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Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 629–638
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous micellar LC determination of lidocaine and tolperisone

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Received 28 May 2002; received in revised form 18 September 2002; accepted 10 October 2002

Abstract

A micellar liquid chromatography (MLC) procedure was developed for the simultaneous separation and determination of lidocaine hydrochloride (LD HCl) and tolperisone hydrochloride (TP HCl) using a short-column C18 (12.5 mm × 4.6 mm, 5 μm), sodium dodecyl sulfate (SDS) with a small amount of isopropanol, and diode array detector. The optimum conditions for the simultaneous determination of both drugs were 0.075 mol l^{-1} SDS–7.5% (v/v) isopropanol with a flow rate of 0.7 ml min^{-1} and detection at 210 nm. The LOD (2S/N) of LD HCl was 0.73 ng 20 μl^{-1}, whereas that of TP HCl was 1.43 ng 20 μl^{-1}. The calibration curves for LD HCl and TP HCl were linear over the ranges 0.125–500 μg ml^{-1} ($r^2 = 0.9999$) and 1.00–500 μg ml^{-1} ($r^2 = 0.9997$), respectively. The %recoveries of both drugs were in the range 98–103% and the %RSD values were less than 2. The proposed method has been successfully applied to the simultaneous determination of TP HCl and LD HCl in various pharmaceutical preparations.
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Keywords: Micellar liquid chromatography; Short column; Lidocaine; Tolperisone

1. Introduction

In recent years, interest in the use of micellar mobile phase in reverse-phase high-performance liquid chromatography (RPLC), instead of conventional organic mobile phases, has grown rapidly because of the biodegradability and lower toxicity of surfactants than the conventional organic mobile phases. This technique is termed micellar liquid chromatography (MLC). A number of papers have appeared focussing on the chromatographic behavior of various solutes [1–4] and there is a report reviewed by Nishi [5] related to MLC in pharmaceutical applications such as cold medicines (phenylpropanolamine, tipepidine, and chlorpheniramine) or ointments (prednisolone, crotamiton, dibucaine, chlorhexidine, and glycyrrhetinic acid) and some narcotics (codeine and morphine). Moreover, Gilabert et al. [6] have

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doi:10.1016/S0731-7085(02)00693-3

applied a mixture consisting of 0.15 mol l^{-1} of sodium dodecyl sulfate (SDS) and 10% of 1-propanol (adjusted to pH 3 with phosphate buffer) as a mobile phase and a Spherisorb ODS-2 C18 column (5 μm, 120 mm × 4.0 mm i.d.) as stationary phase (SP) to determine local anesthetics, including lidocaine, with 230 nm detection. A calibration curve for lidocaine hydrochloride (LD HCl) was linear in the range 5–25 μg ml^{-1} with regression coefficient $r > 0.99$ and the retention time of LD HCl was about 7 min. The reproducibilities ($n = 7$) expressed as the variation coefficients were 5 and 4% at concentrations 5 and 15 μg ml^{-1}, respectively. However, the MLC method has some restrictions in varying amounts of surfactants in the mobile phase, more than critical micelle concentration (CMC) but not too large amount used due to the effect of viscosity. Moreover, the common organic modifier used in MLC for increasing the elution power is short-chain alcohol such as propanol. The more recent development in using longer chain alcohol such as butanol and pentanol has been demonstrated by Agusti et al. [7] for the analysis of some cough–cold drugs. The selected mobile phase was a mixture containing 0.15 mM SDS and 0.6% pentanol.

Tolperisone hydrochloride (TP HCl), a muscle relaxant, and LD HCl, local anesthetic, in general dosage forms of these drugs they can contain either TP HCl or LD HCl or both depending on the therapeutic purposes. There are several reported methods for the determination of LD HCl but few assays for TP HCl in pharmaceuticals and biological samples as mentioned by Liawruangrath et al. [8]. The reported methods for analyzing both drugs are spectrophotometry for the determination of LD HCl [9–12] or TP HCl [13], high-performance liquid chromatography (HPLC) for LD [14–19] or TP [20], high-performance thin layer chromatography (HPTLC) for TP [21], electrochemical analysis for LD [22] or TP [23], and MLC for the analysis of six local anesthetics including LD HCl [6]. However, there is only one paper that has reported on simultaneous determination of these two drugs by HPLC using a mixture of acetonitrile and water (70:30) and 5.5% triethylamine as a mobile phase, and separating on a Spherisorb ODS column (250 mm × 4.6 mm, 5 μm) with UV detection at 254 nm [8]. The detection limits (S/N = 2) for TP HCl and LD HCl were 0.20 and 100 ng 20 μl^{-1} and the quantitation limits (S/N = 5) were 0.50 and 250 ng 20 μl^{-1}, respectively. No published paper has mentioned about the simultaneous determination of TP HCl and LD HCl by MLC technique. For environmental protection and human safety purposes, it is interesting to develop the MLC method for simultaneous determination of TP HCl and LD HCl.

This paper describes the MLC procedure for the simultaneous determination of TP HCl and LD HCl in pharmaceutical preparations. Due to the amine structure and pK_a of TP HCl and LD HCl ($pK_a = 4–5$ and 7.9, respectively), they present the positive charge of protonated amine in acidic and neutral aqueous systems. An anionic surfactant, SDS, was chosen for separating these two drugs. In addition, the purpose of this work was to develop an HPLC method with minimizing organic mobile phase consumption; therefore, the short-column C18 (5 μm, 12.5 mm length and 4.6 mm i.d.) was applied to the separation and determination of both drugs instead of using large amount of surfactant and alcohol in a long column, and to overcome the weak elution power of micellar mobile phase. As far as the authors know, this is the first report using short column in MLC for the simultaneous determination of both drugs.

2. Experimental

2.1. Apparatus and reagents

HPLC analyses were carried out with Hewlett-Packard 1090 with tertiary pump, diode array detector, and 20 μl loop injector. Data acquisition and processing have been controlled by HP chemstation software from Hewlett-Packard. The short column (12.5 mm × 4.6 mm i.d.) was packed with Zorbax C18 (5 μm). Mobile phase was vacuum-filtered through 0.45 μm nylon membranes (Hewlett-Packard, Germany) and degassed

by ultrasonicator (Elma, Germany) and aspirator (Yamato, Japan).

2.2. Reagents

TP HCl, LD HCl, prilocaine hydrochloride, polyoxyethylene, and saccharin sodium (Sigma, St. Louis, MO), methylparaben and propylparaben (Lancaster, UK), and SDS and isopropanol (Carlo Erba, Italy) were of analytical reagent grade. The deionized distilled water was used throughout the experiment.

3. Procedure

3.1. Sample preparation

3.1.1. Tablets

Two commercial tolperisone tablet preparations were chosen with the sample codes A and B for film-coated and uncoated tablets, respectively. Twenty tablets of TP HCl were accurately weighed, ground, and mixed. The powder was accurately weighed equivalent to 100 mg of TP HCl, transferred into 100 ml volumetric flask, dissolved in approximately 50 ml of water, sonicated for 10 min, diluted to the volume with water, and filtered. Then, a solution containing 20 μg ml^{-1} was prepared from this solution using 0.075 mol l^{-1} SDS as a solvent.

3.1.2. Injectables

Three commercial injection samples were selected for the determination of either LD HCl or TP HCl. The sample codes A, B, and C were labeled for lidocaine injection, lidocaine with adrenaline injection, and lidocaine mixed with tolperisone injection, respectively.

For injections A and B, 1 ml of the sample (equivalent to LD HCl of 20 mg) was pipetted and transferred to 100 ml volumetric flask and diluted to the volume with water. Then, 10 μg ml^{-1} LD HCl was prepared from 200 μg ml^{-1} LD HCl by dilution using 0.075 mol l^{-1} SDS as a solvent.

A 100 μl of injection C was transferred into a 50 ml volumetric flask and diluted to volume with 0.075 mol l^{-1} SDS resulting in the final concentrations of 5 and 200 μg ml^{-1} for LD HCl and TP HCl, respectively.

3.1.3. Cream, jelly, and viscous

Other three pharmaceutical preparations of lidocaine (cream, jelly, and viscous) were determined. About 1 g of sample was accurately weighed, dissolved in mobile phase by sonication for 10–15 min, transferred to 100 ml volumetric flask, and diluted to volume with mobile phase. The drug solution containing about 10 μg ml^{-1} of LD HCl was prepared by dilution with the mobile phase. However, for the viscous sample, the final concentration was 10 μg ml^{-1} of LD base, and the density of viscous sample was determined by weighing 10 ml of viscous sample (in 10 ml volumetric flask).

3.2. Standard preparation

Stock standard solutions of LD HCl and TP HCl were prepared in 0.075 mol l^{-1} SDS with 1000 μg ml^{-1}. Other lower concentrations of these two drugs were prepared from these stock solutions by dilution with the medium of 0.075 mol l^{-1} SDS.

3.3. The chromatographic conditions

The chromatographic conditions were carried out in the isocratic mode using a mixture of aqueous SDS solution (0.75 mol l^{-1}) and isopropanol (7.5%, v/v) as mobile phase. The short column packed with Zorbax C18 (4.6 mm i.d. × 12.5 mm, 5 μm) was equilibrated with the mobile phase for 30 min at a flow rate of 1.0 ml min^{-1}. Each sample and/or standard solution was injected by using 20 μl loop injector (three replicate injections). The signal was monitored by diode array detector by scanning the wavelength between 200 and 400 nm. The dead volume was determined by the first perturbation of each chromatogram.

4. Results and discussion

The aim of this work was to develop a micellar HPLC procedure using a short column for the

determination of LD HCl and TP HCl simultaneously using a UV detector; therefore, the suitable wavelength for the detection of both drugs was investigated in order to achieve the high sensitivity. A standard mixture of LD HCl (20 μg ml^{-1}) and TP HCl (50 μg ml^{-1}) was injected and separated on the short column (12.5 mm × 4.6 mm i.d.) packed with Zorbax C18 (5 μm) using 0.10 M SDS–10% isopropanol as mobile phase with a flow rate of 1.0 ml min^{-1}. The UV spectrum of each standard drug showed the absorption maxima about 254 nm and at approximately 200–210 nm with the greater absorbance. Therefore, 210 and 254 nm were taken into consideration. After investigation by MLC, it was found that the peak areas of LD and TP at 210 nm were 1146 and 1748, respectively, whereas at 254 nm the peak areas of both drugs were 28 and 1789, respectively. So, 210 nm was selected to be the suitable wavelength for monitoring both drugs.

To demonstrate the performance of the short-column C18 (12.5 mm length) used for the separation of LD HCl and TP HCl, the chromatographic behavior in terms of capacity factor has been studied using 0.025–0.15 mol l^{-1} SDS mixed with 5–15% (v/v) of isopropanol, flow rate of 1.0 ml min^{-1}, and 210 nm detection. Mixed solution of LD HCl (20 μg ml^{-1}) and TP HCl (50 μg ml^{-1}) was injected into the HPLC system under identical chromatographic conditions. The chromatographic conditions were optimized using the univariate method [24].

4.1. Effect of SDS concentration

The effect of SDS concentration was studied by varying at higher concentration than CMC, 0.025–0.150 mol l^{-1}, while other conditions were maintained at their constant values such as 10% (v/v) of isopropanol and 1.0 ml min^{-1} flow rate. Concentration higher than 0.150 mol l^{-1} SDS was not investigated to avoid viscosity effect. It was seen that the higher the concentration of SDS present in the mobile phase, the lower the capacity factors were obtained. Due to the positive charge of protonated amine of these two drugs, which can be associated with the SDS molecules in SP, the micelle can be associated with solutes more rapidly. The reciprocal values of capacity factors k_1 and k_2 for LD HCl and TP HCl are linear function of concentration of SDS in the mobile phase (Fig. 1), as indicated in Eqs. (1) and (2) for LD HCl and TP HCl, respectively:

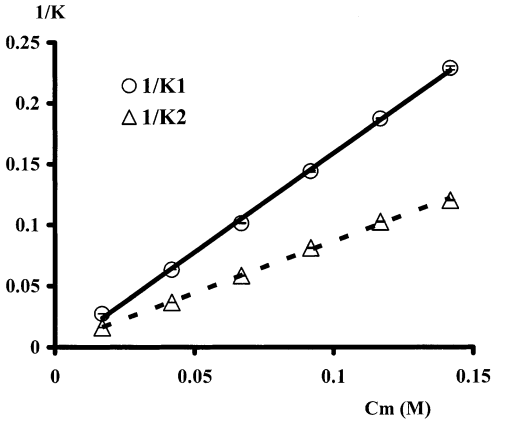

Fig. 1. Influences of SDS in mobile phase (mixed with 10% isopropanol and flow rate of 1 ml min^{-1}) on capacity factors of LD HCl (k_1) and TP HCl (k_2) in short column SB-C18 12.5 mm length.

$$\frac{1}{k_1} = 1.626C_m - 0.004, \qquad r^2 = 0.999, \tag{1}$$

and

$$\frac{1}{k_1} = 0.847C_m - 0.002, \qquad r^2 = 0.999. \tag{2}$$

Both equations are corresponding to the review on chromatographic behavior of solutes in micelle system by Jandera and Fischer [3] according to the following equation:

$$\frac{1}{k} = A + BC_m, \tag{3}$$

where k is the capacity factor, A and B are fitting parameters, and C_m the concentration of micelle in mobile phase, which is equal to the difference between total concentration of SDS (C_s) and CMC.

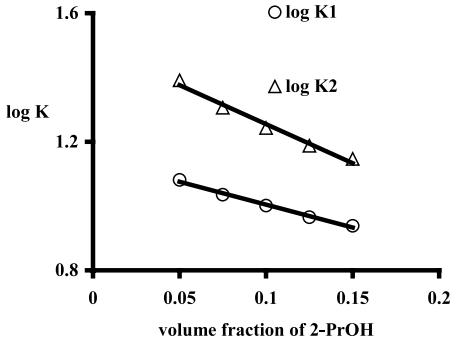

Fig. 2. Influence of volume fraction of isopropanol in micellar mobile phase (0.075 M SDS) on capacity factors of LD HCl (k_1) and TP HCl (k_2) at flow rate of 1 ml min^{-1}.

4.2. Effect of isopropanol

The effects of various concentrations of isopropanol on the retention behavior in micelle mobile phase, and the capacity factors of LD HCl and TP HCl were examined in the mobile phase containing 0.075 mol l^{-1} SDS and various concentrations of isopropanol (5–15%, v/v). Concentrations higher than 15% (v/v) were not used to avoid deaggregation of micelle in high amount of organic solvent. The plots of logarithms of capacity factors of LD HCl and TP HCl versus concentrations of isopropanol in the mobile phase are almost linear as Eqs. (4) and (5) for LD HCl and TP HCl, respectively (Fig. 2), which correspond to the study on the effect of organic modifier in micellar system by Fischer and Jandera [2], which can be expressed as in Eq. (6):

$$\log k_1 = 1.147 - 0.0143\psi, \qquad r = 0.996, \tag{4}$$

$$\log k_2 = 1.4977 - 0.0243\psi, \qquad r = 0.991, \tag{5}$$

$$\log k = a_m - m_{hyb}\varphi, \tag{6}$$

where parameter a_m is the logarithm of the capacity factor in aqueous micellar mobile phase without organic solvent, the slope parameter m_{hyb} can be taken as the solvent strength parameter of the organic solvent in the hybrid aqueous–organic micellar mobile phase, and φ is the volume fraction of the organic solvent in the mobile phase. This behavior can be described in a similar way as dependence in the absence of the surfactants or in organic mobile phase in RPLC.

Upon studying the effects of SDS and alcohol (isopropanol) concentrations on the micellar LC chromatographic behavior using the short column, it was shown that this short column was suitable to separate LD HCl and TP HCl in micellar system with the sufficient capacity factors in the ranges 8.7–12.1 and 14.0–24.6, respectively.

4.3. Selection of suitable chromatographic conditions

The suitable concentration of SDS was studied with various concentrations (0.025–0.150 mol l^{-1}) of SDS at higher than CMC, and using 10% (v/v) isopropanol as modifier at a flow rate of 1 ml min^{-1}. It was found that when the concentration of SDS was higher than 0.075 mol l^{-1}, the resolution factor (R_s) was less than 1.5, whereas for the mobile phases containing 0.025, 0.05, and 0.075 mol l^{-1} SDS, the R_s values were 2.3, 1.8, and 1.52, respectively. However, although 0.025 mol l^{-1} SDS exhibited the greatest R_s value, the separation took a rather long analysis time for TP ($t_r = 13.3$ min). Regarding to analyte peaks obtained using the mobile phases that contained 0.05 and 0.075 mol l^{-1} SDS, it was found that at 0.075 mol l^{-1} SDS, the two analyte peaks were thinner and provided the higher sensitivity than those obtained at 0.05 mol l^{-1} SDS. Therefore, as a compromise among the sensitivity speed with a reasonable resolution, the concentration of 0.075 mol l^{-1} SDS was selected as suitable for the separation of these two drugs.

The appropriate concentration of isopropanol was investigated using the mobile phases containing 0.075 mol l^{-1} SDS in the presence of varying amounts of isopropanol with the flow rate of 1 ml min^{-1}. The results demonstrated that when the isopropanol concentration exceeded 10% (v/v), then R_s was less than 1.5, whereas at concentration less than 7.5% (v/v) isopropanol the sensitivities decrease by reducing the amount of alcohol in the mobile phases. Comparing the results obtained from the presence of 7.5 and 10% (v/v) isopropanol in the mobile phases, TP HCl signal as peak areas at 7.5% (v/v) was smaller than that obtained at 10% (v/v) isopropanol. However, R_s of the system at 7.5% (v/v) was better than that obtained

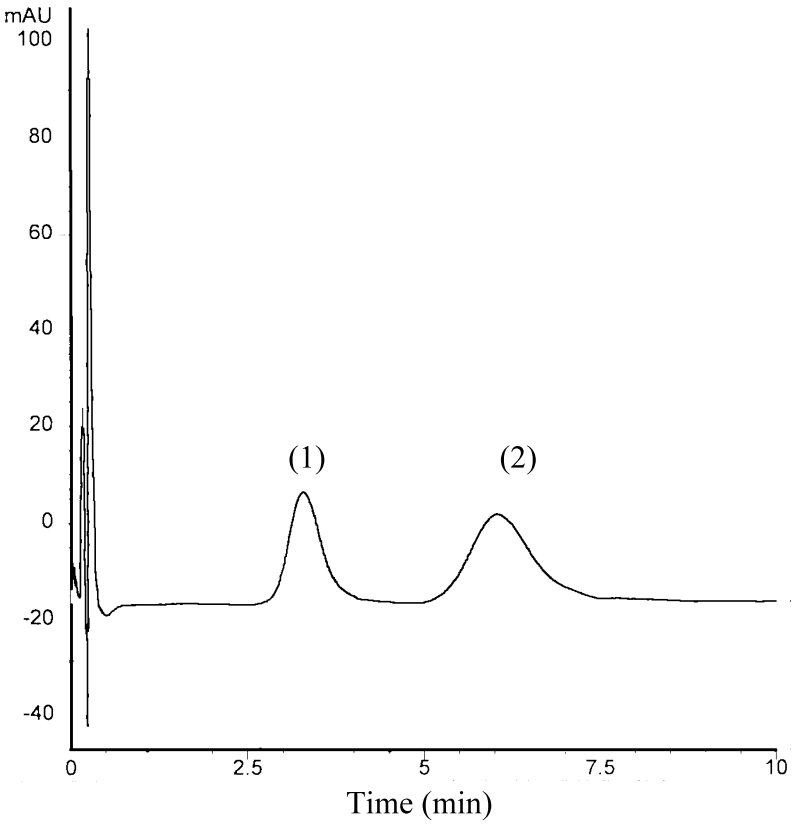

Fig. 3. Chromatogram of a standard mixture of LD HCl (10 μg ml^{-1}) and TP HCl (20 μg ml^{-1}) obtained with 0.075 M SDS–7.5% isopropanol in Zorbax SB-C18 column (12.5 mm length × 4.6 mm i.d., 5 μm) and flow rate of 0.7 ml min^{-1} at 210 nm detection. Compounds: (1) lidocaine and (2) tolperisone.

at 10% (v/v), 1.84 and 1.60, respectively. In order to minimize the organic solvent consumption in the HPLC system, the micellar mobile phase containing 7.5% (v/v) of isopropanol was selected as optimum mobile phase with the acceptable resolution and analysis time.

The mobile-phase flow rate for LD and TP determination was optimized using 0.075 mol l^{-1} SDS in the presence of 7.5% (v/v) isopropanol with following flow rates: 0.5, 0.7, 0.9, 1.0, 1.1, and 1.2 ml min^{-1}. It was clear that the higher mobile-phase flow rate, the shorter retention time, and the worse R_s were obtained, whereas the capacity factors of both drugs were independent of the mobile-phase flow rate. At the flow rate higher than 0.7 ml min^{-1}, the resolution factor was less than 2. Although R_s and the sensitivity of the analyte peak at 0.5 ml min^{-1} are better than those obtained at 0.7 ml min^{-1}, the analysis time is longer than 10 min. The t_r values of LD HCl and TP HCl at 0.5 ml min^{-1} were 4.6 and 8.2 min, respectively. To compromise among resolution, sensitivity, and analysis time, the suitable flow rate was 0.7 ml min^{-1}, with the retention time of 3.3 and 5.8 min for LD HCl and TP HCl, respectively, and the R_s of 2.0 was obtained. The capacity factors for LD HCl and TP HCl were 11.3 and 20.8, respectively. The time taken for complete separation and determination of both drugs was within 8 min. The chromatogram of standard mixture of both drugs was demonstrated in Fig. 3.

The selected conditions for the simultaneous separation and determination of LD HCl and TP HCl on the Zorbax SB-C18 short column (12.5 mm × 4.6 mm, 5 μm) using a mixture of 0.075 mol l^{-1} SDS and 7.5% (v/v) isopropanol as mobile

Table 1
Linear regression analysis ($n = 3$) and limits of detection (S/N = 2) and quantitation (S/N = 5)

Drugs	Concentration range (μg ml^{-1})	Number of solution	Slope (%RSD)	Intercept (%RSD)	r^2	LOD (ng 20 μl^{-1})	LOQ (ng 20 μl^{-1})
LD HCl	0.125–500	20	77.46 (0.09)	−60.91 (3.89)	0.9999	0.73	1.82
TP HCl	1–500	14	60.97 (0.14)	−180.11 (1.28)	0.9997	1.43	3.59

phase with a flow rate of 0.7 ml min^{-1} and detection at 210 nm with diode array detector (scanning wavelength: 400–200 nm) were used for further studies.

4.4. Validation of the method

The limit of detection (LOD) of LD HCl and TP HCl was determined by injecting 20 μl solution into the MLC system. LOD is defined as that concentration of the analyte producing a peak height and/or peak area signal which is at least twice that of the baseline noise (S/N = 2) measured from peak to peak [25]. The LOD values for LD HCl and TP HCl were 0.73 and 1.43 ng 20 μl^{-1}, respectively. Then, the limits of quantitation (LOQs, S/N = 5) investigated at S/N = 5 [25] were 1.82 ng 20 μl^{-1} for LD HCl and 3.59 ng 20 μl^{-1} for TP HCl (Table 1).

Calibration curves were constructed by triplicate injections of each set of standard solutions with higher concentration than that of the quantitation limit of each analyte in the ranges 0.125–500 μg ml^{-1} for LD HCl ($n = 20$) and 1–500 μg ml^{-1} for TP HCl ($n = 14$), and plotting the peak areas (y) versus various concentrations (x). The linear regression analysis showed excellent correlation coefficients ($r^2 > 0.999$), and the linearity data are presented in Table 1.

The accuracy and intra-day precision of the proposed method were determined by analyzing injection C sample spiked with five different concentrations of standard LD HCl and TP HCl (by preparing three solutions for each concentration in the same day) using the proposed method. The %recoveries of the spiked LD HCl and TP HCl were in the range 98–103 and the %RSDs were less than 2. The detailed results are shown in Table 2.

Table 2
Intra-day precision and accuracy by standard addition in sample injection C

Added (μg ml^{-1})	Found ± S.D.[a] (μg ml^{-1})	%Recovery
Standard LD HCl		
2.09	2.16 ± 0.02	103.17 ± 0.96
3.14	3.08 ± 0.04	98.04 ± 1.27
4.19	4.16 ± 0.06	99.47 ± 1.43
5.23	5.17 ± 0.07	98.73 ± 1.34
6.28	6.19 ± 0.02	98.54 ± 0.32
Standard TP HCl		
80.56	82.31 ± 0.43	102.17 ± 0.53
120.84	118.69 ± 0.32	98.22 ± 0.26
161.12	162.92 ± 0.54	101.12 ± 0.34
201.40	203.50 ± 0.46	101.04 ± 0.23
241.68	238.01 ± 0.47	98.48 ± 0.19

[a] S.D. is standard deviation for intra-day repeatability; $n = 3$.

The system precision was determined by 10 replicate injections of aliquot of a mixture containing standard LD HCl (10 μg ml^{-1}) and TP HCl (20 μg ml^{-1}). The %RSDs of the retention time and the area of LD HCl peak were 0.31 and 1.32, whereas those of the TP HCl peak were 0.40 and 0.96, respectively.

Interferences were studied by injecting the solution of other drugs and other ingredients in formulation such as methylparaben, propylparaben, polyoxyethylene, saccharin, and prilocaine hydrochloride. The chromatograms showed that these peaks did not interfere with the analyte peaks.

The peak purity was evaluated using diode array UV detector to consider the specificity by comparing the spectra at the peak up-slope, apex, and

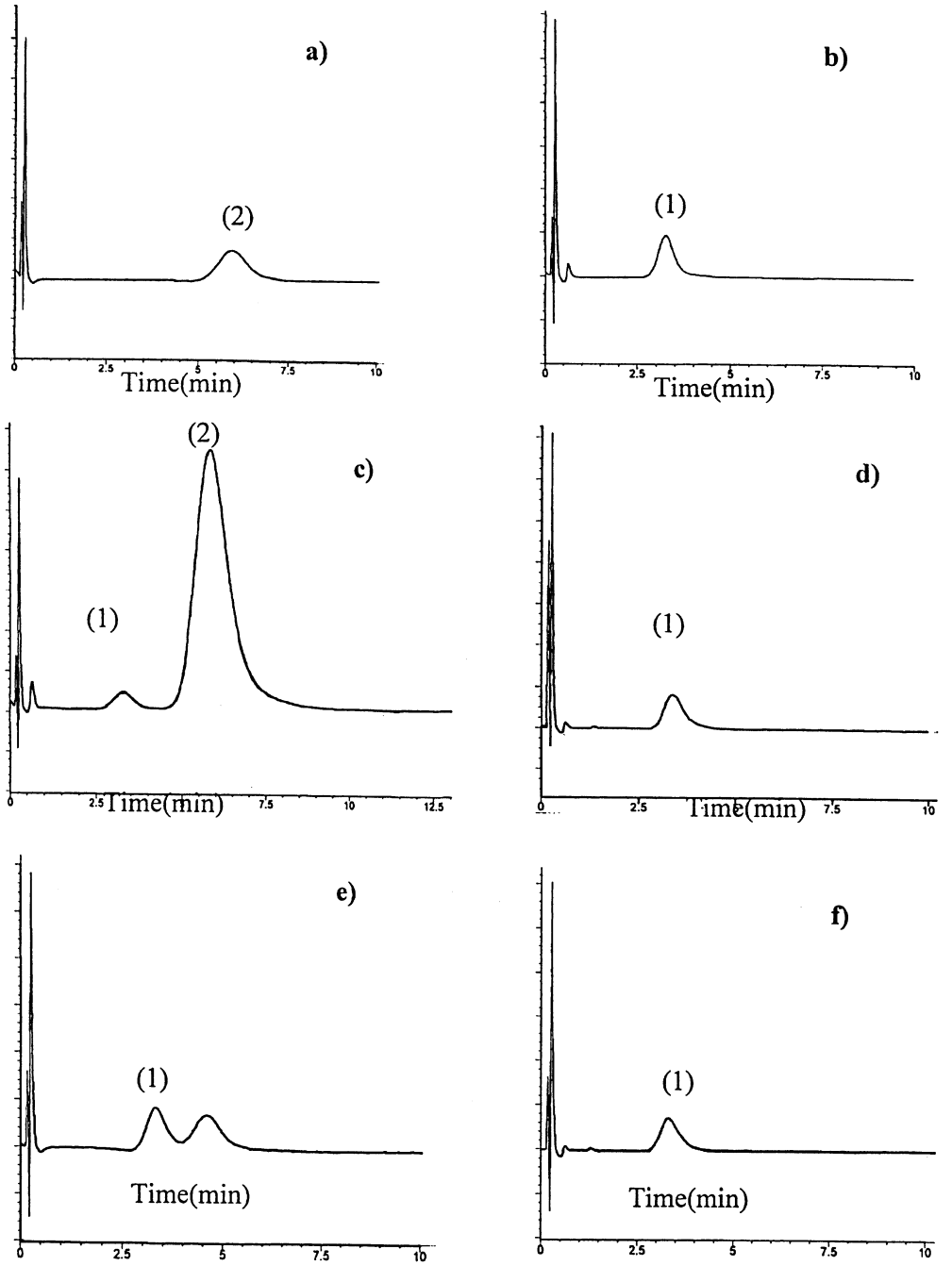

Fig. 4. Chromatograms of some pharmaceutical preparations (see Table 3): (a) film-coated tablet A; (b) injection B; (c) injection C; (d) jelly; (e) cream; and (f) viscous. Compounds: (1) lidocaine and (2) tolperisone.

Table 3
Analysis of pharmaceutical preparations

Samples	Declared	Amount found ± S.D.[a]	
		MLC	RPLC
Film-coated tablet A	Each tablet contains TP HCl: 50 mg	45.25 ± 0.77	45.70 ± 2.97
Uncoated tablet B	Each tablet contains TP HCl: 50 mg	48.17 ± 0.84	48.56 ± 0.67
Injection A	Each 100 ml contains LD HCl: 2.0 g Preservative and water	2.00 ± 0.02	1.99 ± 0.02
Injection B	Each 100 ml contains LD HCl: 2.0 g Adrenaline, preservative, and water	2.00 ± 0.03	2.01 ± 0.01
Injection C	Each 1 ml contains TP HCl: 100 mg LD HCl: 2.5 mg Methylparaben, diethyleneglycol, monoethylether, and water	95.66 ± 1.79 3.04 ± 0.01	95.75 ± 1.85 3.15 ± 0.06
Jelly	Each 1 g contains LD HCl anhydrous: 2.0 mg Methylparaben, propylparaben, hypromellos, sodium hydroxide, hydrochloric acid, and water	1.91 ± 0.03	1.91 ± 0.04
Cream	Each 1 g contains Lidocaine base: 25 mg Prilocaine base: 25 mg Polyoxyethylene, carboxypolymethylene, sodium hydroxide, and water	26.61 ± 0.08	26.17 ± 0.52
Viscous	Each 100 ml contains LD HCl anhydrous: 20 mg Methylparaben, propylparaben, sodium carboxymethylcellulose, saccharin sodium, aroma, and water	19.45 ± 0.11	19.08 ± 0.25

[a] Average from five determinations in the same unit of declared.

down-slope of the analytical peaks of sample and standard chromatogram. The identical spectra are found for each point on the peak. It was demonstrated that no interferences were found from other substances.

The stability of stock standard solutions of LD HCl (1052 μg ml^{-1}) and TP HCl (1044 μg ml^{-1}) was studied by storing at room temperature (25–28 °C) for 10 days. The mixtures were prepared for the analysis daily by diluting to 10.52 and 20.88 μg ml^{-1} for LD HCl and TP HCl, respectively. The amounts found at 0, 1, 3, 5, and 10 days at room temperature for LD HCl were 10.51, 10.52, 10.48, 10.49, and 10.63 μg ml^{-1} and for TP HCl were 20.77, 21.03, 20.83, 20.75, and 20.99 μg ml^{-1}, respectively. Therefore, the stock solutions were stable over a period of at least 10 days at room temperature (%error from expected value was less than 2).

In order to investigate whether the proposed method can be used as a stability indicating method, stress studies for force degradation of both drugs were performed by storing 2 ml of each stock standard solution at 70 °C for 3 h. Subsequently, each solution was analyzed after diluting to 80 μg ml^{-1}. The peak area of TP HCl decreased by 10.4% and two degradation peaks appeared at 2.3 and 2.8 min but no degradation peaks for LD HCl were observed. Therefore, this method can be used as stability indicating method for TP HCl.

However, in the sample mixture of LD HCl and TP HCl, the degradation peaks of TP may interfere with the analyte peak of LD if large amount of the degradation products is present. To solve this problem, dilution or standard addition methods can be used.

4.5. Application

The proposed MLC method has been applied to the simultaneous determination of LD and TP in pharmaceutical preparations (Fig. 4). The results obtained in the analysis of eight pharmaceuticals, which contain either LD or TP and the mixture of both drugs, are given in Table 3 comparing to the published organic RPLC method [8] using the mixture of acetonitrile and water (70:30), and 5.5% triethylamine as mobile phase in column with flow rate of 0.7 ml min^{-1} and detection at 254 nm. The reproducibility of each sample determined by the proposed method was demonstrated in Table 3 and the %RSD of each formulation was less than 2 ($n = 5$). There was no significant difference by t-test between the mean value obtained from both methods at 95% confidential limit [25], except in cream preparation.

5. Conclusion

This proposed micellar LC method using the short column (12.5 mm length) was reliable and suitable for routine analysis of LD HCl and TP HCl in various pharmaceutical preparations with accurate, rapid, and precise results. Due to the advantages of MCL and the short column, this method is superior to previous HPLC [8] and MLC methods (using long-column C18 for LD HCl) [6] in that it is much simpler, faster, lower cost, wider dynamic range, and better detection limit. This method can be employed as stability indicating method for TP HCl. Moreover, for environmental protection and human safety purposes, it can avoid the toxicity of using organic solvents by replacing the technique of organic RPLC by the proposed MLC technique.

Acknowledgements

The authors thank the Ministry of University Affair for funding and the Faculty of Science and Technology, Department of Chemistry, Thammasat University, for providing the equipment. They also wish to express their appreciation to the Postgraduate Education and Research Program in Chemistry (PERCH) for partial support.

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