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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STABILITY-INDICATING ANALYSIS OF ACETAMINOPHEN AND CODEINE PHOSPHATE IN TABLETS AND CAPSULES

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SUMMARY

A high-performance liquid chromatographic method has been developed for the simultaneous determination of acetaminophen and codeine phosphate for product stability studies, and release and dissolution testing of tablets and capsules. The reversed-phase method utilizes UV detection at 214 nm, a C_{18} column and requires a maximum of 10 min per analysis. The method has been validated for use with products containing as much as 500 mg of acetaminophen and as little as 7.5 mg of codeine phosphate. The known potential degradation products, *p*-aminophenol, codeine N-oxide, and codeinone are separated for quantitation simultaneous with the parent compounds. The method has been shown to be linear, reproducible, specific, sensitive and rugged.

INTRODUCTION

Analysis of acetaminophen and codeine phosphate has been reported as individual ingredients and in combination products. Analytical methods have included UV[1,2], colorimetric[3,4], nonaqueous titration[5], gas–liquid chromatography (GLC)[6–8], and high-performance liquid chromatography (HPLC)[9–11]. However, these methods have not been used for the simultaneous quantitation of acetaminophen, codeine phosphate, or their potential known degradation products *p*-aminophenol, codeine N-oxide, and codeinone. The goal of the simultaneous quantitation of large amounts of acetaminophen and small amounts of codeine phosphate, which had previously been unsuccessful[12] has now been achieved.

This report documents the methodology, including sample preparation and chromatography, for the simultaneous quantitative analysis of each of the active components as well as potential degradation products. This method has been successfully employed in stability studies, dissolution evaluation and product release.

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EXPERIMENTAL

Materials, equipment and liquid chromatographic conditions

A μBondapak® C_{18} column 30 cm × 3.9 mm (Waters) was used with a mobile phase of pH 2.35 buffer–methanol (93:7) at 3.0 ml/min. The mobile phase reservoir was maintained at ambient temperature, while the column was heated to 40°C. The chromatographic hardware consisted of a DuPont Model 850 high performance liquid chromatograph equipped with a DuPont automatic sampler with a 20-μl loop, a DuPont column oven, a Waters Model 440 absorbance detector equipped with a 214 nm wavelength extension kit, and a DuPont 4100 integrator. The recorder sensitivity was adjusted to 0.04 a.u.f.s. A Hewlett-Packard Model 1040A diode-array detector was used in specificity studies with stressed and nonstressed samples.

Reagents and solutions

Methanol, water, potassium phosphate monobasic (KH_2PO_4), phosphoric acid, and triethylamine were all HPLC grade. The buffer was made by adding 2.04 g potassium phosphate monobasic (15 m*M*) and 2 ml triethylamine per liter of water. The pH of this solution was adjusted to 2.35 ± 0.1 with concentrated phosphoric acid. The solution was filtered before use with a Millipore® filter (Catalog No. HAW P04700, 0.5 μm pore diameter). The mobile phase was made by adding 70 ml of methanol to 930 ml of pH 2.35 buffer and mixing thoroughly.

The extraction solvent was made by adding 500 ml of methanol to 500 ml of water which had been adjusted to pH 4.0 ± 0.1 with phosphoric acid. The sample solvent was made by adjusting water to pH 3.2 ± 0.1 with phosphoric acid; the standard solvent was made by mixing 50 ml of methanol with 450 ml of water which had previously been adjusted to pH 4.0 ± 0.1 with phosphoric acid.

Standards and samples preparation — assay and content uniformity for product release

For 300-mg doses of acetaminophen, weigh accurately (in duplicate) approximately 33.33 mg of codeine phosphate standard into a 100-ml volumetric flask. Into separate 100-ml volumetric flasks weigh accurately (in duplicate) approximately 66.67 mg of acetaminophen standard. Dissolve the codeine phosphate standards in standard solvent (pH 4.0 water–methanol, 9:1) and dilute to volume. Transfer by pipet, the aliquot of codeine phosphate standard solution, corresponding with the tablet or capsule codeine phosphate strength being analyzed, to the volumetric flask(s) containing the acetaminophen standard. Dissolve and dilute to volume with standard solvent. For a 7.5-mg dose of codeine phosphate, pipet a 5.0-ml aliquot of codeine phosphate standard solution, for a 15-mg dose pipet a 10.0-ml aliquot of codeine phosphate standard solution, 20.0 ml for a 30-mg dose, and 40.0 ml for a 60-mg dose. For dosage strengths of 500 mg of acetaminophen, weigh accurately (in duplicate) approximately 20 mg of codeine phosphate standard into a 100-ml volumetric flask. Proceed as stated in the instructions for 300-mg doses.

Composite sample. Accurately weigh 20 tablets or capsules and calculate the average tablet or capsule fill weight (W_a). Triturate the tablet or capsule granulation to a fine powder. Accurately weigh, in duplicate, an amount of granulation equivalent to 500 mg of acetaminophen (W_x, see *Calculation* section) into a 6 oz. bottle and add 150.0 ml of extraction solvent. Shake for 1 h. Pipet 10.0 ml of this solution into a

50-ml volumetric flask. Mix and dilute to volume with sample solvent. Filter 20 ml (Rainin Nylon-66 filter unit, 0.45 μm or equivalent) and collect the last 5–6 ml for analysis.

Spiked standard. Weigh approximately 66.66 mg of acetaminophen standard, 13 mg of codeine phosphate standard and approximately 0.5–1.0 mg of codeine N-oxide into the same 100-ml volumetric flask. Dissolve and dilute to volume with standard solvent.

Content uniformity sample. A minimum of ten units must be individually analyzed.

Acetaminophen 300 mg with codeine phosphate. Place one tablet or the contents of one capsule into a 4 oz. bottle, add 100.0 ml of extraction solvent. Shake tablets for 4 h and shake the capsules for 1 h. Add 10.0 ml of each sample solution to a separate 50-ml volumetric flask. Mix and dilute to volume with sample solvent (final acetaminophen (APAP) concentration = 0.6 mg/ml). Filter 20 ml (Rainin Nylon-66 filter unit, 0.45 μm or equivalent) and collect the last 5–6 ml for analysis.

Acetaminophen 500 mg with codeine phosphate. Place the contents of one capsule into a 6 oz. bottle, add 150.0 ml of extraction solvent and shake for 1 h. Add 10.0 ml of each sample solution to a separate 50-ml volumetric flask. Mix and dilute to volume with sample solvent (final APAP concentration = 0.667 mg/ml). Filter 20 ml (Rainin Nylon-66 filter unit, 0.45 μm or equivalent) and collect the last 5–6 ml for analysis.

Standards for content uniformity. The volumes and dilutions are the same as for the assay method; however, the standard weights are as follows:

Acetaminophen 300 mg with codeine: weigh accurately, approximately 30 mg of codeine phosphate standard and 60 mg of acetaminophen standard.

Acetaminophen 500 mg with codeine: weigh accurately, approximately 20 mg of codeine phosphate standard and 66.66 mg of acetaminophen standard.

Dissolution samples and standards

Individual dosage units are added to 900 ml of 0.1 *N* hydrochloric acid and agitated using a paddle at 50 rpm (USP Apparatus 2). At 30 min a 20-ml aliquot is withdrawn and filtered (Rainin Nylon-66 filter unit, 0.45 μm or equivalent), with the final 5 ml being collected for direct analysis. Standards are prepared as 100.0% of theoretical concentration by pipeting aliquots of an accurately weighed codeine phosphate standard solution in 0.1 *N* hydrochloric acid into accurately weighed portions of acetaminophen and diluting to volume with 0.1 *N* hydrochloric acid.

Assay procedure

The instrument was assembled as previously indicated and the column equilibrated for at least 20 min with the mobile phase flowing. The system suitability was determined by doubling the recorder chart speed and injecting 20 μl of the spiked standard solution. The resolution between the acetaminophen and the codeine N-oxide and also between the codeine N-oxide and the codeine peaks should be at least 1.25 as determined by using the resolution equation[13].

The precision of the system was determined using the relative standard deviation [R.S.D. (%)] of the response factors (area/μg) for the injections of the standard solutions. Typically the R.S.D. was less than 2.0%.

Samples were analyzed with standard solutions chromatographed before and after and interspersed with the samples if a large number of analyses were to be performed. The retention times for acetaminophen and codeine phosphate were approximately 2.7 min and 5.3, min, respectively.

Calculations

Assay and content uniformity for product release. It is important to correct the weight of codeine phosphate standard for the degree of hydration so that results are reported on an exact hemihydrate basis. The weight of granulation theoretically equivalent to 500 mg of acetaminophen (W_x) is determined using the equation:

$$W_x = \frac{W_a}{L_a} \times W_t$$

where W_a is the average capsule fill or tablet weight (mg); L_a is the label amount of acetaminophen in a capsule or tablet (mg); and W_t is the theoretical amount of acetaminophen to be contained in each weighed sample. The response factor (R) for each weighing of a sample or standard is determined using the equation:

$$R = \frac{A}{W}$$

where A is the average acetaminophen, codeine phosphate or impurity peak area for each weighing; and W is the sample or standard weight (mg). The percent label acetaminophen, codeine phosphate or weight percent impurity is determined using the equation:

$$\% \text{ label} = \frac{R_x}{R_s} \times \frac{W_x}{W_s} \times \frac{100}{F}$$

where R_x is the acetaminophen or codeine phosphate or impurity response factor for each weighing of the sample; R_s is the acetaminophen or codeine phosphate response factor for all weighings of the standard bracketing the sample; W_s is the theoretical amount of acetaminophen or codeine phosphate to be weighed (mg); W_x is the weight (mg) of granulation theoretically equivalent to 500 mg of acetaminophen, and F is the sensitivity factor for an individual compound (Table I).

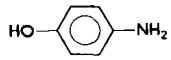
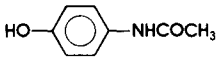
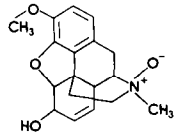
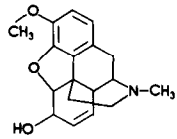
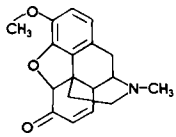
The sensitivity factor for an individual impurity accounts for the difference in sensitivity between either acetaminophen or codeine phosphate (from whichever the impurity is generated) and the individual compound under these analytical conditions (Table I for specific F values). Unidentified peaks are quantified using an F of one and the acetaminophen response factor (R_s). The sensitivity factor is calculated by dividing the response factor (area/μg) of acetaminophen or codeine phosphate into the response factor of the individual impurity.

Dissolution

The standard solutions prepared represent 100.0% of the labeled theoretical

TABLE I

STRUCTURES, NAMES, RETENTION TIMES AND SENSITIVITY FACTORS OF COMPONENTS AND POTENTIAL DEGRADATION PRODUCTS

Structure	*Name*	*Retention times (min)*	*Sensitivity factor (F)*
	*p*-Aminophenol	1.00	1.53*
	Acetaminophen	2.7	1.00*
	Codeine N-oxide	4.2	1.04**
	Codeine	5.3	1.00**
	Codeinone	8.8	1.02**

* Based on the response of acetaminophen.
** Based on the response of codeine phosphate.

amount of acetaminophen and codeine phosphate in the dosage form. Therefore, a direct comparison of the corresponding peak areas translates into percent dissolved:

$$\% \text{ Dissolved} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \times 100$$

Accelerated thermal decomposition

Acetaminophen with codeine phosphate granulation was placed in a beaker and the beaker placed in a sealed vessel containing water. This was placed in an oven at 100°C for approximately two weeks. The granulation significantly darkened and would have failed visual assessment of acceptability. A typical chromatogram of the stressed granulation is shown in Fig. 2. Samples of this material were treated as directed in this procedure and the acetaminophen and codeine peaks were analyzed

on a diode array detector. These UV scans were compared to those obtained from a similar preparation of an unstressed standard.

Precision and recovery analysis

Placebo spiked with 100% of the labeled amounts of acetaminophen and codeine phosphate was weighed as described in the sample preparation section, spiked with an aliquot of a solution containing 0.8% *p*-aminophenol, 4.0% codeine N-oxide and 4.1% codeinone and then diluted to volume. These amounts represented weight percents compared to the parent compound. This experiment was performed six times by three analysts resulting in a total of 18 measurements. The recovery of acetaminophen and codeine phosphate was further evaluated from 80 to 120% of the labeled amount for acetaminophen and from 20 to 240% of the labeled amount of codeine phosphate.

Linearity

The linearity of each component was determined individually by making several dilutions of stock solutions and chromatographing each solution as described in the *Material, equipment and liquid chromatographic conditions section.*

RESULTS AND DISCUSSION

The analytical procedure presented represents a precise, accurate, linear and rugged stability-indicating method for the simultaneous quantitation of acetaminophen, codeine phosphate and their potential degradation products *p*-aminophenol, codeine N-oxide and codeinone[14]. A typical chromatogram of a spiked standard solution containing each of the components listed above is shown in Fig. 1. Thermal stressing of the granulation resulted in the typical chromatogram shown in Fig. 2. Excipients in both stressed and nonstressed samples exhibited no interferences with the quantitation of any of the compounds investigated. Diode-array scans of the acetaminophen and codeine phosphate peaks in the degraded sample were superimposable with those obtained from the standard solution as seen in Fig. 3. This demonstrates the quantitation of the active components of the dosage form to be free of interferences from extraneous degradation peaks.

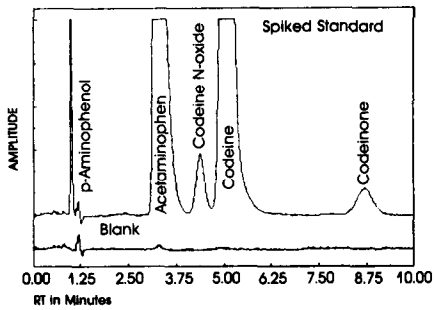


Fig. 1. Typical chromatogram of a spiked standard solution.

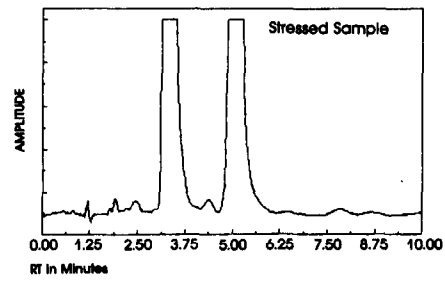


Fig. 2. Typical chromatogram of a thermally stressed granulation of acetaminophen and codeine phosphate.

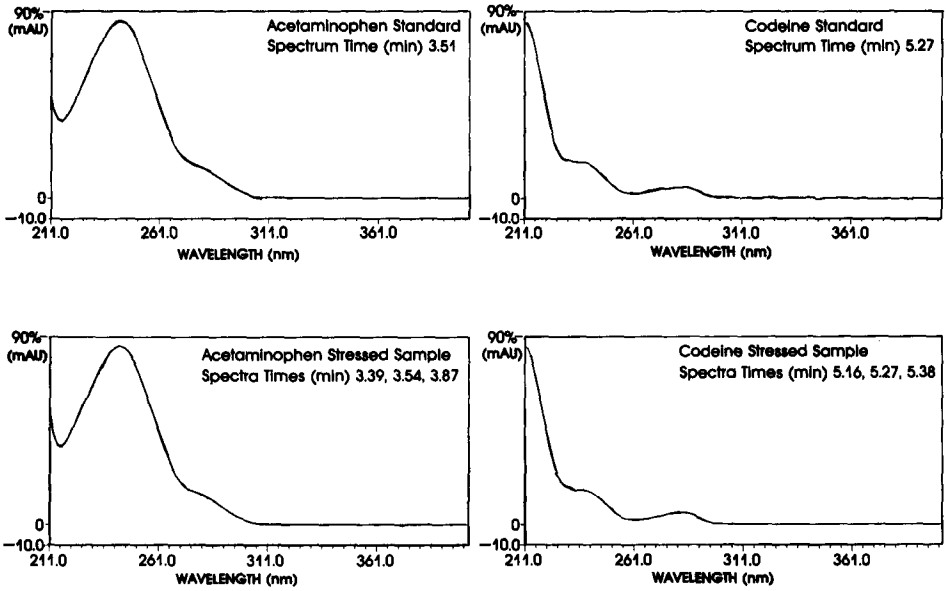


Fig. 3. UV spectra from a diode array detector of acetaminophen and codeine in a stressed sample demonstrating peak purity.

The environment used to force degradation of this dosage does not represent a condition that this dosage form should reasonably experience. This environment does, however, represent a stress condition of temperature and humidity which is considered important. Actual tablet and capsule products stored at room temperature for up to four years showed no evidence of decomposition or diminution of their assay.

The precision of the method was evaluated for each of the components mentioned. The relative standard deviations obtained from 18 individual sample preparations and their chromatographic evaluation are presented in Table II. These data

TABLE II

PRECISION AND RECOVERY DATA FOR ACETAMINOPHEN, CODEINE PHOSPHATE, *p*-AMINOPHENOL, CODEINE N-OXIDE, AND CODEINONE

Compound	*Conc. (mg/ml)*	*Parent (%)*	*Recovery (%)*	*R.S.D. (%)**
p-Aminophenol	0.0055	0.83	100.8	1.05
Acetaminophen	0.5334	80.0	98.9	
	0.6667	100.0	99.4	0.55
	0.8000	120.0	99.8	
Codeine N-oxide	0.0053	4.0	100.5	1.78
Codeine	0.0133	20.0	97.1	
	0.0667	100.0	102.1	0.37
	0.1600	240.0	103.1	
Codeinone	0.0055	4.1	88.7	4.6

* $n = 18$.

demonstrate this method to be sufficiently precise to quantitate each of the components.

The concentrations of active ingredients in the content uniformity preparations are of the same order as the assay preparations and therefore result in similar precision and accuracy.

The final concentration of active ingredients in the dissolution preparation, although lower than those for assay and content uniformity, gave chromatographic precision well within the acceptable limits. A system suitability requirement for standards run every six samples throughout the analysis is a range of less than 4.0%.

The recovery of each of the compounds investigated was determined. The recovery of potential impurities was evaluated at levels appropriate for each, while the recovery of the active components was determined for 80–120% of label for acetaminophen and 20–240% of label for codeine phosphate. These data are also presented in Table II.

The recovery of each of the compounds was also evaluated for different extraction times. Although quantitative extraction for the granulation was routinely achieved at about 40 min, extra time was added to ensure completely recovery.

The area *versus* concentration plots for each component were evaluated and found to be linear over the range of interest. These plots are shown in Fig. 4. The concentration of acetaminophen and codeine phosphate used in the assay are well within their linear range.

The use of 214 nm for detection represents a wavelength where the ratio of the absorbance of codeine phosphate to acetominophen is near a maximum, thereby increasing the sensitivity for codeine while extending the linear response range for acetaminophen. This eliminates the need for changes in either wavelength or detector sensitivity, or multiple injections in order to determine the concentration of acetaminophen or codeine phosphate in a sample solution. This approach should be adaptable to many products which consist of multiple active components.

The quantitative simultaneous extraction of acetaminophen and codeine phosphate has previously been unsuccessful[12]. Further, the simultaneous quantitation of these ingredients has previously proven difficult due to tedious time-consuming procedures or to very large differences in the amount of these ingredients. The use of detection at 214 nm has not only facilitated the quantitation of the active ingredients, but also the potential decomposition products at low levels. The use of controlled pH for the extraction solvent, sample solvent, and standard solvent has resulted in quantitative recovery of acetaminophen, codeine phosphate and codeine N-oxide, and nearly quantitative recovery of codeinone and the accurate quantitation of all. These results are based upon the close control of pH at each step of the procedure.

Acetaminophen, codeine phosphate and codeine N-oxide are quantitatively recovered from the excipient matrix when using methanol–water (pH 4.0) (1:1). At lower water pH (*i.e.*, 3.0) the acetaminophen and codeine phosphate were recovered well, as was the codeinone which gave quantitative recovery; however, codeine N-oxide in the presence of excipients began to degrade to codeine almost immediately resulting in recovery values for codeine phosphate inflated by the amount of codeine N-oxide present in the spiked sample. This phenomenon was confirmed by analyzing aliquots of solution taken at regular intervals beginning at 5 min. The use of pH 4.0 as the final sample solvent, however, is not satisfactory, for the stability of codeinone

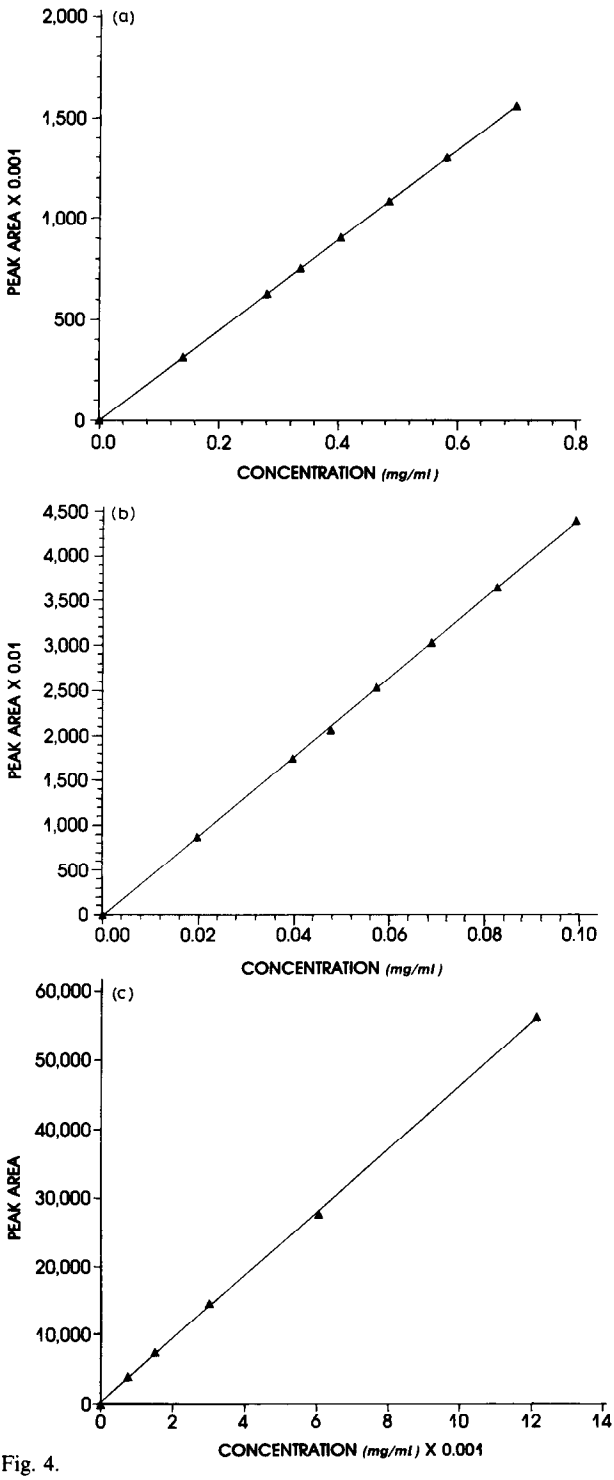


Fig. 4.

(Continued on p. 262)

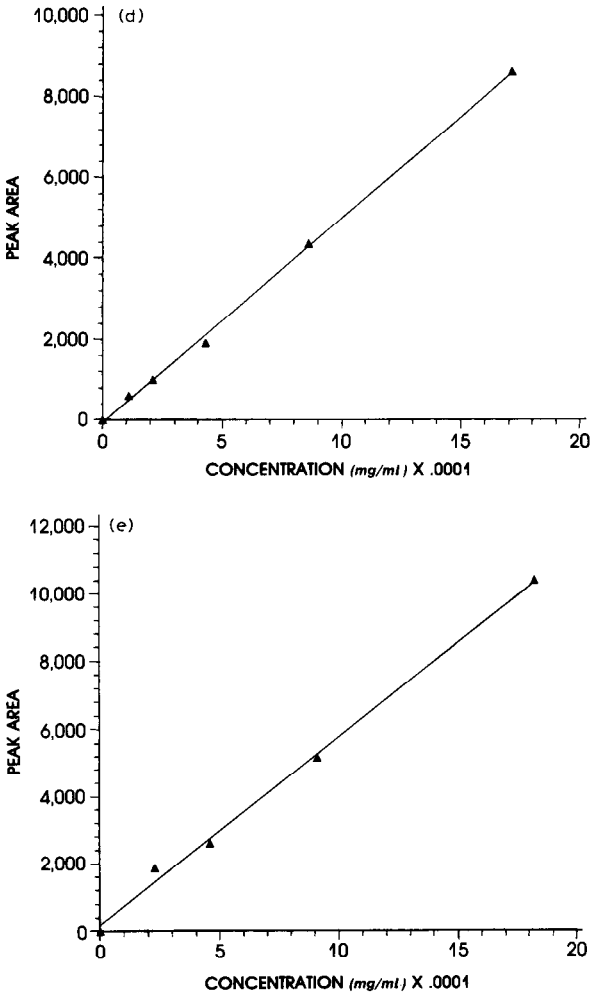


Fig. 4. Area *versus* concentration plots for (A) acetaminophen, (B) codeine phosphate, (C) *p*-aminophenol, (D) codeine N-oxide and (E) codeinone.

is poor. The dilution of a 10.0-ml aliquot of the extraction solution with a 40-ml aliquot of pH 3.2 water resulted in its stabilization. Samples analyzed over a 24-h period showed no decrease in codeinone content when this procedure was followed.

There is, therefore, a need to control the pH of both the final sample solution and the standard solution. In general, it is desirable to adjust the pH of the standard solution close to that of the sample solutions.

The pH of the mobile phase must also be controlled to adequately resolve the components and maintain acceptable column life. At a buffer pH of 3.0 the codeine N-oxide and codeine are not resolved; at pH 2.5 the same peaks are resolved but too broad; at pH 2.0 the peaks are well separated and have good shape but columns void

rapidly. Minor pH adjustments may be made to the mobile phase to affect acceptable chromatography.

The use of a stable elevated temperature enhanced the chromatography and aided in the precision of the analysis and the stability of the capacity factors for all compounds investigated. As long as the temperature was constant, small deviations from 40°C did not significantly alter the chromatography. Large deviations in the temperature from 40°C or an unstable thermal environment resulted in unsatisfactory chromatography. A constant thermal environment is particularly critical when automated analyses are conducted over long periods of time. The effects of temperature and temperature control in liquid chromatography have been documented[15,16].

CONCLUSION

This method represents a rugged stability-indicating analytical procedure for the simultaneous quantitation of acetaminophen, codeine phosphate and their known potential decomposition products. The sample preparation is simple, the analysis time is short and the elution is isocratic. The method is amenable to the analysis of large numbers of samples with precision and accuracy comparable to individual component analysis, resulting in an estimated 100% increase in productivity for both dissolution and stability samples.

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