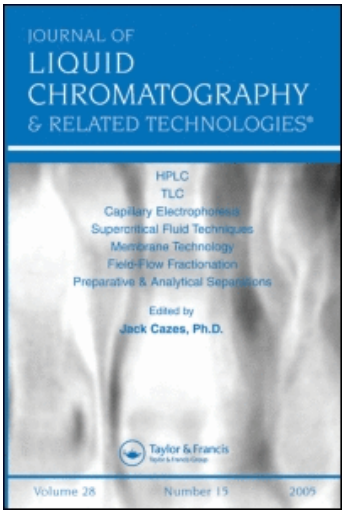

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A STABILITY-INDICATING SIMULTANEOUS ANALYSIS OF ACETAMINOPHEN AND HYDROCODONE BITARTRATE IN TABLETS FORMULATION BY HPLC

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ABSTRACT

A rapid, simple, stability indicating, reversed phase high-performance liquid chromotographic (HPLC) method is presented here for direct and simultaneous quantitation of Acetaminophen and Hydrocodone Bitartrate in tablets dosage form at a ratio of 100:1. This procedure not only separated the two active components but also separated known impurity and degradation product of acetaminophen, p-aminophenol and p-chloroacetanilide. The method described here also separates other opiates, codeine sulfate and hydromorphone. In this procedure a radial pak cartridge packed with chemically bonded octadecylsilane (C18, 4 micron) as a stationary phase and (16:84) acetonitrile with phosphate buffer (pH 3.3) as the mobile phase were utilized. Detection was made with a uv-spectrophotometer at 215 nm. Quantitation was performed by peak height and external standard method. The average recovery at 100% label claim and the relative standard deviation of the two respective components, acetaminophen and hydrocodone were: 99.03%, 1.29% and 98.78%, 0.3%.

INTRODUCTION

Acetaminophen is widely used for its nonsalicylate, analgesic and antipyretic action, while hydrocodone bitartrate is an opioid-analgesic and antitussive in tablet matrix. There are various commercially available prescription drugs which contain narcotics like hydrocodone bitartrate or codeine in combination with acetaminophen. Several methods have been reported for either acetaminophen analysis exclusively (1) or in combination with other pain relievers (2,3) and codeine (4,5) by HPLC. Similarly hydrocodone analysis is separately investigated and reported by gas chromatography (6,7). An official compendial USP XXI method (8) for hydrocodone tablet is potentiometric and spectrophotometric which is not only time consuming but also unreliable because of the possible interference from the excipients in tablets dosage form. However, one method for the combination of acetaminophen and hydrocodone has been reported by Wallo and D'Adamo by HPLC (9) which demonstrate good separation between the two active components. Due to weak absorption and longer retention of hydrocodone on the column, the method reported earlier (9) shows a significant tailing and a small peak height for hydrocodone. This problem is compounded by a strong chromophore present of acetaminophen and the ratio (100:1) of acetaminophen and hydrocodone in the dosage form.

The method proposed here reduces overall analysis time, improves the peak shape and height of hydrocodone and reduces the strong influence of acetaminophen. Furthermore, this procedure also demonstrates good separation of impurity and degradation products.

MATERIALS AND METHODS

Instrumentation: The high-pressure liquid chromatograph (HPLC) consisted of a constant flow pump[1], and an automatic

injector[2], a variable wavelength uv-spectrophotometer[3] and a printer/plotter/integrator[4]. A radial pak cartridge column (8mmx 10cm) packed with octadecylsilane[5] (C18) 4μm particle material and a Z-Module[6] equipped with a temperature controller[7] for the radial pak cartridge. A guard column[8] packed with reversed phase C-18[9] material was attached before the analytical column.

Materials: All the reagents and solvents were of HPLC grade except phosphoric acid. Potassium phosphate monobasic[10], acetonitrile[11], water[12] and triethylamine[13], p-Chloroacetanilide and p-Aminophenol[14] reagent grade. Reference standards acetaminophen USP and hydrocodone bitartrate USP[15].

Mobile Phase: Aqueous portion of mobile phase consisted of 0.02 M potassium phosphate monobasic containing 0.2 mL triethylamine and 0.2 mL phosphoric acid (85%), adjusted to pH 3.3 with 3N phosphoric acid. 84 volumes of aqueous solution and 16 volumes of acetonitrile were filtered through 0.45 micron membrane[16], mixed and degassed prior to use.

Solvent Water: Each liter of water contained 0.5 mL of 3N phosphoric acid.

Chromatographic Conditions: Flow rate, about 2.0 mL/min and column temperature 30°C. Detector range 0.02 aufs measured at 215 nm. Quantitation by peak height and external standard at a chart speed 0.6 cm/min.

Standard Preparation: An initial 0.25 mg/mL stock standard solution of previously dried hydrocodone bitartrate (II) was prepared in solvent water. Accurately weighed, approximately 50 mg of standard acetaminophen USP (I) was transferred to a 100 mL volumetric flask. The weighed quantity of I was dissolved in solvent water then 2 mL of stock solution of II was added into this flask, diluted to mark with solvent water, mixed and filtered.

Resolution Standard: A standard solution containing component I and II was prepared in 0.01 mg/mL final concentration in solvent water.

Spiked Impurities and Other Opiates Solution: A mixture of solution containing 0.01 mg/mL of II, p-aminophenol (III), hydro-

morphone HCL (IV) and codeine sulfate (V), 0.1 mg/mL of p-Chloroacetanilide (VI) and 0.5 mg/mL of I was prepared in solvent water.

Sample Preparation: No less than 20 Vocodin[17] tablets were weighed and average tablet weight was determined. All the tablets were finely powdered and mixed. A portion of powder equivalent to one average tablet weight was weighed and quantitatively transferred into a 100 mL volumetric flask. Fifty milliliters (50mL) of solvent water were added and the mixture was shaken on a mechanical shaker for about 45 minutes, diluted to mark with solvent water, mixed and filtered. A five milliters of filtrate were transferred to a 50 mL volumetric flask, diluted to volume with solvent water and mixed. A portion of this solution was filtered through a 0.45 micron disposable disc filter[18]into a HPLC vial[19].

System Suitability-Procedure: The column temperature was maintained at 30°C and equilibrated with mobile phase prior to making an injection. Six replicate injections of equal volumes (10µ L) of standard preparation and one injection (10µ L) of resolution standard were introduced separately by an automatic injector into the HPLC. The relative standard deviation (% RSD) for each component I and II are calculated using peak height. The resolution factor, R, calculated according to the USP XXI (8) from the equation $2 (t_2 - t_1)/(W_1 + W_2)$, where t_1, t_2 are the retention times and W_1, W_2 are the widths at the base of the two respective peaks. The resolution, R between the two neighboring peaks of interest should not be less than 6 and base line separation should be achieved. The relative standard deviation (% RSD) of six replicate injections of standard solution should be less than 3.0% for individual components.

Assay-Procedure: Equal volumes (10µ L) of standard solution and sample solution were introduced separately by means of automatic injector into the previously equilibrated HPLC.

Calculations: The amount of I and II were calculated in milligrams per tablet from respective equation.

$$\frac{Hu}{Hs} \times \frac{Ws}{Wu} \times A \times 10 \text{ and } \frac{Hu}{Hs} \times A \times F \times 20$$

where Hu and Hs are the respective response of peak heights of sample and standard. Ws and Wu are the weights in milligrams of standard and sample, 10 and 20 are the dilution factors for I and II, respectively. A is the average tablet weight and F^{20} is the conversion factor for hydrocodone bitartrate.

RESULTS AND DISCUSSIONS

The two peaks of interest corresponding to component I and II were eluted in the increasing order of 2.6 and 5.0 minutes, respectively and complete base line separation was achieved as shown in Figure 1, a typical chromatogram of standard solution. The resolution factor, R calculated between the two peaks of interest, I and II was 8.37 and capacity factor, K' for the corresponding components were 0.78 and 2.50, respectively. The separation factor, α, was found to be 3.20. The relative standard deviation (% RSD) of six replicate injections were: 0.72% and 0.89% for I and II, respectively. In addition, retention times recorded from standard solution spiked with impurities and other opiates were in the ascending order of 1.6, 2.3, 2.6, 3.3, 5 and 34.6 minutes corresponding to compounds III, IV, I, V, II and VI, respectively with an adequate separation. A chromatogram of all the six compounds is shown in Figure 2.

The system suitability data and the separation of all the stated compounds, indicated that the column was efficient, suitable and the mobile phase had been optimized for this particular application. In addition, since all the six compounds had different retention times, the specificity of the method was also established.

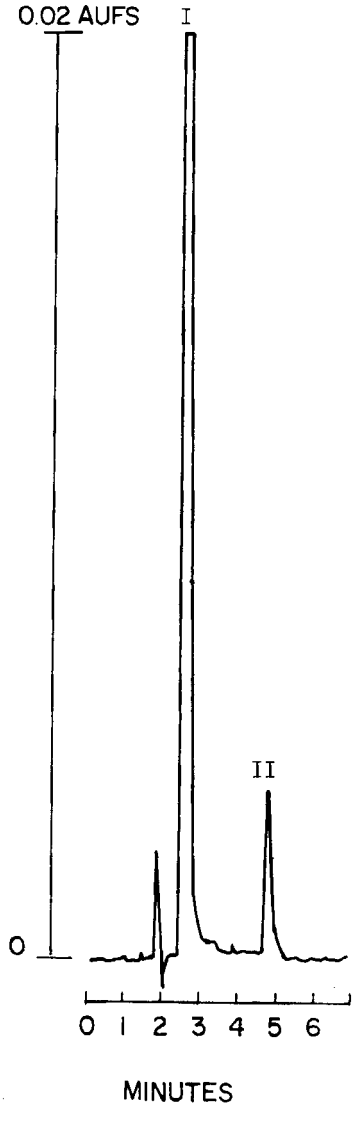

Figure 1 A Liquid Chromatogram of Standard
Key: (I) Acetaminophen
(II) Hydrocodone

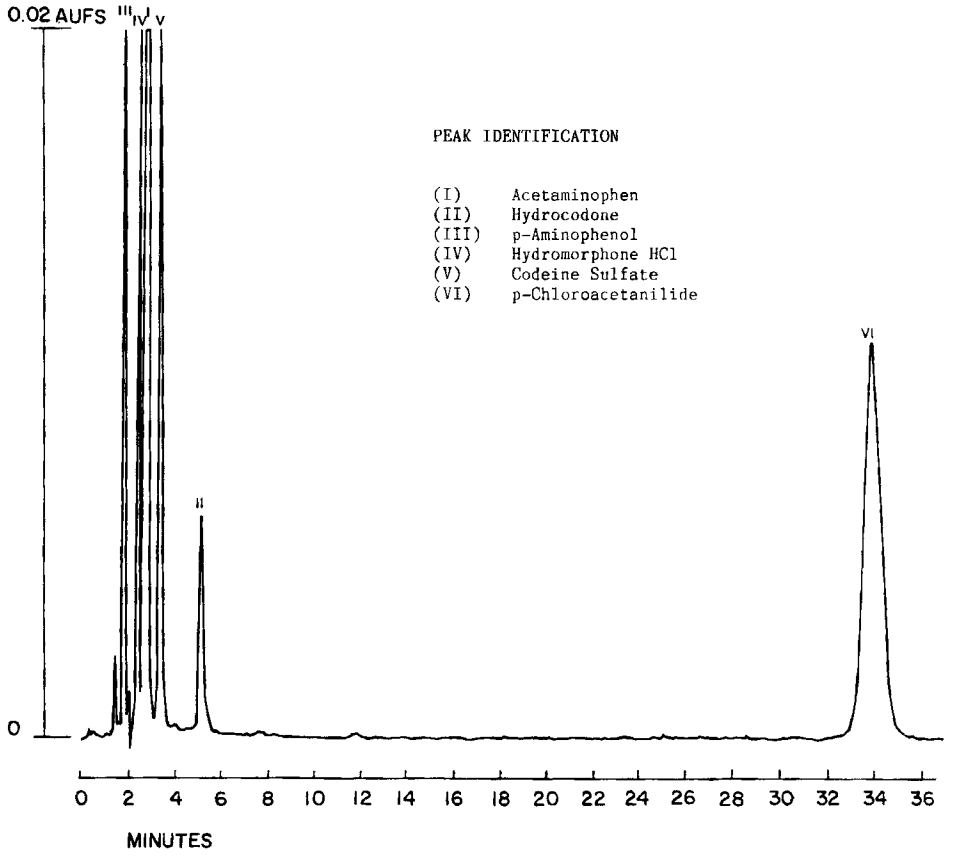

Figure 2 A Liquid Chromatogram of all the six compounds

Linearity: Standard solutions ranging from 60% to 140% at 20% intervals were prepared and introduced into the chromatograph according to the described assay procedure. The peaks response were plotted against the respective concentration for each active component. A straight line was obtained for individual component as evidenced by the correlation coefficient 0.9995 and 0.9993 corresponding to amount injected from 0.2998 mg/mL to 0.6996 mg/mL for I and 0.0031 mg/mL to 0.0072 mg/mL for II, respectively.

Since each active component gave a straight line and correlation coefficient was very close to unity, the method proved to be linear for the expected analysis range (85%-115%) of the label claim.

Recovery: This test was performed to demonstrate the recovery of active ingredients from tablet matrix. The recovery of active components were determined at 50%, 100% and 150% of label claim by adding the known amounts of I and II in the placebo of vicodin tablets[17] and analyzing the mixture according to the assay procedure. The recovery studies of 100% added amount of I were 99.03% with 1.29%, RSD and 98.78% with 0.3%, RSD for component II. Since no interference from excipients was observed in the chromatograms and the recovery of active ingredients at each level was within ± 5% of the theoretical amount as summarized in Table 1, the accuracy of the procedure was demonstrated.

Precision Analysis: This test was conducted to ensure the reproducibility of the system as well as of method. Six different

TABLE 1
RECOVERY DATA

Percent Claimed	Component I		Component II	
	Percent Recoverd (a)	% RSD	Percent Recovered (a)	% RSD
50%	49.36	1.18	50.14	1.07
100%	99.03	1.29	98.78	0.30
150%	145.19	0.05	145.39	0.20

(a) Average of two samples or four injections

samples were prepared from a composite sample according to the described assay procedure and six replicate injections from each sample were chromatographed. The data generated from this test showed an excellent precision and reproducibility as summarized in Table 2. The relative standard deviation (% RSD) of six replicate injections for each sample was found to be <1.5% for each active ingredient.

Sensitivity: Standard solutions of active component I and II corresponding to 0.01% to 0.0004% and 0.2% to 0.04% of 100% were introduced into the HPLC according to the assay-procedure. Component I was detected up to 0.0004% level with % RSD 2.02% Component II at 0.04% level showed % RSD 9.24%. Solutions of Component III and VI were also injected and detected at 0.0002% level with % RSD 6.1% and 1.48%, respectively. The minimum amount detected at a sensitivity of 0.005 aufs and ratio peak to noise (S/N) greater than 8 were 2 ng for Component I and II and 1 ng and 20 ng and corresponding to III and VI, respectively.

Forced Decompostion Studies - Solution Phase: The purpose of this study was to obtain information regarding the degradation of acetaminophen (I) and hydrocodone (II) solutions under extreme conditions, upon treatment with acid, base and oxidizing agent. In order to perform this test solutions of I and II were prepared in 2.5mg/mL and 0.025mg/mL concentration, respectively, in 1N hydrochloric acid, 1N sodium hydroxide and 10% hydrogen peroxide. An aliquot of each solution was refluxed separately for the specified time interval then the dilutions were made with water to obtain final concentration of 0.5mg/mL and 0.005mg/mL corresponding to I and II, prior to introduction into the chromatograph.
An aqueous control of each component was also prepared and refluxed for the same specified time interval, and then diluted to the same concentration as the other samples before making an injection. Each refluxed base solution was neutralized with an equal volume and strength of acid prior to final dilution with water. All the samples were collected at the initial hour before refluxing and at every hour for four hours after refluxing.

TABLE 2
PRECISION ANALYSIS DATA

Acetaminophen						
Injection No.	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5	Sample No. 6
1	96.06	98.28	97.68	98.43	94.72	97.07
2	97.45	99.93	95.57	97.05	95.74	95.80
3	95.51	99.23	98.49	98.07	96.66	96.97
4	98.29	99.17	97.64	96.79	96.00	96.47
5	98.33	101.33	97.13	97.21	95.40	96.19
6	96.80	101.27	96.71	99.93	95.04	95.59
Average	97.07	99.87	97.20	97.91	95.59	96.35
% RSD	1.20	1.23	1.03	1.20	0.73	0.63
Hydrocodone Bitartrate						
1	98.84	98.97	99.02	101.20	98.39	101.86
2	99.91	97.61	98.88	100.91	96.84	101.49
3	101.13	99.83	100.85	100.03	100.09	101.94
4	101.70	97.96	100.40	101.20	98.84	101.35
5	98.56	99.54	100.19	99.95	99.28	102.16
6	99.13	100.54	100.49	99.44	99.35	102.60
Average	99.88	99.08	99.97	100.46	98.80	101.90
% RSD	1.29	1.14	0.82	0.74	1.13	0.45

TABLE 3

FORCED DECOMPOSITION DATA

Acetaminophen Remaining				
Time Interval	Aqueous Control	1N HCL	1N NaOH	10% H_2O_2
Initial	100%	100%	98.13%	100%
1 Hour	100%	64.82%	45.09%	100%
2 Hour	100%	26.14%	18.62%	100%
3 Hour	100%	10.13%	3.58%	91.66%
4 Hour	100%	4.93%	3.60%	90.62%
Hydrocodone Remaining				
Initial	100%	100%	96.29%	62.77%
1 Hour	100%	100%	80.38%	38.11%
2 Hour	100%	100%	79.51%	16.37%
3 Hour	100%	99.86%	71.79%	0%
4 Hour	100%	97.46%	71.65%	0%

No decomposition or extraneous peak was observed in the aqueous control for either component. The acid hydrolysis showed a significant decomposition for Component I, however, II showed minor decomposition at the last hour. Base solution affected both the component but component I was decomposed almost 96.4% at the end of fourth hour. In the initial two hours Component I recovered 100% and showed a loss of 10% at the end of last hour when treated with hydrogenperoxide and chromatographed. Contrary to that compound II was decomposed more than 83% in the first two hours and total decomposition occurred in the third hour with 0% of II remaining. The data presented in Table 3 is an evidenced that the method is sensitive and capable of detecting and quantitating the decom-

position products of the two active compounds in small amounts. However, no attempt was made to identify the decomposed products formed under these extreme conditions.

In conclusion this procedure provides a solution for rapid and accurate quantitation of two active components in a short period of time. As the retention time and peak response of component II are dependent of pH, while I is unaffected by the pH, a significant improvement in peak height and shape of component II is achieved by lowering the pH of the mobile phase to more acidic (pH 3.3) and by shifting away from the maximum wavelength of II to the lower wavelength (215 nm), and thereby enhancing the peak response of II at a much lower concentration. The later change also considerably diminished the influence of a strong chromophore of I, and thus alleviated the absorbance to well within the linear detector range. These changes also eliminated the problem reported in the previous method (9) related to the ratio of component I and II which are present in 100:1 ratio in the dosage form.

The known impurity and degradation product of component I, p-chloroacetanilide and p-aminophenol have different retention times and separate adequately from the active components, therefore, this method proved to be a stability-indicating. In addition, under extreme stress conditions, the decomposition products separated and the remaining active components gave sufficient quantitative data for stability purposes.

In essence this HPLC method is specific, accurate and stability indicating for simultaneous routine analysis of acetaminophen and hydrocodone combination in tablets formulation.

FOOTNOTES

[1] M-6000A, Waters Associates, Milford, MA 01757.

[2] WISP 710B/712B, Waters Associates, Milford, MA.

[3] 490 - Programmable, multiwave length detector, Waters Associates, Milford, MA.

[4] 730 Data module, Waters Associates, Milford, MA.

[5] Nova Pak C-18, 4 micron cartridge, Waters Associates.

[6] Z-module, Waters Associates, Milford, MA.

[7] CH-1448 dual zone temperature controller, Systec, Inc., Minneapolis, MN 55421.

[8] Uptight pre-column kit #135B, Up Church Scientific, Inc., Oak Harbor, Washington 98277.

[9] Bondapak C18/Corasil, 37-50 micron, Waters Associates.

[10] Potassium phosphate monobasic HPLC grade, Fisher Scientifc.

[11] Acetonitrile HPLC grade, J.T. Baker Co., NJ.

[12] Milli-Q-System, Millipore, Bedford, MA.

[13] Triethylamine, HPLC grade, Fisher Scientific.

[14] Aldrich Chemicals, Inc., Milwaukee, WI 53233.

[15] United States Pharmacopeal Convention, Rockville, MD.

[16] Zetapore, AMF, filtration products, Meriden, CT 06450.

[17] Knoll Pharmaceuticals. Contain 500 mg acetaminophen USP and 5 mg hydrocodone bitartrate USP per tablet.

[18] Part #SM16555k, Sartorius Filters, Haywood, CA 94545.

[19] HPLC vial assembly, part nos. 404802, 408032, 408036, Phase Separations, Norwalk, CT 06854.

[20] The ratio of the molecular weights of hydrocodone bitartrate hydrous versus hydrocodone bitartrate anhydrous (494.50/449.46) is 1.1. This ratio is used as a factor.

REFERENCES

1. Sena, F.J., Piechocki, J.T. and LI, K.L., J. Pharm. Sci., 68(11), 1465 (1979).
2. Carneva, L., J. Pharm. Sci. 72(2), 196 (1983).
3. McSharry, W.O. and Savage, I.V.E., J. Pharm. Sci., 69(2), 212 (1980).
4. Ko, C.Y., Marziani and Janicki, C.A., J. Pharm. Sci. 69(9), 1081 (1980).

5. Das Gupta, V., J. Pharm. Sci. 69(1), 110 (1980).
6. Barnhart, S.W., Caldwell, W.J., J. Chromatogr. 130, 243 (1977).
7. Hoffman, D.J., Leveque, M.J., Thomson, T., J. Pharm. Sci., 72, 1342 (1983).
8. "The United States Pharmacopeia" 21st rev., Mack Publishing Co., Easton., PA 18042, p. 498, and pp. 1228-1230.
9. Wallo, W.E. and D'Adamo, A., J. Pharm. Sci. 71(10), 1115 (1982).