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A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ASSAY OF ASPIRIN, CAFFEINE, DIHYDROCODEINE BITARTRATE AND PROMETHAZINE HYDROCHLORIDE IN A CAPSULE FORMULATION

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ABSTRACT

A method for the simultaneous quantitation of aspirin, caffeine, dihydrocodeine bitartrate and promethazine HCl was developed using reversed-phase, solvent-programming high-performance liquid chromatography. Linear gradient elution with acetonitrile in phosphate buffer (from 15:85 to 56:44) allowed all compounds to elute from a C-18 column within 7 min with adequate resolution. This method also allowed the resolution and quantitation of salicylic acid, the principle decomposition product of aspirin. CV% of eight determinations each of aspirin, caffeine, dihydrocodeine bitartrate and promethazine HCl are 0.64, 1.25, 3.37 and 1.72, respectively, and percent recoveries from spiked preparations were 100.25, 100.03, 100.45 and 98.00, respectively. This assay method was shown also to be suitable for dissolution studies with water or acetate buffer as the dissolution medium.

INTRODUCTION

Some oral combination pharmaceuticals designed for symptomatic relief from the discomfort associated with common colds contain drugs having analgesic, antihistaminic, antitussive, and stimulant effects. Some of the nonprescription analgesic preparations or pain relievers contain aspirin and caffeine whereas some of the prescription products contain codeine phosphate or dihydrocodeine

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bitartrate and promethazine hydrochloride in combination with aspirin or acetaminophen (1,2). Several such commercial preparations have been available and are now marketed. The simultaneous assay of mixtures of these and related drugs presents many difficulties and can be very time consuming, especially if the NF procedures are followed (3).

The instability of aspirin and its ready hydrolysis to salicylic acid, the major decomposition product, cause difficulty with some proposed assays for the analgesic. A fluorometric method (4) and a colorimetric method (5) for aspirin determinations depend upon hydrolysis of aspirin to salicylic acid. These methods lack specificity for aspirin.

Other investigators have reported the application of HPLC techniques for the separation and quantitation of aspirin-containing combination drug dosage forms (6-9). One of these methods was reported to be very successful in the simultaneous quantitation of six active ingredients without interference from salicylic acid (8). The method reported herein is a reversed-phase gradient elution procedure applied to the assay of a capsule formulation containing dihydrocodeine bitartrate, caffeine, aspirin and promethazine hydrochloride. The formulation ingredients, salicylic acid and acetanilide (internal standard) were eluted and detected with adequate resolution in a reasonably short period of time.

EXPERIMENTAL

Apparatus

A Varian 4200 liquid chromatograph consisting of two constant displacement pumps and controllers, a mixer module, a multilinear

solvent programmer, a septumless injector and a Varian UV-50 variable wavelength UV detector was used in this study. The retention times and peak areas were obtained with the aid of a Varian CDS-111 data system and the chromatograms were recorded with a Varian 9176 recorder.

Chemicals and Reagents

Acetonitrile was HPLC grade. Aspirin, caffeine and phoshoric acid were U.S.P. or N.F. grade. Glacial acetic acid, sodium acetate, salicylic acid and potassium phosphate (monobasic) were A.C.S Reagent grade. The acetanilide, promethazine hydrochloride and dihydrocodeine bitartrate were used as received from Amend Drug and Chemical Co., Sigma Chemical Co. and Mallinckrodt Chemical Works, respectively. Double distilled water was used for solution preparation at all times.

Chromatographic Conditions

A reversed-phase μBondapak C_{18} column (Waters Assoc., Milford, Mass.), dp = 10 μm, 300x4mm, preceded by a Whatman (Clifton, NJ) guard column containing a similar stationary phase but of dp = 40 μm, was used for all analyses. The mobile phase was delivered to the column by two pumps and a solvent programmer.

Solvent A was 0.01M KH_2PO_4 in double distilled water, adjusted to pH 2.30 with phosphoric acid (approximately 1.3 ml is required per 2 liters) and filtered through a 0.45 μm membrane filter (Millipore Corporation, Bedford, MA). Solvent B was a 60% mixture of acetonitrile in Solvent A. The solvent programmer was adjusted

so that the initial concentration of solvent B in the mobile phase was 25% (equivalent to 15% acetonitrile) and the final concentration of solvent B was 93% (equivalent to 56% acetonitrile), with a linear gradient slope of 9.5% of solvent B per minute. The flow rate was 2 ml/minute.

The injection volume was 5.0 µl for the determination of dihydrocodeine bitartrate, caffeine, and promethazine hydrochloride; 1.0 µl for the determination of aspirin.

The UV detector was operated at a wavelength of 247 nm and a sensitivity of 0.1 AUFS. The entire HPLC system was operated at ambient temperature at all times.

Preparation of Stock and Standard Solutions

The stock solution of the internal standard, acetanilide, was prepared at a concentration of 1.0 mg/ml in a 10% mixture of ethanol in water. The stock solution of promethazine hydrochloride in water was prepared at a concentration of 0.625 mg/ml.

The standard solution was prepared by dissolving 357.0 mg aspirin and 50.0 mg of salicylic acid in 15 ml of ethanol, then exactly 10 ml of the stock solution of the internal standard and exactly 10 ml of the stock solution of promethazine hydrochloride were each pipetted into this aspirin solution. Then 16.0 mg of dihydrocodeine bitartrate and 30.0 mg of caffeine were dissolved in the resulting solution which was then diluted with water to approximately 70 ml and quantitatively filtered through a sintered glass filter of medium porosity. The filtrate was combined with all rinsing solutions and diluted to exactly 100 ml with water.

This resulting standard solution should contain 0.16 mg/ml of dihydrocodeine bitartrate, 0.30 mg/ml of caffeine, 3.57 mg/ml of aspirin, 0.50 mg/ml of salicylic acid, 0.0625 mg/ml of promethazine hydrochloride and 0.10 mg/ml of internal standard if there is no loss of ingredients during filtration.

Similar solutions containing known quantities of each of the ingredients and internal standard were prepared without filtration and analyzed by the HPLC procedure. No significant differences in the integrated results were observed as compared with the filtered standard solution, indicating that filtration does not remove the ingredients from the solution.

Preparation of Sample Solutions From Capsules

The powder in each of the randomly selected capsules was quantitatively transferred and dissolved in a mixture of approximately 10 ml of ethanol and 50 ml of water into which 10 ml of the stock solution of the internal standard was added. This mixture was quantitatively filtered through a sintered glass filter of medium porosity and the filtrate was diluted with water to exactly 100 ml. The solution thus prepared from a capsule with 100% of label amounts should contain the same concentrations of each ingredient as those of the standard solution except that no salicylic acid would be present.

Preparation of Placebo Solution

Powdered excipients of the amounts contained in one capsule were placed in 70 ml of a 10% mixture of ethanol in water. This

mixture was then filtered and diluted to exactly 100 ml in a manner similar to that of the preparation of the sample solutions.

No interference from this blank solution was detected under the chromatographic conditions previously described.

Recovery of Drugs from the Capsule Formulation in the HPLC Assay

The excipients and drug ingredients of 100% of the label amounts in one capsule were mixed with 10 ml of ethanol and 50 ml of water to which was added 10 ml of the internal standard solution. This mixture was then filtered and diluted to 100 ml in a manner similar to that in the preparation of the standard solution. The resulting spiked solution was injected and the recovery rate of each ingredient was calculated by comparing the chromatographic results with those of the standard solution.

Application of Assay to Dissolution Study

The recovery of each drug ingredient in the HPLC analyses for a proposed dissolution study was investigated. Double distilled water and 0.05M acetate buffer of pH 4.50 (10) were each used as the dissolution media.

The standard solution was prepared by dissolving the label amounts of the drug ingredients of one capsule in 500 ml of a 2% mixture of ethanol in water. An aliquot of 7 ml of this mixture was filtered through a 0.22 µm membrane filter (Millipore Corp.) and exactly 5 ml of the filtrate was mixed with 1 ml of the internal standard solution, which was prepared at 0.1 mg/ml, and diluted to 10 ml with water.

Spiked solutions were prepared in a similar manner except that the capsule excipients and an empty gelatin capsule were included in the preparation, and water and acetate buffer were each used as diluents. The spiked solutions were assayed chromatographically with the injection volume being 40 µl. The results were compared to those of the standard solution which contained similar concentrations of all ingredients. Placebo solutions of 500 ml each were prepared from the excipients and an empty gelatin capsule both in water and in acetate buffer. No assay interferences from these placebo solutions were detected under the chromatographic conditions previously described.

RESULTS AND DISCUSSION

The Assay Method

The HPLC method described herein for the simultaneous assay of aspirin, caffeine, dihydrocodeine bitartrate and promethazine hydrochloride in a capsule formulation is rapid, simple, reproducible, accurate and convenient. The use of the gradient elution technique resulted in definite advantages over the isocratic method of Das Gupta (8). The gradient elution method reduced total assay time from more than 30 min. to 7.5 min. and, at the same time, greatly narrowed the promethazine peak. Also the resolution of all peaks was excellent and the degradation of aspirin to salicylic acid could be followed by quantitating the salicylic acid peaks. This method enables the simultaneous measurement of small quantities of salicylic acid (0.5mg/capsule) in such formulation.

This HPLC method elutes dihydrocodeine in approximately 2.4 min, caffeine in approximately 2.9 min, acetanilide in approximately 4.1 min, aspirin in approximately 4.6 min and promethazine in approximately 6.7 min. Salicylic acid, the principal known decomposition product of aspirin is eluted in approximately 5.3 min. The low pH (2.3) of the mobile phase is important for satisfactory resolution between aspirin and salicylic acid (8).

Quantitation of small amounts of salicylic acid is possible due to its high UV absorptivity which is almost 4 times that of aspirin. Promethazine was found to have a much stronger affinity to the stationary phase of this column material than the other drug ingredients. Isocratic elution of this formulation with mobile phases of different acetonitrile strengths was not successful. In order to quantitate all ingredients simultaneously, gradient elution techniques were necessary. FIGURE 1 shows the chromatogram of a standard solution and identical chromatograms were obtained from solutions of placebo formulation spiked with drug ingredients. FIGURE 2 shows the chromatogram of a sample solution prepared from a capsule taken from a container which was exposed to 75% relative humidity at 35°C for four weeks.

Calculation of the Unknown in the Sample Solution

The amount of each ingredient in the sample solution was calculated by a programmable chromatographic integrator. A Relative Response Factor (RRF) was determined for each ingredient by successive injections of the standard solution. The formula in the program is

Conditions:
Mobile phase: Solvent A - 0.1 M KH_2PO_4 (pH 2.3)
Solvent B - acetonitrile and 0.1 M KH_2PO_4 (pH 2.3) (60:40)
Gradient = 25 - 93% linear of Solvent B at 9.5%/min
Flow rate: 2 ml/min
Detection: UV @ 247 nm
Back pressure: 1500 psi
Injection volume: 5 µl

Peak Identification:

a.	solvent peak	
b.	dihydrocodeine bitartrate	0.16 mg/ml
c.	caffeine	0.30 mg/ml
d.	acetanilide	0.10 mg/ml
e.	aspirin	3.57 mg/ml
f.	salicylic acid	0.50 mg/ml
g.	promethazine HCl	0.0625 mg/ml

Chart speed: 0.5 cm/min

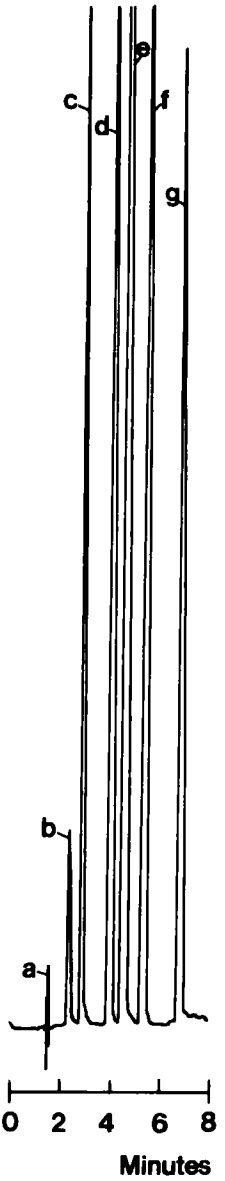

FIGURE 1.

HPLC separation of standard solution on a µBondapak C_{18} column (dp = 10 µm, 300 X 4 mm).

Conditions:
Column: μBondapak C_{18}
(dp = 10 m, 300 X 4 mm)
Mobile phase: Solvent A -
0.1 M KH_2PO_4 (pH 2.3)
Solvent B - acetonitrile
and 0.1 M KH_2PO_4 (pH 2.3)
(60:40)
Gradient = 25 - 93%
linear of Solvent B
at 9.5%/min
Flow rate: 2 ml/min
Detection: UV @ 247 nm
Back pressure: 1500 psi
Injection volume: 5 μl

Peak identification:

a.	solvent peak	
b.	dihydrocodeine bitartrate	0.17 mg/ml
c.	caffeine	0.30 mg/ml
d.	acetanilide	0.10 mg/ml
e.	aspirin	3.61 mg/ml
f.	salicylic acid	0.03 mg/ml
g.	promethazine HCl	0.0582 mg/ml

Chart speed: 0.5 cm/min

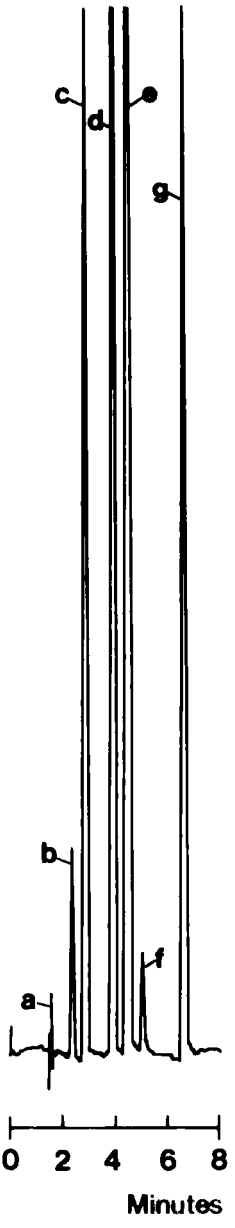

FIGURE 2.

HPLC separation of a sample solution prepared from a capsule selected from a container stored at 35°C and 75% relative humidity for 4 weeks.

$$\frac{\text{Area Counts}_{(\text{internal standard})}}{\text{Amount}_{(\text{internal standard})}} = \frac{\text{Area Counts}_{(\text{drug})}}{\text{Amount}_{(\text{drug})}} \times \text{RRF}$$

The calculated RRF of each ingredient was then programmed into the integrator for the determination of the unknown amount in the sample solution:

$$\text{Amount}_{(\text{drug})} = \frac{\text{RRF} \times \text{Area Counts}_{(\text{drug})} \times \text{Amount}_{(\text{internal standard})}}{\text{Area Counts}_{(\text{internal standard})}}$$

A minimum of triplicate injections was performed for each determination.

Standard Linearity

Injections of four different volumes, 2.5, 5.0, 7.5 and 10µl, were made from the standard solution. The amounts of drugs contained in such injection volumes covered a range of 0.400 - 1.600 µg for dihydrocodeine bitartrate, 0.750 - 3.000 µg for caffeine, 8.930 - 35.700 µg for aspirin and 0.156 - 0.625 µg for promethazine hydrochloride. For each injection volume, a minimum of triplicate injections were performed. Standard linearity was examined over these ranges.

High linear correlation was found for all ingredients over the ranges examined. The least square regression analysis of the peak area responses gave correlation coefficients greater than 0.999 for all four ingredients.

Reproducibility of the Chromatographic Procedures

Eight determinations of each of the ingredients in the standard solution were performed according to the chromatographic procedures

described. The coefficient of variability (CV) was calculated as :

$$CV = \frac{100\ s}{\bar{y}} \quad \%$$

where s is the standard deviation of the eight determinations of each ingredient, $\bar{y}$ is the mean. The reproducibility of this HPLC assay is shown in TABLE 1.

The CV values for dihydrocodeine bitartrate, caffeine, aspirin and promethazine hydrochloride were 3.37, 1.25, 0.64 and 1.72%, respectively. The higher CV of dihydrocodeine bitartrate is the result of its low UV absorptivity at 247 nm, which is even lower at 254 nm. If the peak areas are determined manually, it is desirable to change the detector wavelength to 285 nm during the elution

TABLE 1

Reproducibility of HPLC Assay

Ingredient	Mean of Eight Determinations (mg/100ml) ± SD[a]	Range of Eight Determinations (mg/100ml)	CV %
Dihydrocodeine bitartrate	15.99 ± 0.54[a]	15.23 - 16.99	3.37
Caffeine	30.42 ± 0.38	29.96 - 31.20	1.25
Aspirin	359.85 ± 2.31	355.63 - 362.08	0.64
Promethazine hydrochloride	6.38 ± 0.11	6.23 - 6.52	1.72

[a]Standard deviation, n = 8

of dihydrocodeine bitartrate in order to obtain a stronger absorption. The other ingredients should still be detected at 254 nm or 247 nm because of their poor absorptivity at 285 nm. Ten capsules were randomly selected from one lot and individually assayed for their contents with this procedure. A minimum of triplicate analyses was performed for each determination. The results are listed in TABLE 2.

Recovery Rates

The recovery rates of each ingredient from the prepared solutions for capsule contents assay and for dissolution study are shown

TABLE 2

Assays of 10 Capsules

Ingredient	Average Amount per Capsule[a] (mg) ± SD[b]	Range for Contents of 10 Capsules (mg)	Acceptable Range (85-115% of Label Amount) (mg)
Dihydrocodeine bitartrate	16.19 ± 0.87	14.83 - 17.63	13.60 - 18.40
Caffeine	31.37 ± 0.92	30.69 - 33.64	25.50 - 34.50
Aspirin	369.00 ± 8.02	352.87 - 379.10	303.45 - 410.55
Promethazine hydrochloride	6.68 ± 0.25	6.35 - 6.99	5.31 - 7.19

[a]Minimum of triplicate determinations for each capsule.

[b]Standard deviation, n = 10

TABLE 3

Percent Recovery from Prepared Solutions

Ingredient	Capsule Content Assay[a,b]	Dissolution Assay[b,c]	
	Water[d]	Water[d]	Acetate Buffer[d] (pH=4.50)
Dihydrocodeine bitartrate	100.45	99.87	97.97
Caffeine	100.03	98.98	98.28
Aspirin	100.25	98.03	97.76
Promethazine hydrochloride	98.00	101.44	92.07

[a]Spiked solutions were prepared from 100% label amounts and excipients.

[b]Each value is the mean of a minimum of triplicate determinations.

[c]Spiked solutions were prepared from 100% label amounts, excipients and an empty gelatin capsule.

[d]Solvent for the spiked solutions.

in TABLE 3. Both water and acetate buffer can be used as the dissolution media for the HPLC assay of the sample solutions. If acetate buffer is chosen as the dissolution medium, the standard solution must also be prepared in acetate buffer in order to improve the recovery rate. Because aspirin is the dissolution-rate-limiting ingredient in this formulation, the acetate buffer as described in the monograph of Aspirin Capsules of USPXX may be selected as the dissolution medium for this capsule formulation.

The solvent-programming reversed-phase HPLC procedures reported in this paper provided a rapid, simple and quantitative assay method for the simultaneous determination of aspirin, caffeine,

dihydrocodeine bitartrate and promethyazine hydrochloride in a gelatin capsule formulation.

CONCLUSIONS

An HPLC method for the simultaneous quantitative determination of aspirin, caffeine, dehydrocodeine bitartrate, promethazine hydrochloride and salicylic acid in a gelatin capsule formulation was developed. The method uses acetanilide as an internal standard.

The advantages of the method include the following:

1. Excellent resolution of the six substances is obtained with gradient elution.
2. The method is rapid, requiring approximately 7 minutes for the completion of each assay.
3. The method allows the quantitation of salicylic acid, the major degradation product of aspirin, in the presence of the other substances.
4. The recovery and precision data show that the method is suitable for the analysis of solid pharmaceutical dosage forms.

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