Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001, Pages 191-198 藥物食品分析 第九卷 第四期

Simultaneous Quantitation of Cationic Disinfectants by High-Performance Liquid Chromatography on a Silica Gel Column Using Aqueous Eluents

FU-AN CHEN[1], KUN-SHAN WU[2], MING-CHUNG HUANG[3], CHAU-YANG CHEN[1] AND AN-BANG WU[4]*

[1.] *Department of Pharmacy, Ta-jen Institute of Technology, 20, Wei-Shin Road, Yan-Puu Hsiang, Pingtung Hsien 907, Taiwan, R.O.C.*

[2.] *Tainan County Health Bureau, 72, San Min Road, Hsinyiang City, Tainan Hsien 732, Taiwan, R.O.C.*

[3.] *National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2, Kuen Yang Street, Nankang 115, Taipei, Taiwan, R.O.C.*

[4.] *Graduate Institute of Pharmaceutical Sciences, Taipei Medical University, 250, Wu Hsing Street, Taipei 110, Taiwan, R.O.C.*

(Received: June 29, 2001; Accepted: August 15, 2001)

ABSTRACT

A simple, rapid, and accurate high-performance liquid chromatographic (HPLC) method for quantitation of cationic disinfectants was developed using a silica gel column with aqueous mobile phases. The disinfectants are classified into two categories, both of which can be separated simultaneously on a μ-Porasil silica gel column (3.9 × 300 mm). An aqueous mobile phase (1), composed of CH_3CN: H_2O (70/30, v/v), triethylamine (0.04%), and acetic acid (0.1%), with UV detection at 262 nm, provides quantitation of benzalkonium chloride (**1**) and chlorhexidine gluconate (**2**) in cosmetics. The identification of two cationic disinfectants in each cosmetic sample can be achieved in 9 min with dextromethorphan HBr as an internal standard. With the mobile phase (2) of CH_3CN: H_2O (90/10, v/v), triethylamine (0.04%), and acetic acid (0.1%), setting the UV detection at 269 nm can identify the constituents of domiphen bromide (**3**), benzethonium chloride (**4**), cetylpyridium chloride (**5**), and dequalinium chloride (**6**) in pharmaceuticals. When propantheline bromide was used as an internal standard, the quantitation of four cationic disinfectants in each pharmaceutical sample could be completed within 16 min. The method provides a reliable, prompt way to analyze cationic disinfectants in both household cosmetics and pharmaceutical preparations available on the market.

Regression equations of six standard curves revealed the related coefficients, r^2, of between 0.9997 and 1.0000. The HPLC method can be carried out with a high level of precision and accuracy. From the intra- and interday tests, the coefficients of variation (CV) were between 0.13% and 2.04% for cosmetics, and 0.08% and 0.87% for pharmaceuticals. The recoveries were found to be 96.75%~99.95% for cosmetics with CV of 0.45%~2.88%, and 99.17%~99.92% for pharmaceuticals with CV of 0.21%~0.47%.

Key words: HPLC, cationic disinfectant, silica column, cosmetics, pharmaceuticals

INTRODUCTION

Benzalkonium chloride (**1**) and chlorhexidine gluconate (**2**), commonly used in cosmetics as preservatives or bactericides, should not exceed 0.05% according to the *Drug Components Standard of Cosmetics*[(1)] as regulated by the Department of Health, Executive Yuan, R.O.C. Benzalkonium chloride is often added to cosmetics such as shampoos and hair conditioners, while chlorhexidine gluconate is an active ingredient in compounding facial creams. In general, the preservatives, i.e., benzalkonium chloride, domiphen bromide (**3**), benzethonium chloride (**4**), cetylpyridium chloride (**5**), and dequalinium chloride (**6**), are classified as quaternary amine drugs, which share a few common properties, e.g., they can adhere to the surfaces of bacteria, destroying their cell walls and membranes, and inactivating their enzymatic activities. These drugs are often used as effective ingredients for manufacturing certain pharmaceutical preparations such as tablets and lotions. Chlorhexidine gluconate does not belong to the same family as it does not have a quaternary ammonium functional group. However, it has similar physical, chemical, and anti-bacterial properties[(2)], and thus it is regarded as an effective anti-bacterial biguanide.

In using reversed-phase HPLC to analyze basic amine drugs, the most common disadvantages encountered are peak tailing and lengthy time consumption[(3-6)]. In the present study, we selected a silica gel column combined with aqueous mobile phases for HPLC analysis of six basic amine drugs with the objective of improving of quantitation techniques.

MATERIALS AND METHODS

I. *Materials*

(I) *Cosmetics and Pharmaceuticals*

The cosmetics tested included various brands of shampoo and facial cream available on the market. Pharmaceuticals were in the forms of tablets and lotions. Both categories of samples (trade names are substituted by codes) were pur-

* Author for correspondence. Tel: 02-27361661 ext. 692; Fax: 02-27366518; E-mail:anbangwu@tmu.edu.tw

chased from a local drugstore in suburban Taipei in 1997.

(II) *Reagents*

Methanol and acetonitrile (LC grade) were purchased from Labscan (Dublin, Ireland). Triethylamine (reagent grade) was from Merck (Gibbstown, NY, USA). Acetic acid (reagent grade) was from Nacalai Tesque (Kyoto, Japan). Tetramethylammonium chloride (reagent grade) was purchased from Sigma Chemical (St. Louis, MO, USA). Sodium chloride (reagent grade) was from Wako Pure Chemical Industries (Osaka, Japan).

(III) *Standard Chemicals and Internal Standards*

The chlorhexidine gluconate (20%) standard was purchased from Sigma Chemical. Benzalkonium chloride (C16), domiphen bromide, benzethonium chloride, cetylpyridium chloride, dequalinium chloride, propantheline bromide, and dextromethorphan HBr were from USP Standards (Rockville, MD, USA).

(IV) *Apparatus*

The high-performance liquid chromatograph included a Waters (Milford, MA, USA) Model 600E system controller, Waters Model 510 pump, Waters 717 auto-sampler, Waters 486 tunable absorbance auto-sampler detector, and a computer integrator. A Waters μ-Porasil 125A, 10-μm silica gel column (3.9 mm i.d. × 300 mm) and a Milli-Q-Waters purification system (Milli-Pore, Milford, MA, USA) were used.

II. *Methods*

(I) *HPLC Analysis Conditions*

1. Mobile phase (1) (for the analysis of cosmetics): CH_3CN: H_2O (70/30, v/v) with triethylamine (0.04%) and acetic acid (0.1%).

2. Mobile phase (2) (for the analysis of pharmaceuticals): CH_3CN: H_2O (90/10, v/v) with triethylamine (0.04%) and acetic acid (0.1%).

The solvents were mixed well, filtered through the Milli-Q purification system, and degassed for further use. The flow rate was 1.0 mL/min.

(II) *Preparation of Standard Solutions*

1. Stock Solutions (1)

An amount of 100 mg of benzalkonium chloride was weighed accurately and placed in a 100-mL volumetric flask. The content was dissolved in mobile phase (1) and diluted to mark, making a solution with a concentration of 1.0 mg/mL. Accordingly, 100 mg of a 20% solution of chlorhexidine gluconate was taken, dissolved in mobile phase (1), and diluted to make a concentration of 0.20 mg/mL.

2. Stock Solutions (2)

Amounts of 100 mg each of domiphen bromide, benzethonium chloride, cetylpyridium chloride, and dequalinium chloride were weighed separately and accurately in a 100-mL volumetric flask. Each solution was dissolved in methanol one by one, and diluted to mark to make a concentration of 1.0 mg/mL.

(III) *Preparation of Standard Solutions*

Five different concentrations of benzalkonium chloride and chlorhexidine gluconate solutions (Table 1) were prepared from stock solution (1). An amount of 20 μg/mL of dextromethorphan HBr, as an internal standard, was added to each solution. Accordingly, five different concentrations of domiphen bromide, benzethonium chloride, cetylpyridium chloride, and dequalinium chloride solutions (Table 1) were prepared from stock solutions (2). Each solution was supplemented with 100 μg/mL propantheline HBr as an internal standard.

For HPLC analysis, 20 μL of each sample of the six prepared standard solutions (each in five different concentrations) was withdrawn and injected. Each sample was determined with six replicates to obtain the average area ratio of spiked disinfectant to the internal standard. Finally, linear regression equations and correlation coefficients were obtained from plots of concentration vs. area ratio.

(IV) *Intra- and Interday Tests*

Three batches of each disinfectant in solutions were pre-

Table 1. Linear regression equations obtained from the standard curves of six cationic disinfectants

Cationic disinfectant	Concentration of standard solutions (μg/mL)	Regression equation	r^2
Benzalkonium chloride[a]	10,20,30,40,50	Y = 0.02001X-0.00747	0.9997
Chlorhexidine gluconate[a]	2.0,4.0,6.0,8.0,10	Y = 0.9575X-0.3732	0.9999
Domiphen bromide[b]	20,50,100,150,200	Y = 0.00629X-0.00934	1.0000
Benzethonium chloride[b]	20,50,100,150,200	Y = 0.00647X-0.01133	1.0000
Dequalinium chloride[b]	20,50,100,150,200	Y = 0.01433X-0.01946	1.0000
Cetylpyridium chloride[b]	20,50,100,150,200	Y = 0.00684X+0.00243	1.0000

[a] Dextromethorphan HBr (conc.= 20 μg/mL), [b] Propantheline bromide (conc.= 100 μg/mL) as an internal standard.

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pared and used to carry out intraday tests. The same procedure was repeated for 5 consecutive days to conduct the interday tests, and standard deviations (S.D.) and coefficients of variation (CV) were calculated (Table 2). The intraday and interday variations were used to test the precision of the method.

(V) *Recovery Experiment*

1. Cosmetics

(1) Recovery of Benzalkonium Chloride

An amount of 50 mg of tetramethylammonium chloride was carefully weighed and placed in a 25-mL volumetric flask, which contained 2.5 mL of distilled water. By adding CH_3CN to mark, the concentration of the solution was exactly 0.20%. An amount of 0.40 g each of three different brands of shampoo were weighed and placed in a 10-mL volumetric flask. Then, 4 mL of 50 μg/mL benzalkonium chloride standard solution (spiked amount equivalent to 0.05% w/w), 4 mL of 0.2% tetramethylammonium chloride, and 200 μg of dextromethorphan HBr were added. Finally, mobile phase (1) was added to mark. The mixture was shaken for 1 minute to ensure complete dissolution. The solution was then centrifuged at 3000 rpm for 15 minutes. With 20 μL of the supernatant solution injected to HPLC, the recovery ratio of benzalkonium chloride was calculated from its peak-area to standard ($A_{\text{benzalkonium chloride}}/A_{\text{internal standard}}$) ratio by the obtained linear equation.

(2) Recovery of Chlorhexidine Gluconate

A sample of 0.20 g facial cream was carefully weighed and placed in a 10-mL volumetric flask. Next, 4 mL of 25 μg/mL chlorhexidine gluconate standard solution (spiked amount equivalent to 0.05% w/w), 2 mL of 0.7% NaCl solution, and 200 μg of dextromethorphan HBr were added. Finally, mobile phase (2) was added to mark. The mixture was shaken for 1 minute, then transferred to a 15-mL glass

Table 2. Interday and intraday tests of six cationic disinfectans in the cosmetic and pharmaceutical samples

Cationic disinfectant	concentration (μg/mL)	Mean ± S.D.	CV(%)
Benzalkonium chloride	25[a]	24.8 ± 0.08	0.34
	45[a]	44.8 ± 0.17	0.37
	25[b]	24.9 ± 0.10	0.40
	45[b]	44.8 ± 0.06	0.13
Chlorhexidine gluconate	5.0[a]	5.0 ± 0.08	1.68
	9.0[a]	9.0 ± 0.09	1.00
	5.0[b]	4.9 ± 0.10	2.04
	9.0[b]	8.8 ± 0.06	0.65
Domiphen bromide	60[a]	59.9 ± 0.43	0.72
	120[a]	120.0 ± 0.45	0.37
	180[a]	180.1 ± 0.35	0.19
	60[b]	60.0 ± 0.26	0.44
	120[b]	120.0 ± 0.31	0.25
	180[b]	180.1 ± 0.25	0.14
Benzethonium chloride	60a	60.0 ± 0.47	0.79
	120[a]	120.2 ± 0.41	0.34
	180[a]	180.3 ± 0.28	0.15
	60[b]	60.1 ± 0.36	0.60
	120[b]	120.1 ± 0.32	0.27
	180[b]	180.3 ± 0.15	0.08
Dequalinium chloride	60[a]	60.1 ± 0.52	0.87
	120[a]	120.2 ± 0.29	0.24
	180[a]	180.2 ± 0.63	0.35
	60[b]	59.9 ± 0.31	0.51
	120[b]	119.9 ± 0.47	0.39
	180[b]	180.2 ± 0.82	0.45
Cetylpyridium chloride	60[a]	60.0 ± 0.30	0.51
	120[a]	120.1 ± 0.48	0.40
	180[a]	179.9 ± 0.23	0.13
	60[b]	59.8 ± 0.15	0.26
	120[b]	120.2 ± 0.32	0.27
	180[b]	180.1 ± 0.42	0.23

[a] Interday test (n=5); [b] Intraday test (n=3).

tube for centrifugation at 3000 rpm for 15 minutes. For each sample, 20 μL of the supernatant solution was injected to HPLC for analysis, and the recovery ratio of chlorhexidine gluconate was calculated from its peak-area to internal standard ($A_{\text{chlorhexidine gluconate}}/ A_{\text{internal standard}}$) ratio by the obtained linear equation.

2. Pharmaceuticals

(1) Recovery of Domiphen Bromide, Cetylpyridium Chloride, and Dequalinium Chloride

Twenty tablets containing domiphen bromide, or cetylpyridium chloride, or dequalinium chloride were taken and ground to a fine powder. Proper amounts of the three powdered pharmaceutical samples with the active ingredients equivalent to 1.0 mg were taken. After adding 7.0 mL of methanol, each sample was sonicated for 20 min. One mL of propantheline bromide with a concentration of 1.0 mg/mL was added as the internal standard. The samples were well mixed, sonicated, and centrifuged. Then, 20 μL each of the supernatant solutions was injected into HPLC for analysis. The percent recovery of each ingredient was calculated according to the peak-area to internal standard ratio.

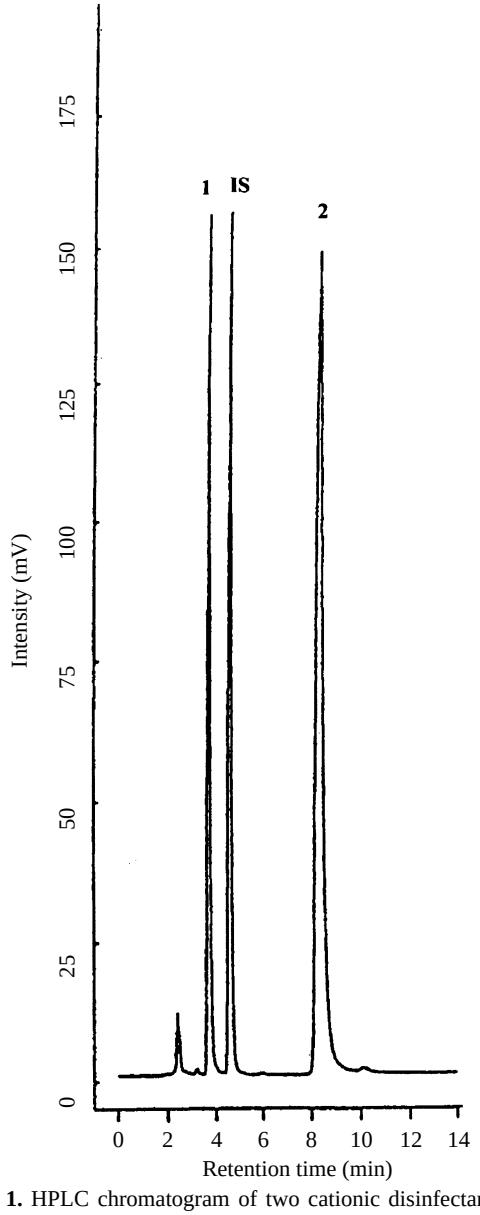

Figure 1. HPLC chromatogram of two cationic disinfectants in cosmetics:
1. Benzalkonium chloride (conc. = 45 μg/mL).
2. Chlorhexidine gluconate (conc. = 3.0 μg/mL).
IS. Dextromethorphan HBr (conc. = 20 μg/mL) as the internal standard.

(2) Recovery of Benzethonium Chloride

The fourth sample containing benzethonium chloride was in the form of a lotion. An amount of active ingredient equivalent to 1.0 mg was taken and diluted with methanol to exactly 10 mL. One mL of propantheline bromide with a concentration of 1.0 mg/mL was also added as the internal standard. Then, 20 μL of each of the supernatant solutions was injected into HPLC for analysis. The percent recovery of the ingredient was calculated according to the peak-area to internal standard ratio.

In an attempt to determine the contents of the above active ingredients which are added to marketed cosmetics and pharmaceutical preparations as sold in drugstores, a few samples were purchased at random and labeled with codes. By using our developed HPLC quantitation method, the amount of the active ingredient determined can be compared to the labeled content of the samples to calculate the percent detection.

RESULTS AND DISCUSSION

I. *Analytical Conditions*

The cationic disinfectants were classified into two categories using a μ-Porasil silica gel column (3.9 × 300 mm) for HPLC analysis: an aqueous mobile phase (1), composed of CH_3CN: H_2O (70/30, v/v), triethylamine (0.04%), and acetic acid (0.1%), with UV detection at 262 nm, benzalkonium chloride (**1**) and chlorhexidine gluconate (**2**) in cosmetics can be separated simultaneously. Compounds **1** and **2** were resolved and eluted at 3.74, and 8.26 min, respectively (Figure 1). The identification of two cationic disinfectants in each cosmetic sample can be achieved within 9 min with dextromethorphan HBr as the internal standard. With mobile phase (2) of CH_3CN: H_2O (90/10, v/v), triethylamine (0.04%), and acetic acid (0.1%), the constituents of domiphen bromide (**3**), benzethonium chloride (**4**), cetylpyridium chloride (**5**), and dequalinium chloride (**6**) in pharmaceutical samples can be identified at 269 nm simultaneously. Compounds **3**, **4**, **5**, and **6** were resolved at 8.47, 9.59, 11.4, and 15.3 min, respectively (Figure 2). When propantheline bromide was used as the internal standard, the quantitation of four cationic disinfectants in each pharmaceutical sample could be completed within 16 min.

II. *Linearity and Limit of Detection*

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Table 3. Recoveries of spiked cationic disinfectants in cosmetic and pharmaceutical samples

Cationic disinfectant	Spiked amount [a]	Recovery (%)	Mean ± S.D.[b]	CV(%)
1		100.4		
	0.05	99.95	99.95 ± 0.45	0.45
		99.50		
2		99.58		
	0.05	94.02	96.75 ± 2.78	2.88
		96.64		
3	50	99.84		
	100	100.1	99.92 ± 0.39	0.39
	150	99.34		
4	50	100.4		
	100	99.78	99.92 ± 0.43	0.43
	150	99.58		
5	50	98.98		
	100	99.15	99.70 ± 0.47	0.47
	150	99.39		
6	50	100.24		
	100	99.39	99.17 ± 0.21	0.21
	150	99.47		

[a] **1** and **2** in %w/w, **3**~**6** in μg/mL concentration. [b] Sample size n = 3.

Table 4. The content of six cationic disinfectants detected in marketed cosmetics and pharmaceuticals

Sample	Code name	Labeled ingredient/amount		Amount detected	Detection (%)
Cosmetics	Shampoo A	**1**,	0.05[a]	0.05020	100.4
	Shampoo B		0.05[a]	0.04998	99.95
	Shampoo C		0.05[a]	0.04975	99.50
	Facial Cream A	**2**,	0.05[a]	0.04978	99.58
	Facial Cream B		0.05[a]	0.04701	94.02
	Facial Cream C		0.05[a]	0.04832	96.64
Pharmaceuticals	Tablet A	**3**,	0.5[b]	0.4851	97.02
	Lotion A	**4**,	0.1[c]	0.1037	103.71
	Tablet B	**5**,	1.0[b]	0.9901	99.01
	Tablet C	**6**,	0.5[b]	0.5205	104.10

[a] % w/w; [b] mg/tablet; [c] mg/100g sample.

Benzalkonium chloride in the concentration range from 10 to 50 μg/mL shows a good linear relationship with a related coefficient of r^2 = 0.99973, and the limit of detection (LOD) is 2.0 μg/mL with signal/noise ≧ 5. Chlorhexidine gluconate in the range between 2.0 and 10 μg/mL also has a good linear relationship with a related coefficient, r^2 = 0.99993 (Table 1), and the LOD is 0.15 μg/mL. In the analysis of pharmaceuticals, the four compounds of domiphen bromide, benzethonium chloride, cetylpyridium chloride, and dequalinium chloride in the concentration range of 20~200 μg/mL exhibit good linear relationships, and with related coefficients of r^2 = 0.99996~1.0000 (Table 1). The LOD for dequalinium chloride was 1.25 μg/mL; those for the other ingredients were all 2.0 μg/mL.

III. *Precision and Accuracy*

From the observed inter- and intraday data, the precision of this method is excellent, judging from the coefficients of variation (CV) for the cosmetics being 0.13%~2.04%, and the CVs of pharmaceuticals being between 0.08% and 0.87% (Table 2).

To estimate the percent recovery in cosmetics, a simulation method was used by which a standard solution was added along with the internal standard solution to each sample of the shampoo or facial cream. The average recovery of **1** was 99.95% among three samples of shampoo with a CV of 0.45%; the average recovery of **2** in three facial cream samples was 96.75% with a CV of 2.88%. As for the pharmaceutical samples, the spiked amounts were all 50, 100, and 150 μg/mL of the respective ingredient. The average recoveries of **3**, **4**, **5**, and **6** were between 99.17% and 99.76% with CVs of 0.21%~0.47% (Table 3). The accuracy of the method, based on the above recovery tests, is also very satisfactory.

By applying our developed HPLC quantitation method to survey the contents of cationic disinfectants in marketed cosmetics (3 brands of shampoo and 3 facial cream marked with codes) and pharmaceutical preparations (3 brands of tablet and 1 lotion), the percent detections were found to be 94.02%~104.10% (Table 4).

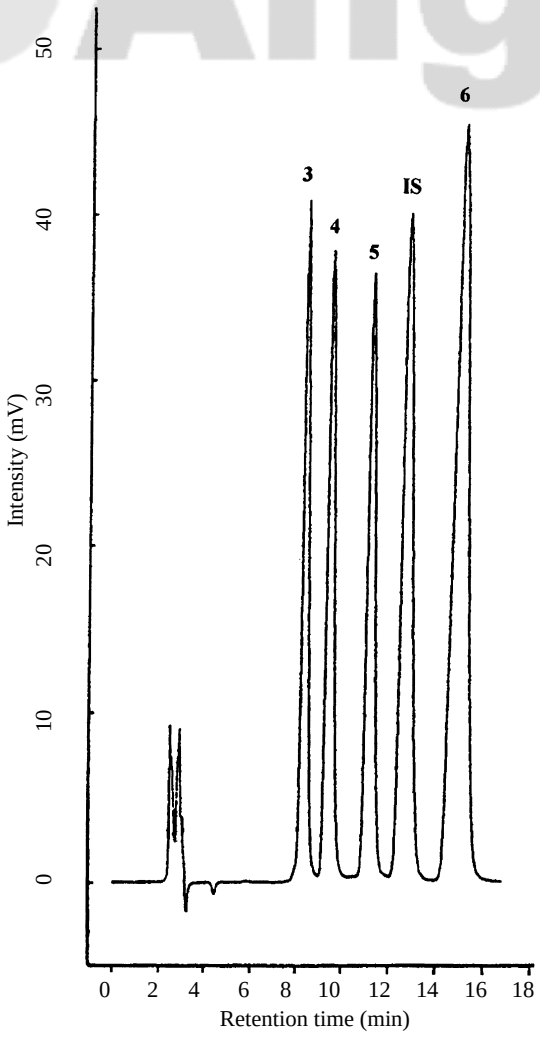

Figure 2. HPLC chromatogram of four cationic disinfectants in pharmaceuticals:
3. Domiphen bromide (conc. = 100 μg/mL).
4. Benzethonium chloride (conc. = 120 μg/mL).
5. Cetylpyridium chloride (conc. = 80 μg/mL).
6. Dequalinium chloride (conc. = 100 μg/mL).
IS. Propantheline bromide (conc. = 100 μg/mL) as the internal standard.

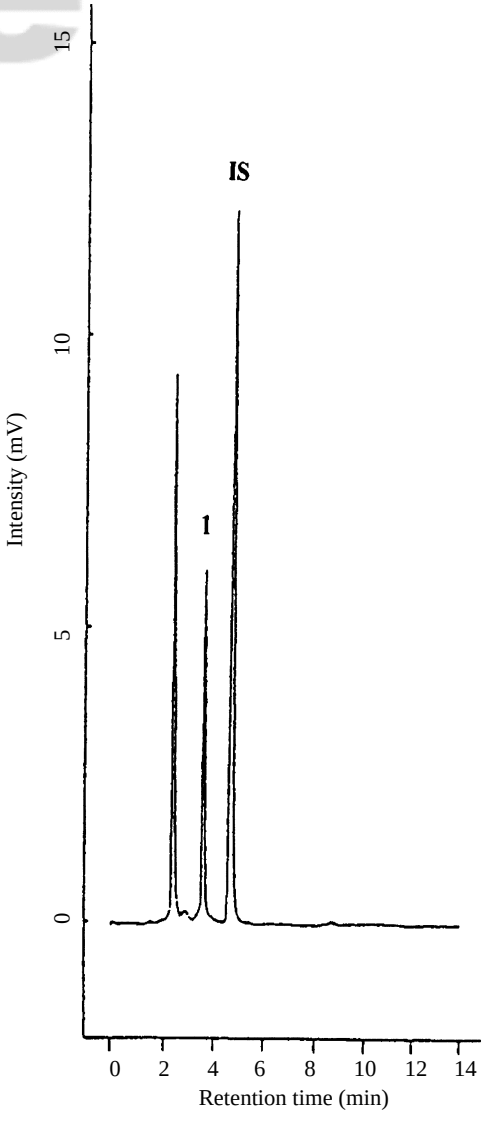

Figure 3. HPLC chromatogram of a cosmetic sample spiked with
1. Benzalkonium chloride (conc. = 20 μg/mL).
IS. Dextromethorphan HBr (conc. = 20 μg/mL) as the internal standard.

IV. *Advantages of the Improved HPLC Method*

The most common disadvantages of using reversed-phase chromatography to analyze amine compounds are peak tailing and it is time consuming. Bidlingmeyer *et al*. reported that many separations of lipophilic amines, which are very difficult on bonded reversed-phase packings, are easily accomplished with good peak symmetry on silica. The key to good peak symmetry is not the presence or absence of residual silanols but more probably the assessibility of the surface groups. If surface silanols are freely assessible, there is good symmetry[7]. In order to improve these possible disadvantages, the present study thus selected a silica gel column combined with an aqueous mobile phase for HPLC quantitation. The principle is similar to the ion exchange method, i.e., an ingredient must carry positive charge(s) for successful detection, and thus can be isolated. The components in cosmetics are usually very complex; nonetheless, the non-ionized, or anionic compounds in the sample were indeed not retained, which eliminated disturbance to the cationic ingredients[8] present. Simultaneously, the chromatograms exhibit steady baselines of sharp and symmetrical signals with improved signal-to-noise ratio and very clean chromatograms can be obtained. Additionally, the time consumed is not lengthy. Our developed HPLC method is able to complete the separation of all components in cosmetics within 9 minutes (Figure 1), while for pharmaceuticals, the overall time required was less than 16 minutes (Figure 2).

From the recovery tests of cosmetics, when benzalkonium chloride, **1**, was added to tetramethylammonium chloride solutions, the samples were found to be less colloidlike. Benzalkonium chloride is easily dissolved in the mobile phase, which can then be separated from the undissolved

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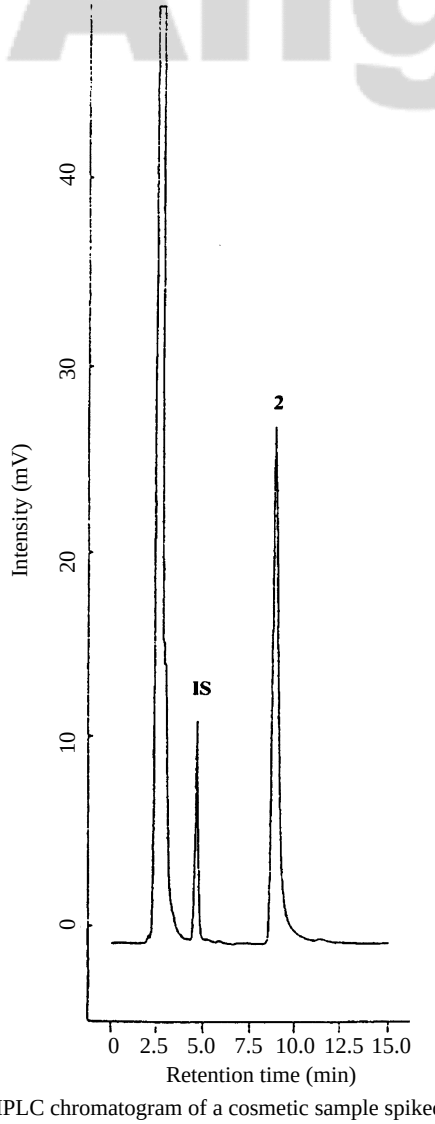

Figure 4. HPLC chromatogram of a cosmetic sample spiked with **2**. Chlorhexidine gluconate (conc. = 10 μg/mL).
IS. Dextromethorphan HBr (conc. = 20 μg/mL) as the internal standard.

vehicle of shampoo (Figure 3). Chlorhexidine gluconate carries a positive charge in neutral or weakly acidic media. On the contrary, if any sample carries a negative charge, a 0.7% NaCl solution can be added. The Na^+ ions will attract the anions, which decreases the possibility of interaction between chlorhexidine gluconate and the anions[9]. A typical HPLC chromatogram of **2** detected in a shampoo sample is shown in Figure 4. In the recovery of pharmaceuticals, the samples were dissolved in methanol, and the analyses proceeded accordingly. The above analytical method gives satisfactory results no matter what kind of vehicle is used. The HPLC chromatograms of **3** and **6** detected in tablets are shown in Figures 5 and 6.

ACKNOWLEDGEMENTS

The authors thank Mr. Hwi-Chang Chen of the National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, for his help in recalculation of some statistical data.

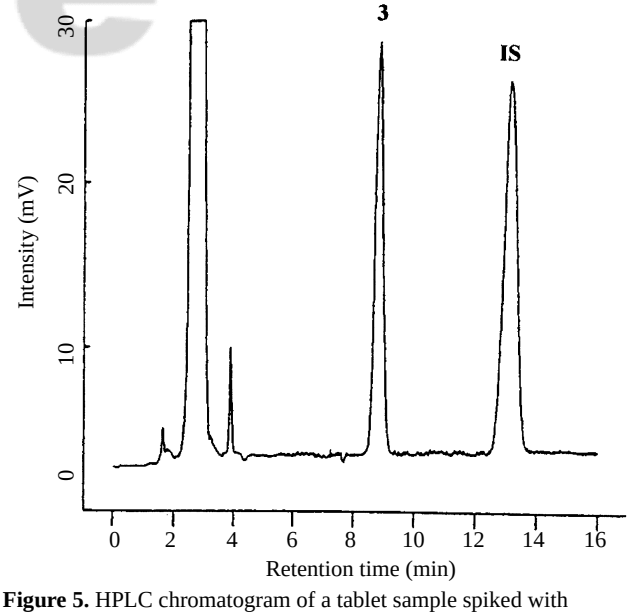

Figure 5. HPLC chromatogram of a tablet sample spiked with **3**. Domiphen bromide (conc. = 100 μg/mL).
IS. Propantheline bromide (conc. = 100 μg/mL) as the internal standard.

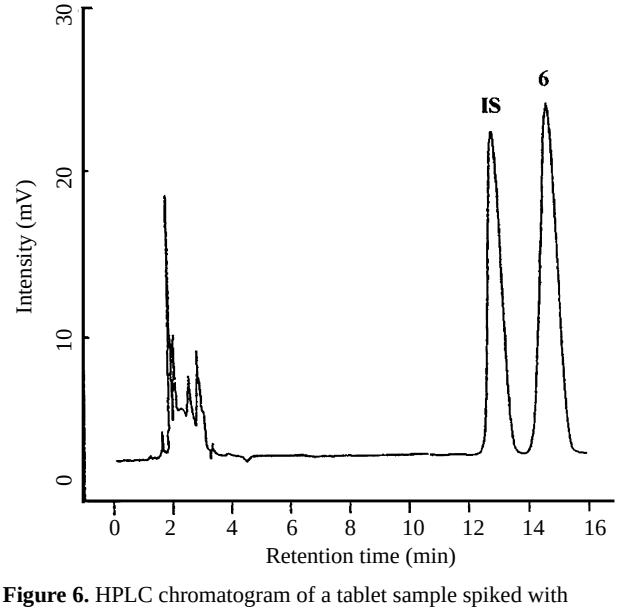

Figure 6. HPLC chromatogram of a tablet sample spiked with **6**. Dequalinium chloride (conc. = 100 μg/mL).
IS. Propantheline bromide (conc. = 100 μg/mL) as the internal standard.

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以矽膠管柱搭配含水移動相之高壓液相層析法同時定量陽離子殺菌劑成分

陳福安[1]　吳坤憲[2]　黃明權[3]　陳朝洋[1]　吳安邦[4]*

[1] 大仁技術學院　藥學系　屏東縣鹽埔鄉新二村維新路20號
[2] 台南縣衛生局　台南縣新營市三民路72號
[3] 行政院衛生署　藥物食品檢驗局　台北市南港區昆陽街161-2號
[4] 台北醫學大學　藥學研究所　台北市吳興街250號

（收稿：June 29, 2001 ；接受：August 15, 2001）

摘　要

本研究已發展出一種簡單、快速及正確之高壓液相層析法以矽膠管柱搭配含水移動相用以定量陽離子殺菌劑成分。首先將這些殺菌劑區分為兩類，使用μ-Porasil矽膠管柱（3.9公釐內徑×300公釐）皆可同時分離所添加之殺菌劑。使用第一種含水移動相，其組成為乙腈：水（70/30，v/v），0.04%三乙基胺及0.1%醋酸，紫外光檢測波長設定為262 nm，可定量添加於化妝品中之氯化苄二甲烴銨**(1)**及葡萄糖酸洛赫西定**(2)**。以氫溴酸右旋美索芬為內在標準品，可於九分鐘內偵測出每一種化妝品檢品中所含之上列兩種陽離子殺菌劑。第二種含水移動相之組成為乙腈：水（90/10，v/v），0.04%三乙基胺及0.1%醋酸，紫外光檢測波長為269 nm，可同時定量添加於藥品中之溴化多米芬**(3)**、氯化本索寧**(4)**、氯化鯨臘吡啶**(5)**及氯化氨狹那**(6)**。以溴化普泮夕林為內在標準品，可於16分鐘內定量每一種藥物檢品中所含之上列四種陽離子殺菌劑。此分析法提供一種可靠及快速的方式以分析日常家用之化妝品及藥品中陽離子殺菌劑成分。

由六種陽離子殺菌劑標準品所得之六條標準直線之線性回歸顯示相關係數，r^2，介於0.9997~1.0000之間。本高壓液相層析法具有高標準之精密度及準確度，由日內及日間誤差分析結果：化妝品檢品之變異係數介於0.13~2.04%之間；藥物檢品之變異係數則介於0.08~0.87%。化妝品檢品之回收率為96.75~99.75%，變異係數為0.45~2.88%；藥物檢品之回收率為99.17~99.92%，變異係數為0.21~0.47%。

關鍵詞：高壓液相層析法，陽離子殺菌劑，矽膠管柱，化妝品，藥品