLC assays were also developed for several other procaine drug combinations since their monographs are present in the USP 24; however, quantitation was not investigated since these combinations are not commercially available. A mobile phase consisting of 80:20% v/v 25 mM phosphate buffer (pH 3.0) containing 50 mM heptanesulfonic acid–acetonitrile was utilized for the levonordefrin–tetracaine–procaine drug combination, while a mobile phase consisting of 70:30% v/v 25 mM phosphate buffer (pH 3.0) containing 50 mM heptanesulfonic acid sodium salt–acetonitrile was utilized for the separation of levonordefrin–procaine–propoxycaine and norepinephrine–procaine–propoxycaine. All separations were achieved on a phenyl column at a flow rate of 1 ml min^{-1} and UV detection at 254 nm.

Keywords: Prilocaine; Procaine; Epinephrine; Norepinephrine; Levonordefrin; Propoxycaine; Tetracaine; HPLC; Stability-indicating

1. Introduction

Local anesthetics play an important role clinically in dentistry and minor surgery for temporary relief of pain. Local anesthetics may be applied

* Corresponding author. Tel.: +1-706-542-5770; fax: +1-706-542-5358
E-mail address: jstewart@rx.uga.edu (J.T. Stewart).

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either topically or parenterally to a localized area to produce a state of local anesthesia by reversibly blocking the nerve conductances that transmit the feeling of pain [1]. Prilocaine hydrochloride, procaine hydrochloride, tetracaine, and propoxycaine hydrochloride contain in their chemical structure a secondary or tertiary amino group connected to an aromatic residue. The aromatic residue is connected to the intermediate group by an ester or amide linkage for which local anesthetics are typically classified. The type of linkage along with other structural changes in the molecule affects potency, duration of action, rate of metabolism, and toxicity [2]. Since the duration of action of a local anesthetic is proportional to the time during which it is in contact with the nerve, vasoconstrictors are often combined with local anesthetic preparations to decrease the rate at which the local anesthetics are absorbed into the circulation [1]. Thus, epinephrine, norepinephrine, and levonordefrin have all been used in conjunction with local anesthetics by various medical professions.

A literature survey revealed that a spectrophotometric assay was reported for the determination of epinephrine and procaine hydrochloride [3,4]; however, no high-performance liquid chromatographic (HPLC) method was available for this combination. For the epinephrine–prilocaine combination, a USP 24 monograph using HPLC is available, but the method involves two different mobile phases as well as two detectors (Electrochemical detector (ECD) for epinephrine and UV for prilocaine hydrochloride) [5]. The separation and determination of procaine–tetracaine with other local anesthetics by micellar liquid chromatography [6,7], GC-MS [8], HPLC with UV detection [9], gas chromatography-nitrogen phosphorous detector (GC-NPD) [10], and spectrophotometry [11] have also been reported. Norepinephrine and epinephrine have been determined via HPLC-ECD [5,12], HPLC with fluorescence detection [13], ion-pair HPLC [14], thin layer chromatography (TLC) [15], and GC-MS [16]. Levonordefrin has been determined utilizing ion-pair HPLC [17] and spectrophotometry [18,19]. The existing USP 24 monographs for levonordefrin–tetracaine–procaine involve three different spectrophotometric assays to determine each analyte individually [19]. The USP 24 monographs for levonordefrin–procaine–propoxycaine and norepinephrine–procaine–propoxycaine each involve two different spectrophotometric assays for the determination of the analytes [18,20].

In this article, stability-indicating assays have been developed for the determination of epinephrine–prilocaine, epinephrine–procaine, levonordefrin–tetracaine–procaine, norepinephrine–procaine–propoxycaine, and levonordefrin–procaine–propoxycaine combinations using HPLC with UV detection. The compounds are separated on a phenyl column using an ion-pair mobile phase. The assays were applied to the commercially available epinephrine–prilocaine (4% Citanest® Forte) combination and to a laboratory-compounded injection solution of 20 μg ml^{-1} epinephrine and 10 mg ml^{-1} procaine. The separations were achieved within 20 min for all analytes in each drug mixture. The HPLC separations for the other procaine drug combinations are also shown since their monographs are included in the USP 24 [18–20].

2. Experimental

2.1. Reagents and chemicals

The structures and formulae of the compounds studied are shown in Fig. 1. Tetracaine, propoxycaine hydrochloride, procaine hydrochloride, and levonordefrin reference standards were purchased from the United States Pharmacopoeial Convention, Inc. (Rockville, MD). Norepinephrine bitartrate, epinephrine bitartrate, prilocaine hydrochloride and heptanesulfonic acid sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO). 4% Citanest® Forte (40 mg ml^{-1} prilocaine hydrochloride and 5 μg ml^{-1} epinephrine injection, USP) (Lot: 009061, Expiration: 09/01) was purchased from Astra (Westborough, MA). A compounded solution of epinephrine (20 μg ml^{-1}) and procaine hydrochloride (10 mg ml^{-1}) was prepared in the laboratory. Acetonitrile (J.T. Baker, Phillipsburg, NJ) was HPLC grade. Monobasic potassium dihydro-

gen phosphate (KH_2PO_4) and concentrated phosphoric acid were Baker analyzed reagents.

2.2. Apparatus

A Beckman Model 110-B pump (Fullerton, CA), a Rheodyne Model 7125 injection valve equipped with a 20 μl loop (Rheodyne, Cotati, CA), a Waters 486 UV–VIS detector (Waters Corp., Milford, MA), and a Shimadzu C-R3A chromatopac integrator (Shimadzu Corp., Columbia, MD) constituted the HPLC system used in this study. Separations were accomplished on a μ-Bondapak™ phenyl column (300 × 3.9 mm i.d., Waters, Millford, MA).

2.3. Chromatographic conditions

Chromatographic analysis was carried out at ambient temperature. The isocratic separations of the epinephrine–prilocaine, epinephrine–procaine, and levonordefrin–tetracaine–procaine combinations were achieved with a buffered ion-pair mobile phase (25 mM potassium phosphate

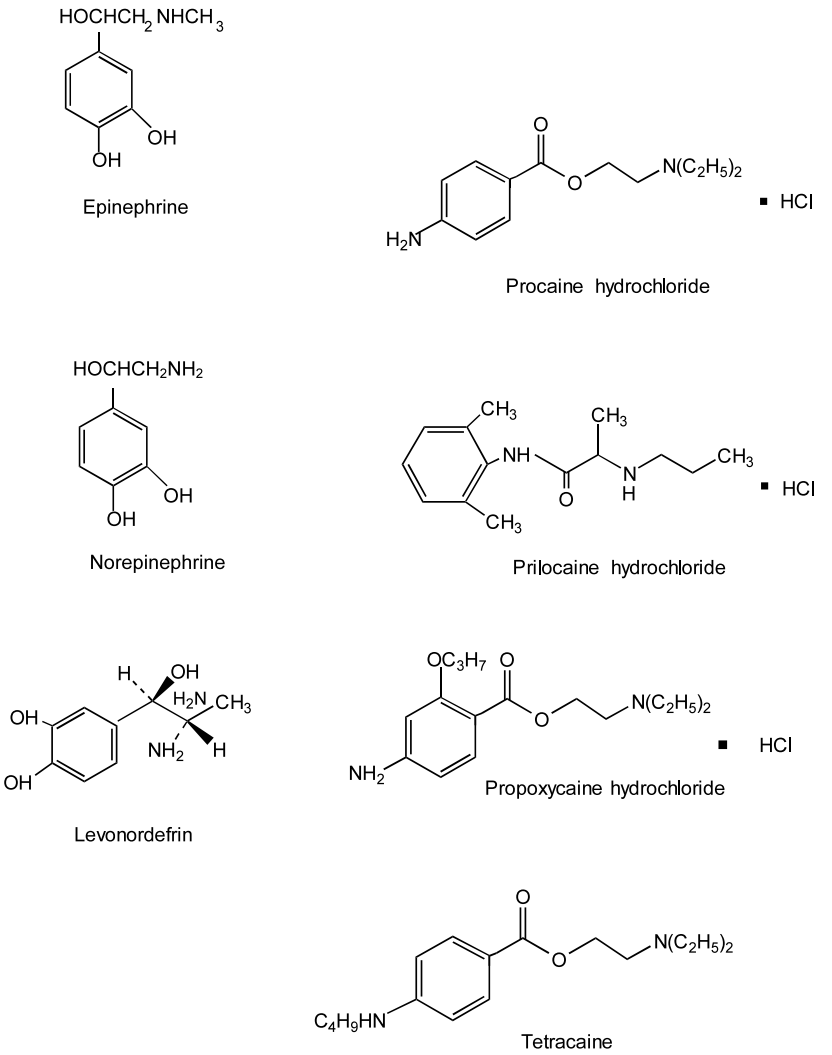

Fig. 1. Chemical structures of compounds studied.

monobasic in water (pH 3.0) with 50 mM heptanesulfonic acid sodium salt–acetonitrile (80:20% v/v)). The mobile phase for the separation of norepinephrine–procaine–propoxycaine and levonordefrin–procaine–propoxycaine drug combinations was composed of a buffer solution (25 mM potassium phosphate monobasic in water (pH 3.0) with 50 mM heptanesulfonic acid sodium salt–acetonitrile (70:30% v/v)). Each mobile phase was filtered through a 0.45 μm nylon-66 filter (Alltech, Deerfield, IL) and degassed by sonication prior to use. The flow rate was set at 1 ml min^{-1}. The UV detector was set at 254 nm.

2.4. Standard solutions

A combined standard solution containing epinephrine and prilocaine hydrochloride was prepared by accurately weighing 1.0 mg of epinephrine and transferring to a 5 ml volumetric flask with mobile phase added to volume. A 1:40 dilution was made in mobile phase to give a 5 μg ml^{-1} epinephrine solution. Then, 40 mg of prilocaine hydrochloride was added to result in the combined standard solution. Appropriate dilutions were made in mobile phase to obtain solutions containing 2.5, 1, 0.5, 0.33, and 0.25 μg ml^{-1} of epinephrine and 200, 80, 40, 20, and 8 μg ml^{-1} of prilocaine hydrochloride.

A combined standard solution containing epinephrine and procaine hydrochloride was prepared by accurately weighing 1 mg of epinephrine and transferring to a 5 ml volumetric flask with mobile phase added to volume. A 1:10 dilution was made in mobile phase to result in a 20 μg ml^{-1} epinephrine solution. Then, 10 mg of procaine hydrochloride was added to result in the combined standard solution. Appropriate dilutions were made in mobile phase to obtain solutions containing 2, 1, 0.8, 0.5, 0.25 μg ml^{-1} of epinephrine and 100, 50, 20, 10, and 5 μg ml^{-1} of procaine hydrochloride.

A combined standard solution containing levonordefrin, tetracaine, and procaine hydrochloride was prepared by accurately weighing 250 μg of levonordefrin, 7.5 mg of tetracaine, and 100 mg of procaine hydrochloride and transferring to a 5 ml volumetric flask with mobile phase added to volume. For the separation, a 1:250 dilution was made in mobile phase prior to the HPLC injection.

A combined standard solution containing levonordefrin, procaine hydrochloride, and propoxycaine hydrochloride was prepared by accurately weighing 250 μg of levonordefrin, 100 mg of procaine hydrochloride, and 20 mg of propoxycaine hydrochloride and transferring to a 5 ml volumetric flask with mobile phase added to volume. A 1:250 dilution was made in mobile phase prior to separation by HPLC.

A combined standard solution containing norepinephrine, procaine hydrochloride, and propoxycaine hydrochloride was prepared by accurately weighing 167 μg of norepinephrine, 100 mg of procaine hydrochloride, and 20 mg of propoxycaine hydrochloride and transferring to a 5 ml volumetric flask with mobile phase added to volume. For the separation, a 1:250 dilution was made in mobile phase prior to the HPLC injection.

2.5. Application of the proposed method to dosage forms

A 1 ml aliquot of the commercially available injection (Citanest® Forte) equivalent to 5 μg of epinephrine and 40 mg of prilocaine hydrochloride was transferred to a 5 ml test tube. For the analysis of epinephrine, a 1:12 dilution was made utilizing mobile phase to result in an epinephrine concentration of 0.42 μg ml^{-1}. A 1:800 dilution in mobile phase resulted in a concentration of 50 μg ml^{-1} for the analysis of prilocaine hydrochloride.

Since epinephrine and procaine hydrochloride are not commercially available prepackaged in the United States, an injection solution was prepared in the laboratory. This solution was prepared by accurately weighing 100 μg epinephrine and 50 mg of procaine hydrochloride USP reference standard and transferring to a 5 ml volumetric flask and filled to volume with mobile phase to result in a combined solution containing 10 mg ml^{-1} of procaine hydrochloride and 20 μg ml^{-1} of epinephrine. For the analysis of epinephrine, a 1:22 dilution was made in mobile phase. A 1:300

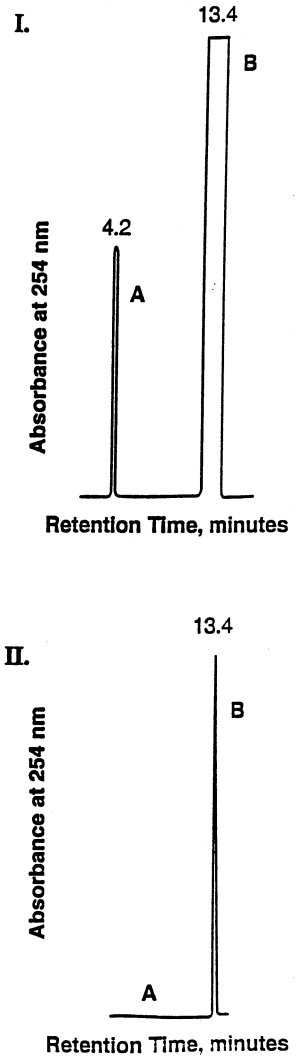

Fig. 2. Typical HPLC chromatogram of epinephrine (A) and prilocaine hydrochloride (B). (I) Typical HPLC chromatogram for quantitation of A. (II) Typical HPLC chromatogram for quantitation of B. See Section 2 for assay conditions.

dilution in mobile phase was utilized for the analysis of procaine hydrochloride.

3. Results and discussion

The goal of this study was to develop stability-indicating HPLC assays for the analysis of five local anesthetic drug combinations: epinephrine–prilocaine, epinephrine–procaine, levonordefrin–tetracaine–procaine, levonordefrin–procaine–propoxycaine, and norepinephrine–procaine–propoxycaine. Initial studies to develop HPLC assays involved the use of C_{18}, C_8, and phenyl columns with various mobile phases containing acetonitrile– or methanol–aqueous phosphate buffers. In addition, a bare silica column with a buffered aqueous–organic mobile phase was investigated since this laboratory has previously reported HPLC methods to analyze basic, acidic, and neutral compounds in pharmaceutical dosage form and biological samples using underivatized silica [21–24]. In each study, epinephrine, levonordefrin and norepinephrine eluted too close to the solvent front. However, the phenyl column was chosen for further ion-pair studies since it produced sharp and symmetrical peaks.

Thus, our attention turned to the use of heptanesulfonic acid sodium salt as an ion-pair reagent to increase the retention time of epinephrine, norepinephrine, and levonordefrin. An HPLC assay utilizing an ion-pair mobile phase for the determination of levonordefrin has been reported [17]. In addition, the USP 24 monograph for the epinephrine and prilocaine injection uses an ion-pair mobile phase for the determination of epinephrine by HPLC-ECD [5]. Since there were no reports describing the separation of the selected drug mixtures utilizing HPLC with UV detection, we investigated various ion-pair concentrations in the mobile phase with the phenyl column.

The final selective HPLC mobile phase consisting of 80:20% v/v phosphate buffer (pH 3.0) with heptanesulfonic acid sodium salt–acetonitrile and a phenyl column provided chromatograms (Figs. 2–4) with a steady base line and the specificity required for the separation of epinephrine–prilocaine, epinephrine–procaine, and levonordefrin–tetracaine–procaine drug combinations. The HPLC mobile phase consisting of 70:30% v/v phosphate buffer (pH 3.0) with heptanesulfonic acid sodium salt–acetonitrile and a phenyl column provided chromatograms (Figs. 5 and 6) with the specificity required for the separation of norepinephrine–procaine–propoxycaine and levonordefrin–procaine–propoxycaine drug combinations.

3.1. Linearity

Linearities were demonstrated for the epinephrine–prilocaine combination from 20 μl injections of solutions containing quantities of epinephrine (2.5, 1, 0.5, 0.33, and 0.25 μg ml^{-1}) and prilocaine hydrochloride (200, 80, 40, 20, and 8 μg ml^{-1}). Linearities were demonstrated for the epinephrine–procaine combination from 20 μl injections of solutions containing quantities of

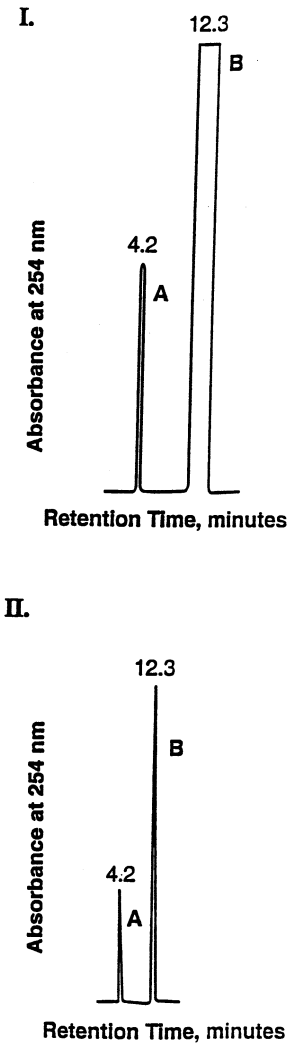

Fig. 3. Typical HPLC chromatogram of epinephrine (A) and procaine hydrochloride (B). (I) Typical HPLC chromatogram for quantitation of A. (II) Typical HPLC chromatogram for quantitation of B. See Section 2 for assay conditions.

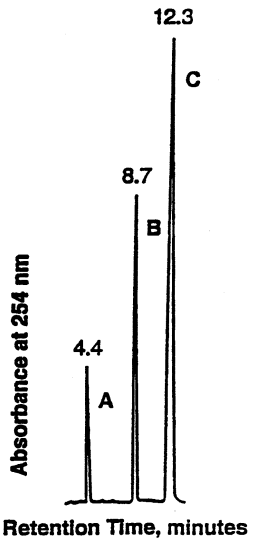

Fig. 4. Typical HPLC chromatogram of levonordefrin (A), tetracaine (B), and procaine hydrochloride (C) based on 1:150 dilution. See section 2 for assay conditions.

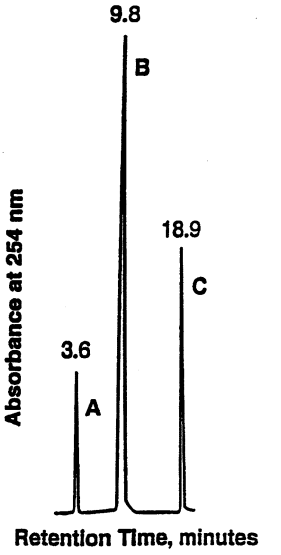

Fig. 5. Typical HPLC chromatogram of levonordefrin (A), procaine hydrochloride (B), and propoxycaine hydrochloride (C) based on 1:150 dilution. See section 2 for assay conditions.

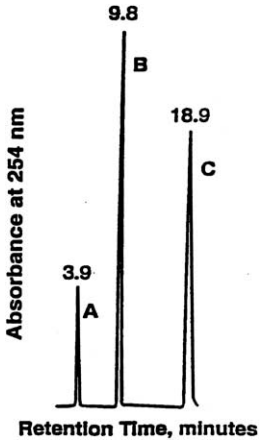

Fig. 6. Typical HPLC chromatogram of norepinephrine (A), procaine hydrochloride (B), and propoxycaine hydrochloride (C) based on 1:150 dilution. See section 2 for assay conditions.

epinephrine (2, 1, 0.8, 0.5, and 0.25 μg ml^{-1}) and procaine hydrochloride (100, 50, 20, 10, and 5 μg ml^{-1}). Linearities were demonstrated for the levonordefrin–tetracaine–procaine combination from 20 μl injections of solutions containing quantities of levonordefrin (2.5, 1.25, 1, 0.63, 0.5, and 0.31 μg ml^{-1}), tetracaine (75, 37.5, 30, 20, 15, and 10 μg ml^{-1}), and procaine hydrochloride (100, 50, 20, 10, and 5 μg ml^{-1}). Linearities were demonstrated for the levonordefrin–procaine–propoxycaine combination from 20 μl injections of solutions containing quantities of levonordefrin (2.5, 1.25, 1, 0.63, 0.5, and 0.31 μg ml^{-1}), procaine hydrochloride (100, 50, 20, 10, and 5 μg ml^{-1}), and propoxycaine (20, 10, 5, 3.33, and 2 μg ml^{-1}). Linearities were demonstrated for the norepinephrine–procaine–propoxycaine combination from 20 μl injections of solutions containing quantities of norepinephrine (3.34, 1.67, 1.11, 0.56, and 0.33 μg ml^{-1}), procaine hydrochloride (100, 50, 20, 10, and 5 μg ml^{-1}), and propoxycaine (20, 10, 5, 3.33, and 2 μg ml^{-1}). The resulting data were plotted as peak height versus concentration and studied by linear regression analysis.

3.2. Precision and accuracy

To obtain intra- and inter-day precision data for the epinephrine–prilocaine and epinephrine–procaine combinations, five standard curves for each analyte in each drug mixture were prepared over 3 days. The results are presented in Table 1. The values obtained in all cases were lower than 3.23%. Percent error was also evaluated for both the prilocaine–epinephrine and procaine–epinephrine combinations using spiked samples containing each analyte. Table 1 shows that the

Table 1
Accuracy and precision using spiked drug samples

Analyte	Concentration added (μg ml^{-1})	Concentration found[a] (μg ml^{-1})	Percent error	%RSD
Prilocaine–epinephrine				
Prilocaine	100.00	100.04 ± 0.02	0.04	0.02
	25.00	25.02 ± 0.04	0.20	0.16
	10.00	10.01 ± 0.02	0.10	0.20
Epinephrine	1.25	1.26 ± 0.03	0.80	2.56
	0.63	0.62 ± 0.02	1.59	3.23
	0.31	0.32 ± 0.00	3.23	1.82
Procaine–epinephrine				
Procaine	62.50	62.51 ± 0.02	0.02	0.03
	25.00	24.99 ± 0.04	0.04	0.16
	6.25	6.26 ± 0.02	0.16	0.24
Epinephrine	1.33	1.32 ± 0.02	0.75	1.24
	0.67	0.69 ± 0.02	2.99	1.19
	0.40	0.41 ± 0.01	2.56	2.56

[a] Each value represents mean ± S.D. of three replicates.

Table 2
Stability-indicating nature of assays

Drug	Treatment	Duration	%Δ In peak height
Epinephrine–prilocaine			
Epinephrine	0.1 N HCl (90 °C)	1 h	−25
	0.1 N NaOH	45 min	−15
	3% H_2O_2	2 h	−12
Prilocaine	0.1 N HCl (90 °C)	1 h	−20
	0.1 N NaOH	30 min	−15
	3% H_2O_2 (90 °C)	1 h	−17
Epinephrine–procaine			
Epinephrine	0.1 N HCl (90 °C)	1 h	−18
	0.1 N NaOH	45 min	−11
	3% H_2O_2	2 h	−16
Procaine	0.1 N HCl (90 °C)	1 h	−25
	0.1 N NaOH (90 °C)	15 min	−10
	3% H_2O_2 (90 °C)	1 h	−14
Levonordefrin–tetracaine–procaine			
Levonordefrin	0.1 N HCl	1 h	−25
	0.1 N NaOH	1 h	−10
	3% H_2O_2	2 h	−11
Tetracaine	0.1 N HCl (90 °C)	1 h	−15
	0.1 N NaOH (90 °C)	30 min	−20
	3% H_2O_2	45 min	−13
Procaine	0.1 N HCl (90 °C)	1 h	−22
	0.1 N NaOH (90 °C)	15 min	−13
	3% H_2O_2 (90 °C)	1 h	−16

percent error of the method was always less than 3.23%; therefore, it was concluded that the procedure gives acceptable accuracy and precision for all of the analytes in each drug mixture.

3.3. Stability

To show that the methods are stability-indicating, it was necessary to subject the analytes to extreme conditions to cause them to degrade [25]. In each case, 1 mg ml^{-1} of each analyte was mixed with the appropriate degradation solution such as 0.1 N HCl (ambient temperature and 90 °C), 0.1 N NaOH (ambient temperature and 90 °C), and 3% H_2O_2 (ambient temperature and 90 °C). Acid- and base-degraded samples were first neutralized with equal volumes and concentrations of either acid or base and diluted prior to injection into the HPLC system. Samples degraded with peroxide were diluted and injected into HPLC. For comparison, a 1 ml aliquot of each stock solution was heated to 90 °C without the addition of acid, base, or peroxide. The stability studies were designed such that 10–30% of the original sample was degraded. This enabled the degradation of each analyte without the risk of secondary degradation products. When present, degradation peaks did not interfere with the analytes of interest. Results are presented in Tables 2 and 3.

3.4. Assay of dosage forms

The combination standards of epinephrine–prilocaine were injected in duplicate to obtain a standard curve for each analyte. The correlation coefficients for the curves were 0.9996 and 0.9998 for epinephrine and prilocaine hydrochloride, respectively ($n = 10$ for each curve). The injection solution was injected three times and the data subjected to linear regression analysis. The percent label claim for the commercial injection (Citanest® Forte) was found to be $98.41 \pm 1.37\%$ ($n = 3$, %RSD = 1.39) or 4.92 μg ml^{-1} for epinephrine and $100.03 \pm 0.04\%$ ($n = 3$, %RSD = 0.04) or 40.01 mg ml^{-1} for prilocaine hydrochloride.

The combination standards of epinephrine–procaine were injected in duplicate to obtain a standard curve for each analyte. The correlation coefficients for the curves were 0.9994 and 0.9996 for epinephrine and procaine hydrochloride, respectively ($n = 10$ for each curve). The laboratory-prepared injection solution was injected three times and data subjected to linear regression analysis. The percent label claim for the laboratory prepared injection containing epinephrine and procaine hydrochloride was found to be $101.10 \pm$

1.10% ($n = 3$, %RSD = 0.95) or 20.22 μg ml^{-1} for epinephrine and 100.02 ± 0.05% ($n = 3$, %RSD = 0.05) or 10.00 mg ml^{-1} for procaine hydrochloride.

4. Conclusion

The proposed stability-indicating HPLC methods in this study have the advantage of simplicity, precision, accuracy, and convenience for the separation and quantitation of prilocaine–epinephrine and procaine–epinephrine drug combinations and can be employed for the assay of their respective dosage forms. Although different dilutions are necessary to quantitate the analytes in each drug mixture, there are significant advantages of this method. For the prilocaine and epinephrine combination, this method is advantageous over the existing USP 24 HPLC assays, which employ different mobile phases as well as two detectors [5]. The method is also an advantage over the existing USP HPLC assays for the procaine hydrochloride combinations since these methods typically involve spectrophotometric assays which require a sequence of sample preparation steps as well as the preparation of a variety of reagents [4,18–20]. Moreover, the proposed stability-indicating HPLC methods use simple reagents, with minimal preparation procedures, encouraging application in routine analysis.

Table 3
Stability-indicating nature of assays

Drug	Treatment	Duration	%Δ In peak height
Levonordefrin–procaine–propoxycaine			
Levonordefrin	0.1 N HCl	1 h	−25
	0.1 N NaOH	1 h	−10
	3% H_2O_2	2 h	−11
Procaine	0.1 N HCl (90 °C)	1 h	−18
	0.1 N NaOH (90 °C)	30 min	−20
	3% H_2O_2 (90 °C)	1 h	−14
Propoxycaine	0.1 N HCl (90 °C)	1 h	−25
	0.1 N NaOH (90 °C)	45 min	−20
	3% H_2O_2	1 h	−12
Norepinephrine–procaine–propoxycaine			
Norepinephrine	0.1 N HCl (90 °C)	1 h	−12
	0.1 N NaOH	30 min	−25
	3% H_2O_2	2 h	−10
Procaine	0.1 N HCl (90 °C)	1 h	−21
	0.1 N NaOH (90 °C)	45 min	−24
	3% H_2O_2 (90 °C)	1 h	−16
Propoxycaine	0.1 N HCl (90 °C)	1 h	−21
	0.1 N NaOH (90 °C)	30 min	−16
	3% H_2O_2	1 h	−13

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