Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 381–386
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Determination of cetylpyridinium chloride and tetracaine hydrochloride in buccal tablets by RP-HPLC

Jiansong Wang, Jinrong Lu, Lingxia Zhang, Yuzhu Hu *

Department of Analytical Chemistry, China Pharmaceutical University, Nanjing 210009, China

Received 15 August 2002; received in revised form 23 February 2003; accepted 23 February 2003

Abstract

The HPLC method for simultaneous determination of cetylpyridinium chloride (CPC), tetracaine hydrochloride (TTC) in Xipiluan buccal tablets was developed and validated. The HPLC method was performed on a CN column (150 × 4.6 mm i.d., 5 μm particle size); the mobile phase was methanol–tetramethylammonium hydroxide (20 mM)–potassium dihydrogen phosphate (3 mM) (90:10:3, v/v/v) (pH* 5.0), pumped at a flow rate 1.5 ml min^{-1}. The UV detector was set at 230 nm. The retention time for CPC and TTC was 3.52 and 3.10 min, respectively. Calibration curves were linear ($r = 0.9999$, $n = 6$) in the range of 5–2000 μg ml^{-1} for CPC and 1–500 μg ml^{-1} for TTC. Limit of detection and quantitation for CPC was 0.033 and 0.11 μg ml^{-1}, for TTC were 0.0056 and 0.019 μg ml^{-1}. The R.S.D. of repeatability and intermediate precision for CPC and TTC were less than 2.0%.

Keywords: Cetylpyridinium chloride; Tetracaine hydrochloride; Determination; Buccal tablet

1. Introduction

A tablet formulation, "Xipiluan buccal tablet", was produced by Nanjing Lifenergy Health Co., Ltd. of China. It is a kind of compound preparations for the treatment of common infections of the mouth and throat. Each tablet which weighs 1.5 g contains 1.5 mg of cetylpyridinium chloride (CPC), 0.1 mg of tetracaine hydrochloride (TTC) and 50 mg of Vitamin C (Vc) according to the labeled composition provided. To our knowledge, no similar formulation was found in the USP25 (United States Pharmacopoeia), BP2002 (British Pharmacopoeia), CP 2000 (Chinese Pharmacopoeia).

CPC is a cationic surfant widely used as antibacterial agent. Some methods, such as titration method [1,2], spectrophotometric estimation [3,4], thin-layer chromatography (TLC) [5,6], gas chromatography (GC) [7,8] and high-performance liquid chromatography (HPLC) [9–12], were developed for the determination of CPC. TTC is widely used as an ester-type local anaesthetic to reversibly block nerve function. TTC can be determined by sequential injection analysis with chemiluminescence [13], GC-MS [14,15] and

* Corresponding author. Tel.: +86-25-327-1280; fax: +86-25-539-1161.
E-mail address: njhuyuzu@jlonline.com (Y. Hu).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00108-0

HPLC [16]. Vc in this tablets has anti-scorbutic activity, which is easy to determine by the titrimetric methods [17–19], as well as the HPLC methods [20–24].

We also did not find publications related to simultaneous determination of the three active substances of the buccal tablet in pharmaceutical dosage forms, although each of them has official standard in USP, BP and CP. Thus, the focus of present study was concerned with the objective to develop a HPLC method for the simultaneous determination of CPC and TTC.

2. Experimental

2.1. Chemicals and reagents

Methanol was of chromatographic grade and was purchased from Huaiyin Han-Bang Science & Technology Co., (Jiangsu, China). Tetramethylammonium hydroxide (25% aqueous, w/w) was analytical-reagent grade from Shanghai Reagent Co., (Shanghai, China). TTC (CP reference standard) was purchased from Yanjing Pharmaceutical Factory (Beijing, China). Vc (CP reference standard) was purchased from Jiangshan pharmaceutical Co., Ltd. (Jiangsu, China). CPC (CP reference standard) and the buccal tablets were supplied by Nanjing Lifenergy Health Co., Ltd., (Jingsu, China). All other reagents were analytical-reagent grade and purchased from Nanjing Chemical Reagent Factory (Jiangsu, China). Water was demineralized and bidistilled.

2.2. Apparatus and chromatographic conditions

The chromatograph used in this study consisted of a LC-10A pump (Shimadzu, Japan), a SIL-10A injection valve with a 20 μl loop, a SPD-10A UV–Vis detector operated at 230 nm. Data acquisition was performed using N2000 chromatography software from Zhejiang University (Zhejiang, China). The pH measurement was performed on a pH meter (Orion, model 818, Shanghai, China). A Shimadzu UV-2100 spectrophotometer was used for scanning and selecting working wavelength of detection.

Chromatographic separation was performed on a Hypersil CN column (150 × 4.6 mm i.d., 5 μm particle size) packed and supplied by Dalian Elite Scientific Instruments Co., Ltd. (Liaoning, China). The mobile phase was methanol–tetramethylammonium hydroxide (20 mM)–potassium dihydrogen phosphate (3 mM) (90:10:3, v/v/v). The apparent pH was adjusted to 5.0 using acetic acid. The flow rate was maintained at 1.5 ml min^{-1}. Prior to use the mobile phase was filtered through a 0.45 μm membrane and degassed for 10 min. Peak areas were measured and all separations were carried out at ambient temperature.

2.3. Stock and working standard solutions

Standard stock solutions of CPC (2 mg ml^{-1}) and TTC (0.5 mg ml^{-1}) were prepared by dissolving 200 mg standard CPC and 50 mg standard TTC in 100 ml mobile phase. The stock solutions were diluted with mobile phase to obtain the final concentration of CPC 0.5, 5, 15, 50, 100 and 200 μg ml^{-1}; TTC 0.1, 0.5, 1.0, 5, 10 and 25 μg ml^{-1}.

Standard solution was prepared with the concentration of CPC 60 μg ml^{-1} and TTC 4 μg ml^{-1}. This solution was used as the working standard for the determination of CPC and TTC. Standard solution was found to be stable during the analysis time.

2.4. Calibration procedure

Calibration curves of CPC and TTC were conducted using the standard solutions described previously. Triplicate 20 μl injections were made of each standard solution and each calibration curve was fitted by linear regression according to the peak area against the corresponding concentration of CPC and TTC.

2.5. Assay of pharmaceutical preparations

Twenty tablets were weighed and finely pulverized. An appropriate of this powder, equivalent to 3.0 mg of CPC and 0.2 mg of TTC was placed in 50 ml volumetric flask with 40 ml of mobile phase. The solution was sonicated for 3 min and diluted to volume with mobile phase. A portion of this

solution was centrifuged at 2500 rppm for 5 min. The aliquot was filtered through the 0.45 μm membrane and used for the analysis containing 60 μg ml^{-1} of CPC and 4 μg ml^{-1} of TTC. 20 μl sample was injected into the HPLC system as mentioned above.

3. Results and discussion

3.1. Method development

Initial separations were conducted with a Hypersil C18 column (Dalian Elite, China), methanol–water–triethylamine (70:30:0.5, v/v) as mobile phase. It was confirmed as reported [25] that the CPC had about 5-fold stronger affinity to the C18 surface than the structurally different, symmetric, spheric molecule of tetrabutilammonium (TBA), mainly for the long alkyl (C16) chain of CPC. The peak shape of CPC seemed to be bad with tailing factor (T_f) over 3.0. Changing the amount of methanol from 70 to 90% and triethylamine from 0.5 to 1.0% did not decrease the affinity of CPC to the C18 surface.

Afterwards, the separation was tested on a CN column with competitive binding reagent contained in the mobile phase. Initially, cetyltrimethylammonium bromide (CTAB) (from 1 to 10 mM) ion pairing agent in methanol–water (70:30, v/v) was chosen as mobile phase. The value of T_f was over 2.0. When the mobile phase adopted methanol–methylammonium hydroxide (70: 30, v/v), the pH was adjusted from 3.0 to 6.0 with acetic acid, the T_f of CPC could be reduced significantly and the pH 5.0 gave the best results with good peak shape and suitable retentions of CPC and TTC. Methanol in the mobile phase could reduce T_f and make the peak symmetrical. When the ration of methanol reached to 90%, T_f of CPC was 1.24, but CPC and TTC could not reach baseline separation ($R < 1.5$). Adding 3 ml of potassium dihydrogen phosphate (3 mM) to 100 ml mobile phase could improve the separation ($R = 2.1$), but over 3 ml the T_f of CPC became bad again.

The flow rate also affected the separation and the tailing factor of CPC. At flow rate of 1 ml min^{-1} T_f and R_s was 1.52 and 2.3, respectively; at 1.5 ml min^{-1} R_s 2.1, T_f 1.34; at 2.0 ml min^{-1}, R_s 1.88, T_f 1.26. Therefore, we choose the flow rate of 1.5 ml min^{-1} to have satisfactory separation.

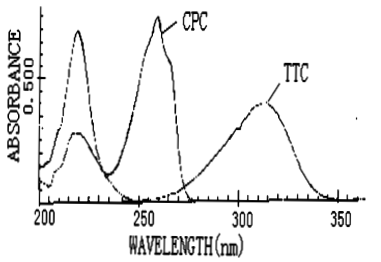

Fig. 1. Absorption spectra for CPC (dotted line) and TTC (continuous line).

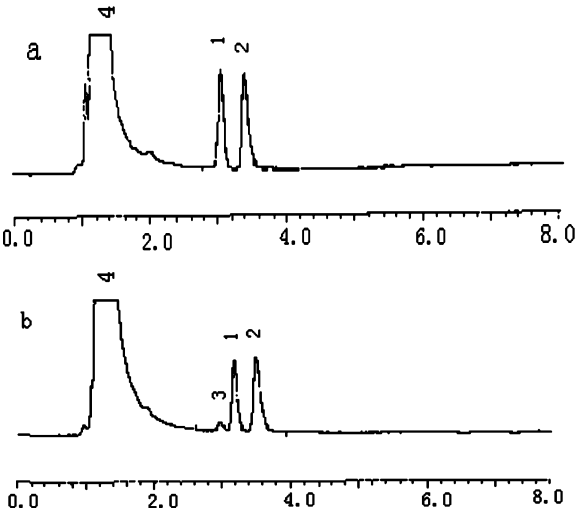

Fig. 2. HPLC profiles. (a) The solution of real sample; (b) the solution of real sample after degradation solution under 1 M NaOH. 1, TTC; 2, CPC; 3, degradation of TTC; 4, excipients and Vc.

The UV spectrum of CPC and TTC is shown in Fig. 1. In the buccal tablets, the label claim for CPC and TTC was 1.5 and 0.1 mg. In order to simultaneously determine the amount of CPC and TTC, a wavelength of 230 nm was chosen as the common wavelength to match the concentration ratio of the drug presented in the formulation, because the position of 230 nm was peak valley for spectrum of CPC and peak top for spectrum of TTC. In the typical chromatogram with such

Table 1
System suitability and limits of detection/quantification in CPC and TTC determination ($n = 5$)

Parameters	CPC	TTC
Retention time (min)	3.52	3.10
R.S.D. (%)	1.0	0.6
Capacity factor	2.91	2.44
Tailing factor	1.34	1.09
Resolution factor	2.1	
R.S.D. (%)	0.9	0.6
LOD (μg ml^{-1})	0.033	0.0056
LOQ (μg ml^{-1})	0.11	0.019
R.S.D. (%)	4.4	1.9

HPLC system, the absorbance of CPC nearly equaled to that of TTC for the real formulation sample as shown in Fig. 2.

3.2. Selectivity

In order to confirm the selectivity of proposed method, forced degradation studies were performed under various stress conditions. Thus, appropriate amounts of powdered tablets equivalent to two tablets weight were deal with 1.0 M HCl, 1.0 M NaOH for 4 h at room temperature. Moreover, samples of powdered tablets were exposed to daylight and heated in 100 °C for 4 h, respectively. After the degradation treatments were completed, the samples were analyzed according to assay sample preparations. Under the stressed conditions, CPC was very stable and no degradation was found while TTC was also stable except in the 1 M NaOH solution and one unknown degradation peak ($t_r = 2.85$ min) appeared. Fig. 2 shows HPLC profiles of the degradation solution of real formulation sample under 1 M NaOH solution.

CPC ($t_r = 3.52$ min), TTC ($t_r = 3.10$ min) could be well separated with R_s 2.1. The blank excipient, and the peak produced by degradation did not interfere with CPC and TTC, but Vc and blank excipient peaks were almost at the same time, so Vc could not be separated well from the excipients. Thus, the HPLC method was selective and could be used for simultaneous determination of CPC and TTC.

3.3. System suitability

To ascertain the resolution and reproducibility of the HPLC method, system suitability tests were carried out using working standard solution of CPC and TTC. This solution was injected five times, parameters such as retention time, tailing factor, theoretical plate number, resolution factor, capacity factor, limit of detection (LOD) (S/N 3:1) and limit of quantification (LOQ) (S/N 10:1) were studied. Their average values, along with relative standard deviation (R.S.D.) values, were presented in Table 1.

3.4. Linearity

The linearity of this method was investigated for CPC and TTC at six concentration levels. The calibration curves for CPC and TTC were linear in

Table 2
Accuracy evaluation in CPC and TTC analysis of pharmaceutical formulations ($n = 6$)

Drug	Amount of drug added (mg)	Amount found (mg)	Recovery (%)	R.S.D. (%)
CPC	2.2500	2.290	101.8	0.89
	3.0000	3.023	100.8	1.2
	3.7500	3.802	101.4	1.0
TTC	0.1500	0.1495	99.67	0.82
	0.2000	0.1972	98.60	1.1
	0.2500	0.2530	101.2	1.4

Table 3
The evaluation of intermediate precision ($n = 3$)

	Batch No.1		Batch No.2		Batch No.3	
	Amount of CPC (mg per tablet) (label claim 1.5 mg per tablet)	Amount of TTC (mg per tablet) (label claim 0.1 mg per tablet)	Amount of CPC (mg per tablet) (label claim 1.5 mg per tablet)	Amount of TTC (mg per tablet) (label claim 0.1 mg per tablet)	Amount of CPC (mg per tablet) (label claim 1.5 mg per tablet)	Amount of TTC (mg per tablet) (label claim 0.1 mg per tablet)
Lab 1						
Analyst 1	1.4670	0.09815	1.4834	0.09988	1.4722	0.09655
Analyst 2	1.4819	0.09947	1.4681	0.1113	1.4809	0.09973
Analyst 3	1.4789	0.1006	1.5060	0.1022	1.4789	0.09716
Lab 2						
Analyst 1	1.4852	0.09858	1.4953	0.1004	1.4669	0.1082
Analyst 2	1.5121	0.1022	1.5100	0.1108	1.4928	0.09816
Analyst 3	1.5080	0.09872	1.5219	0.09804	1.4590	0.1002
Mean	1.4888	0.09962	1.4974	0.1038	1.4751	0.1020
R.S.D. (%)	1.2	1.6	1.3	0.6	0.8	0.5

the range 5–2000 and 1–500 μg ml^{-1}, respectively. The representative linear equations relating y (peak area) to x (concentration μg ml^{-1}) were CPC $y = 1512 + 260\,300x$ ($r = 0.9999$) and TTC $y = 6416 + 2\,866\,000x$ ($r = 0.9999$).

3.5. Stability of the test solution

The stability of the real sample solution of CPC and TTC was monitored by measuring the areas of response of injections kept at room temperature over a period of 7 days: 0, 1, 2, 3, 4, 5, 6, 7 days. In 7 days, the R.S.D. (%) values of CPC and TTC were 0.4 and 0.3, respectively.

3.6. Accuracy and precision

To confirm the accuracy of the proposed methods, recovery experiments were carried out by adding a known amount of standard to the blank excipient at three different levels. Each level was repeated six times ($n = 6$) and the amounts of drug were found by the assay methods. The recovery was calculated by dividing the amount of found by the added, then multiplied by 100%. Results and statistical parameters were shown in Table 2.

The precision of HPLC method was determined by repeatability and intermediate precision experiments. The repeatability was evaluated by preparing six real sample solutions for determination of the samples of same batch number according to the assay of pharmaceutical preparations. The R.S.D.(%) values for repeatability of CPC and TTC were 0.4 and 0.3, respectively. Three different analysts in two labs on two instruments performed intermediate precision experiments with separate mobile phase according to the assay of pharmaceutical preparations. Each real sample solution was assayed in triplicate times. The results showed in Table 3. The results show that the method is precise and accurate.

4. Conclusion

The HPLC method for simultaneous assay of CPC and TTC in Xipiluan buccal tablets was developed and validated. It was easy to perform, precise and accurate. The whole procedure may be extended to the applications on quality control of commercial products.

References

[1] The United States Pharmacopeia, National Formula, US Pharmacopeial Convention, Reckville, MD, 2000, p. 370.
[2] The British Pharmacopeia, British High Commission, London, 2002, p. 350.
[3] L.J. Schep, D.S. Jones, I. Tucker, M.G. Shepherd, Int. J. Pharm. 113 (1995) 263–266.
[4] M. Benamor, N. Aguersif, M.T. Braa, J. Pharm. Biomed. Anal. 26 (2001) 151–154.
[5] H.T. Rasmussen, A.M. Pinto, M.W. DeMouth, P. Touretzky, B.P. McPherson, J. High Resolution Chromatogr. 17 (1994) 593–596.
[6] J. Paesen, I. Quintens, G. Thoithi, E. Roets, G. Reybrouck, J. Hoogmartens, J. Chromatogr. A 677 (1994) 377–384.
[7] R.B. Tayler, S. Toasaksiri, R.G. Reid, J. Chromatogr. A 798 (1998) 335–343.
[8] J.M. Herrero-Martinez, E.F. Sim-Alfonso, C. Mongay-Fernandez, G. Ramis-Ramos, J. Chromatogr. A 895 (2000) 227–235.
[9] J.E. Parkin, J. Chromatogr. 635 (1993) 75–80.
[10] K. Heinig, C. Vogt, G. Werner, J. Chromatogr. A 745 (1996) 281–292.
[11] R.B. Tayler, S. Toasaksiri, R.G. Reid, J. Capillary Electrophoresis 5 (1998) 45–50.
[12] L.M. Nair, R. Saari-Nordhaus, J. Chromatogr. A 804 (1998) 233–239.
[13] H. Pasekova, M. Polaseka, Talanta 52 (2000) 67–75.
[14] J. Klein, D. Fernandes, M. Gazarrian, G. Kent, G. Koren, J. Chromatogr. B 655 (1994) 83–90.
[15] T. Ohshima, T. Takayasu, J. Chromatogr. B 726 (1999) 185–194.
[16] R. Gill, R.W. Abbott, A.C. Moffat, J. Chromatogr. 301 (1984) 155–163.
[17] N.K. Pandey, Anal. Chem. 54 (1982) 793–796.
[18] R. Men, S. Liu, S. Yan, Fenxi Huaxue 17 (1989) 903–905.
[19] W. Zhou, M. Ji, Z. Mao, Zhongguo yaoxue Zazhi 25 (1990) 95–96.
[20] H.R. Kim, P.A. Seib, J. Chromatogr. A 803 (1998) 141–145.
[21] H. Iwase, I. Ono, J. Chromatogr. A 806 (1998) 361–364.
[22] M.G. Pauloa, H.M.C. Marques, J.A.G. Morais, A.J. Almeida, J. Pharm. Biomed. Anal. 21 (1999) 399–406.
[23] V. Gokmen, N. Kahraman, N. Demir, J. Acar, J. Chromatogr. A 881 (2000) 309–316.
[24] T. Deima, C. Akay, K. Buyukafar, J. Pharm. Biomed. Anal. 26 (2001) 15–21.
[25] G. Szasz, B.Z. Budvarine, F.K. Gyimesine, Acta Pharm. Hung. 66 (1996) 101–104.