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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STABILITY-INDICATING ANALYSIS OF ACETAMINOPHEN, CODEINE PHOSPHATE, AND SODIUM BENZOATE IN ELIXIRS

WILLIAM R. SISCO*, C. TODD RITTENHOUSE, LISA A. EVERHART and ANNA M. McLAUGHLIN
Analytical Development Department, McNeil Pharmaceutical, Spring House, PA 19477 (U.S.A.)
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SUMMARY

A stability-indicating high-performance liquid chromatographic method has been developed for the simultaneous determination of acetaminophen, codeine phosphate and sodium benzoate in an elixir formulation. The reversed-phase paired-ion method utilizes UV detection at 214 nm, a C_{18} column at 50°C and requires *ca.* 10 min per analysis. The method has been validated for use with elixirs containing 120 mg of acetaminophen, 12 mg of codeine phosphate and 7.5 mg sodium benzoate preservative per 5 ml. 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

The analysis of liquid dosage forms of pharmaceutical preparations is a difficult task, since many of these preparations contain more than one active constituent, along with various dyes, flavors, preservatives and sweeteners. The use of high-performance liquid chromatography (HPLC) has become a powerful technique in the successful analysis of these dosage forms. The HPLC techniques employed include normal-phase[1], reversed-phase[2] and paired-ion chromatography[3–5].

Simultaneous quantitative determinations of all active components, especially in multicomponent preparations have been difficult. One procedure[6] required separate spectrophotometric methods to determine two antihistamines which would not separate by HPLC. Other procedures[1,7] have used HPLC for the analysis of only one of the active constituents.

Further, differences in amounts of active constituents or their absorptivities or their position in the chromatogram have necessitated either shifts in detector sensitivity or wavelength during the analysis to accurately and precisely quantitate the constituents[2,8,9].

Pharmaceutically active moieties which have, nonetheless, been analyzed in

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elixirs by HPLC, include acetaminophen, codeine phosphate, pheniramine maleate, pyrilamine maleate, phenylpropanolamine, guaifenesin, and dextromethorphan hydrobromide. Preservatives such as sodium benzoate and the parabens have also been quantified using HPLC.

The simultaneous quantitation of more than one active constituent along with potential degradation products in liquid dosage forms has not been previously reported. This report documents the methodology used for the simultaneous analysis of acetaminophen, codeine phosphate, and their potential degradation products[10] as well as the preservative sodium benzoate. This method has been successfully employed in stability studies and product release.

EXPERIMENTAL

Materials, equipment and liquid chromatographic conditions

A μBondapak™ C_{18} column 30 cm × 3.9 mm I.D. (Waters Assoc.) was used with a mobile phase of 80:20 pH 4.8 buffer–methanol at a flow-rate of 2.0 ml/min. The mobile phase reservoir was maintained at ambient temperature, while the column was heated to 50°C. The chromatographic hardware consisted of a DuPont model 850 high-pressure liquid chromatograph equipped with a DuPont automatic sampler with a 20-μl loop, a DuPont column oven, a Waters Assoc. Model 440 absorbance detector equipped with a 214-nm wavelength extension kit, and a DuPont 4100 integrator. The integrator sensitivity was adjusted to 0.08 a.u.f.s. A Hewlett-Packard Model 1040A diode-array detector was used for specificity studies of stressed and unstressed samples.

Reagents and solutions

The methanol, water, potassium phosphate monobasic (KH_2PO_4), phosphoric acid, and triethylamine were all HPLC grade or equivalent. The 1-butane sulfonic acid sodium salt was reagent grade. The buffer was made by adding 2.4 g of 1-butane sulfonic acid sodium salt (0.015 *M*) and 2.04 g of potassium phosphate monobasic (0.015 *M*) and 2 ml triethylamine per liter of water. The pH of this solution was adjusted to 4.8 ± 0.1 with dilute phosphoric acid. The solution was filtered before use with a 0.45-μm pore diameter Millipore® filter (Catalogue No. HAWP04700). The pH of the filtered buffer was rechecked and readjusted if necessary. The mobile phase was made by adding 200 ml of methanol to 800 ml of buffer and mixing thoroughly.

The standard solvent was prepared by adjusting distilled water to pH 3.7 ± 0.1 with dilute phosphoric acid.

Standards and sample preparation

The standards were prepared in duplicate in the following manner: accurately weigh *ca.* 30 mg of codeine phosphate standard into a 100-ml volumetric flask; accurately weigh *ca.* 30 mg of acetaminophen standard into a 50-ml volumetric flask; accurately weigh *ca.* 46.8 mg of sodium benzoate standard into a 25-ml volumetric flask; dissolve the sodium benzoate standards in distilled water and dilute to volume; transfer by pipet a 10.0-ml aliquot of a sodium benzoate standard to the 100-ml volumetric flask containing the codeine phosphate standard and dilute to volume

with distilled water; transfer by pipet 10.0 ml of the codeine phosphate–sodium benzoate solution to the 50-ml volumetric flask containing the acetaminophen standard and dilute to volume with standard solvent. The pH of the final solution was checked to ensure it was close (±0.3) to the pH of the sample solutions.

The samples were prepared using a "To Contain" pipet. A 5.0-ml aliquot of elixir was accurately transferred to a 200-ml volumetric flask and diluted to volume with distilled water.

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 integrator chart speed and injecting 20 μl of the standard solution. The resolution between sodium benzoate and the codeine peaks should be at least 1.25 as determined using the resolution equation[11].

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

Samples were analyzed with standard solutions chromatographed before, after and interspersed with the samples if a large number of analyses were to be performed. The typical retention times for acetaminophen, sodium benzoate and codeine phosphate were 2.6, 3.8 and 4.7 min, respectively.

Calculations

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. A response factor (R) for each weighing of a standard is determined using the equation $R = A/W$ where A is the average acetaminophen, sodium benzoate or codeine phosphate peak area for each individual weighing, and W (mg) is the weight of each individual standard compound. The percent label acetaminophen, sodium benzoate, or codeine phosphate or weight percent impurity is determined using the equation:

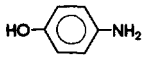
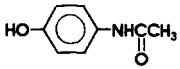
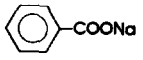
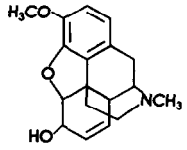
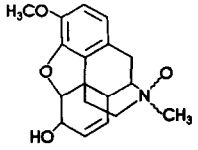
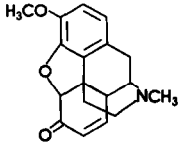
$$\% \text{ label} = \frac{A_u}{R_s} \cdot \frac{100}{W_{ts} \cdot F}$$

where A_u is the average acetaminophen, sodium benzoate, codeine phosphate or impurity peak area for each pipetting of the sample; R_s is the average acetaminophen, sodium benzoate, or codeine phosphate response factor for all weighings of the standard bracketing the sample; W_{ts} is the theoretical amount of acetaminophen, sodium benzoate, or codeine phosphate standard to be initially weighed for the preparation of a standard; and F is the sensitivity factor for an individual compound (see Table I for specific F values).

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. Unidentified peaks are quantified using an F value of 1 and the acetaminophen response factor. The sensitivity factor is calculated by dividing the response factor

TABLE I

STRUCTURES, SENSITIVITY FACTORS, RETENTION TIMES AND DETECTION LIMITS FOR THE INVESTIGATED COMPOUNDS

Structure	*Name*	*Retention time (min)*	*Sensitivity factor (F)**	*Detection limit (wt. %)**
	*p*-Aminophenol	1.8	7.23	0.02
	Acetaminophen	2.8	1.00	NA**
	Sodium benzoate	3.9	NA	NA**
	Codeine phosphate	4.9	1.00	NA**
	Codeine N-oxide	6.4	1.12	0.25
	Codeinone	8.8	1.15	0.25

* With respect to parent compound.
** Not applicable.

(area/μg) of acetaminophen or codeine phosphate into the response factor of the individual impurity.

Accelerated thermal decomposition

Acetaminophen with codeine phosphate elixir was placed in a clear flint-glass bottle, capped and placed in a 60°C oven for two weeks. The elixir darkened and would have failed visual assessment of acceptability. Samples of this material were treated as directed in this procedure and the acetaminophen, sodium benzoate, and codeine phosphate peaks were analyzed on a diode-array detector. These scans were compared with those obtained from a similar preparation of an unstressed sample.

Precision and recovery analysis

Precision was evaluated using a stock placebo containing sodium benzoate spiked with *ca.* 1% *p*-aminophenol, 1% codeinone, 1.4% codeine N-oxide, 100% acetaminophen and 100% codeine phosphate. The impurity compound amounts represent weight percents compared with the parent compound. Aliquots were drawn and samples prepared from this stock solution by three analysts, with each analyst making six preparations. The recovery of acetaminophen and codeine phosphate was further evaluated by adding 80, 100 and 120% of the labeled amounts of these constituents to a placebo, while sodium benzoate recovery was also evaluated by adding 10 and 20% excess to the same spiked placebo.

Linearity

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

Ruggedness

The ruggedness of the chromatographic system was determined by evaluating alterations of several key parameters. The results of these experiments were used to establish variation limits for each of the parameters investigated. The parameters evaluated included temperature, pH, paired-ion concentration, potassium phosphate monobasic salt concentration, and organic concentration of the mobile phase.

RESULTS AND DISCUSSION

The analytical procedure presented represents a specific, precise, accurate, linear and stability-indicating method for the simultaneous quantitation of acetaminophen, sodium benzoate and codeine phosphate, as well as potential degradation products *p*-aminophenol, codeine N-oxide and codeinone. A typical chromatogram of a spiked standard solution containing each of the components given above in a placebo matrix is shown in Fig. 1. A standard solution and a thermally stressed elixir sample were chromatographed and detected using a diode-array detector as seen in Fig. 2. Excipients in both stressed and non-stressed samples exhibited no interferences with the quantitation of any of the compounds investigated. Diode-array scans of the acetaminophen, codeine phosphate and sodium benzoate peaks in the degraded sample were superimposable with those obtained from the standard preparation as seen in Figs. 3–5. This demonstrates the quantitation of the major investigated components in the elixir dosage form to be free of interferences, thereby establishing specificity.

The precision of the method was evaluated for each of the components mentioned. The relative standard deviations or the standard deviations obtained from individual sample preparations and their chromatographic evaluation are presented in Tables II and III. These data demonstrate this method to be sufficiently precise to quantitate each of these components.

The recovery of each component investigated was determined. The recovery of potential impurities was evaluated at *ca.* 1% and 3% by weight, while the recovery of the main components was determined at 80–120% label for acetaminophen and

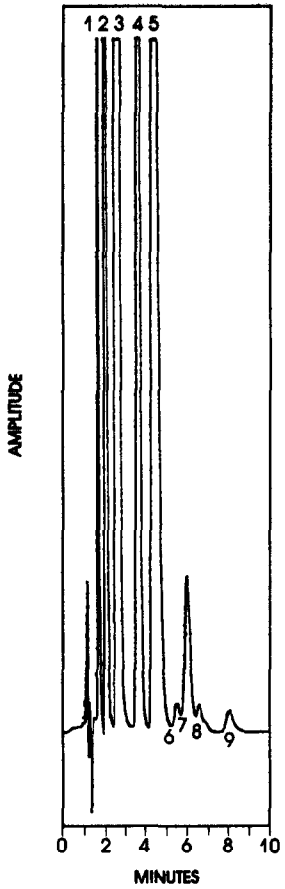


Fig. 1. Typical chromatogram of a spiked standard solution. Peaks: 1 = *p*-aminophenol; 2 = saccharin; 3 = acetaminophen; 4 = sodium benzoate; 5 = codeine phosphate; 6 = codeine N-oxide; 7 = excipient; 8 = excipient; 9 = codeinone.

codeine phosphate and at 100–120% label for sodium benzoate. These data are presented in Table IV.

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 Figs. 6 and 7. The concentrations used in the assay for each of the major components are well within their investigated linear range.

The use of 214 nm for detection represents a wavelength where the ratio of the absorbance of codeine phosphate to acetaminophen is near a maximum, thereby increasing the sensitivity for codeine while extending the linear response range for acetaminophen. This, as a result, necessitates no adjustments in either wavelength or detector sensitivity in order to accurately and precisely quantitate any of the investigated components. This approach has been previously used and reported[12].

The ruggedness of the method was evaluated by varying certain chromatographic parameters, evaluating their qualitative effects on the analysis and extrapo-

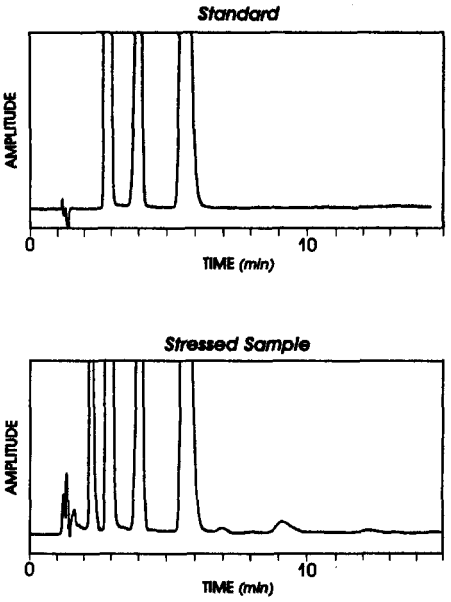


Fig. 2. High-performance liquid chromatograms of acetaminophen with codeine elixir standard and stressed samples.

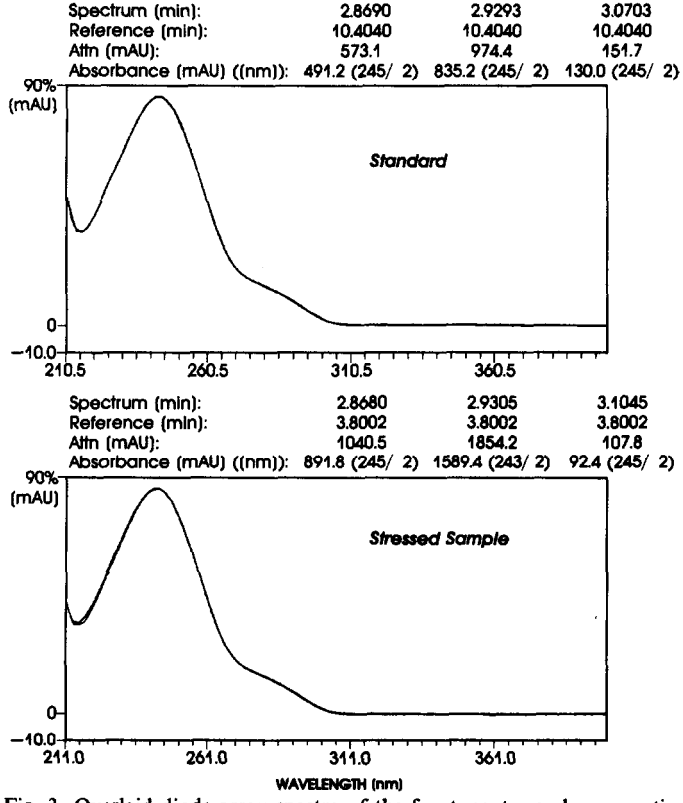


Fig. 3. Overlaid diode-array spectra of the front, center and rear portions of the acetaminophen component of acetaminophen with codeine elixir standard and stressed sample.

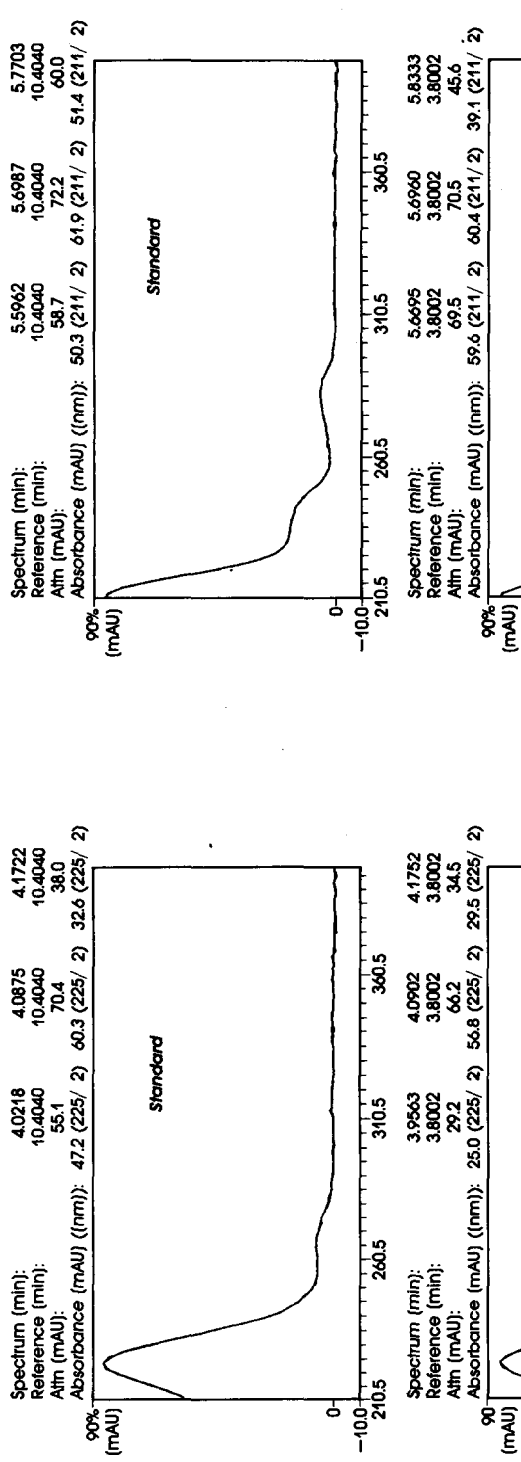


Fig. 4. Overlaid diode-array spectra of the front, center and rear portions of the sodium benzoate component of acetaminophen with codeine elixir standard and stressed sample.

Fig. 5. Overlaid diode-array spectra of the front, center and rear portions of the codeine phosphate component of acetaminophen with codeine elixir standard and stressed sample.

TABLE II

PRECISION DATA: POTENTIAL DEGRADATION PRODUCTS

Values are weight percent of the label.

Sample	*p-Aminophenol*		*Codeine N-oxide*		*Codeinone*
	Inst 1	*Inst 2*	*Inst 1*	*Inst 2*	*Inst 1*
1	0.92	0.94	1.28	1.82	1.14
2	0.94	0.95	1.77	1.87	1.10
3	0.94	0.95	1.54	1.82	1.12
4	0.94	0.95	1.55	1.55	1.19
5	0.94	0.94	1.72	1.84	1.05
6	*	0.95	*	1.75	*
7	0.92	0.92	1.32	1.60	1.10
8	0.93	0.94	1.45	1.29	1.06
9	0.92	0.92	1.60	1.69	1.21
10	0.93	0.91	1.46	1.79	1.10
11	0.93	0.93	1.42	1.05	1.15
12	0.94	0.91	1.42	1.70	1.01
13	0.92	0.92	1.03	1.45	1.22
14	0.92	0.93	1.23	1.62	1.07
15	0.93	0.94	1.17	1.44	1.18
16	0.92	0.93	1.25	1.50	1.02
17	0.92	0.91	1.08	1.71	1.21
18	0.91	0.90	1.27	1.54	1.12
$\bar{x}$	0.93	0.93	1.38	1.61	1.12
S.D.	0.0097	0.00161	0.210	0.214	0.066
Overall S.D.	0.013		0.212		0.067

* Sample was not injected by autosampler.

lating these to quantitative implications. The extent of each individual variation was chosen to represent extreme experimental deviations from the prescribed conditions. The criteria used in the qualitative evaluation consisted of ascertaining the resolution between peak pairs and the positioning in the chromatogram of the peaks of interest. The quantitative extrapolation of these data was based on the acceptability of the chromatography to assay adequately the samples for acetaminophen, sodium benzoate, codeine phosphate, and any potential decomposition products.

The pH of the aqueous buffer was the most critical parameter to control for acceptable resolution of the major compounds of interest. The pH should be controlled between 4.7 and 4.9, for outside this range the codeine phosphate and sodium benzoate coelute. The potential decomposition products are also acceptably resolved within the 4.7–4.9 range. Below pH 4.7 a flavor peak merges with codeine N-oxide, while above 4.9 a dye begins to coelute with codeine N-oxide.

The temperature of the column was varied over the 45–55°C range, with the retention times moving predictably, *i.e.*, a decrease in retention time with an increase in temperature. Within this range, all majors were resolved from each other, excipients and potential decomposition products. Above 50°C the dye and codeine N-

TABLE III

PRECISION DATA: MAJOR COMPONENTS

Values are weight percent of the label.

Sample	*Acetaminophen Inst 1*	*Sodium benzoate*		*Codeine phosphate*	
		Inst 1	*Inst 2*	*Inst 1*	*Inst 2*
1	95.1	100.0	101.1	109.9	110.4
2	95.3	100.1	101.0	110.2	110.7
3	95.2	100.2	101.4	109.7	110.6
4	95.2	100.5	100.7	110.1	110.2
5	95.6	100.3	101.3	110.4	110.1
6	*	*	101.2	*	110.3
7	93.7	98.6	99.4	108.1	108.3
8	93.6	98.5	99.5	107.9	108.4
9	93.4	98.0	98.4	107.7	108.0
10	93.8	98.4	98.4	108.0	108.0
11	94.8	99.6	99.1	109.4	108.2
12	95.0	99.8	99.9	109.3	109.9
13	94.4	99.8	99.4	109.3	108.6
14	94.2	99.6	100.1	109.0	109.0
15	94.1	100.1	99.4	109.5	106.6
16	93.8	99.3	99.6	109.2	108.5
17	94.1	99.7	99.5	109.0	108.2
18	94.0	99.3	98.6	109.4	108.0
$\bar{x}$	94.4	99.5	99.9	109.2	109.0
S.D.	0.696	0.737	1.006	0.822	1.185
R.S.D. (%)	0.74	0.74	1.01	0.75	1.09
Overall R.S.D. (%)	0.74	0.90		0.93	

* Sample was not injected by autosampler.

TABLE IV

RECOVERY DATA

Sample	*Recovery (%)*					
	Acetamin-ophen	*Sodium benzoate*	*Codeine phosphate*	*p-Amino-phenol*	*Codeine N-oxide*	*Codeinone*
Placebo	–	100.0	–	–	–	–
80% Spike	101.7	100.6	101.0	–	–	–
100% Spike	100.8	100.2	100.6	–	–	–
125% Spike	100.4	100.6	101.4	–	–	–
1% Impurity Spike	100.8	99.6	100.2	96.0	93.6	115.1
3% Impurity Spike	100.7	99.6	99.8	96.2	96.0	113.9
Average recovery	100.9	100.1	100.6	96.1	94.8	114.5

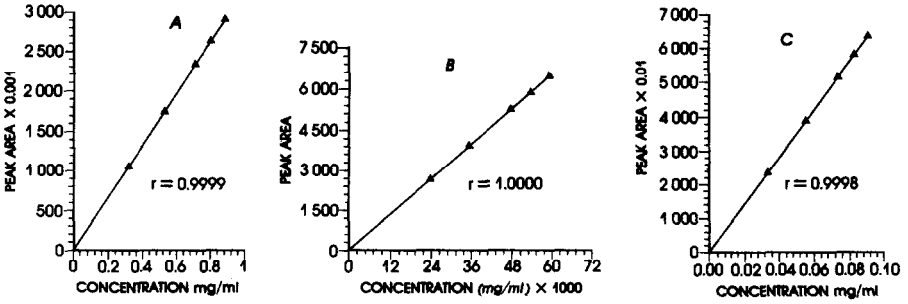


Fig. 6. Linearity plots of major components. (A) Linearity of acetaminophen at 214 nm; (B) linearity of sodium benzoate at 214 nm; (C) linearity of codeine phosphate at 214 nm.

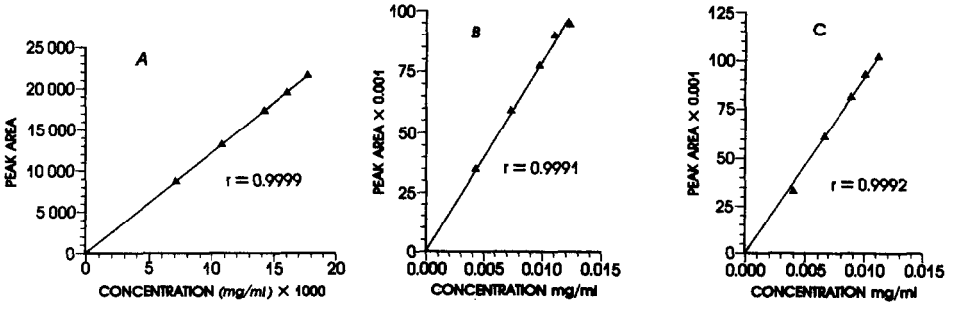


Fig. 7. Linearity plots of potential degradation products. (A) Linearity of *p*-aminophenol at 214 nm; (B) linearity of codeine N-oxide at 214 nm; (C) linearity of codeinone at 214 nm.

oxide would begin to lose resolution, and at *ca.* 55°C would reverse their elution order. The potential decomposition product *p*-aminophenol may have a problem being properly quantified above 50°C, owing to its rapid elution. Likewise, codeine N-oxide, if present, may not be adequately assayed above 50°C. However, at 45°C the resolution is more than adequate.

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. A constant thermal environment is particularly useful when automated analyses are conducted over long periods of time. The effects of temperature and temperature control in liquid chromatography have been documented[13,14].

The concentration of the 1-butane sulfonic acid sodium salt was varied ±0.1 g/l. All major components were adequately resolved over this range, with no problem observed in the assay due to inadequate resolution. However, codeine N-oxide and codeinone, if present, would not be adequately resolved from the dye at either extreme.

The proportion of methanol in the mobile phase was varied from 15 to 24%, with the major components always being resolved from each other, the excipients, and the potential decomposition products. At 25% methanol the sodium benzoate and codeine peaks were not completely resolved. The codeine N-oxide and dye are not completely baseline resolved at 20% methanol; the resolution of the codeine

N-oxide and codeinone from the dye and a flavor excipient is very sensitive. However, no corresponding sensitivity was noted for the resolution of quantitation of the major components.

The concentration of potassium phosphate monobasic was varied by ±0.1 g/l. At each extreme all major components were baseline resolved with only a slight change in retention times. No apparent quantitation problems should be encountered for the major components.

CONCLUSION

This method represents a rugged stability-indicating analytical procedure for the simultaneous quantitation of acetaminophen, codeine phosphate, their known potential decomposition products, and sodium benzoate. 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 with individual component analyses. Overall, this analytical procedure is extremely time- and cost-effective, resulting in significant increases in productivity.

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