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VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD OF ASSAY FOR ACETAMINOPHEN, BUTALBITAL AND CAFFEINE IN SOLID DOSAGE FORMS

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ABSTRACT

The validation of a liquid chromatographic procedure for the determination of acetaminophen, butalbital and caffeine in solid dosage forms is described. The dosage content of tablets or capsules is diluted and chromatographed on a Radialpak Cyanopropylsilane Cartridge with a mobile phase of water-acetonitrile-1M dibutylamine phosphate (90+9+1, V/V) with detection at 215 nm. The calibration curve is linear with correlation coefficients of 0.999 for each component. Recoveries of spiked excipient blend averaged 99.5% for acetaminophen, 102.5% for butalbital and 101.0% for caffeine. The method met USP requirements for system suitability with proper resolution between two adjacent peaks. The relative standard deviation (RSD) of peak response of each component (obtained by chromatographing six replicates of standard solution) is less than 2.0% and the tailing factor of each component is not greater than 1.5. The method can be used for composite, content uniformity and dissolution assay of acetaminophen, butalbital and caffeine in tablet and capsule formulations.

INTRODUCTION

Acetaminophen, butalbital and caffeine combination dosage forms are available as tablets and capsules. These dosage forms

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are used for the relief of moderate to moderately severe pain. There is a compendial High Performance Liquid Chromatographic (HPLC) method for the simultaneous determination of aspirin and butalbital in tablets (1) but there is no compendial method at this time for the simultaneous determination of acetaminophen, butalbital and caffeine in tablets and capsules. This paper describes a method for simultaneous quantitation of acetaminophen, butalbital and caffeine in tablets and capsules. The method is used for the composite, content uniformity and dissolution assay of these products. The validation is carried out following the published Food and Drug Administration (FDA) guidelines (2) and USP system suitability criteria (3).

MATERIALS

Chemicals and Reagents

Acetonitrile and water were HPLC grade. Dibutylamine phosphate solution (1M) purchased as D_4 from Waters Associates, Milford, Mass. Acetaminophen, butalbital and caffeine were USP reference standards.

Apparatus

(a) Liquid Chromatograph - A liquid chromatograph equipped with 710B autosampler, model 510B pumps, model 481 variable wavelength UV detector (Waters, Milford, Mass.)

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

(c) Column - Radialpak Cyanopropylsilane Cartridge, 10 micron, 10 cm x 8 mm i.d. (Waters, Milford, Mass.)

METHODS

Mobile Phase

The mobile phase was composed of 90:10:1 solution of water + acetonitrile + 1M dibutylamine phosphate. The ratio of the solvents in mobile phase may be adjusted to achieve optimum resolution between major peaks. The mobile phase was vacuum filtered and deaerated by ultrasonication before use.

Standard Solution

Accurately weighed quantities of USP acetaminophen RS, USP butalbital RS and USP caffeine RS were dissolved in water to obtain a solution having known concentrations of 0.325 mg/ml, 0.05 mg/ml and 0.04 mg/ml, respectively.

A one to ten dilution of this solution was prepared in water for the analysis of dissolution samples.

Sample Solution

For composite and content uniformity assays, an amount equivalent to one average tablet or capsule weight, or a single unit, was transferred to a 100-mL volumetric flask, diluted with 50 mL water, sonicated for 10 minutes to dissolve and made upto volume with water. A 1 mL portion of this solution is further diluted to 10 mL in a volumetric flask.

Dissolution samples were filtered and chromatographed without further dilution.

Chromatographic Conditions

With all components of the system in place, the mobile phase was passed through the column at a flow rate of 1.5 mL per minute. The detector was set at 215 nm with a sensitivity of 0.05 AUFS. The temperature was ambient. The integrator (HP 3390 A) parameters were set to a chart speed of 0.5 cm/minute,

peak width of 0.16, threshold of 6 or 7 and an attenuation of 2^8. The speed of the chart recorder was 0.5 cm/minute.

Procedure

All solutions were filtered through a membrane filter of 0.45 micron porosity (Gelman Sciences, Ann Arbor, Michigan) for chromatographic determination. Equal volumes of about 10 uL of standard and sample solutions were chromatographed and peak area responses recorded. For the analysis of dissolution samples, 50 uL portions of standard and sample solutions were chromatographed.

Calculations

Quantities in mg =

$$\frac{\text{Peak response from sample solution}}{\text{Peak response from standard solution}} \times \text{Conc. of standard (mg/mL)}$$

$$\times \frac{\text{Average Weight (g) (tablet or capsule)}}{\text{Sample Weight (g)}}$$

Percent Dissolved =

$$\frac{\text{Peak response from sample solution}}{\text{Peak response from standard solution}} \times \text{Conc. of Standard (mg/mL)}$$

$$\times \frac{\text{Volume of dissolution medium (mL)}}{\text{Label claim (mg)}} \times 100$$

Method Validation

System Suitability

With all system components in place, the column was equilibrated with 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, 10 uL aliquots of standard solution were chromatographed. The peak responses of acetaminophen, butalbital and caffeine were recorded. The resolution factor, relative retention times, relative standard

deviation (RSD) and tailing factors for each peak were calculated according to criteria described in USP XXI (3).

Linearity

Five concentrations of each component ranging from 50 - 150% of the label claim were prepared in water and chromatographed. The linearity of each component was established by linear regression analysis of peak area responses versus concentrations.

Accuracy

An amount of acetaminophen, butalbital, caffeine and excipient mix equal to one tablet or capsule weight was dissolved in water and the resulting solution was assayed in triplicate. The results obtained were compared with label claim.

A solution containing only excipients was also prepared in water and chromatographed to establish any interference that may occur due to excipients.

Recovery

Solutions of excipients were spiked with each active component from 50 - 150% of the label claim and assayed. The recovery of each component was calculated and compared with the amount added.

RESULTS AND DISCUSSION

The assay, content uniformity and dissolution results for capsule dosage form are summarized in Tables 1, 2 and 3.

The results indicate that acetaminophen, butalbital and caffeine can be quantified in pharmaceutical dosage forms using the proposed HPLC method. Figure 1 shows proper resolution between any two peaks. The reproducibility of the method is indicated by the relative standard deviation of less than 2.0% for

TABLE 1

Assay Results of Acetaminophen, Butalbital and Caffeine in Capsules

Acetaminophen	Butalbital	Caffeine
323.43 mg (99.52%)	51.26 mg (102.51%)	40.44 mg (101.09%)

TABLE 2

Content Uniformity Results of

Acetaminophen, Butalbital and Caffeine in Capsules

	Acetaminophen		Butalbital		Caffeine	
	(mg)	(%)	(mg)	(%)	(mg)	(%)
1	326.76	100.5	49.69	99.4	38.68	96.7
2	329.10	101.3	50.19	100.4	40.11	100.3
3	330.71	101.8	50.55	101.1	40.20	100.5
4	318.40	98.0	48.97	97.9	40.06	100.1
5	321.52	98.9	48.87	97.7	38.65	96.6
6	332.14	102.2	49.75	99.5	39.50	98.8
7	331.94	102.1	48.65	97.3	40.21	100.5
8	322.69	99.3	48.11	96.2	38.90	97.3
9	328.71	101.1	48.05	96.1	38.95	97.4
10	324.93	100.0	49.80	99.6	39.68	99.2
Mean	326.69	100.5	49.26	98.5	39.49	98.7
± SD	4.69	1.4	0.86	1.73	0.65	1.61
RSD	1.44	1.4	1.75	1.75	1.65	1.63

TABLE 3

Dissolution Results of

Acetaminophen, Butalbital and Caffeine in Capsules

	% Dissolved		
	Acetaminophen	Butalbital	Caffeine
1	91.3	96.0	95.6
2	91.6	101.0	98.7
3	81.5	97.0	83.5
4	99.6	101.1	102.9
5	106.7	91.2	93.1
6	93.7	94.4	105.5
7	84.5	80.9	88.9
8	94.2	100.9	100.7
9	84.3	82.0	92.8
10	87.7	97.4	102.4
11	93.9	90.3	100.6
12	97.1	87.6	96.5
Mean	92.2	93.3	96.8
± SD	7.1	7.0	6.4
RSD	7.7	7.5	6.6

five replicate injections of standard solution as evident from Table 4.

The relative retention times are 0.21 for acetaminophen, 1.0 for butalbital and 0.30 for caffeine. A typical chromatographic run is complete in about 12 minutes. The tailing factors for each peak are calculated to be not greater than 1.5.

A linear response is obtained for acetaminophen with correlation coefficient of 0.998, for butalbital and caffeine with a correlation coefficient of 0.999. The results are summarized in Table 5.

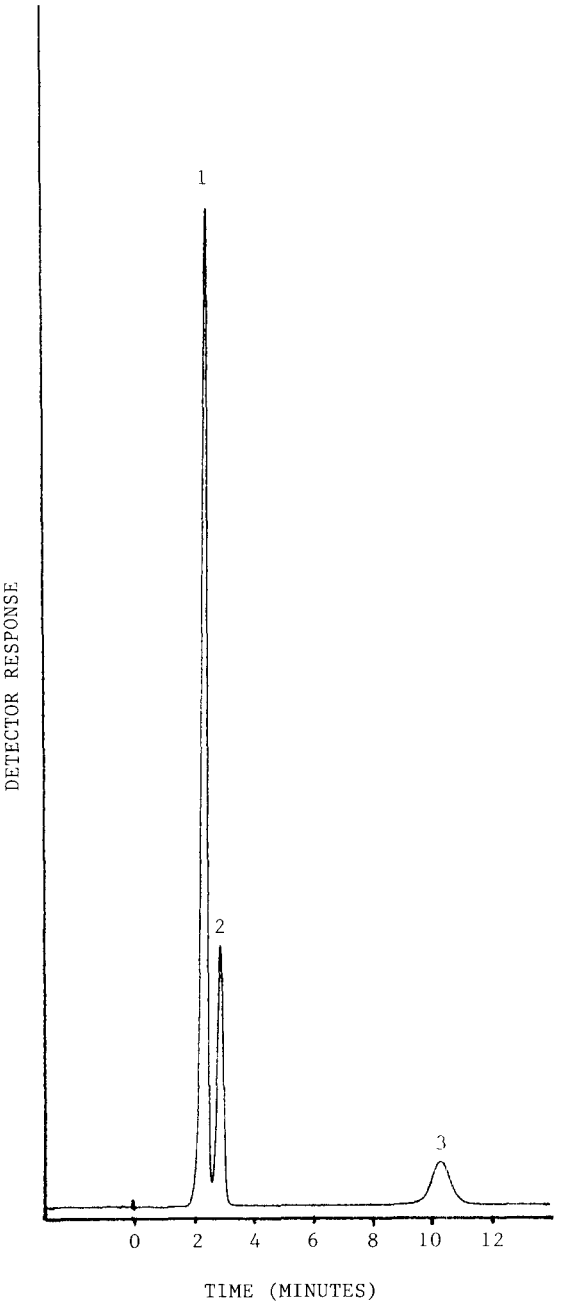

FIGURE 1 - A typical chromatogram of acetaminophen, butalbital and caffeine from standard solution. Peaks 1 - 3 are acetaminophen, caffeine and butalbital, respectively.

TABLE 4

Reproducibility of Standard Solution

Injection Number	Peak Height Responses		
	Acetaminophen	Butalbital	Caffeine
1	3064296	289952	688489
2	3106642	289751	682045
3	3068404	284594	676174
4	3080148	286113	678993
5	3109612	288326	680985
Mean	3085820	287747	681337
± SD	21203	2336	4580
RSD	0.68	0.81	0.67

TABLE 5

Linearity of Response for Acetaminophen, Butalbital and Caffeine

Conc. (mg/mL)	Acetaminophen	Conc. (mg/mL)	Butalbital	Conc. (mg/mL)	Caffeine
0	0	0	0	0	0
160.95	1603477	25.55	141439	20.13	341944
257.52	2604870	40.88	231509	32.21	545331
321.90	3111436	51.10	288302	40.26	652344
386.28	3581146	61.32	333804	48.31	793529
482.85	4412725	76.65	417762	60.39	989646
Slope	8569.16		534181		15995.33
Y-intcpt	304318.39		9476.82		20586
Correlation coefficient	0.998		0.999		0.999

TABLE 6

Accuracy of Method for Acetaminophen, Butalbital and Caffeine

	Label Claim (mg/dose)	Assay Results* (mg)	% of Label
Acetaminophen	325	323.43 ± 1.68	99.52 ± 0.50
Butalbital	50	51.26 ± 0.39	102.51 ± 0.78
Caffeine	40	40.44 ± 0.30	101.10 ± 0.70

* Mean of assays performed in triplicate.

TABLE 7

Recovery of Actaminophen, Butalbital and Caffeine

from Spiked Excipient Solutions

Acetaminophen		Butalbital		Caffeine	
mg Amount		mg Amount		mg Amount	
Added	Recovered	Added	Recovered	Added	Recovered
161	165.76 (102.96%)	25.6	25.18 (98.3%)	20.1	20.15 (100.27%)
322	323.69 (100.52)	51.1	51.21 (100.2)	40.3	40.40 (100.27)
483	471.41 (95.04)	76.6	73.93 (96.51)	60.4	58.47 (96.80)

The accuracy of the method is established by achieving reproducible results of 99.52% ± 0.5 for acetaminophen, 102.51 ± 0.8 for butalbital and 101.1 ± 0.70 for caffeine (Table 6).

The excipient solution showed no peaks. The recovery of active components from a spiked excipient solution afforded excellent results as summarized in Table 7.

The proposed HPLC mehtod is simple, accurate and reproducible. In the absence of any compendial method for the simultaneous

determination of acetaminophen, butalbital or caffeine, the proposed method can be used for routine quality control and stability evaluations of these products.

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