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EJ087199900013

Journal of Food and Drug Analysis
1999. 7(1) : 13-22

藥物食品分析
第七卷　第一期

Simultaneous Determination of Complex Cold Medicine Formulations by HPLC

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ABSTRACT

A simple, rapid and reliable high performance liquid chromatographic method for simultaneous determination of compounds typically found in complex cold medicines (i.e. acetaminophen, bromvalerylurea, bucetin, caffeine, chlorpheniramine maleate, dextromethorphan HBr, ethoxybenzamide, guaifenesin, methylephedrine HCl, noscapine HCl) has been developed. Isocratic separation was performed using an Inertsil ODS-3V column (5 μm, 25 cm x 4.6 mm i.d.) with a mobile phase: 0.2% triethylamine, 2% tetrahydrofuran in 0.1% H_3PO_4: CH_3CN (85: 15; pH=2.70), flow rate 1.0 ml/min. An ultraviolet spectrophotometer was used as the detector at the wavelength of 205 nm. The precision and accuracy were satisfactory. The system can be used to assay the above compounds at typical cold medicine dosage forms.

Key words: **complex cold medicine formulations, HPLC.**

INTRODUCTION

Department of Health in Taiwan, ROC has announced that it will allow ten types of over-the-counter drugs in our country to be imported without product inspection, which will be replaced with documentation review by related authorities. The quality of these over-the-counter drugs will be controlled by inspection as normally conducted for commercial products. Complex cold medicines, commercially available and widely used to alleviate the symptom of colds[1], have been listed as one of the over-the-counter drugs. Most cold medicines contain some combination of antipyretics or analgesics (e.g. acetaminophen, bromvalerylurea, bucetin, and ethoxybenzamide), antitussives (e.g. dextromethorphan hydrobromide, guaifenesin, and noscapine hydrochloride), bronchodilators (e.g. methylephedrine hydrochloride), antihistamines (e.g. chlorpheniramine maleate) which usually causes somnolence, and some amount of central nerve system stimulant (e.g. caffeine) for reducing this side effect. Several analytical methods for some of the above compounds have been reported in literature[2, 3, 4]. Those methods, however, are not suitable for the simultaneous determination of our formulations by chromatography. This paper describes a rapid and accurate reversed-phase HPLC method using tertiary amine as a competing base for simultaneous

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Accepted for Publication: Aug. 17, 1998

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determination of the ten compounds frequently found in combination cold medicine formulas.

MATERIALS AND METHODS

HPLC analysis was carried out by using a Waters HPLC system equipped with a Waters Model 600E System Controller, a Waters Model 510 pump, a Waters Model 717 autosampler, a Waters 486 Tunable Absorbance detector, and a Leo 486 DX2-33 computer. The separation was performed on a GL Sciences Inertsil ODS-3V column (5 μm, 250 X 4.6 mm i.d.). A Milli-Q SP Reagent Water System (Milli-pore) was used to filter the mobile phase. The mobile phase was prepared as follows. We transferred 20 ml of tetrahydrofuran, 2 ml of triethylamine, and 1 ml of phosphoric acid to a 1 l-volumetric flask, and diluted

Table 1. Validation of HPLC assay methods

Chromatogram		Standard curve			Precision				
						Between day (n=5)		Within day (n=3)	
Compounds	k'[a] Rs LOD (μg/ml)	Conc. range (μg/ml)	Regression line[b]	γ^2	Added (μg/ml)	Mean±S.D. (μg/ml)	C.V. (%)	Mean±S.D. (μg/ml)	C.V. (%)
Methylephedrine HCl	2.27 3.60 0.050	10~70	Y=0.011X+0.012	0.9998	20 50 60	19.96±0.13 50.13±0.34 59.91±0.14	0.64 0.68 0.23	19.96±0.04 50.62±0.10 59.82±0.12	0.19 0.20 0.20
Acetaminophen	2.99 1.63 0.025	5~30	Y=0.0247X+0.018	0.9999	10 20 30	10.05±0.07 19.91±0.17 30.07±0.26	0.71 0.87 0.88	10.12±0.04 19.69±0.02 59.82±0.12	0.37 0.10 0.23
Caffeine Anhydrous	3.32 9.08 0.015	3~25	Y=0.0392X-0.0003	0.9999	5 10 20	4.98±0.05 10.06±0.07 19.99±0.10	0.91 0.66 0.49	5.05±0.01 10.05±0.01 20.04±0.02	0.26 0.12 0.10
Chlorpheniramine Maleate	6.04 8.49 0.070	10~100	Y=0.0112X-0.0021	0.9999	20 60 80	20.00±0.11 59.98±0.13 79.94±0.04	0.56 0.22 0.06	20.01±0.09 60.02±0.05 79.96±0.07	0.46 0.08 0.08
Guaifenesin	9.65 8.43 0.050	20~100	Y=0.0171X+0.0034	0.9998	20 50 80	19.72±0.19 50.07±0.19 80.14±0.39	0.95 0.39 0.48	20.00±0.10 50.01±0.17 80.31±0.06	0.51 0.34 0.08
Noscapine HCl	14.19 5.03 0.050	10~80	Y=0.0347X-0.0054	0.9999	20 40 60	19.98±0.15 40.02±0.19 60.05±0.06	0.77 0.48 0.11	20.00±0.08 40.00±0.05 60.12±0.04	0.41 0.13 0.07
Ethoxybenzamide	18.52 4.21 0.050	10~60	Y=0.0577X+0.006	0.9999	20 40 60	20.03±0.13 40.04±0.07 60.09±0.19	0.64 0.18 0.31	19.93±0.04 40.03±0.031 60.17±0.05	0.18 0.03 0.08
Bromvaleryulurea	24.90 2.73 0.500	25~300	Y=0.0117X-0.0012	0.9999	50 150 300	49.96±0.19 150.08±0.46 300.32±0.63	0.38 0.31 0.21	50.15±0.04 149.50±0.13 299.53±0.20	0.08 0.09 0.07
Dextromethorphan HBr	29.68 4.16 0.500	25~150	Y=0.0141X+0.0002	0.9999	50 100 150	49.99±0.18 100.05±0.53 150.51±0.67	0.37 0.53 0.45	49.92±0.12 99.68±0.21 150.38±1.27	0.24 0.22 0.85

[a] System suitability k': Capacity factor. Rs: Resolution. LOD: Limited of detection. γ^2: Correlation coefficient.

[b] Bucetin (50 μg/ml) as internal standard.

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with water to volume, then mixed well. We mixed 850 ml of resulting solution with 150 ml of acetonitrile, and adjusted with phosphoric acid to a pH=2.70 ± 0.05, and then filtered through a Milli-Q filtration system and degassed with an ultrasonic bath.

HPLC grade methanol and acetonitrile were purchased from BDH. An analytical grade triethylamine and a reagent grade sodium hydroxide were of Merck brand. Phosphoric acid (reagent grade) and tetrahydrofuran (HPLC grade) were purchased from Kanto Chemical Co. and Labscan Co., respectively.

Acetaminophen standard was supplied by Hen-Ta Chemical Co. (Taiwan). Bromvalerylurea and caffeine anhydrous were purchased from Knoll (Australia). Bucetin, chlorpheniramine maleate, dextromethorphan hydrobromide, guaifenesin and methylephedrine hydrochloride were obtained from Sigma (USA). Ethoxybenzamide standard was supplied by National Laboratories of Foods and Drugs and noscapine hydrochloride was obtained from Narcotics Bureau of Department of Health.

Test samples A~E contained the following active ingredients as each label claims.

I. *Sample A*

100 mg of acetaminophen, 12.5 mg of caffeine anhydrous, 1.25 mg of chlorpheniramine maleate, 5 mg of dextromethorphan hydrobromide, 83 mg of ethoxybenzamide, and 4 mg of dl-methylephedrine hydrochloride.

II. *Sample B*

200 mg of acetaminophen, 30 mg of caffeine anhydrous, 2.5 mg of chlorpheniramine maleate, 166.6 mg of ethoxybenzamide, 4 mg of dl-methylephedrine hydrochloride, and 10 mg of noscapine hydrochloride.

III. *Sample C*

300 mg of acetaminophen, 25 mg of caffeine anhydrous, 2.5 mg of chlorpheniramine maleate, 15 mg of dextromethorphan hydrobromide, and 14 mg of dl-methylephedrine hydrochloride.

IV. *Sample D*

80 mg of acetaminophen, 100 mg of bromvalerylurea, 25 mg of caffeine anhydrous, and 200 mg of ethoxybenzamide.

V. *Sample E*

200 mg of guaifenesin.

Internal standard stock solution was prepared by dissolving a suitable quantity of bucetin in 5 ml of methanol followed by mobile-phase diluting to obtain a solution containing ca. bucetin of 1 mg/ml.

The standard stock solutions were prepared as

Table 2. Evalution of recovery and determination of the compounds in commercial products

Compounds	Evaluation	Commercial products				
	Recovery (%)	Tablet A	Capsule B	Capsule C	Tablet D	Tablet E
Methylephedrine HCl	100.4	4.0(104.1)[a]	14.0(95.6)[a]	10.0(101.6)[a]		
Acetaminophen	100.0	100.0(93.1)	300.0(96.3)	200.0(99.2)	80.0(90.2)[a]	
Caffeine anhydrous	99.4	12.5(94.3)		30.0(104.5)	25.0(99.8)	
Chloropheniramine Maleate	101.0	1.25(95.6)	25.0(109.5)	2.5(106.7)		
Ethoxybenzamide	102.7	83.0(92.1)		166.6(103.1)	200.0(92.9)	
Dextromethorphan HBr	100.5	5.0(99.7)	15.0(104.0)			
Noscapine HCl	101.9			10.0(109.2)		
Bromvalerylurea	101.9				100.0(109.3)	
Guaifenesin	99.5					200.0(100.0)[a]

Bucetin (50 mg/ml) as internal standard.

[a]: Listed content in mg/tablet or mg/capsule (retrieved quantity in %).

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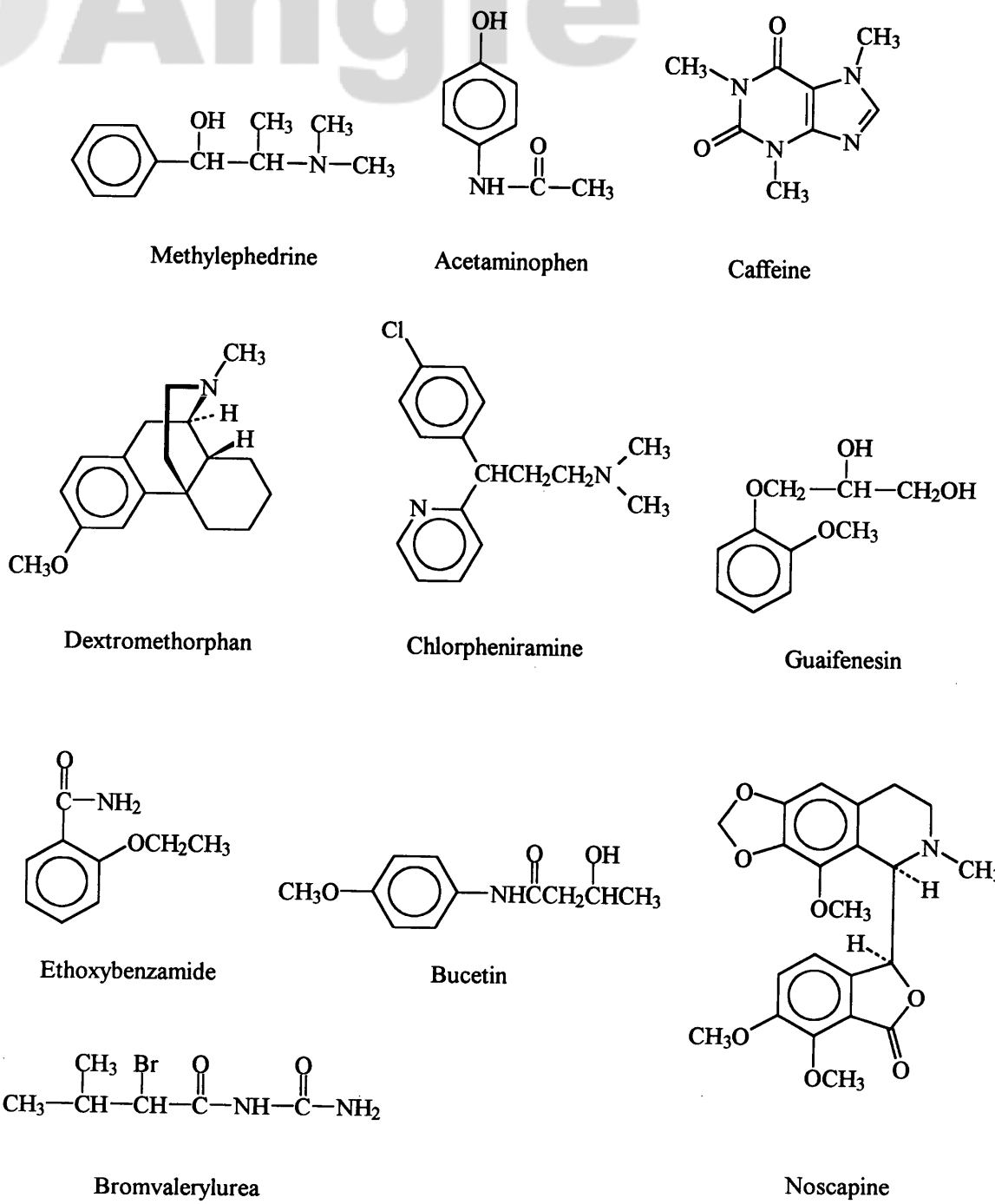

Figure 1. The structures of compounds commonly found in cold medicines.

follows. We transferred an accurately-weighed quantity of acetaminophen, bromvalerylurea, caffeine anhydrous, chlorpheniramine maleate, dextromethorphan hydrobromide, ethoxybenzamide, guaifenesin, dl-methylephedrine hydrochloride, and noscapine hydrochloride each to a suitable volumetric-flask respectively. Then, we dissolved them in 5 ml of methanol followed by mobile-phase diluting to make each solution reach a fixed concentration of 1 mg/ml.

Linearity was studied over the concentration range as described in Table 1 with 50 μg/ml of bucetin as internal standard. We separately injected equal volume (about 20 μl) of above solutions

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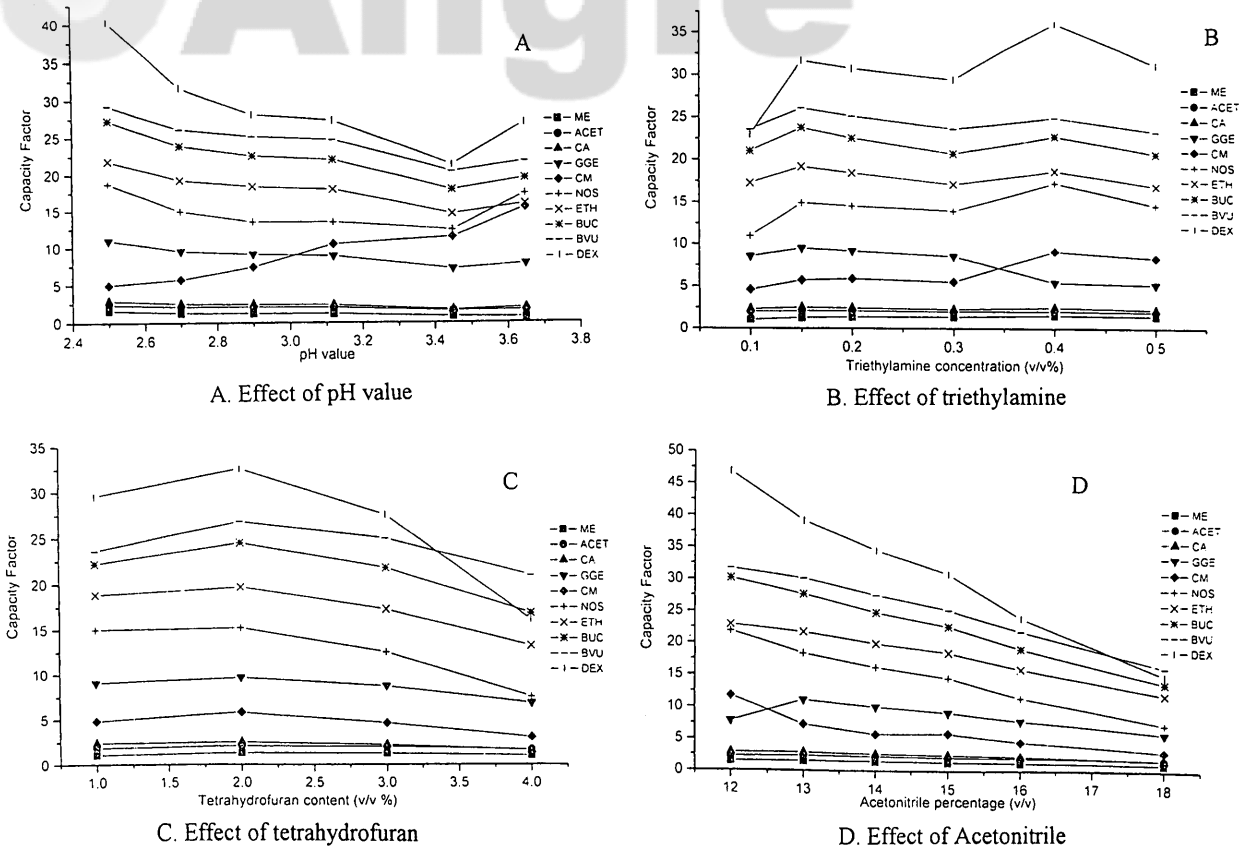

A. Effect of pH value

B. Effect of triethylamine

C. Effect of tetrahydrofuran

D. Effect of Acetonitrile

Figure 2. The effect on capacity factors of the compounds.
condition: THF, TEA, 0.1%H_3PO_4 in aqueous solution: CH_3CN.

into the HPLC column and recorded the response ratios of standard, and internal standard peak obtained from chromatograms. The mathematical expression of the curves and correlation coefficient of each individual curve were determined.

The precision of this method was assessed by carrying out within-day and between-day assays. The within-day assay was performed on three replicate samples of three concentrations (as listed in Table 1) of each drug through the entire procedure in one analysis day. The same procedure was carried out for five days to perform between-day assay. Both standard deviation and coefficient of variation for within-day and between-day assays were calculated.

The reproducibility (accuracy and recovery) was evaluated by standard addition method[5]. We ground sample A as a placebo and spiked various amounts of each active ingredient at approximately 50%, 80%, 100%, 150% of normal dosage level, respectively (as shown in Table 2). We dissolved them in methanol and followed by mobile-phase diluting to achieve a series of suitable concentrations (the final concentration of internal standard was 50 μg/ml), to be injected to a HPLC column. The injection was performed in triplicate and the active ingredients were quantified according to the peak area ratio to internal standard. Recovery was calculated on the basis of the labeled amount of active ingredients in sample A (as a placebo).

For the analysis of commercial products, we weighed and finely powdered not less than 20 of sample A~E tablets, respectively. We transferred an accurately weighed portion of the powder, equivalent to the labeled claim of active ingredients in test sample. We dissolved them in methanol and the procedures were similar to the

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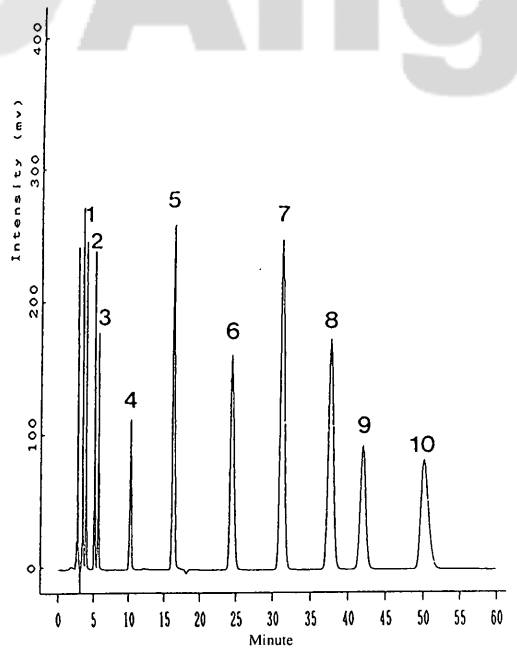

Figure 3. The chromatogram of ten compounds tested.

1. Methylephedrine HCl 2. Acetaminophen
3. Caffeine anhydrous 4. Chlorpheniramine maleate
5. Guaifenesin 6. Noscapine HCl
7. Ethoxybenzamide 8. Bucetin (internal standard)
9. Bromvalerylurea 10. Dextromethorphan HBr

Column: Inertsil ODS-3V column (5μm, 250 mm x 4.6 mm).

Mobile phase: 2% Tetrahydrofuran, 0.2% Triethylamine, 0.1% H_3PO_4 in aqueous slotion: CH_3CN (85: 15) pH=2.70.

Detector: UV 205 nm.

Flow rate: 1.0 ml/min.

reproducibility test. The quantity of active ingredients were calculated by measuring the peak responses obtained from the chromatogram of standard solution and sample solution, respectively.

RESULTS AND DISCUSSION

The structures of these ten compounds are shown in Figure 1. Most of them are amine drugs except for guaifenesin. It is general known that amine compounds give severely tailing peaks in reversed-phase HPLC[9]. Residual silanol groups and metal impurities in column packing materials can be the cause of peak tailing in reversed-phase HPLC. Strongly tailing appears when we used conventional C_8 or C_{18} columns. To prevent this problem, we used highly purified silica gel with a low content metal impurities and full end-capping column[8]. Thus, when an Inertsil ODS-3V column (5 μm, 250 x 4.6 mm) was used for the testing, the peak tailing problem could be prevented.

Optimization of the separation of the ten components in HPLC was achieved by controlling the difference in the capacity factor (k') and resolution factor (R_S) of them. The effects of pH value, organic modifier, and additives of mobile phase on k' and R_S of the ten components were systemically studied by changing one of these parameters in turn while keeping the others constant. Ternary solvent system was performed to decrease retention time and improve resolution. THF demonstrated to be a very selective organic modifier in our preliminary test.

The pH value of the buffer normally plays an important role in the separation since it determines the extent of ionization of each analytes. Thus, manipulation of buffer pH value is usually a key strategy for optimizing the separation in HPLC. The pH value of the aqueous solution in mobile phase was adjusted with phosphoric acid from 2.5 to 3.65, while keeping the acetonitrile and aqueous solution at the constant level of 15 and 85% (v/v) respectively. Similarly, the TEA concentration was constant at 0.15% and the THF concentration was maintained at 2% in 0.1% phosphoric acid solution. The elution orders of some components were observed to cause change in the k' values. This is illustrated clearly in Fig.2A. It is fair to conclude that the best HPLC resolution for these ten components occurs around pH 2.70.

Alkylamines act primarily by hydrogen bonding to non-derivatized silanol sites, thereby reducing adsorption and/or ion-exchange effects. The addition of an alkylamine to a mobile phase can dramatically improve peak shapes with little loss of retention. In addition to their ability to reduce peak tailing, alkylamines are also useful as selec-

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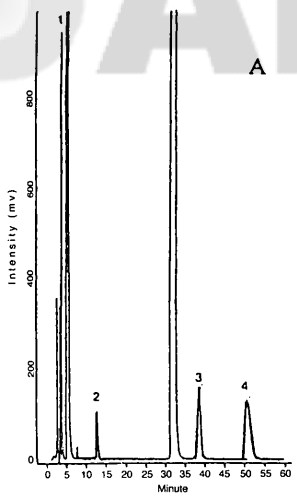
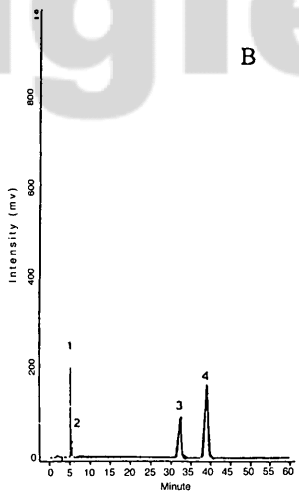
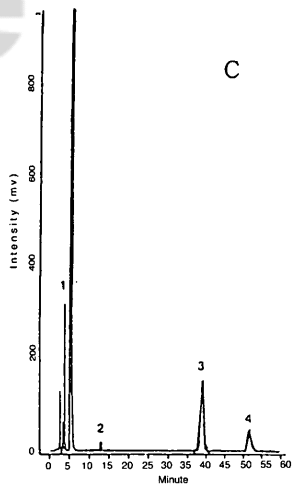


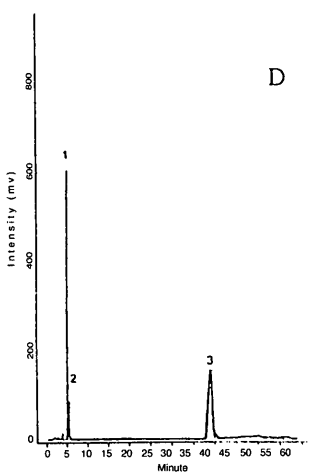
A. Product A(concentration)
1. Methylephedrine HCl 2. Chlorpheniramine maleate
3. Bucetin (internal standard) 4. Dextromethorphan HBr



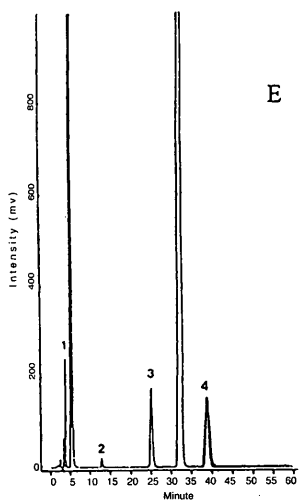
B. Product A (dilution)
1. Acetaminophen 2. Caffeine anhydrous
3. Ethoxybenzamide 4. Bucetin (internal standard)



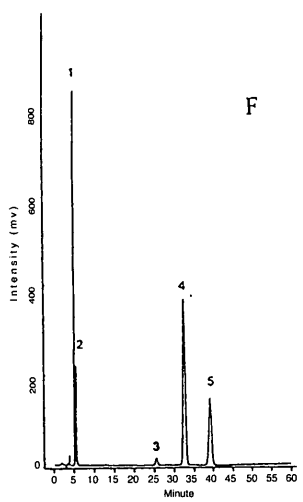
C. Product B(concentration)
1. Methylephedrine HCl 2. Chlorpheniramine maleate
3. Bucetin (internal standard) 4. Dextromethorphan HBr



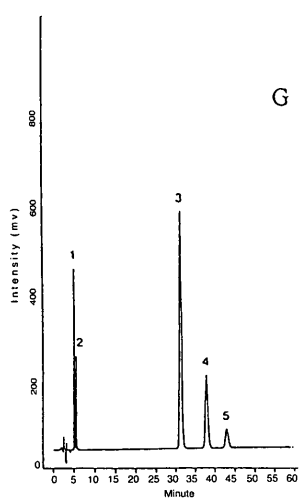
D. Product B(dilution)
1. Acetaminophen 2. Caffeine anhydrous
3. Bucetin (internal standard)



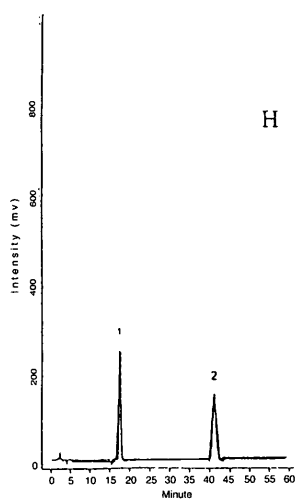
E. Product C(concentration)
1. Methylephedrine HCl 2. Chlorpheniramine maleate
3. Noscapine HCl 4. Bucetin (internal standard)



F. Product C(dilution)
1. Acetaminophen 2. Caffeine anhydrous
3. Noscapine HCl 4. Ethoxybenzamide
5. Bucetin (internal standard)



G. Product D
1. Acetaminophen 2. Caffeine anhydrous
3. Ethoxybenzamide 4. Bucetin (internal standard)



H. Product E
1. Acetaminophen 2. Caffeine anhydrous

Figure 4. The chromatogram of commercial product. Condition is similar to Figure 3.

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tivity-enhancing agents. Short chain tertiary amine modifiers like TEA are highly effective in reducing or eliminating silanophilic interactions. Triethylamine was added to the mobile phase to improve the peak shape of these amine analytes by reducing peak tailing. The effect of TEA concentration on *k'* values was studied from 0.1 to 0.5% (v/v) in 0.1% phosphoric acid solution while keeping the other parameters constant: at 15% acetonitrile, 2% THF in 0.1% phosphoric acid solution, pH 2.70, and aqueous solution at the constant level of 85% (v/v) respectively. The effect of THF concentration on *k'* values was studied from 1 to 4% (v/v) in 0.1% phosphoric acid solution while keeping the other parameters constant : at 15% acetonitrile, 0.2% TEA in 0.1% phosphoric acid solution, pH 2.70, and aqueous solution at the constant level of 85% (v/v) respectively. These are illustrated in Fig.2B and Fig.2C. No major changes in the *k'* values of the ten components were observed, but resolution(R_S) and shapes of peaks of the ten components were improved.

As shown in Fig.2D, the effect of acetonitrile on *k'* was studied from 12 to 18% (v/v). Significant decrease in the *k'* values of all ten components were found as the concentration of acetonitrile was increased. The optimum acetonitrile concentration for the separation of these ten components is about 15% (Fig.2D). As a result, a suitable mobile phase of 2% THF, 0.2% TEA in 0.1% phosphoric acid solution: acetonitrile (85:15) adjusted to pH 2.70 with phosphoric acid was thus determined.

An analysis of drug formulas was accomplished in a reversed-phase Inertsil ODS-3V column (5 μ, 250 x 4.6 mm), using as the mobile phase 2% THF, 0.2% TEA in 0.1% phosphoric acid solution : acetonitrile (85 : 15, v/v) adjusted to pH 2.70 with phosphoric acid at a flow-rate of 1.0 ml/min, and a ultraviolet detector operated at 205 nm. Figure 3 showed a typical chromatogram of the ten components, the capacity factors, resolution factors, and limits of detection (LOD, signal to noise ratio=3) were described in Table 1.

The calibration curves showed linearity and the mathematical expression of the curves were showed in Table 1, respectively, using bucetin as the internal standard. The correlation coefficient of each individual curve was greater than 0.9998, showing good proportionality between the concentration and detector response. The precisions were also shown in Table 1. The within-day precision had the coefficients of variation (CV) between 0.03% to 0.85% and the between-day precision ranging from 0.06 to 0.95%. The evaluation of recovery and application of this method in commercial products were shown in Table 2. The accuracy were from 99.4% to 102.7% for the recovery test. The HPLC chromatograms of five commercial products were shown in Figure 4 and no interference was observed in these chromatograms by excipients from these commercial products. It demonstrated that this method might be applied to qualitative and quantitative analysis of complex cold medicines.

In conclusion, a practical method has been developed and validated for simultaneous qualitative and quantitative determination of acetaminophen, bromvalerylurea, bucetin, caffeine, chlorpheniramine maleate, dextromethorphan hydrobromide, ethoxybenzamide, guaifenesin, methylephedrine hydrochloride, and noscapine hydrochloride in solid pharmaceutical dosage forms. The method can be optimized for maximum resolution and minimum elution time for the ten components in various matrices by adjusting the key mobile phase parameters such as pH, TEA and the organic modifiers. This procedure does not involve any extraction or reaction steps. It can be easily mixed with an internal standard solution and analyzed *via* HPLC. The results indicate that this analytical method is simple, specific, accurate, sensitive, reproducible, and reliable.

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Journal of Food and Drug Analysis. 1999. 7(1)

利用高效液相層析法分析定量綜合感冒藥等十種成分

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摘 要

本文提供一簡單、快速之逆相高效液相層析法，可同時鑑別十種感冒藥：acetaminophen、bromvalerylurea、bucetin、caffeine、chlorpheniramine maleate、dextromethorphan HBr、ethoxybenzamide、guaifenesin、methylephedrine HCl及noscapine HCl等成分。

本法採用之分析管柱爲Inertsil ODS-3V column (5 μm, 250 x 4.6 mm)，移動相組成爲含2% tetrahydrofuran、0.2% triethylamine之0.1% H_3PO_4水溶液：CH_3CN(85：15)混液，以磷酸調整pH至2.70，移動相之流速爲1.0 ml/min，偵測器係利用紫外光分光光度計，波長設至205 nm。

本法以bucetin爲內部標準品，可以於50分鐘內完成上述十種感冒藥成分之分離，經確效結果，分離率(Rs)爲1.63～9.08，其標準曲線線性良好、精密度及準確度頗佳，可應用此方法分析、定量市售檢體，足見其實用性。

關鍵詞：綜合感冒藥，高效液相層析法。