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Simultaneous Quantitation of Caffeine, Ethoxybenzamide and Propyphenazone in Oral Analgesic Tablets by High-Pressure Liquid Chromatography

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ABSTRACT

A method for simultaneous quantitation of caffeine, ethoxybenzamide and propyphenazone is described. The method was based on reversed-phase high-pressure liquid chromatography with a mobile phase of methanol-acetonitrile-3% aqueous acetic acid solution (24 : 16 : 60, v/v). The compounds were well separated from each other by the propcedure, using salicylamide as internal standard. The method gave excellent results and provided a simple, rapid and accurate method for separation and quantitation of synthetic mixtures and commercial products.

Key words : High-pressure liquid chromatography, Caffeine, Ethoxybenzamide, Propyphenazone.

INTRODUCTION

Most commercially available analgesics contain one or more pain relievers such as acetaminophen, aspirin, propyphenazone, ethoxybenzamide, salicylamide or phenacetin. Many of them also contain caffeine and buffering agents such as aluminum hydroxide[1]. One of the most popular commercial drugs in Taiwan contains a combination of propyphenazone, caffeine and ethoxybenzamide.

Caffeine in mixtures has been analyzed by high-pressure liquid chromatography (HPLC)[2-4], gas-liquid chromatography (GLC)[5],and near-infrared (near-IR) spectrometry[6]. A GLC method was also reported for quantitation of propyphenazone. No reports could, however, be found in the literature for analysis of ethoxybenzamide in either synthetic mixtures or multicomponent dosage forms.

The present report describes a simple, rapid and accurate method for simultaneous quantitation of caffeine, ethoxybenzamide and propyphenazone in commercial tablets reversed-phase HPLC.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

Propyphenazone, caffeine, ethoxybenzamide and salicylamide were kindly provided by Analytical Center of Medical Supply, National Defense Medical Center, Taipei, R.O.C. and used

directly without further purification. All solvents were of analytical grade (E. Merck Co.).

II. *Chromatogaphic Conditions*

The high-pressure liquid chromatograph was connected to a Kratos solvent delivery system equipped with a U6K universal injector (Waters Associates, Milford, Mass., U.S.A), a multiple wavelength detector (Model 450 variable wavelength detector. Waters Associates, Milford, Mass., U.S.A), an integrator (Chromatocorded II, System Instruments Co., Tokyo, Japan), and a μ bondapak C 18 column (Waters Associates, Milford, Mass., U.S.A.). The nonpolar column(30 cm $\times$4 mm I.D.) consisted of a mono-molecular layer of octadecyltrichorosilane permanently bonded by silicone-carbon bonds.

The mobile phase consisted of methanol-acetonitrile-3% aqueous acetic acid solution (24 : 16 : 60, v/v). The flow rate was 1 ml min^{-1}. The detector sensitivity was 0.04 (254 nm). The integrator attenuation was 8, except for propyphenazone peaks where it was 64. The temperature was ambient.

III. *Internal Standard Solution*

The internal standard solution (2.5 mg ml^{-1}) was prepared by dissolving salicylamide in methanol-water (20 : 80, v/v).

IV. *Standard Solutions for Calibration Curves*

Stock solutions of propyphenazone (9.0 mg ml^{-1}), caffeine (3.0 mg ml^{-1}) or ethoxybenzamide (10.5 mg ml^{-1}) were separately prepared by dissolving the reagents in methanol-water (50 : 50, v/v) solution. The standard solutions were prepared by diluting the stock solutions with methanol to concentrations of 10.5, 9.0, 7.5, 6.0, 4.5 mg ml^{-1} for ethoxybenzamide, 9.0, 7.5, 6.0, 4 .5, 3.0 mg ml^{-1} for propyphenazone, and 3.0, 2.4 , 1.8, 1.2, 0.6 mg ml^{-1} for caffeine, respectively. For each drug, a standard solution with appropriate concentration was chosen and a volume of 0.5 ml of each was accurately pipetted into a test tube and mixed with those of other drugs. Accurately, 0.5 ml of each of mixed standard solutions was mixed with 0.5 ml of the internal standard prior to assay.

The five concentrations of each drug were subjected to linear regression analysis. Calibration graphs were established by plotting the peak-area ratios of drugs to internal standard against their concentrations(μg).

V. *Synthetic Mixture Preparation*

A synthetic mixture was prepared by mixing 150 mg of propyphenazone, 45.8 mg of caffeine and 250 mg of ethoxybenzamide.

VI. *Preparation of Assay Solutions of Commercial Tablets and Synthetic Mixtures*

Not less than twenty tablets were weighed and finely powdered. An accurately weighed portion equivalent to about 150 mg of propyphenazone, 45.8 mg of caffeine and 250 mg of ethoxybenzamide was transferred into a 100-ml volumetric flask and diluted to volume with methanol-water (50 : 50, v/v). The solution was mixed vigorously for 30 min, transferred into a screw-capped centrifuge tube and centrifuged at 2000 rpm for 10 min. Accurately pipetted volume of 0.5 ml of the upper clean solution was placed in a screw-capped test tube and mixed with 0.5 ml of internal standard solution prior to assay.

The assay solution of synthetic mixture was prepared in the same manner as those of commercial tablets.

VII. *Assay*

For all standard solutions and assay solutions, a 20.0 μl aliquot was injected and chromagraphed, using the described mobile phase.

VIII. *Calculations*

Peak-area ratios versus concentrations were linear between 2 and 10 μg for caffeine, between 10 and 30 mg for propyphenazone and between 15 and 35 mg for ethoxybenzamide by exami-

ning the calibration curves of standard solutions . The drug amount which was injected into the chromatograph was within those linear concentration ranges. As a result, the drug amount found in the commercial tablets and synthetic mixtures could be calculated from the linear regression equations of calibration curves.

The recovery of synthetic mixtures and the percent recovery of commercial tablets as compared to the claimed label were calculated using:

$$\% \text{ Recovery} = \frac{\text{Amount found (mg)}}{\text{Amount added (mg)}} \times 100$$

$$\% \text{ Label Claim} = \frac{\text{Amount found (mg)}}{\text{Amount labelled (mg)}} \times 100$$

RESULTS AND DISCUSSION

Figure 1 clearly indicated that it is possible to separate the three active ingredients and internal standard from each other using a mobile phase of methanol-acetonitrile-3% aqueous acetic acid solution (24 : 16 :60, v/v). Separation of the same ingredients from each other with other solvent systems is possible, but needs a longer time to elute all the ingredients.

From Figures. 2, 3, and 4, it is clear that the peak-area ratios versus concentrations tested (in μg : caffeine, 2.0-10.0; ethoxybenzamide, 15.0 -35.0; propyphenazone, 10.0-30.0) are linear. Linear regession equations are $Y=0.982\times X-0.514$ for caffeine, $Y=0.0262\times X+0.0949$ for ethoxybenzamide, $Y=0.0300\times X-0.1641$ for propyphenazone. The calculated correlation coefficients (r) of least-square regression were 0.9976, 0.9990, 0.9948 for caffeine, ethoxybenzamide, and propyphenazone, respectively. Figure 5 illustrates a chromatogram of the assayed drugs. The active ingredients of the tablets dissolved in methanol-water (50 : 50, v/v) were chromatographed. Salicylamide was added to the solution as the internal standard. The area under the curve for each peak on the chromatograms was determined with a digital integrator. The ratio of each peak area to the area of the internal standard was calculated for each chromatogram. The drug amount found in a known synthetic mixture and commercial tablets could be calculated from those linear regression equations,

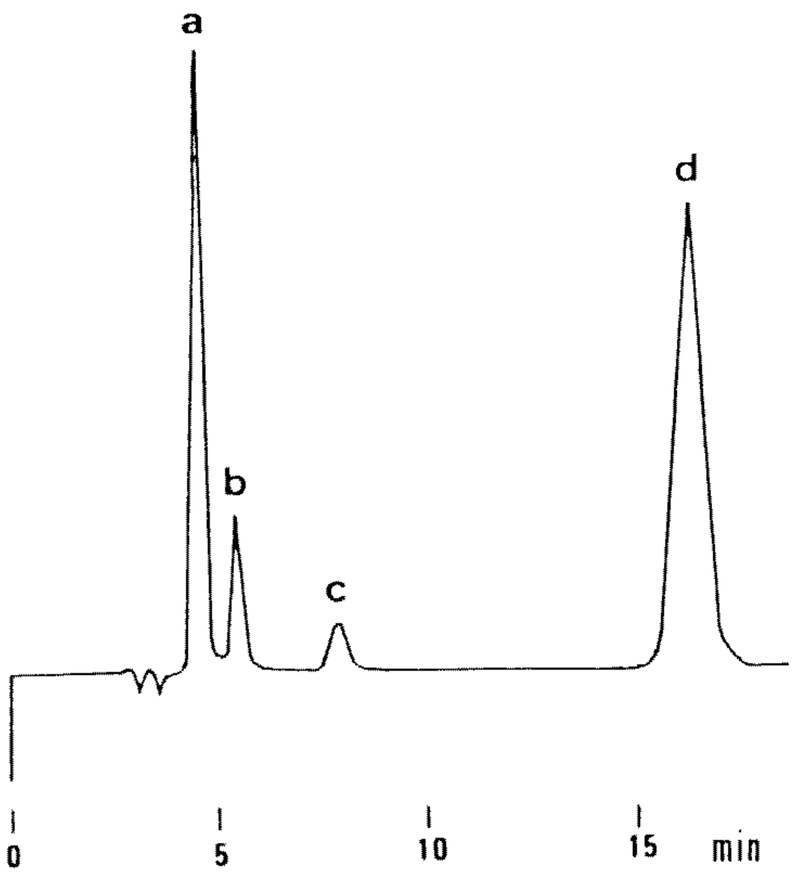

Figure. 1 Liquid chromatogram of a caffeine-ethoxybenzamide-propyphenazone mixture in methanol-acetonitrile 3% aqueous acetic acid (24 : 16 : 60) on a μ bondapak C18 column at a flow rate of 1ml min^{-1}. Key : a, caffeine; b, salicylamide (internal standard); c, ethoxybenzamide; and d, propyphenazone.

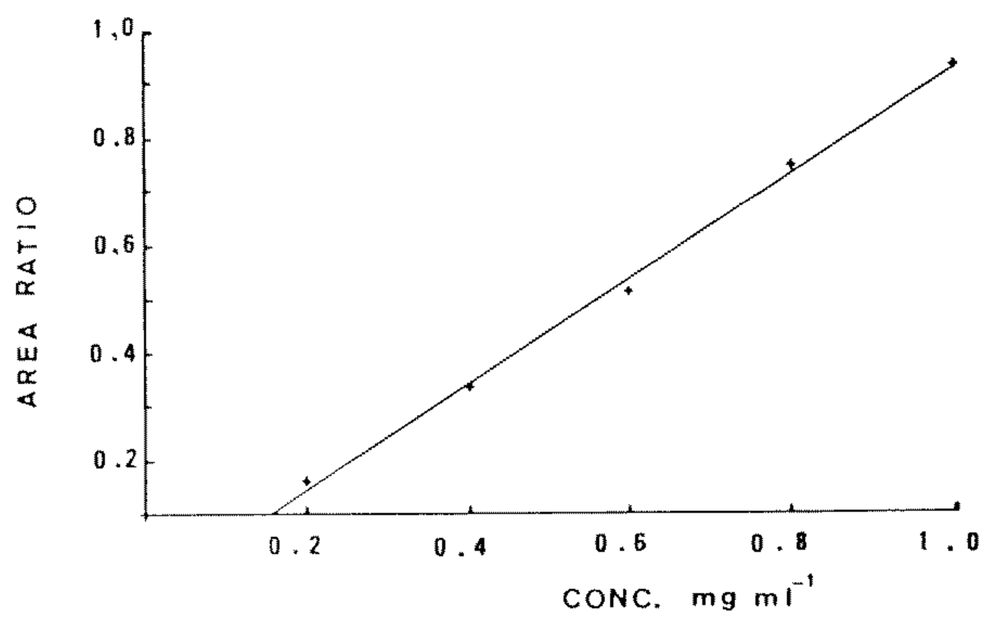

Figure. 2 Calibration graph for caffeine analysis.

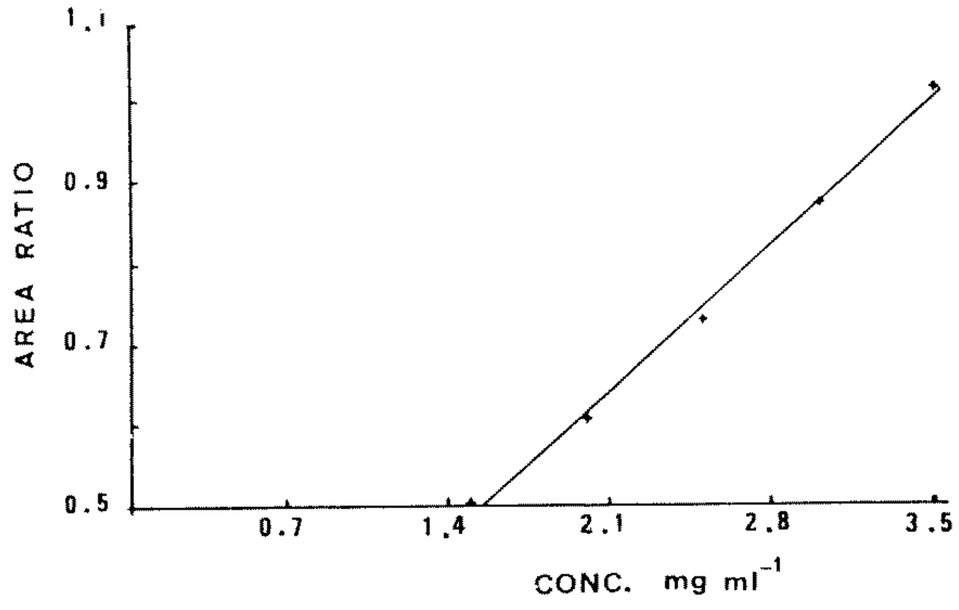

Figure. 3 Calibration graph for ethoxybenzamide analysis.

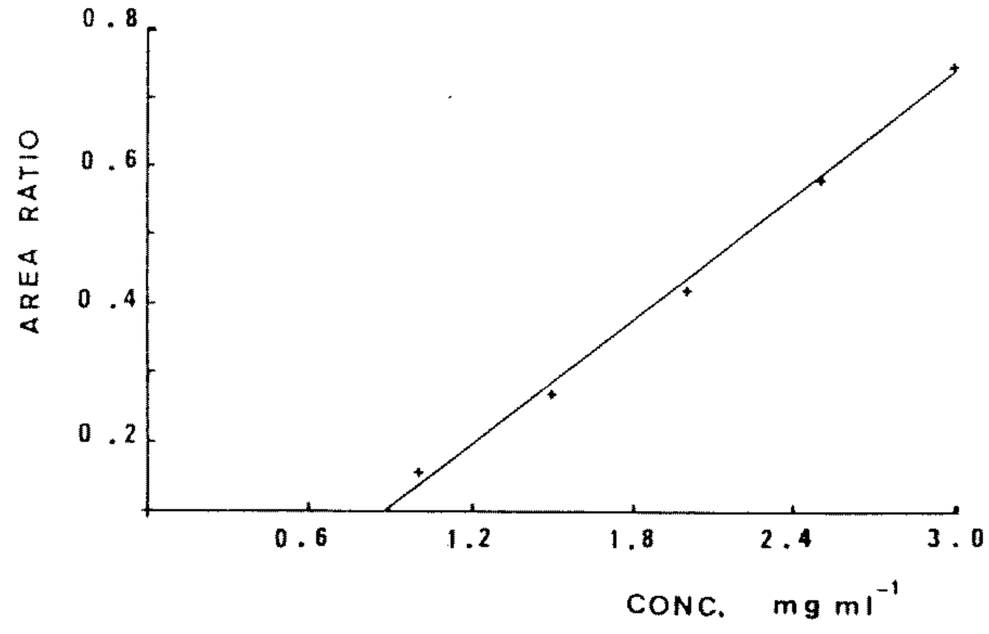

Figure. 4 Calibration graph for propyphenazone analysis.

Table 1. HPLC Assay of Caffeine, Ethoxybenzamide and Propyphenazone in the Synthetic Mixture.

Ingredient	Synthetic mixture			
	Amount added mg	Amount found* mg	Recovery %	RSD** %
Ethoxybenzamide	250.0	249.6	99.8	0.34
Propyphenazone	150.0	146.8	97.9	0.46
Caffeine	45.8	45.4	99.1	0.59

*Average of six analyses.

**Relative standard deviation.

Table 2. HPLC Assay of Caffeine, Ethoxybenzamide and Propyphenazone in the Commercial Tablets

Ingredient	Commercial tablets	
	Amount labelled mg	Amount found* mg
Ethoxybenzamide	250.0	252.6
Propyphenazone	150.0	141.7
Caffeine	45.8	46.5

*Average of two analyses.

with results as shown in Table 1 and 2, respectively. The utility of HPLC in the analysis of caffeine-ethoxybenzamide-propyphenazone is clearly demonstrated with a precision of 0.34-0.59% RSD.

In summary, the analytical results of a synthetic mixture and commercial tablets (Figure 5, Table 1 and 2) indicate that the proposed method can be used for simultaneous quantitation of caffeine, ethoxybenzamide and propyphenazone in commercial products and synthetic mixtures.

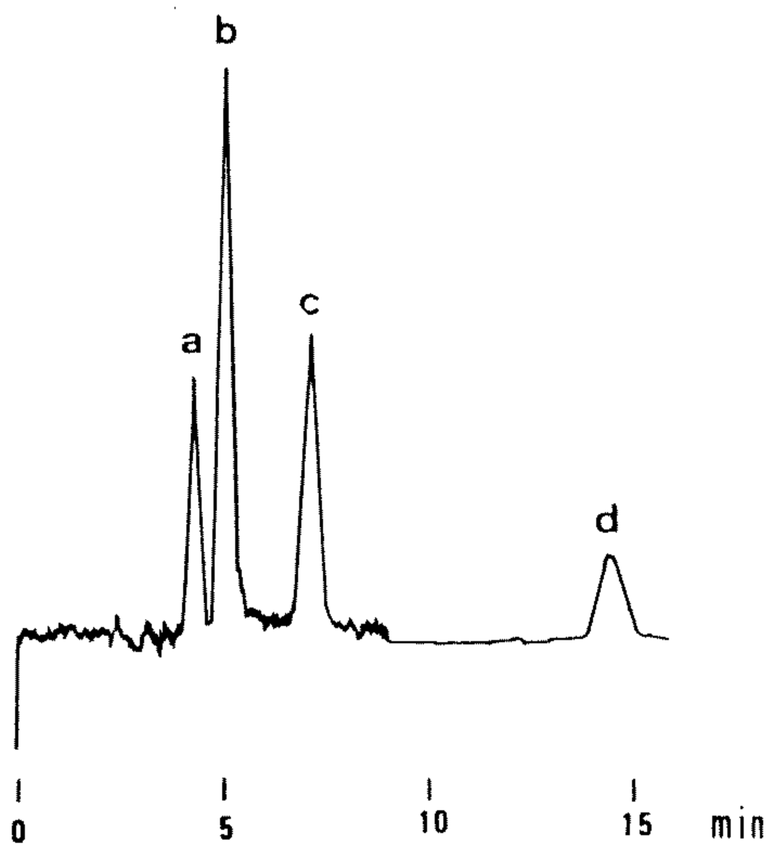

Figure 5 Sample chromatogram of a caffeine-ethoxybenzamide-propyphenazone solution in a tablet combination in methanol-acetonitrile-3% aqueous acetic acid (24 : 16 : 60) on a μ bondapak C18 column at a flow rate of 1ml min^{-1}. The lines before peak a and after peak c indicate attenuation 8 and 64, respectively. Key : a, caffeine; b, salicylamide (internal standard); c, ethoxybenzamide; d, propyphenazone.

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高壓液相層析法定量口服鎮痛錠劑成分咖啡鹼、乙氧基苄醯胺及異丙安替比林

李安榮　宋光生　黃文鑫

國防醫學院藥學系國軍衛材藥品檢驗中心

摘　要

本報告敍述以柳醯胺(salicylamide)爲內部標準,利用高壓液相層析法,以μ bondapak C18層析柱及甲醇-乙腈-3%醋酸(24：16：60)爲移動相,進行市售口服鎮痛錠劑成分咖啡鹼(caffeine),乙氧基苄醯胺(ethoxybenzamide)及異丙安替比林(propyphenazone)之定量分析。研究顯示本法爲一簡單,快速且準確的方法。