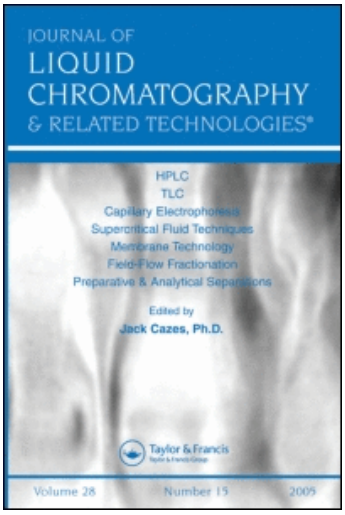

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SIMULTANEOUS DETERMINATION OF ACETAMINOPHEN AND HYDROCODONE BITARTRATE IN SOLID DOSAGE FORMS BY HPLC

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ABSTRACT

A reversed-phase, high performance liquid chromatographic method is developed and validated for the simultaneous determination of acetaminophen and hydrocodone bitartrate in pharmaceutical dosage forms. The method employs a Radialpak Cyanopropylsilane Cartridge with a mobile phase containing water, acetonitrile and dibutylamine phosphate (90:10:1). The detection is carried out using a variable wavelength UV-detector set at 215 nm. The acetaminophen showed linearity from 2.0 to 8.0 mg/mL range with a correlation coefficient of 0.999. The hydrocodone bitartrate showed linearity from 0.020 to 0.080 mg/mL range with a correlation coefficient of 0.999. Recoveries for acetaminophen averaged about 100.2% and hydrocodone bitartrate about 102.0% with a standard deviation of about 2.0%. System suitability showed excellent resolution of major peaks. The method is suitable for composite, content uniformity and dissolution assay of capsules and tablets.

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Acetaminophen and hydrocodone bitartrate combination dosage forms are available for the relief of moderate to moderately severe pain. There are a number of procedures that describe the determination of acetaminophen and hydrocodone bitartrate (1-16). However, with the exception of one method (15), none of these procedures describe simultaneous determination of acetaminophen and hydrocodone bitartrate in capsules or tablets.

This paper describes a method for the simultaneous determination of acetaminophen and hydrocodone bitartrate in capsules and tablets. In addition to composite and content uniformity, dissolution assay is also feasible with this method.

MATERIALS

Apparatus

a) Liquid Chromatograph - Waters system equipped with 710B autosampler, model 6000A solvent delivery system, model 480 variable wavelength UV detector (Waters, Milford, Mass.)

b) Integrator and Recorder - HP 3390A integrator (Hewlett-Packard, Palo Alto, CA) and Fisher Recordall®, series 5000 chart recorder (Fisher Scientific Co., Fair Lawn, NJ)

c) Columns - Radialpak Cyanopropylsilane Cartridge, 10 micron, 10 cm x 8 mm i.d., and u-Bondapak Cyano 10 micron, 30 cm x 3.9 mm i.d. (Waters, Milford, Mass.)

Chemicals and Reagents

Acetaminophen and hydrocodone bitartrate were USP reference standards. Water and acetonitrile were HPLC grade. Dibutylamine phosphate was reagent grade. (Purchased as D_4 from Waters, Milford, Mass.). Dibutylamine phosphate (1M) can be prepared in the laboratory by the following procedure:

Pipet 16.8 mL of dibutylamine (Eastman Kodak Co., Rochester, NY) into a 100 mL beaker. Add 70 mL deionized water and adjust the pH to 2.5 with concentrated phosphoric acid, reagent grade (Fisher Scientific Co., Fair Lawn, NJ). Transfer to a 100 mL volumetric flask and dilute to volume with deionized water.

METHODS

Mobile Phase

The mobile phase was composed of a 90:10:1 solution of water, acetonitrile and 1M dibutylamine phosphate, respectively. The final concentration of dubutylamine phosphate in the mobile phase was approximately 0.01M. The ratio of the solvents in mobile phase may be adjusted as necessary to achieve optimum resolution and symmetry between major peaks. The mobile phase was vacuum filtered and deaerated by ultrasonication before use.

Standard Solution

Standard solution containing 5 mg/mL of acetaminophen and 0.05 mg/mL of hydrocodone bitartrate were prepared in water for composite and content uniformity test. One to ten dilution of this standard solution was prepared in water for dissolution test.

Sample Solution

For composite assay and content uniformity test, an amount equivalent to 1 average capsule or tablet weight or a single unit was transferred to a 100-mL volumetric flask. About 50 mL of water was added and the solution sonicated for 5 minutes followed by mechanical shaking for 30 minutes. The solution was diluted to volume with water. A portion of this solution was filtered through a membrane filter of 0.45 micron porosity (Gelman Sciences, Inc., Ann Arbor, Mich.) and placed in a vial for chromatographic determination. The dissolution samples were

filtered through a membrane filter of 0.45 micron porosity and chromatographed without further dilution.

Chromatography

System Suitability

With all system components in place, the column was equilibrated by passing the mobile phase at a flow rate of 1.5 mL/minute for at least 30 minutes or until a steady baseline was obtained. Five replicate injections of 5 - 10 uL portions of standard solution were made. The major peaks were completely resolved. The resolution factor, R, was equal to or greater than 2.0. The tailing factor for each component was not greater than 1.5. The relative standard deviation of five replicate injections for each peak was not more than 2.0%.

Note: Upon recommendation of a reviewer of this paper, the equilibration time for a new Cyanopropylsilane column was determined. It was found that for a new column, equilibration was attained by passing the mobile phase at a flow rate of 1.5 mL/minute for about 4 hours. Subsequent use of this column required only about 30 minutes to obtain a steady baseline. Upon completion of analysis, the column was washed with about 30 mL of water and stored. For consistent results, a dedicated column is desirable.

Procedure

Equal volumes (about 5 uL) of the standard solution and sample solution were injected into the chromatograph for composite assay and content uniformity test and the peak area response was obtained from the integrator for acetaminophen and hydrocodone bitartrate for quantitation.

For dissolution samples, a 50 uL aliquot of standard solution and sample solutions was chromatographed.

Calculation

The milligram quantity of acetaminophen and hydrocodone bitartrate, per capsule or tablet, was determined by the following equation:

$$mg = \frac{Au}{As} \times \text{Concentration of Standard Solution} \times \frac{\text{Average weight (g) (capsule or tablet)}}{\text{Sample weight (g)}}$$

where:

Au = Peak area response from sample solution

As = Peak area response from standard solution

The percent of each drug dissolved in dissolution test, was determined by the following equation:

$$\% \text{ Drug dissolved} = \frac{Au}{As} \times \text{Concentration of Standard} \times \frac{\text{Volume of dissolution medium}}{\text{Label claim of drug}} \times 100$$

Symbols Au and As represent peak area response from sample and standard solutions respectively.

Validation of Assay Method

Linearity

Five concentrations of standard solutions ranging from 40 - 160% of label claim were prepared in water. Triplicate injections of each concentration were made and the data obtained were plotted.

Reproducibility and Recovery

Reproducibility of the method was established by analyzing 5 uL replicate portions of a solution that contained acetaminophen and hydrocodone bitartrate in amounts equal to the label claim of each drug per 100 mL. Recovery was determined by addition of amounts ranging from 40 - 160% of label claim to a solution that contained excipient mixture.

RESULTS AND DISCUSSION

The chromatography indicated good separation between acetaminophen and hydrocodone bitartrate (Figure 1). A plot of peak response versus the concentration of two components injected was linear with a correlation coefficient of 0.999 for each component (Figure 2, Table 1).

The relative standard deviations for six injections of a sample were less than 2.0% for both acetaminophen and hydrocodone bitartrate (Table 2).

The recovery from a spiked placebo gave reproducible results with a mean accuracy of 100.2% for acetaminophen and 102.05% for hydrocodone bitartrate (Table 3). No interference due to the placebo (excipients) could be detected.

The proposed HPLC method is simple and less time consuming as compared to USP XXI methods for acetaminophen and for hydrocodone bitartrate (16) where acetaminophen has to be separated by tedious column chromatography and measured spectrophotometrically. Then, hydrocodone bitartrate has to be

TABLE-1

Linearity of Acetaminophen and Hydrocodone Bitartrate

Acetaminophen		Hydrocodone Bitartrate	
Concentration (mg/mL)	Peak Area Response (10^7)	Concentration (mg/mL)	Peak Area Response
2.001	1.7150	0.0205	1042100
4.002	3.4796	0.0410	2178550
5.0025	4.3284	0.0512	2694350
6.003	5.1024	0.0615	3205600
8.004	6.7631	0.0820	4444300
Correlation Coefficient = 0.999		Correlation Coefficient = 0.999	

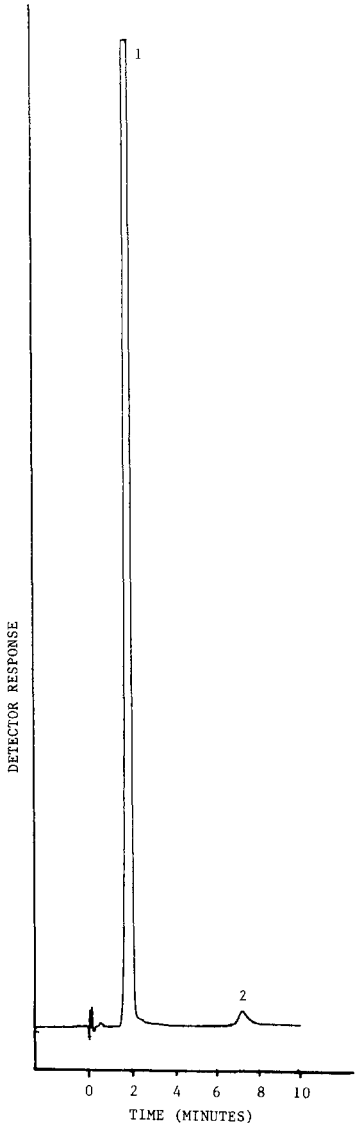

FIGURE 1 - A typical chromatogram of acetaminophen and hydrocodone bitartrate from standard solution. Peaks 1 - 2 are from acetaminophen and hydrocodone bitartrate, respectively.

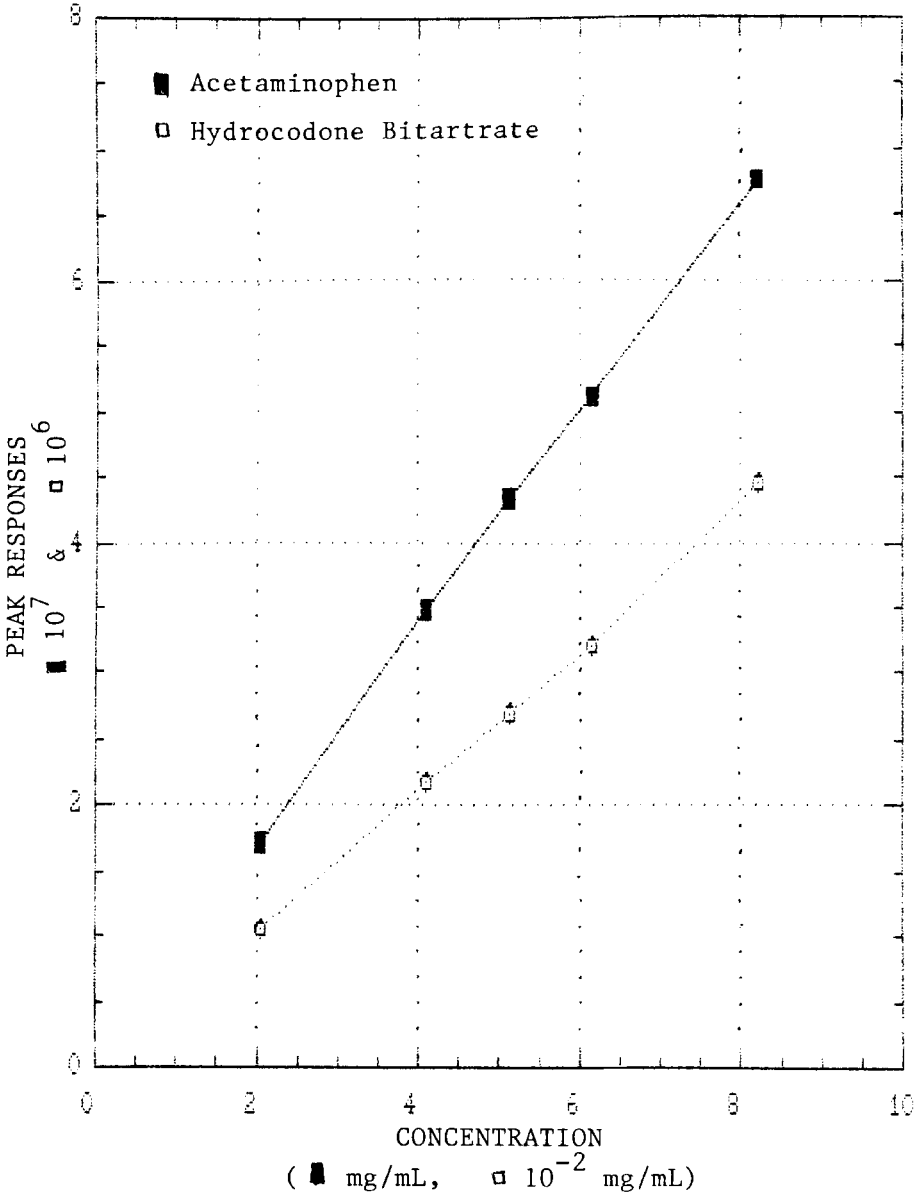

Figure 2: Linearity of Acetaminophen and Hydrocodone Bitartrate

TABLE-2

Reproducibility of Acetaminophen and Hydrocodone Bitartrate

	Acetaminophen		Hydrocodone Bitartrate	
	mg	% Label	mg	% Label
1.	500.07	99.96	5.16	100.68
2.	499.46	99.84	5.19	101.27
3.	500.48	100.05	5.20	101.46
4.	500.37	100.02	5.17	100.88
5.	500.37	100.02	5.24	102.24
6.	499.93	99.94	5.17	100.88
Mean	500.11	99.97	5.19	101.23
± SD	0.38	0.08	0.03	0.57
RSD	0.076	0.08	0.58	0.56

TABLE-3

Recovery of Acetaminophen and Hydrocodone Bitartrate from Spiked Placebo

Acetaminophen			Hydrocodone Bitartrate		
Amount Added (mg)	Amount Recovered (mg)	% Recovered	Amount Added (mg)	Amount Recovered (mg)	% Recovered
200.10	200.37	100.13	2.05	2.0518	100.09
400.20	406.55	101.59	4.10	4.1894	102.18
500.25	505.70	101.10	5.125	5.3049	103.51
600.30	596.13	99.31	6.15	6.3115	102.63
800.40	790.16	98.72	8.20	8.3504	101.83
Mean		100.17	Mean		102.05
±SD		1.2	±SD		1.26
RSD		1.2	RSD		1.24

extracted and quantitated titrimetrically. The proposed method offers simultaneous determination of both active ingredients with easy sample preparation. The results are compared in Table 4.

A previous paper (15) describes the simultaneous determination of acetaminophen and hydrocodone bitartrate using a C_{18} column. The mobile phase consisted of 25% methanol and 75% of an aqueous solution containing 0.01N monobasic potassium phosphate and 0.05N potassium nitrate, adjusted to a pH of 4.5 by dropwise addition of 3N phosphoric acid solution. In this method, the absorbances of the eluted peaks were measured at 283 nm with detector sensitivity adjustments for each peak. In the proposed method, the analysis is performed using a Radialpak Cyano Cartridge and a simpler mobile phase made up of water, acetonitrile and dibutylamine phosphate. The mobile phase is easier to prepare and is quite economical. The absorbances of the eluted peaks are measured at 215 nm with fixed detector

TABLE-4

Comparison of HPLC vs USP Assay

for Acetaminophen and for Hydrocodone Bitartrate

Sample Number	Acetaminophen (%)		Hydrocodone Bitartrate (%)	
	HPLC	USP XXI	HPLC	USP XXI
1	99.96	101.2	100.68	100.6
2	99.84	99.7	101.27	98.7
3	100.05	99.3	101.46	96.8
4	100.02	98.4	100.88	99.1
5	100.02	98.9	102.24	100.2
6	99.94	96.2	100.88	97.8
Mean:	99.97	98.95	101.24	98.87
± SD:	0.077	1.65	0.57	1.43
RSD:	0.077	1.67	0.56	1.45

sensitivity of 0.05 AUFS. In addition to composite assay and content uniformity, dissolution samples can also be analyzed providing simultaneous quantitation of each component dissolved using the proposed method.

The HPLC method described in this report allows for a rapid, reproducible and simultaneous determination of acetaminophen and hydrocodone bitartrate in solid dosage forms. The method is quite suitable for quality control assays of such combination products.

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