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# The simultaneous assay of triprolidine, pseudoephedrine and dextromethorphan in combined preparations by derivative-difference spectrophotometry

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**Abstract**: Difference spectrophotometric procedures are described for the assay of triprolidine hydrochloride, dextromethorphan hydrobromide and pseudoephedrine hydrochloride in Actifed preparations. Triprolidine is assayed by the measurement of the difference absorbance at 301 nm between equimolar solutions of the sample extract in 0.1 M sulphuric acid and 0.1 M sodium hydroxide in ethanol (20% v/v). Dextromethorphan and pseudoephedrine are assayed by measurement of the amplitudes in the second and fourth derivative spectra of the difference absorption spectrum of the sample solutions. The measured values are proportional to the concentrations of the drugs. The accuracy, precision and selectivity of the procedures are discussed. Applications of the assay are described for Actifed Compound Linctus, Actifed Syrup and Actifed Tablets.

**Keywords**: *Difference spectrophotometry; derivative spectrophotometry; triprolidine; dextromethorphan; pseudoephedrine; Actifed preparations.*

## Introduction

As a result of the overlapping absorption bands of triprolidine hydrochloride, pseudoephedrine hydrochloride and dextromethorphan hydrobromide, the spectrophotometric assay of these substances in Actifed cough preparations presents an interesting challenge to the pharmaceutical analyst. The assay of the syrup preparations is further complicated by their formulation in a highly coloured and flavoured syrup matrix.

Derivative spectrophotometry has been used previously to assay the pseudoephedrine content of the preparations [1, 2]. A high-pass digital filtering technique has also been described for the spectrophotometric assay of pseudoephedrine [3]; dextromethorphan and triprolidine have been assayed using a least-squares method which involves the treatment of the zero-order spectral data by a computer program [2]. The present paper describes an alternative technique that involves a combination of derivative and

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difference spectrophotometry to assay all three components directly by using readily available spectrophotometric instrumentation.

The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance ($\Delta A$) between two equimolar solutions of the analyte in different chemical forms that exhibit different spectral characteristics. pH-induced difference spectrophotometry may be applied to the assay of a substance in the presence of other absorbing substances provided that reproducible changes can be induced in the spectrum of the analyte by the addition of reagents of different pH and that the absorbance of the interfering substances is not altered by the reagents. The $\Delta A$, which is normally measured at a wavelength of maximum difference absorption between solutions whose pH values provide the maximum difference in absorbance, is selective for the analyte since the contribution of the interfering substances to the difference absorbance of the mixture is eliminated.

In derivative spectrophotometry, the enhanced resolution and bandwidth discrimination, which are the principal advantages of the technique, often result in the complete elimination of the interference by broad bandwidth substances from the derivative spectra of narrow bandwidth substances [4]. Instrumental and chemical parameters are usually selected to provide optimum selectivity and precision of the measured amplitude. In practice, second and fourth derivative spectra offer a satisfactory compromise between the superior selectivity and lower precision of the higher order spectra.

The method described in this report involves the selective assay of triprolidine by the measurement of the pH-induced difference absorbance at 301 nm and the simultaneous assay of pseudoephedrine and dextromethorphan by the measurement of selected amplitudes in the second and/or fourth derivative spectra of the difference spectrum.

## Experimental

*Drug substances*

Pseudoephedrine hydrochloride was obtained from Sigma Chemical Co. Dextromethorphan hydrobromide was a gift from Roche Products and triprolidine hydrochloride was purchased from Burroughs Wellcome.

*Reagents*

Sulphuric acid (1 M) and sodium hydroxide (1 M) were prepared from analytical reagent grade substances (BDH Chemicals). Chloroform A.R. (BDH Chemicals) and absolute ethanol (Burroughs) were used.

*Spectrophotometer*

Absorption (Fig. 1) and difference absorption (Fig. 2) spectra in 1-cm silica quartz cells were recorded over the wavelength range 350–230 nm using a Perkin–Elmer 552 double-beam recording ultraviolet-visible spectrophotometer. The scan speed was 1 nm $s^{-1}$, the response (time constant) was 0.5 s, and the spectral bandwidth was 1 nm. Second derivative difference ($d^2\Delta A/d\lambda^2$) spectra (Fig. 3) were recorded at a scan speed of 2 nm $s^{-1}$ with the spectrophotometer operating in the second derivative mode. Fourth derivative difference ($d^4\Delta A/d\lambda^4$) spectra (Fig. 4) were recorded simultaneously with the $d^2\Delta A/d\lambda^2$ spectra by use of a Hitachi analogue derivative accessory operating in the second derivative mode (mode 5) in series with the spectrophotometer operating in the second derivative mode.

*Standard solutions*

Standard solutions of pseudoephedrine hydrochloride (3 mg $ml^{-1}$), dextromethorphan hydrobromide (1 mg $ml^{-1}$) and triprolidine hydrochloride (0.125 mg $ml^{-1}$) were prepared by dissolving in water and diluting to 200 ml in separate flasks accurately weighed quantities of the reference compounds. A 5-ml volume of each solution was transferred by pipette into two 50-ml volumetric flasks, one containing 5 ml of 1 M sodium hydroxide and 10 ml of ethanol and the other containing 5 ml of 1 M sulphuric acid, and the solutions were diluted to volume with water.

*Sample solutions*

*Syrups*. The weight per ml of the syrup was determined by weighing 10 ml of the sample in a 10-ml volumetric flask. A sample of the syrup equivalent to about 10 ml was accurately weighed into a 100-ml separating funnel containing 30 ml of water. The solution was made alkaline by the addition of 2 ml of 5 M sodium hydroxide and then extracted successively with three 20-ml volumes of chloroform. The extracts were combined in a second separating funnel and then extracted successively with 20, 15 and 10 ml of 0.1 M sulphuric acid. The acidic extracts were combined and diluted to 50 ml in a volumetric flask. A 5-ml volume of the acidic extract was transferred to two 20-ml volumetric flasks, one containing 1.5 ml of 1 M sulphuric acid and the other containing 2.5 ml of 1 M sodium hydroxide and 4 ml of ethanol, and the contents of both flasks were diluted to 20 ml with water.

*Tablets*. Twenty tablets were weighed and powdered. A weight of powder equivalent to a tablet of average weight was shaken for 15 min with 40 ml of 0.1 M sulphuric acid and then diluted to 50 ml with the same solvent. The extract was filtered through Whatman No. 1 filter-paper and then the assay was completed as described for the syrups from the words "a 5-ml volume of the acidic extract was transferred . . .".

*Procedure*

The zero-order difference spectra of the sample solutions and that of the standard solutions of triprolidine were recorded with the acidic solution in the sample beam of the spectrophotometer and the alkaline solution in the reference beam (Fig. 2). The difference absorbance at the maximum in the difference spectrum at 301 nm ($\Delta A_{301}$) was measured and corrected for the absorbance difference between 0.1 M sulphuric acid and 0.1 M sodium hydroxide in ethanol (20% v/v). The concentration of triprolidine hydrochloride in the sample solutions and hence in the sample was calculated from the proportional relationship that exists between the corrected $\Delta A_{301}$ and concentration.

The second and fourth derivative difference absorption spectra ($d^2\Delta A/d\lambda^2$ and $d^4\Delta A/d\lambda^4$, respectively) of the sample solutions and of the standard solutions of pseudoephedrine and dextromethorphan were then recorded. The amplitudes in the $d^2\Delta A/d\lambda^2$ spectrum of the sample solutions from the maximum at 288.5 nm to its shorter wavelength satellite (shown in Fig. 3b) and in the $d^4\Delta A/d\lambda^4$ spectrum from the minimum at 288.5 nm to its shorter wavelength satellite (shown in Fig. 4b) were measured; the concentration of dextromethorphan hydrobromide was calculated by reference to the corresponding amplitudes in the spectra of the standard solutions of dextromethorphan and the proportional relationship that exists between the measured amplitudes and concentration. Similarly, the amplitudes in the $d^2\Delta A/d\lambda^2$ spectrum of the sample solutions measured from the minimum at 261.5 nm to its shorter wavelength satellite

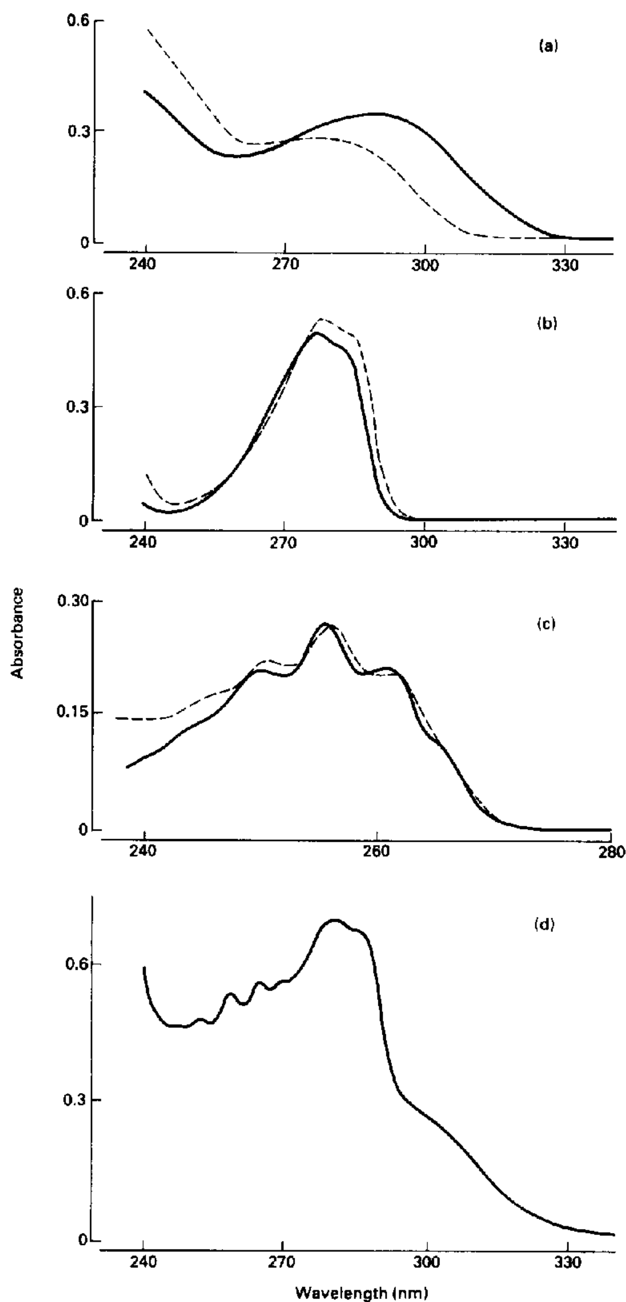


**Figure 1**
The absorption spectra of (a) triprolidine hydrochloride (12.5 μg $ml^{-1}$); (b) dextromethorphan hydrobromide (100 μg $ml^{-1}$); (c) pseudoephedrine hydrochloride (300 μg $ml^{-1}$) in 0.1 M sulphuric acid (solid line) and in 0.1 M sodium hydroxide (broken line); (d) an extract of Actifed Compound Linctus in 0.1 M sulphuric acid. Note the expanded wavelength and absorbance scales in Fig. 1c to show the very small spectral changes that occur when a solution of pseudoephedrine is made alkaline.

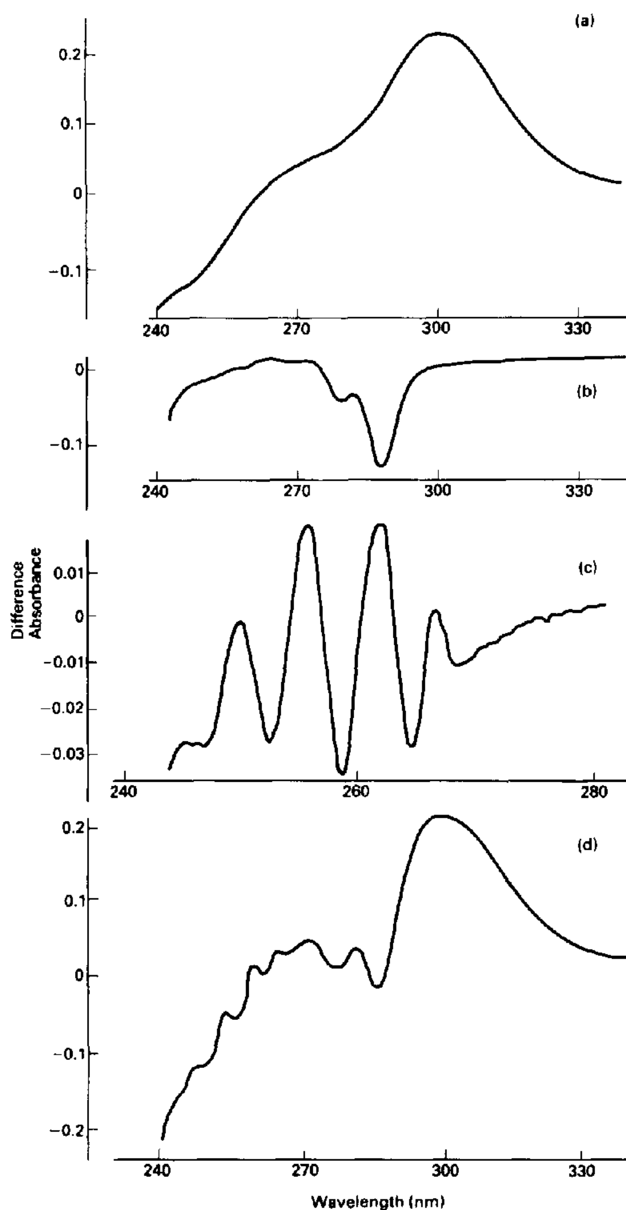


**Figure 2**
The zero-order difference absorption ($\Delta A$) spectra of (a) triprolidine hydrochloride (12.5 μg $ml^{-1}$); (b) dextromethorphan hydrobromide (100 μg $ml^{-1}$) and pseudoephedrine hydrochloride (300 μg $ml^{-1}$). Note the expanded wavelength and absorbance scales in Fig. 2c to show the weak difference absorbance of pseudoephedrine.

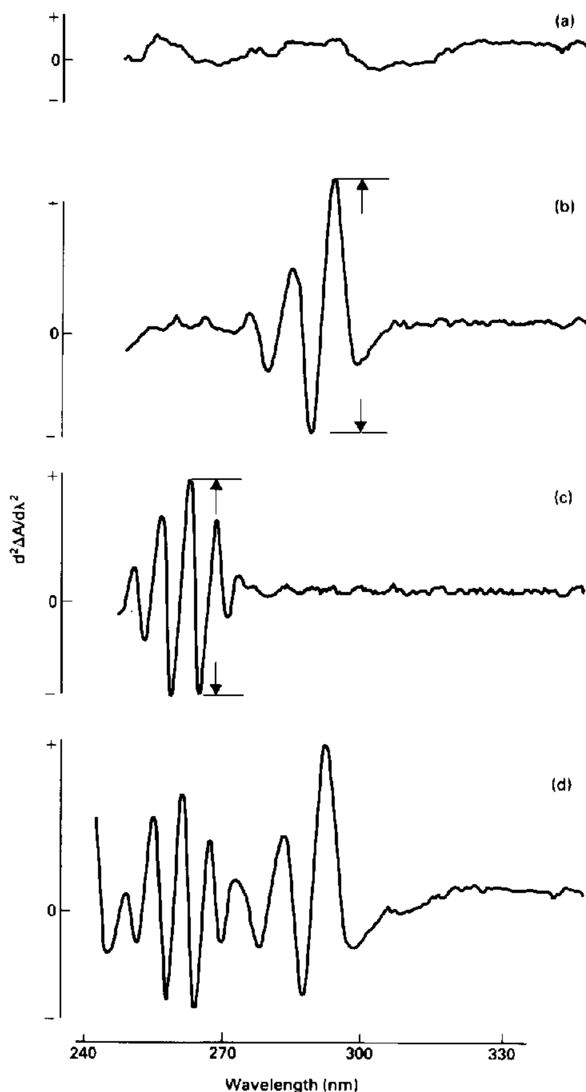


**Figure 3**
The second derivative spectra of the difference absorption spectra shown in Fig. 2. The ordinate ($d^2\Delta A/d\lambda^2$) scale in each case is the same.

(shown in Fig. 3c) and in the $d^4\Delta A/d\lambda^4$ spectrum from the maximum at 261.5 nm to its shorter wavelength satellite (shown in Fig. 4c) were used to calculate the concentration of pseudoephedrine by reference to the corresponding amplitudes in the spectra of the standard solutions of pseudoephedrine hydrochloride.

## Results and Discussion

### *Development of the method*

The individual spectra of triprolidine, dextromethorphan and pseudoephedrine in acidic solution are shown in Fig. 1a, b and c, respectively. Actifed Compound Linctus is a

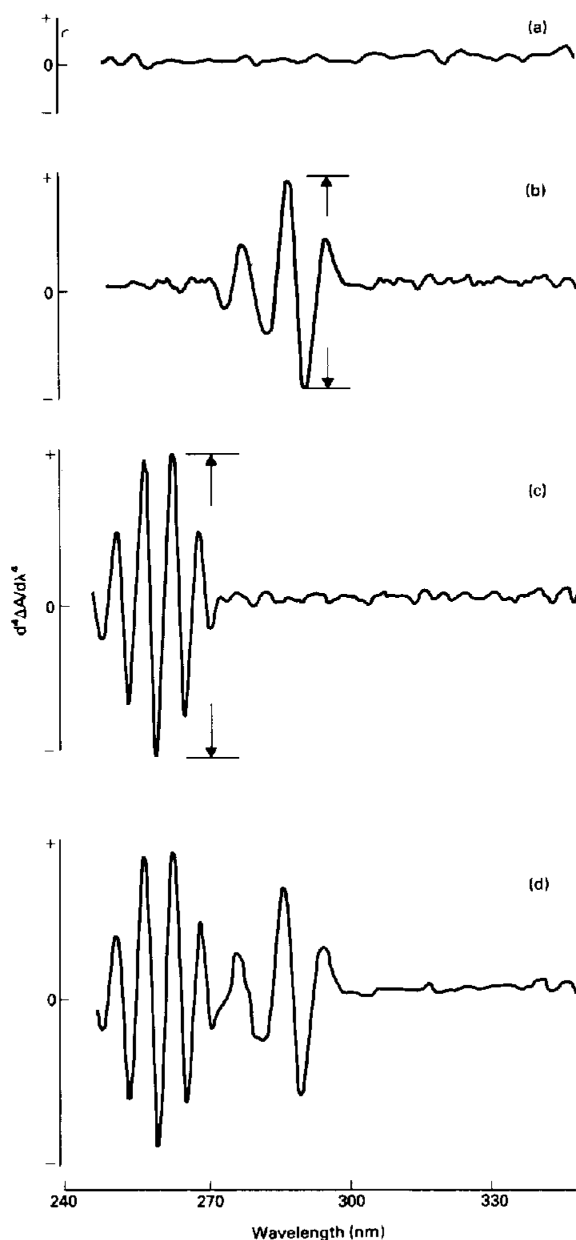


**Figure 4**
The fourth derivative spectra of the difference absorption spectra shown in Fig. 2. The ordinate ($d^4\Delta A/d\lambda^4$) scale in each case is the same.

coloured syrup containing 0.25 mg $ml^{-1}$ of triprolidine hydrochloride, 2 mg $ml^{-1}$ of dextromethorphan hydrobromide and 6 mg $ml^{-1}$ of pseudoephedrine hydrochloride. The spectrum of the colourless acidic extract of the linctus, that is obtained after extraction of the active ingredients into chloroform, is a combination of the spectra of the individual substances, in which the vibrational bands of the weakly absorbing

pseudoephedrine are barely discernible on the slope of the fused broad bands of the more strongly absorbing triprolidine and dextromethorphan (Fig. 1d).

Figure 1a, b and c also shows the effect of alkali on the spectra of the three compounds. Triprolidine undergoes a major hypsochromic (blue) shift from the maximum in acidic solution at 290 nm to 279 nm and a concomitant hypochromic effect due to deprotonation of its pyridinium nitrogen atom. Pseudoephedrine displays small wavelength shifts and a slight reduction of the resolution of the vibrational bands between 250 and 265 nm; dextromethorphan shows a small increase in absorbance at the maximum at 278 nm and its adjacent shoulder.

The resultant zero-order difference spectrum ($D^{\circ}\Delta A$) of triprolidine (Fig. 2a) shows an intense broad difference absorption band with its maximum at 301 nm; at this wavelength the other two components give zero difference absorption. The assay of triprolidine in the samples is based on the measurement of the $\Delta A_{301}$. Figure 2b and c show that the small spectral changes displayed by dextromethorphan and pseudoephedrine in acid and alkali result in weak difference absorption bands which are of a narrower bandwidth than that of triprolidine. Furthermore, the wavelength regions of the $D^{\circ}\Delta A$ bands of the two compounds are well separated although both occur in the region of the short wavelength slope of the $\Delta A$ band of triprolidine, which is almost devoid of spectral structure (Fig. 2d).

Figure 1a, b and c show the $d^2\Delta A/d\lambda^2$ spectra of the three substances. The broad difference absorption band of triprolidine and its shoulder near the isosbestic wavelength at 265 nm give rise to very weak derivative bands whereas the narrow $D^{\circ}\Delta A$ bands of pseudoephedrine and dextromethorphan give rise to much more intense derivative absorption spectra. The separate derivative bands of pseudoephedrine and dextromethorphan are clearly distinguished in the $d^2\Delta A/d\lambda^2$ spectrum of the syrup extract (Fig. 3d). Close examination of the derivative bands of pseudoephedrine in the standard solution and in a sample solution of all three components indicates that slight distortion of the $d^2\Delta A/d\lambda^2$ spectrum occurs owing to the weak derivative bands of triprolidine in the wavelength region of the bands of pseudoephedrine.

The effect of further differentiation in the $d^4\Delta A/d\lambda^4$ spectra of the three substances is shown in Fig. 4a, b and c. The spectrum of triprolidine (Fig. 4a) is identical to that of the solvent blanks showing that the broad band interference from triprolidine in the $D^{\circ}\Delta A$ spectra is completely eliminated in the $d^4\Delta A/d\lambda^4$ spectra. In contrast, the narrow $D^{\circ}\Delta A$ bands of dextromethorphan and pseudoephedrine are further intensified in the $d^4\Delta A/d\lambda^4$ spectra (Fig. 4b and c, respectively) and are also easily distinguished in the $d^4\Delta A/d\lambda^4$ spectrum of the syrup extract (Fig. 4d). The elimination of the interference of triprolidine in the $d^4\Delta A/d\lambda^4$ spectrum of the mixture is confirmed by the observation that the relative amplitudes of the derivative bands of pseudoephedrine are not affected by the presence of triprolidine.

The assays of pseudoephedrine and dextromethorphan are based on the measurement of the amplitudes in the $d^2\Delta A/d\lambda^2$ and $d^4\Delta A/d\lambda^4$ spectra, that are shown in Figs 2 and 4, respectively. Since the accuracy and precision of derivative spectral measurements are markedly affected by temperature [5, 6], it was necessary to ensure that all the solutions were at the same temperature during the recording of the spectra.

The $pK_a$ values of triprolidine, pseudoephedrine and dextromethorphan are 6.5, 9.8 and 8.3, respectively [7]. The choice of 0.1 M sulphuric acid (pH approximately 1) and 0.1 M sodium hydroxide (pH approximately 13) is expected on theoretical grounds to provide the maximum $\Delta A$ of all three substances. Although it may be possible to avoid

the distortion of the $d^2\Delta A/d\lambda^2$ spectrum of pseudoephedrine by triprolidine by recording the $d^2\Delta A/d\lambda^2$ spectrum of a solution at pH 8, rather than the 0.1 M sulphuric acid solution, against an equimolar solution in 0.1 M sodium hydroxide, this was not investigated in the present study.

The extraction procedure for the separation of the drug bases from the excipients in the coloured syrup matrix is based upon a published procedure [1] for the assay of pseudoephedrine in an earlier formulation of Actifed Compound Linctus in which codeine phosphate rather than dextromethorphan was used as the cough suppressant. However, it was necessary to replace the hydrochloric acid used in the back-extraction of the bases from chloroform with sulphuric acid since it was found that dextromethorphan is not fully extracted into hydrochloric acid, possibly as a result of the formation of a lipophilic ion-pair with chloride anion. It was also found to be necessary to incorporate 20% (v/v) of ethanol in the alkaline solutions of dextromethorphan for the spectral measurements to overcome the problem of precipitation of the base in a completely aqueous alkaline solution.

*Validation*

To test the selectivity of the procedure, three series of solutions were prepared. In each series the concentration of one of the drugs was constant at its nominal concentration in Actifed Compound Linctus and the concentration of the other two components was varied systematically to be 0, 40, 80, 100, 120 and 160% of their nominal concentration in the linctus. These drugs were added, separately and together, to the constant concentration of the third compound. The solutions were diluted with 0.1 M sodium hydroxide in ethanol (20%, v/v) and with 0.1 M sulphuric acid and determined spectrophotometrically as described for the linctus samples.

The results in Table 1 show that the difference absorbance of triprolidine at 301 nm is not affected by concentrations of dextromethorphan and pseudoephedrine up to 160% of their nominal concentration. Similarly the $d^2\Delta A/d\lambda^2$ and $d^4\Delta A/d\lambda^4$ amplitudes of dextromethorphan are not affected by the presence of pseudoephedrine and triprolidine. However, the $d^2\Delta A/d\lambda^2$ spectra of pseudoephedrine show a very slight but increasing distortion in the presence of increasing concentrations of triprolidine. This is confirmed by the significant linear relationship between the measured $d^2\Delta A/d\lambda^2$ amplitude of pseudoephedrine and the concentration of triprolidine. Calculation of the interference in the assay of pseudoephedrine at the nominal concentration of triprolidine in the linctus, from the regression statistics in Table 1, indicates that the triprolidine gives a systematic error of +1.2% in the measured $d^2\Delta A/d\lambda^2$ amplitude of pseudoephedrine. All the other amplitudes of pseudoephedrine suffer similar levels of interference. In many analyses, a systematic error of only 1.2% is considered to be negligible; if results of higher accuracy are required, the error is easily corrected by subtraction from the total value. Alternatively, measurement of the $d^4\Delta A/d\lambda^4$ amplitude of pseudoephedrine, which is not affected by the presence of triprolidine, provides a selective assay for pseudoephedrine.

The proportionality of the measured values and concentration was checked by measuring the relevant values in the spectra of the three series of mixtures prepared for the assessment of specificity (*vide supra*). In each case a proportional relationship between the measured value and concentration was observed; this was confirmed by the negligible intercept (less than 0.8% of the value at the nominal assay concentration) and excellent correlation coefficient (not less than 0.998) (Table 2).

**Table 1**
Selectivity data

| Analyte* | Added drug† | Measured value‡ | Mean value§ ±RSD | Linear regression data for added drug‖ a | b | r | linear relationship? ($P$ = 0.95) |
|---|---|---|---|---|---|---|---|
| Triprolidine HCl (12.5 μg ml$^{-1}$) | P | $\Delta A$ | 0.221 ± 1.11% | — | — | −0.085 | No |
| | D | | 0.223 ± 1.17% | — | — | 0.192 | No |
| | P + D | | 0.222 ± 0.90% | — | — | −0.184 | No |
| Dextromethorphan HBr (100 μg ml$^{-1}$) | P | $D^2\Delta A$ | 178.6 ± 0.78% | — | — | −0.111 | No |
| | T | | 177.6 ± 0.70% | — | — | 0.317 | No |
| | P + T | | 178.9 ± 0.91% | — | — | 0.400 | No |
| | P | $D^4\Delta A$ | 133.5 ± 0.97% | — | — | 0.373 | No |
| | T | | 134.1 ± 0.74% | — | — | −0.061 | No |
| | P + T | | 134.6 ± 0.76% | — | — | −0.084 | No |
| Pseudoephedrine HCl | D | $D^2\Delta A$ | 155.5 ± 0.55% | — | — | 0.429 | No |
| | T | | 157.2 ± 0.77% | 155.6 | 0.0192 | 0.827 | Yes |
| | D + T | | 156.9 ± 0.86% | 154.2 | 0.0208 | 0.841 | Yes |
| | D | $D^4\Delta A$ | 190.1 ± 0.94% | — | — | 0.484 | No |
| | T | | 191.2 ± 1.00% | — | — | −0.189 | No |
| | D + T | | 190.8 ± 1.10% | — | — | 0.219 | No |

*Present in solutions at the nominal assay concentration stated in brackets.
†P = pseudoephedrine, D = dextromethorphan, T = triprolidine; present at 0–160% of their nominal assay concentration ($N$ = 6).
‡$\Delta A$, $D^2\Delta A$ and $D^4\Delta A$ measurements in the zero-order, second and fourth derivative spectrum respectively.
§$\Delta A$ is measured in difference absorbance at 301 nm. $D^2\Delta A$ and $D^4\Delta A$ measured in mm at the wavelengths specified in the text.
‖$y = a + bx$ where $y$ is the measured value and $x$ is the % nominal assay concentration of added drug; $r$ is the correlation coefficient.

**Table 2**
Calibration data

| Analyte* | Measured value† | Linear regression data‡ a | b | $r$ |
|---|---|---|---|---|
| Triprolidine HCl | $\Delta A$ | 0.0018 | 0.0178 | 0.999 |
| Dextromethorphan HBr | $D^2\Delta A$ | −0.061 | 1.775 | 0.998 |
| | $D^4\Delta A$ | 0.18 | 1.330 | 0.999 |
| Pseudoephedrine HCl | $D^2\Delta A$ | 1.07 | 0.521 | 0.999 |
| | $D^4\Delta A$ | −0.74 | 0.631 | 0.998 |

*Each analyte was measured in the concentration range 0–160% of its nominal assay concentration ($N = 6$). Triprolidine solutions contained a constant concentration of pseudoephedrine; dextromethorphan solutions contained a constant concentration of triprolidine; and pseudoephedrine solutions contained a constant concentration of dextromethorphan (see Table 1).

†See footnote ‡ to Table 1.

‡$y = a + bx$ where $y$ is the measured value and $x$ is the concentration of the analyte in $\mu g\ ml^{-1}$; $r$ is the correlation coefficient.

**Table 3**
Assay results

| Sample* | Content of active ingredients (as % stated content) Triprolidine HCl $\Delta A$ | Dextromethorphan HBr $D^2\Delta A$ | Dextromethorphan HBr $D^4\Delta A$ | Pseudoephedrine HCl $D^2\Delta A$ | Pseudoephedrine HCl $D^4\Delta A$ |
|---|---|---|---|---|---|
| Actifed Compound Linctus | 101.6 | 101.4 | 100.5 | 100.1 | 99.5 |
| Actifed Compound Linctus | 99.1 | 99.3 | 100.4 | 100.5 | 100.0 |
| Actifed Syrup | 98.7 | — | — | 99.0 | 98.7 |
| Actifed Syrup | 99.0 | — | — | 99.1 | 100.7 |
| Actifed Tablets | 100.4 | — | — | 99.7 | 99.6 |
| Actifed Tablets | 102.4 | — | — | 101.2 | 101.8 |

*Actifed Compound Linctus is stated to contain triprolidine HCl 0.25 mg $ml^{-1}$, dextromethorphan HBr 2 mg $ml^{-1}$ and pseudoephedrine HCl 6 mg $ml^{-1}$.
Actifed Syrup is stated to contain triprolidine HCl 0.25 mg $ml^{-1}$ and pseudoephedrine HCl 6 mg $ml^{-1}$.
Actifed Tablets are stated to contain triprolidine HCl 2.5 mg and pseudoephedrine HCl 60 mg.

The precision of the analysis of Actifed Compound Linctus was measured by carrying out the assay ten times on a single sample of linctus. The mean assay results expressed in terms of its label composition ± the relative standard deviation were: triprolidine hydrochloride, 99.1 ± 0.97%; pseudoephedrine hydrochloride, 100.5 ± 1.1% by $d^2\Delta A/d\lambda^2$ and 100.0 ± 1.4% by $d^4\Delta A/d\lambda^4$; dextromethorphan hydrobromide, 99.3 ± 1.1% by $d^2\Delta A/d\lambda^2$ and 100.4 ± 1.5% by $d^4\Delta A/d\lambda^4$. Relative standard deviations of the order of these values indicate satisfactory precision of the whole assay procedure.

*Assay results*

A number of Actifed preparations were assayed by the procedures. The results in Table 3 show that the assay results for each of the active substances are in good agreement with the stated content of the formulations and confirm that the combination of derivative and difference spectrophotometry provides a simple, rapid and selective method for the assay of the drugs in the Actifed preparations.

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