The use of derivative and least-squares methods to analyse a polypharmaceutical product by UV spectrophotometry

R. JONES*[1], M. J. ORCHARD[1] and K. HALL[2]

[1] *Central Analytical Laboratories (Chemical), and* [2] *Central Analytical Laboratories (Biological),*
The Wellcome Foundation Ltd., Temple Hill, Dartford, Kent DA1 5AH, UK

Abstract: Derivative UV spectrophotometry is well established for analysing pharmaceutical products containing more than one drug. By contrast, the least-squares method for over-determined systems is rarely used, because it is assumed that measurements at a large number of wavelengths are needed to obtain good results. Both methods have advantages, and their use in combination is useful for analysing polypharmaceuticals.

A combination of derivative and least-squares methods was used to analyse tablets containing pseudoephedrine hydrochloride, triprolidine hydrochloride and dextromethorphan hydrobromide. Pseudoephedrine was determined by derivative spectrophotometry. The other drugs were determined by the least-squares method at higher wavelengths where pseudoephedrine does not absorb. Satisfactory precision for the least-squares method was obtained with a manual spectrometer measuring at six wavelengths and calculating the results with a microcomputer.

Introduction

The availability of inexpensive data processing facilities has greatly increased the usefulness of UV spectrophotometry in the pharmaceutical analysis laboratory. Both derivative measurements [1] and the least-squares method for overdetermined systems [2, 3], which statistically fits standard spectra to a test spectrum, can be used for the quantitative analysis of mixtures. Whilst derivative spectra can be obtained on most scanning UV spectrophotometers, facilities for the least-squares method are available only on a few highly priced instruments with integral or separate computing facilities. In order that the least-squares method should become more generally accepted for quality control of pharmaceuticals, it is necessary to demonstrate that it can be carried out relatively quickly on simple equipment.

The present paper describes the development of spectrophotometric methods for the analysis of a tablet containing pseudoephedrine, triprolidine and dextromethorphan.

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*To whom correspondence should be addressed.

Triprolidine and dextromethorphan are determined by the least-squares method applied to the zero order spectra, measuring at six wavelengths where pseudoephedrine is transparent. The results are calculated with a microcomputer (Commodore Pet). Pseudoephedrine is measured by second derivative UV-spectrophotometry. The methods are rapid and can be carried out on simple spectrophotometers.

Experimental

Computer programs

Computer programs to calculate results by the least-squares method for mixtures were written in Basic for a 32K Commodore Pet microcomputer model 8032 with an 8050 disk drive and 4022 printer.*

The program STANDATA 8032 requests input from the keyboard and writes a sequential data file containing absorbance and concentration data from standard solutions on to disk. An output to the printer is given for checking purposes.

The program SPEC2 8032 loads the file containing standard data and requests keyboard input of test data. The result is printed as the percent m/v of each standard substance in the test solution.

Reagents

Hydrochloric acid was of analytical reagent grade.

Drugs and formulations

The pure drugs complied with the requirements of the *British Pharmacopoeia* [4]. The tablets were manufactured by the Wellcome Foundation Limited (Dartford, UK) and contained 60 mg of pseudoephedrine hydrochloride, 2.5 mg of triprolidine hydrochloride and 20 mg of dextromethorphan hydrobromide.

Apparatus

The computer employed was as described above. Three UV-spectrophotometers were used: Pye Unicam model SP500 Series 2; Pye Unicam model SP8-200; and the Perkin Elmer model 554.

Procedures

Weigh and powder 20 tablets. Dissolve an accurately weighed amount of powdered tablets, equivalent to 10 mg of triprolidine hydrochloride, in 0.1 M hydrochloric acid using an ultrasonic bath and adjust the volume to 100 cm^3. Filter and dilute 10.0 cm^3 of the filtrate to 100 cm^3 with 0.1 M hydrochloric acid. This is the test solution.

Pseudoephedrine hydrochloride. Dissolve about 0.12 g of pseudoephedrine hydrochloride, accurately weighed, in 0.1 M hydrochloric acid and adjust the volume to 100 cm^3. Dilute 20.0 cm^3 to 100 cm^3 with 0.1 M hydrochloric acid. This is the standard solution.

Record the second derivative spectrum of the test and standard solutions in a 1 cm cell over the wavelength range 280–240 nm, using 0.1 M hydrochloric acid in the reference cell. The instrument settings (scan speed, derivative gain and damping, absorbance range

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*Details of the programs written for the Commodore Pet are available from the authors.

and bandwidth) should be optimized to produce a spectrum with about 80% full scale deflection and an acceptable noise level.

Settings for the Pye Unicam SP8-200 spectrometer were: slit width, 2 nm; scan speed, 1 nm s^{-1}; recorder range, 1 absorbance unit full scale; gain, (2nd derivative) setting no. 3 undamped; recorder damping, off.

Record each spectrum in triplicate without refilling the cell. Measure the peak amplitude from the satellite maximum near 250 nm to the trough at about 254 nm.

If D_T is the mean amplitude for the test solution (mm); D_S is the mean amplitude for the standard solution (mm); M_T is the mass of powdered tablets taken for the test solution (g); M_S is the mass of pseudoephedrine hydrochloride taken for the standard solution (g); and MTM is the mean tablet mass (g), then the mass of pseudoephedrine hydrochloride (mg) per tablet is given by:

$$\frac{D_T \times M_S \times MTM \times 2000}{D_S \times M_T}.$$

The percentage of the stated amount of pseudoephedrine hydrochloride is given by:

$$\frac{D_T \times M_S \times MTM \times 3333.3}{D_S \times M_T}.$$

Triprolidine hydrochloride and dextromethorphan hydrobromide. Accurately prepare separate standard solutions in 0.1 M hydrochloric acid containing 0.001% m/v triprolidine hydrochloride and 0.008% m/v dextromethorphan hydrobromide.

Measure the absorbance of each standard solution and of the test solution in a 1 cm cell at 5 nm intervals over the range 275–300 nm, using 0.1 M hydrochloric acid in the reference cell and making any correction necessary for cell error. Calculate the concentration (% m/v) of triprolidine hydrochloride and of dextromethorphan hydrobromide in the test solution using the STANDATA 8032 and SPEC2 8032 computer programs.

Let P be the concentration (% m/v) of either drug found in the test solution. Then the mass of either drug (mg) per tablet is given by:

$$\frac{P \times MTM \times 10\ 000}{M_T}.$$

The percentage of the stated amount of triprolidine hydrochloride is given by:

$$\frac{P \times MTM \times 400\ 000}{M_T}.$$

The percentage of the stated amount of dextromethorphan hydrobromide:

$$\frac{P \times MTM \times 50\ 000}{M_T}.$$

Content uniformity. Since the triprolidine hydrochloride dose in the tablets is low it is desirable to apply a test to confirm the uniformity of drug content. In order to assay

single tablets for triprolidine hydrochloride content prepare the test solutions by treating a single tablet as described for preparation of the test solution but replacing the specified dilution by a dilution of 20.0 cm^3 to 50 cm^3.

Results and Discussion

Computer programs

The calculation is based on the standard least squares treatment of over-determined systems [2] as used previously for Fortran programs [3]. The only problem when programming in the popular versions of Basic is the matrix inversion. An approximate Gauss–Jordan routine converted from Fortran was used, and the accuracy was confirmed by comparative calculations in APL on an IBM 4341 computer. The programs are dimensioned for a maximum of six standards and 250 wavelengths which covers most applications, and have been tested with data for mixtures containing up to six components.

The calculation was split into two parts to allow the use of a set of standard data on several occasions. This is possible only if the wavelength calibration of the spectrometer is unchanged over the period of use. Either a variable or fixed wavelength step can be used. In the latter case, the results can be recalculated with a new wavelength step or limits in order to optimize the method. The squared residuals are printed with the residual at each wavelength if required.

Assay development

In the development stage, the possibility of analysing all three components by the least-squares method was investigated. Mixtures of the three drugs were measured over the wavelength range 240–300 nm at 5 nm intervals (13 data points). Good results were obtained for all three components when a spectrometer with reproducible wavelength setting (Pye Unicam SP8-200) was used. When an instrument with manual wavelength adjustment was used, the pseudoephedrine results were variable, which was attributed to the non-reproducibility of wavelength selection. This is expected to be a general problem for compounds with sharp benzenoid bands. Derivative measurements have been shown to be suitable for determining similar compounds [5]. The method was simplified by recording the second derivative spectrum of pseudoephedrine hydrochloride at lower wavelengths and measuring the two other drugs at higher wavelengths where pseudoephedrine does not absorb. Figure 1 shows a typical second derivative spectrum for a tablet extract. Measuring between the two deflections shown eliminates the interference due to triprolidine and dextromethorphan. Figure 2 shows the spectra of the three drugs' over the wavelength range of interest. There is no significant contribution from the pseudoephedrine above 275 nm. Triprolidine and dextromethorphan are estimated precisely using six wavelengths only. The method can therefore be carried out easily with a manual spectrometer.

Following a suggestion from a referee, it was shown that triprolidine and dextromethorphan could possibly be determined by second derivative spectrometry, though different conditions would be needed for each of the three drugs.

Assay validation

Linearity of response. The linearity of response of standard solutions was checked over the range 0–150% of the assay concentration. For triprolidine and dextromethorphan

Figure 1
The second derivative spectrum of a test solution showing the method of measurement. The deflections over the range 275–300 nm are due to triprolidine and dextromethorphan and are absent from the standard spectrum. Concentration of pseudo-ephedrine hydrochloride — 0.024% m/v.
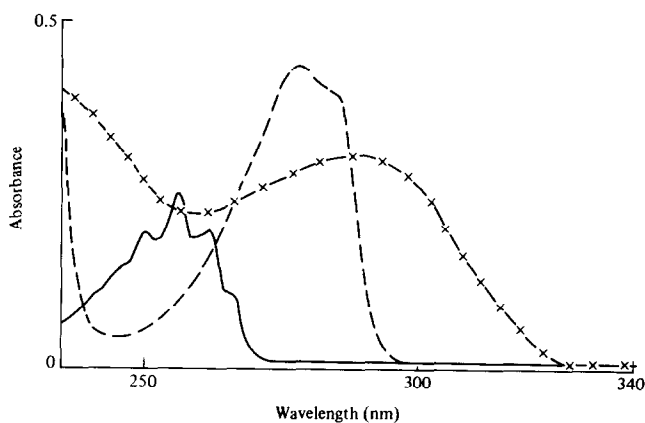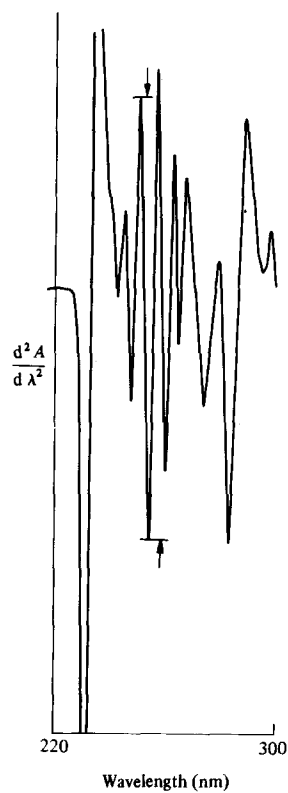
Figure 2
Zero order UV spectra of the three drugs over the wavelength range of interest. — — — — Dextro-methorphan hydrobromide 0.008% m/v; ×—×—×— triprolidine hydrochloride 0.001% m/v; ————— pseudoephedrine hydrochloride 0.024% m/v.

mixed standard solutions containing the drugs in the same proportions were assayed against separate standards.

The following results were obtained for plots of response (as a percentage of 100% standard response) vs concentration (as a percentage of the assay concentration): pseudoephedrine, $y = 0.9926 x + 0.78$ (S.E. $= 0.0094$); triprolidine, $y = 1.0063 x - 0.42$ (S.E. $= 0.0088$) and dextromethorphan, $y = 1.0057 x - 0.64$ (S.E. $= 0.0035$).

Specificity. Recovery experiments were carried out by adding the drugs in solution to mixtures of excipients in the proportions found in tablets. The following excipients were screened: dextrose, lactose, mannitol, hydrolysed starch, starch, povidone, talc, gum acacia, gelatin, magnesium stearate and stearic acid. All results were within 2% of theory. The method was not tested for specificity in the presence of the drug decomposition products because it is intended as a release assay only.

Ruggedness. A ruggedness test for the dextromethorphan and triprolidine assay was carried out by the method of the AOAC [6]. The factors selected for study and the results are shown in Table 1. No factor was significant at the 5% level, confirming that the method is rugged to changes in the procedure expected when the method is transferred to other laboratories. The effect of the spectrometer was investigated separately since it is important to show that the assay can be carried out successfully on a variety of instruments. Test and standard solutions were prepared and assayed in duplicate on three spectrophotometers (the two Pye Unicam instruments used in the AOAC ruggedness test and a Perkin Elmer 554 spectrometer). The six results were in the range 102.6–103.3% of the stated amount for triprolidine, and 97.5–99.1% for dextromethorphan.

Table 1
Results of the ruggedness test

Factor	Initial value specified in assay A	Modified value B	Difference in mean results ($A - B$, % stated amount) for Triprolidine	Dextromethorphan
Weight of powdered tablet	Amount ≡ 10 mg of triprolidine hydrochloride	Amount ≡ 11 mg	+1.28	−0.05
Volume of hydrochloric acid added	60 ml	50 ml	−0.52	−0.30
Strength of acid	0.1 M	0.09 M	+0.28	−0.85
Weight of triprolidine hydrochloride standard	100 mg	90 mg	+0.02	+0.10
Weight of dextromethorphan hydrobromide standard	160 mg	150 mg	+0.33	−0.65
Wavelength settings	As in method	All 0.5 nm higher	−0.87	+1.10
Spectrometer	Pye Unicam SP8–200	Pye Unicam SP 500	+1.58	+1.20

This type of ruggedness test was not applied to the assay of pseudoephedrine because the ruggedness of the derivative UV assay for a compound with a benzenoid chromophore has been addressed in a previous publication [5]. However, the effect of dextromethorphan and triprolidine on the derivative spectrum of pseudoephedrine was measured in separate experiments and the results are plotted in Figs 3 and 4.

Figure 3
Effect of triprolidine on the second derivative assay for pseudoephedrine. Pseudoephedrine is present at the assay concentration and triprolidine at up to 150% of the assay concentration.
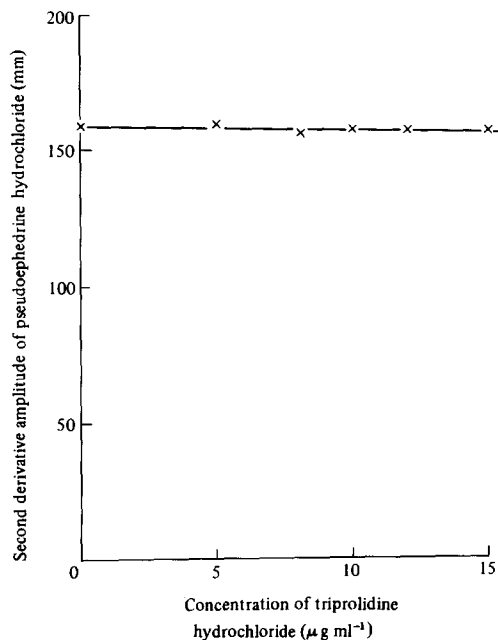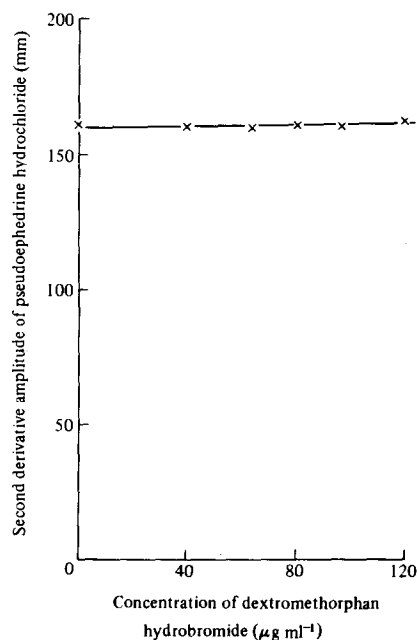
Figure 4
Effect of dextromethorphan on the second derivative assay for pseudoephedrine. Pseudoephedrine is present at the assay concentration and dextro-methorphan at up to 150% of the assay con-centration.

Dextromethorphan and triprolidine have negligible effect at concentrations up to 150% of the formula amount.

Precision of assay. Two operators each carried out four independent assays of a bulk of powdered tablets. The operators worked in the same laboratory but used different instruments and reagents. The following results (percent of stated amount) were obtained: pseudoephedrine hydrochloride — mean 98.2, standard deviation 1.1; triprolidine hydrochloride — mean 101.4, standard deviation 1.1; dextromethorphan hydrobromide — mean 98.0, standard deviation 1.1.

A single assay carried out under these conditions is expected to give a result within 3% of the mean for each component ($p = 0.95$).

Conclusions

The least-squares method for overdetermined systems can be used for pharmaceutical analysis without the purchase of specialized equipment. Comparable precision to separation techniques such as HPLC can be obtained with a manual spectrometer and a limited number of wavelengths. A combination of derivative and multiwavelength measurements gives a rapid assay for pseudoephedrine, triprolidine and dextromethorphan in tablets.

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