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Diode array spectroscopy in pharmaceutical analysis: determination of acetaminophen/ codeine phosphate tablets

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Abstract The use of diode array spectrophotometry for the analysis of tablets containing acetaminophen and codeine has been investigated The technique was found to be applicable to the analysis of tablets which nominally contain 300 mg acetaminophen and 15, 30 or 60 mg codeine phosphate (Tylenol Nos 2–4, respectively) The concentration of codeine in extracts of Tylenol No 1, which contains 7 5 mg codeine phosphate, was found to be too low for precise determination First-derivative spectroscopy is required for the analysis of Tylenol Nos 2 and 3 Tylenol No 4 can be determined using either the zero or the first-derivative spectra, however, the former was found to give consistently lower values for each component The influence of derivative order and analytical wavelength range on the precision was investigated The accuracy of the procedure was assessed by comparing the results from spectroscopy with those from liquid chromatography Finally, some general conclusions on the applicability of diode array spectroscopy for the multicomponent analysis of pharmaceuticals are presented

Keywords *Diode array spectroscopy, codeine phosphate, acetaminophen, pharmaceutical analysis*

Introduction

Ideally, the individual components in a combination product are assayed simultaneously using a single analytical procedure such as gas or liquid chromatography Frequently, this is not possible due to the widely different physicochemical and chromatographic properties of the individual components This is particularly disadvantageous in content uniformity testing and dissolution studies, in which large numbers of samples are to be determined Multiple component analysis complicated by spectral overlap can be aided by derivative spectroscopy [1] The diode array spectrophotometer performs these functions and offers distinct advantages over chromatographic methods in terms of speed of analysis It has become clear during the course of the present studies that at least two vital criteria must be satisfied for the accurate and precise analysis of multiple

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components by ultraviolet (UV) or visible spectrophotometry Firstly, the absorption spectra must be sufficiently different to allow adequate discrimination Secondly, there must be significant contributions to the total absorbance from all components of the mixture over a reasonable range of wavelengths In pharmaceutical analysis the second criterion in particular is not trivial since the dosages to be determined often contain individual components in widely different amounts The present studies are aimed at identifying the potential and limitations of diode array spectrophotometry for the analysis of such systems Acetaminophen/codeine tablets proved to be an ideal model system for these investigations, since they are commercially available in four strengths containing 300 mg of acetaminophen and 7 5, 15, 30 and 60 mg of codeine phosphate (Tylenol Nos 1–4, respectively) Currently, the acetaminophen and codeine phosphate in these products are assayed by UV spectroscopy and gas chromatography (GC), respectively [2] The acetaminophen is determined near 248 nm, after methanolic extraction of the tablets while the codeine is determined by GC with flame ionization detection following a basic extraction into chloroform

Experimental

Materials

Tylenol with codeine tablets (Nos 1–4, McNeil Pharmaceuticals, Spring House, PA) were obtained from a local retail pharmacy The codeine phosphate USP and the acetaminophen used as analytical standards were obtained from Merck (Rahway, NJ) and Eastman Kodak (Rochester, NY), respectively HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Fair Lawn, NJ) All other materials were of reagent grade from various sources Deionized water was used throughout

Sample preparation

The tablets containing acetaminophen and codeine were prepared for analysis in the following manner A single tablet was weighed, transferred to a 250-ml volumetric flask and dispersed into about 100 ml of methanol with the aid of a glass rod Complete disintegration of the tablets in the methanol was achieved by sonication for 15 min in two stages The mixture was allowed to cool for 15 min between, and after, each stage of sonication The volume of the dispersion was adjusted to 250 ml with methanol and a 3-ml aliquot was centrifuged for 15 min A 1-ml aliquot of supernatant was diluted with water to either 10 or 50 ml for analysis by either high-performance liquid chromatography (HPLC) or spectroscopy, respectively

Standard preparation

Standard solutions containing acetaminophen and codeine phosphate were prepared by diluting stock solutions of the drugs dissolved in deionized water For spectroscopic analysis the standard solutions contained 4 and 10 $\mu g\ ml^{-1}$ of codeine phosphate and acetaminophen, respectively, for chromatographic analysis the standard solutions contained 24 and 120 $\mu g\ ml^{-1}$, respectively The methanol concentration in all the standard solutions was adjusted so that it was the same as that in the sample solutions

Liquid chromatography

The acetaminophen and codeine in the tablet extracts were determined by HPLC on a Micropak CN-10 column (30 cm × 4 mm i d , Varian Assoc , Palo Alto, CA) using a

mobile phase of acetonitrile–0 10 M KH_2PO_4 (pH 4 5, 10 90, %v/v) The chromatograph comprised a Constametric IIG pump (LDC, Riveria Beach, FL), a Negretti Model 194 injector (20 µl loop) (HPLC Technology, Palos Verdes Estates, CA) and a Spectromonitor D variable-wavelength detector (LDC) A flow rate of 1 25 ml min^{-1} was employed and the detection wavelength was 217 nm Quantification of the components in the samples was by comparison of their peak areas with those of the external standards The peak areas were measured using a data system which comprised of an HP 87 microcomputer (Hewlett Packard, Palo Alto, CA), a Nelson Analytical Intelligent Interface (Model 761, Nelson Analytical, Cupertino, CA), a Nelson Analytical Software Model 366, version 2 2, and a Hewlett–Packard 3½" dual-disc drive The chromatograms were displayed on the cathode ray tube of the HP 87 and recorded on either a Hewlett Packard printer (Model 82906A) or a Hewlett Packard X–Y plotter (Model 7470A)

Ultraviolet spectroscopy

Ultraviolet spectra (200–300 nm) were obtained using a Hewlett Packard 8451 diode array spectrophotometer and recorded on a Hewlett Packard X–Y plotter Mathematical manipulations of the spectral data were performed using the integrated HP 85 microcomputer and associated software All derivative spectra were obtained using three smoothing points The spectral data were stored on 3½" discs using a dual-disc drive (HP 7470A) The same 1 cm quartz cell was used throughout

Results and Discussion

Selection of wavelength range for analysis

The UV absorption spectra of acetaminophen and codeine phosphate in aqueous solutions, obtained by diode array spectroscopy (Fig 1), were found to be consistent with reference spectra obtained by more conventional techniques and published elsewhere [3, 4] The two compounds exhibit significantly different spectral features in the UV region (200–300 nm) (Fig 1, Table 1), particularly between 200–240 nm Codeine has an absorption maximum at 214 nm whereas acetaminophen exhibits a minimum in this region around 218 nm At higher wavelengths (above 240 nm) the two compounds have somewhat similar absorption spectra Both compounds exhibit two absorption bands centred around 244 and 284 nm, and minima around 264 nm There is negligible absorption by either compound above 325 nm

The differences in spectral properties of acetaminophen and codeine indicate that the most useful region for multicomponent analysis of these substances was between 200–240 nm It was found that there was interference between 200–215 nm from the methanol required to extract the active ingredients from the tablets, leading to poor reproducibility of the spectra in this region Consequently, a wavelength range of 220–240 nm was found to be the most appropriate for the analysis of samples containing both drugs Beer's law was obeyed at 220, 230 and 240 nm for aqueous solutions of codeine and acetaminophen over the concentration ranges 0–1 6 × 10^{-4}M and 0–4 35 × 10^{-4}M, respectively At higher concentrations the absorbances of the solutions were >2 00 and deviations from Beer's law were observed Consequently, all tablet extractions were diluted so that the total absorbance of the solutions was <2 00

The suitability of the chosen wavelength (220–240) region was confirmed by additional studies using simulated spectra (Figs 2–5) in which the individual theoretical contri-

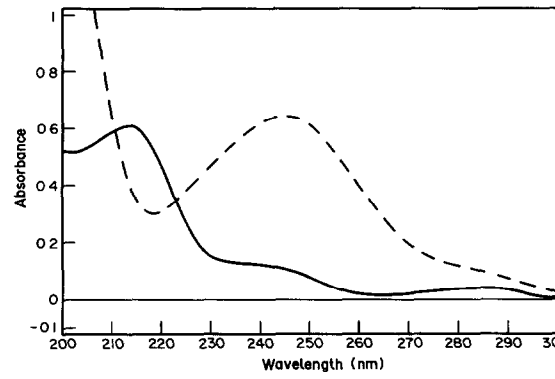

Figure 1
Ultraviolet spectra of acetaminophen (— —) and codeine phosphate (———) Solvent 10% methanol in water Temperature, ambient, path-length, 1 cm, concentration, 6 60 × 10^{-5}M acetaminophen, 2 44 × 10^{-5}M codeine phosphate, wavelength range, 200–300 nm

Table 1
Molar extinction coefficient (ϵ) of acetaminophen and codeine at various wavelengths of significance

Wavelength (nm)	ϵ (l mol^{-1} cm^{-1}) Acetaminophen	Codeine
214	5700	25,000 (max)
218	4500 (min)	23,000
220	4600	17,200
244	9700 (max) (10,000 [3])	4700 (shoulder)
264	4500 (min)	840 (min)
284	1580 (shoulder)	1830 (max) (1585 [4])

butions from the various components in the mixture were calculated, according to equation (1)

$$A^{\lambda} = \sum_{i=1}^{m} \epsilon_i^{\lambda} c_i b, \tag{1}$$

where the subscript, i, refers to the individual components of the tablet extracts, and the path length, b, is assumed to be 1 00 cm The contributions of the extracting solvent and tablet excipients (i = 3 to m) were found to be negligible above 215 nm, in which case equation (1) can be simplified to

$$A^{\lambda} = \epsilon_1^{\lambda} c_1 + \epsilon_2^{\lambda} c_2, \tag{2}$$

where the subscripts 1 and 2 refere to codeine and acetaminophen, respectively Figure 2 shows the absorption spectrum of an extracted tablet containing about 300 mg of acetaminophen and 15 mg of codeine phosphate, superimposed on the simulated spectra calculated from the individual contributions of the two components of each wavelength, λ

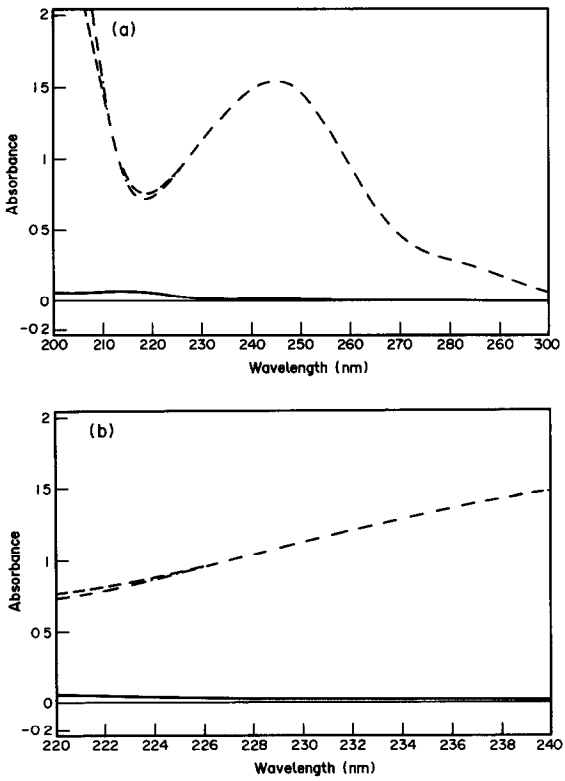

Figure 2
Ultraviolet spectrum of an extract of a Tylenol No 2 tablet (— — —) and of simulated spectra for acetaminophen (— —) and codeine phosphate (———) with similar concentration to that of the extract Wavelength range (a) 200–300 nm, (b) 220–240 nm Other conditions as in Fig 1

$$A_1^{\lambda} = \epsilon_1^{\lambda} c_1, \tag{3}$$

$$A_2^{\lambda} = \epsilon_2^{\lambda} c_2, \tag{4}$$

The actual values of c_1 and c_2 were taken from those obtained subsequently by multicomponent analysis It can be seen that the contribution by codeine to the total absorption spectrum from an extract of Tylenol No 2 is extremely small due to its low concentration compared with acetaminophen ($c_1/c_2 = 20$, by wt), and that the spectrum from the tablet extract is superimposable upon that of acetaminophen itself at $\lambda > 250$ nm

Derivative spectroscopy

Despite the relatively small contribution to the total spectrum arising from codeine between 220–240 nm, it was possible to enhance the spectral differences by transformation to the first and higher order derivative with respect to wavelength, [1] and thereby determine the components in the tablet extracts according to equation (5b)

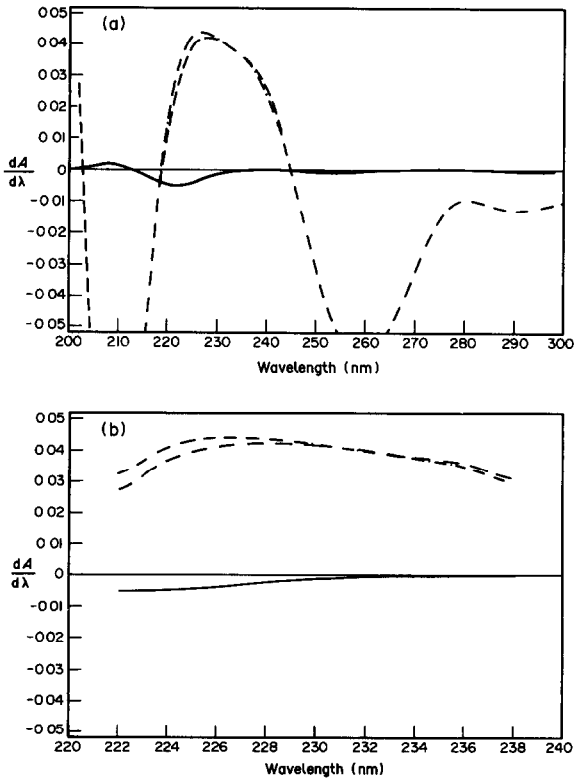

Figure 3
First-derivative spectrum of an extract of a Tylenol No 2 tablet and simulated first-derivative spectra of acetaminophen and codeine phosphate Line types and other conditions as in Figs 1 and 2

$$\frac{d^n A^\lambda}{d\lambda^n} = \frac{d^n A_1^\lambda}{d\lambda^n} + \frac{d^n A_2^\lambda}{d\lambda^n} \tag{5a}$$

$$= \frac{d^n \epsilon_1^\lambda}{d\lambda^n} \; c_1 + \frac{d^n \epsilon_2^\lambda}{d\lambda^n} \cdot c_2, \tag{5b}$$

where n is the derivative order from 0 to 4 (Figs 1–6) Ten tablets, each from single batches of Tylenol Nos 1–4 were analysed for acetaminophen and codeine phosphate using the standard multicomponent analysis software integrated within the microprocessor of the HP 8451 diode array spectrophotometer Each tablet extraction was analysed using the regular spectrum and the four derivative orders available (i e n = 0–4), the results being summarized in Table 2 For n = 0, the narrower optimum wavelength range of 220–226 nm was chosen for deconvolution, since this presents the maximum spectral differences for the two compounds of interest (Figs 1–6) For n = 1 it was necessary to increase the upper wavelength to 240 nm in order to accommodate the truncation in the spectra introduced by the instrumental algorithm used to calculate derivatives (Figs 2b, 3b, 4b, 5b and 6b) Due to the negligible absorption contributions from codeine at $\lambda > 250$ nm, there were no observable differences in the derivative spectra (n = 1–3) of the tablet extraction at wavelengths above 250 nm (Figs 1–4) It is

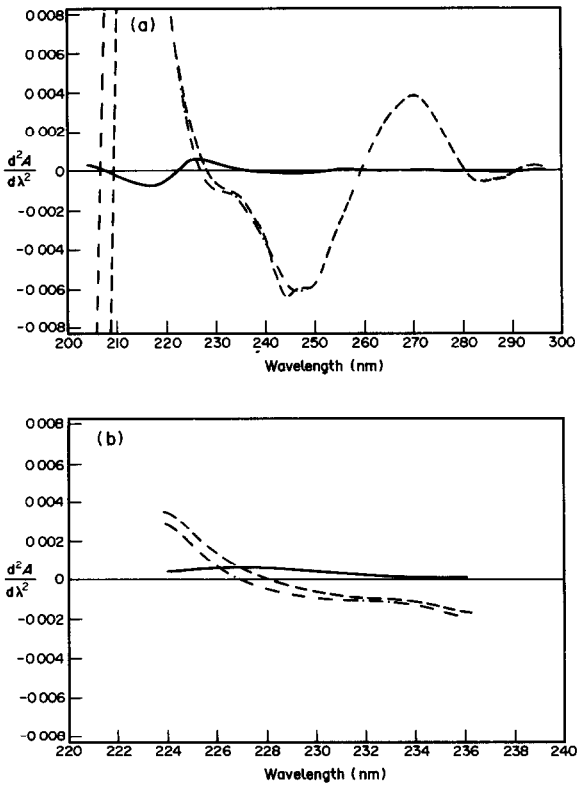

Figure 4
Second-derivative spectrum of an extract of a Tylenol No 2 tablet and simulated second-derivative spectra of acetaminophen and codeine phosphate Line types and other conditions as in Figs 1 and 2

probable that the differences in derivative spectra seen for $n = 3$ or 4 (Figs 5 and 6) may be artefactual, since they are inconsistent with Figs 1–4 and with equations (5a) and (5b) These observations also support the suitability of the choice of 248 nm as the analytical wavelength for the single determination of acetaminophen extracted from combination tablets also containing codeine phosphate [2]

Tablet assays

The amount of codeine phosphate present in Tylenol No 1 (7 5 mg) was too low for it to be determined accurately by multicomponent analysis ($n = 0$–4) using this diode array spectrophotometer (Table 2) The values obtained for first derivative spectral data ($n = 1$) for codeine phosphate and acetaminophen in Tylenol Nos 2 and 3 were within the USP specifications for two-component tablets When data based on the zeroth derivative were used, the concentrations of codeine found in extracts of Tylenol Nos 2 and 3 could not be determined with satisfactory precision (Tables 3 and 4) The results obtained for Tylenol No 4 using the zeroth-derivative data, were compared with those obtained by the first derivative It can be seen (Table 3c) that the values obtained for each tablet extraction were significantly higher for $n = 1$ than for $n = 0$ The percentage relative mean differences (RMD %) for the two derivative techniques were 1 83 and

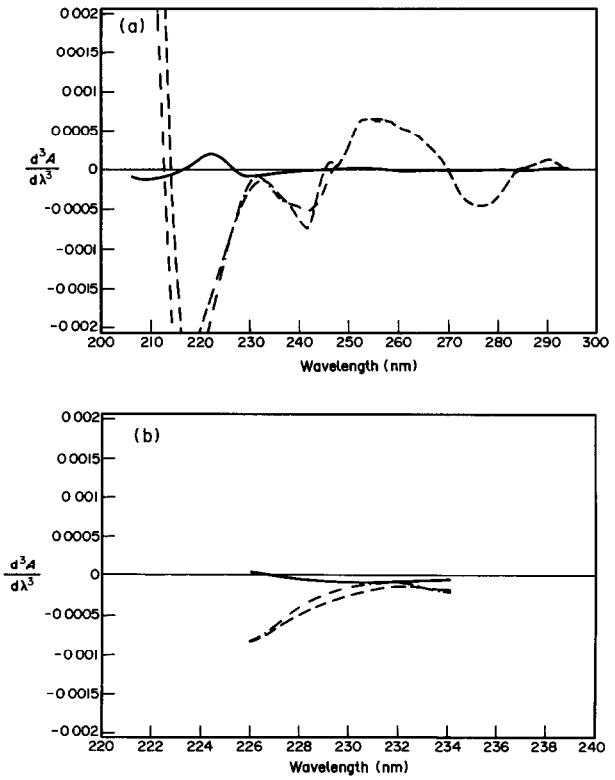

Figure 5
Third-derivative spectrum of an extract of Tylenol No 2 tablet and simulated third-derivative spectra of acetaminophen and codeine phosphate Line types and other conditions as in Figs 1 and 2

3 32% for acetaminophen and codeine phosphate, respectively in Tylenol No 4 (cf Table 3)

Referee analysis accuracy

The accuracy of multicomponent analysis of tablets containing both acetaminophen and codeine phosphate was investigated further by re-assaying the same extract of Tylenol Nos 2 and 4 by HPLC, the appropriate paired comparisons of the results obtained for each individual tablet assay are shown in Table 3 The acetaminophen and codeine were separated and quantified (Fig 7) using a cyanopropyl column and a mobile phase of methanol–0 10 M KH_2PO_4 (10 90, %v/v) This methodology was adapted from that developed for the analysis of codeine in biological fluids [5]

The amounts of codeine phosphate and acetaminophen found in the Tylenol No 2 tablets were consistently lower (RMD = 4 39 and 8 72%, respectively) by HPLC compared with those obtained by first-derivative spectroscopy By contrast, for Tylenol No 4, comparison (Table 3) of the values obtained by spectroscopy (n = 0 and 1) with those obtained by HPLC gave somewhat different results With data based on the zeroth derivative, the values obtained by HPLC were higher than those by spectroscopy for acetaminophen (RMD = 2 09%) However, the corresponding comparison for codeine phosphate in Tylenol No 4 revealed a mean difference of only −0 3 mg/tablet

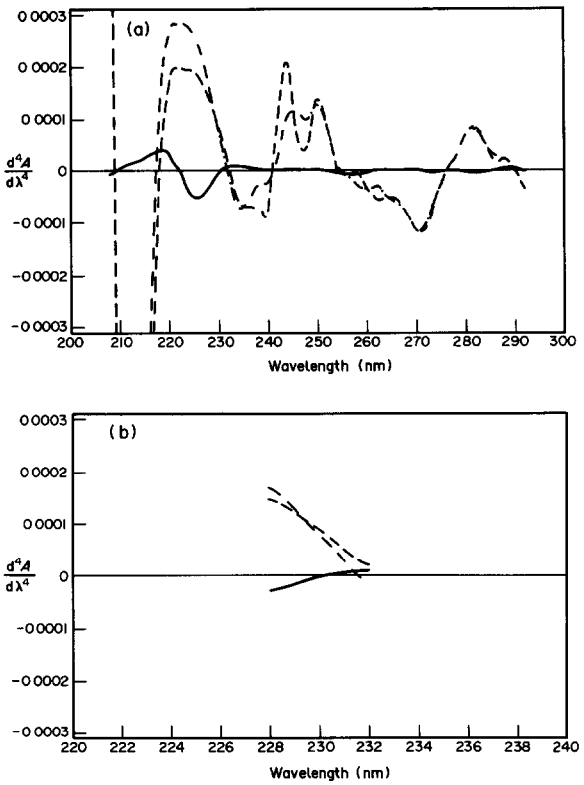

Figure 6
Fourth-derivative spectrum of an extract of a Tylenol No 2 tablet and simulated fourth-derivative spectra of acetaminophen and codeine phosphate Line types and other conditions as in Figs 1 and 2

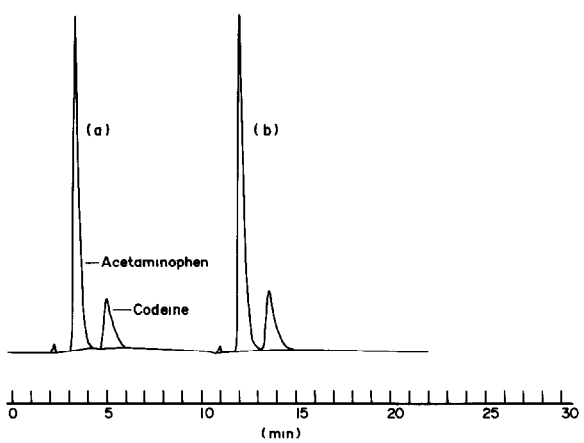

Figure 7
Chromatograms of acetaminophen and codeine phosphate (a) Extract of Tylenol No 4, (b) analytical standard (see text for conditions and additional explanation)

Table 2
Results of the assays for the extracts from each type of Tylenol (Nos 1–4), using diode array spectrophotometry in each derivative order (n) from 0 to 4

Tylenol tablet formulation	Component (nominal mg/tablet)*	$n = 0$		$n = 1$		$n = 2$		$n = 3$		$n = 4$	
		Mean†	mg/Tablet	Mean†	mg/Tablet	Mean†	mg/Tablet	Mean†	mg/Tablet	Mean†	mg/Tablet
1	A (300)	23 63	295 4	25 00	312 8	23 38	317 2	25 91	323 9	25 78	322 2
	C (7 5)	0 194	2 422	0 721	9 008	0 762	9 526	0 546	6 829	0 489	6 110
2	A (300)	23 95	299 3	25 10	313 8	25 63	320 3	25 87	323 3	27 98	349 8
	C (15)	0 836	10 44	1 266	15 82	1 190	14 87	0 792	9 90	1 350	16 87
3	A (300)	23 71	296 4	24 54	306 8	25 08	313 4	24 36	304 5	23 73	296 6
	C (30)	2 147	26 84	2 460	30 75	2 321	29 01	2 275	28 43	2 257	28 21
4	A (300)	24 34	304 3	24 79	309 8	24 30	303 7	23 40	292 4	26 57	332 1
	C (60)	4 679	58 47	4 835	60 44	4 777	59 71	4 654	58 17	5 728	71 59

*A = acetaminophen, C = codeine phosphate
†Mean of 10 replicate extracts per Tylenol formulation ($\mu g\ ml^{-1}$)

Table 3
Comparison of the spectroscopic assay data for acetaminophen and codeine phosphate in Tylenol formulations Nos 2 and 4, with data from HPLC assays of the same extracts

Tylenol tablet formation	Component*	Mean mg/tablet (RSD)†			Mean difference (mg)		RMD (%)§		Mean difference between first and zero order derivative (mg)‖	RMD (%)**
		HPLC	$n = 0$	$n = 1$	$n = 0$	$n = 1$	$n = 0$	$n = 1$		
2	A	300 0 (3 73)	ND	313 8 (0 86)	ND	13 8	ND	4 39	ND	ND
	C	14 44 (3 43)	ND	15 82 (3 14)	ND	1 38	ND	8 72	ND	ND
4	A	310 6 (3 60)	304 3 (0 51)	309 8 (1 08)	−6 3	−0 80	−2 07	−0 25	5 5	1 8
	C	58 19 (4 96)	58 47 (1 49)	60 41 (2 05)	0 28	2 22	0 48	3 67	1 94	3 3

*A = acetaminophen, C = codeine phosphate
†Mean of 10 separate extracts from each formulation
‡Mean difference = (diode array assay in derivative order n) − (HPLC assay)
§RMD (%) = mean difference as a percentage of diode array assay ($n = 0$)
‖Mean difference = (first order derivative) − (zero order derivative)
**RMD (%) = mean difference in derivative assay data as a percentage of zero order assay
ND = not determined

Table 4
Summary of assay data for acetaminophen and codeine phosphate found in Tylenol combination tablets assayed by zero order ($n = 0$) and first-derivative ($n = 1$) diode array spectroscopy and by HPLC

Tylenol tablet formulation	Component (nominal mg/tablet)*	mg/Tablet (RSD)† $n = 0$	$n = 1$	HPLC
1	A (300)	ND‡	ND	ND
	C (7 5)	ND	ND	ND
2	A (300)	ND	313 7 (0 86)	300 (3 73)
	C (15)	ND	15 8 (3 14)	14 4 (3 4)
3	A (300)	ND	306 8 (2 17)	ND
	C (30)	ND	30 8 (3 76)	ND
4	A (300)	304 3 (0 51)	309 8 (1 08)	310 6 (3 60)
	C (60)	58 5 (1 50)	60 4 (2 05)	58 2 (4 96)

*A = acetaminophen, C = codeine phosphate
†Mean of 10 separate extractions from each formulation, RSD as percentage
‡ND = not determined

(RMD = 0 48%), where HPLC values were lower When the HPLC values were compared with those obtained by first-derivative spectroscopy, there was a very small difference for acetaminophen (RMD = 0 25%), whereas the corresponding comparison for codeine phosphate revealed consistently lower values by HPLC (RMD = −3 67%), the diode array values being significantly higher than in the zero order mode (Tables 3 and 4)

Conclusions

Multicomponent analysis using the HP 8451A diode array spectrophotometer is applicable to the determination of acetaminophen and codeine phosphate in tablet combination products in which the amount of codeine phosphate is ≥15 mg/tablet The technique is inapplicable to the analysis of Tylenol No 1 which contains 7 5 mg of codeine phosphate/tablet, due to the unacceptable errors associated with the low spectral contribution of this component in tablet extracts However, further development in instrumental sensitivity can be expected to eliminate these problems

The choice of the analytical wavelength range used for the analysis, must be made carefully in order to eliminate interferences from the background matrix and the extracting solvent In particular, this analytical wavelength range should encompass the region of maximum spectral differences It is likely that the success of the proposed first-derivative procedure for the analysis of codeine phosphate in the presence of a larger amount of acetaminophen, is due to the fact that within the chosen analytical range of wavelengths (220–240 nm), the first-derivative spectrum for codeine is negative, whereas that for acetaminophen is positive

Although an increase in derivative order may simultaneously enhance the resolution, specificity, and sensitivity of the spectroscopic determinations, the derivative technique will increase the intrinsic noise level and therefore the detection limit [6] In the present study, this led to the second, third and fourth derivative spectra, giving unacceptable results for the determination of codeine and acetaminophen in combination tablets The problems arising from the compounding of errors are clearly indicated in the third and fourth-derivative spectra (Figs 5 and 6) which show a peak centred around 244 nm for the tablet extracts This peak is inconsistent with lower order derivative spectra (Figs 1a,

2a, 3a and 4a) for the tablets, as well as with the spectra for the individual components These results suggest that, in general, the lowest derivative order which gives satisfactory results in terms of accuracy and precision should be chosen

The reasons behind the differences in the values obtained by the different analytical techniques (HPLC, zero- and first-derivative spectroscopy) and their significances in future applications of diode array spectroscopy to multicomponent analysis of pharmaceuticals, are not clear and require further study These differences do signal the need for caution when interpreting the results of analysis based on diode array spectroscopy, particularly in situations where derivative techniques are employed to enhance the spectral features of any component which makes only a small contribution to the overall absorbance

The HP 8451A diode array spectrophotometer can be used to rapidly assess the content uniformity of Tylenol with Codeine Nos 2–4 using the first-derivative UV spectra of the active ingredients The tablets in the current investigation were all found to meet the specifications of the USP [7]

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