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Second-order derivative spectrophotometric assay for imipramine hydrochloride and diazepam in pure admixtures and in dosage forms

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Abstract

The determination of imipramine HCl and diazepam in tablets by derivative spectrophotometry is described. The drugs in combined preparations have been quantified using the second-order derivative spectra of their solutions in 0.1 M HCl. The method has been applied to pure drug mixtures as well as commerical preparations and was found to be precise and reproducible. Compliance of Beer's Law was observed in the concentration range of 10–70 μg ml^{-1} for imipramine HCl and 2–8 μg ml^{-1} for diazepam. Lower limits of detection at the 95% confidence level were 1.96 μg ml^{-1} for imipramine HCl and 0.21 μg ml^{-1} for diazepam.

Keywords: Diazepam; Imipramine HCl; Second-order derivative spectrophotometry; Simultaneous quantitation; Tablets

1. Introduction

The term derivative spectrophotometry refers to a technique in which the rate of change of spectral intensity with wavelength, i.e. the slope of the spectrum, is measured. It represents an elegant way of resolving overlapping spectra and has been successfully used for the determination of drugs alone or in mixtures [1–3]. The combination of imipramine HCl with diazepam in tablets is used for the treatment of depression associated with anxiety and agitation. Methods have been reported for the determination of imipramine HCl [4,5] and diazepam [6,7]. The present work investigates the simultaneous determination of the drugs in combined preparations without prior separation from each other or from formulation excipients by second order derivative UV spectrophotometry.

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2. Experimental

2.1. Materials, reagents and equipment

Hydrochloric acid was AR grade (E. Merck India, Ltd.); methanol was spectroscopic grade (Spectrochem. India, Ltd.); pure drug samples of Imipramine HCl U.S.P. and Diazepam U.S.P. were obtained as gifts.

The following tablet preparations were assayed: Brand A — imipramine HCl 25 mg and diazepam 5 mg; Brand B — imipramine HCl 25 mg and diazepam 5 mg; and Brand C — imipramine HCl 25 mg and diazepam 2 mg.

The second-order derivative spectra were recorded with a Jasco 7800 UV/visible double-beam scanning spectro-photometer using 10-mm matched cuvettes.

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Table 1
Selectivity of the method for the determination of imipramine HCl in the presence of diazepam by second-order derivative spectrophotometry

Composition of mixture ($\mu g\ ml^{-1}$) [a]		Mean Value [b] of $d^2A/d\lambda^2$ (at 275 nm)	Confidence Limits (95%) [c]	F test values [d]	
IMP	DIZ			Critical	Calculated
10	05	-0.0018 ± 0.0001	± 0.0001	4.48	1.0
20	05	-0.0036 ± 0.0001	± 0.0001	4.48	1.0
30	05	-0.0054 ± 0.0001	± 0.0001	4.48	1.0
40	05	-0.0072 ± 0.0002	± 0.0001	4.48	1.0
50	05	-0.0090 ± 0.0002	± 0.0001	4.48	0.25
60	05	-0.0109 ± 0.0003	± 0.0002	4.48	0.25
70	05	-0.0127 ± 0.0002	± 0.0002	4.48	0.25

[a] IMP = imipramine HCl; DIZ = diazepam.
[b] Mean value of ten replicate determinations.
[c] Based on Student's t distribution.
[d] Based on the F test for non-linearity; F critical = F_α (5,9) values from % F table for a tail area, $\alpha = 0.025$ (2.5% level of significance); F calculated = (S_y^2/S_s^2), where S_y is the standard error of the estimate and S_s is the standard deviation of a single measurement of y.

2.2. Standard and sample solution

Appropriate volumes of stock solutions of pure imipramine HCl (1 mg ml^{-1}) and diazepam (0.1 mg ml^{-1}) in methanol were used to prepare four series of solutions, seven in each series, in 0.1 M HCl. The first series (Series A) contained various concentrations of pure imipramine HCl (10–70 $\mu g\ ml^{-1}$). The second series (Series B) contained various concentrations of diazepam (2–8 $\mu g\ ml^{-1}$). The third series (Series C) contained a constant concentration of diazepam (5 $\mu g\ ml^{-1}$) and varying concentrations of imipramine HCl (10–70 $\mu g\ ml^{-1}$). The fourth series (Series D) contained a constant concentration of imipramine HCl (40 $\mu g\ ml^{-1}$) and a varying concentration of diazepam (2–8 $\mu g\ ml^{-1}$).

Twenty tablets containing imipramine HCl with diazepam were finely ground, and a weight of the powder equal to the average weight of the tablet was dissolved in methanol and filtered through Whatman No. 1 filter paper. The first and last 5 ml of the filtrate were discarded. Appropriate volumes of the filtrate were used to prepare sample solutions containing approximately 30 $\mu g\ ml^{-1}$ of imipramine HCl and approximately 6 $\mu g\ ml^{-1}$ or 3 $\mu g\ ml^{-1}$ of diazepam, respectively. Solutions of the pure drugs and of the tablet samples were scanned in a Jasco 7800 UV/visible double-beam spectrophotometer; the scan-rate was 240 nm min^{-1} and the spectral bandwidth was 3 mm. The second-order derivative spectra were obtained using digital algorithms. The results of the scan are presented in Tables 1–4.

3. Results and discussion

The technique of derivative spectrophotometry can be used for the quantification of one analyte whose peak is obscured by a larger overlapping peak of some other analyte, with minimum error [8]. The advantage of second-order derivative spectrophotometry in eliminating the background absorption due to formulation excipients has been studied [9]. The first derivative of the absorption spectrum represents the gradient at all points of the spectrum and may be used to locate the hidden peaks as $dA/d\lambda = 0$ at peak maxima; however, the higher even-order derivatives are potentially more useful for analysis. The absorption of two or more compounds in the same wavelength region, which would create inseparable interference in direct absorption spectrophotometry, can often be resolved in the derivative mode by choosing a wavelength at which the derivative signal of one analyte passes through zero.

For quantitative work, the amplitude of the derivative peak can be measured in various ways. In the present investigation, the amplitudes (indicated by h_1, h_2, h_3 and h_4 in Figs. 1, 2, 3 and 4, respectively) have been measured

Table 2
Selectivity of the method for the determination of diazepam in the presence of imipramine HCl by second-order derivative spectrophotometry

Composition of mixture (μg ml^{-1}) [a]		Mean Value [b] of $d^2A/d\lambda^2$ (at 275 nm)	Confidence Limits (95%) [c]	F test values [d]	
IMP	DIZ			Critical	Calculated
02	40	0.0037 ± 0.0001	±0.0002	4.48	0.25
03	40	0.0055 ± 0.0002	±0.0001	4.48	0.25
04	40	0.0074 ± 0.0002	±0.0001	4.48	0.25
05	40	0.0090 ± 0.0003	±0.0001	4.48	1.00
06	40	0.0108 ± 0.0001	±0.0001	4.48	0.25
07	40	0.0128 ± 0.0002	±0.0002	4.48	1.00
08	40	0.0144 ± 0.0003	±0.0002	4.48	0.25

[a] DIZ = diazepam; IMP = imipramine HCl.
[b] Mean value of ten replicate determinations.
[c] Based on Student's t distribution.
[d] Based on the F test for non-linearity at a 2.5% level of significance.

with respect to a derivative of zero, which is the true derivative amplitude [10]. Using the amplitudes of the peak with respect to a derivative of zero of the corresponding second-order derivative spectrum (h_3 in Fig. 3), imipramine HCl was quantified at 275 nm (where the $d^2A/d\lambda^2$ value of the second-derivative spectrum of diazepam is zero); similarly, diazepam was quantified by using the amplitude of the derivative spectrum (indicated by h_4 in Fig. 4) at 258 nm (where the $d^2A/d\lambda^2$ value of the second-derivative spectrum of imipramine HCl is zero). Thus, the measurements made at the zero crossing of the derivative spectrum of one of the two components would be a function only of concentration of the other component. In the case of imipramine HCl and diazepam combination, the heights of the spectra at the wavelengths 275 nm and 258 nm were found to be proportional to the concentrations of imipramine HCl (10–70 μg ml^{-1}) and diazepam (2–8 μg ml^{-1}), respectively (Figs. 3 and 4). It is well known that derivative spectrophotometry requires careful optimisation of experimental parameters, and hence the following operating parameters were carefully chosen for the present investigation: scan-speed of 240 nm min^{-1}; spectral bandwidth of 3 nm; chart expansion of $x1$. Because the zero crossing point may shift depending on the scan-speed, the above-mentioned wavelengths for the quantification of the drugs should be taken as 'working wavelengths' under the specified conditions. On changing the instrumental parameters and/or type of instrument, it would be advisable to verify the working wavelengths.

Table 3
Regression analysis of imipramine HCl and diazepam standard solution

Sample	Composition of sample (μg ml^{-1}) [a]		Regression equation [b]	Correlation coefficient (r)	Test for significance [c] of evidence of correlation	
	IMP	DIZ			Critical	Calculated
Series A	10–70	0	$y = -0.0002x + 0.0002$	0.9996	5.89	81.33
Series B	0	2–8	$y = 0.0018x + 0.0001$	0.9998	5.89	127.98
Series C	10–70	5	$y = -0.0002x + 0.0001$	0.9998	5.89	141.48
Series D	40	2–8	$y = 0.0018x + 0.0001$	0.9997	5.89	99.38

[a] IMP = imipramine HCl; DIZ = diazepam.
[b] Based on seven calibration values; x = concentration of the drug in μg ml^{-1}; measurements at 275 nm for IMP and at 258 nm for DIZ.
[c] Based on Student's t test at a significance level of 0.1% and five degrees of freedom.

Table 4
Assay results for imipramine HCl and diazepam in commercial formulations by second-order derivative spectrophotometry

Sample	Label claim [a] (mg per tablet)		Recovery [b] (% w/w)		RSD [c] (%)	
	IMP	DIZ	IMP	DIZ	IMP	DIZ
Sample A	25	05	98.54 ± 0.64	99.72 ± 1.32	0.64	1.33
Sample B	25	05	99.68 ± 0.66	98.80 ± 0.68	0.66	0.69
Sample C	25	02	100.04 ± 0.63	98.28 ± 0.73	0.63	0.74

[a] IMP = imipramine HCl; DIZ = diazepam.
[b] Mean value of five determinations (±SD), as percentage of label claim.
[c] Relative standard deviation.

The regression equations and correlation coefficients obtained by the statistical analysis of the data given in Table 1 and 2 have been presented in Table 3. The ordinate values of the regression lines were obtained directly form the video monitor by positioning the cursor at the appropriate wavelengths. These values may also be calculated from the recorder output (Figs. 3 and 4). The similarity of the regression equations of pure drug solutions to that of their mixtures, as well as the high correlation coefficient values in the range of 0.9996–0.9998, indicate the non-interference of one drug in the absorption measurements of the other at the chosen wavelengths. In addition, the values of the test for the significance of evidence of correlation based on Student's t test [11] presented in Table 3 show that the calculated t values with five degrees of freedom at a significance level of 0.1% are larger than the critical t values obtained from the t table for the same number of degrees of freedom and level of significance; thus, the results clearly confirm a strong positive correlation between the concentrations of the drugs in solution and the $d^2A/d\lambda^2$ values of the respective derivative spectra. The small values of standard deviation associated with the determination of the amplitudes of the derivative spectra of the drug mixtures (Tables 1 and 2) indicate not only the high level of precision associated with the determination of derivative values at the appropriate wavelengths for quantification, but also the independence of one drug in the absorption measurement of the other. The negligible intercepts of the equations indicate regression through or close to the origin at the chosen wavelengths.

The results of application of the F test for non-linearity [11] to the $d^2A/d\lambda^2$ values in Tables 1 and 2 have been presented in the last columns of the respective Tables. This test gives a quantitative measure of the strength of evidence for non-linearity, and the values show that the standard error of the estimate, S_y, is not too large to be compatible with the standard deviation value associated with repeated determinations at a single concentrations, S_s; this strongly indicates the existence of a linear relationship between $d^2A/d\lambda^2$ values and the drug concentrations at a significance level of 2.5%.

The standard errors of prediction of values [11] in the determination of a given concentration calculated by statistical analysis of the regression equations are presented in Figs. 5 and 6. These calculations clearly indicate that the error is minimal at concentrations of approximately 40 μg ml^{-1} and 5 μg ml^{-1} for imipramine HCl (at 275 nm) and diazepam (at 258 nm), respectively. The lower limits of detection [11] at the 95% confidence level were found to be 1.96 μg ml^{-1} for imipramine HCl and 0.21 μg ml^{-1} for diazepam.

The results of the application of the proposed method to the determination of imipramine HCl and diazepam in three different brands of tablets (brands A, B and C) are presented in Table 4. They indicate that the excipients present in these brands do not interfere in the determination. Nevertheless, it is important to confirm the non-interference of the excipients in a particular brand of tablets, if known, prior to application of the method to the formulation; this is because excipients such as dicalcium hydrogen phosphate are likely to alter the pH of the solutions, resulting in a pH different from that of the pure drug solutions. Similarly, coloring agents such as tartrazine (water soluble) absorb powerfully in the 230–

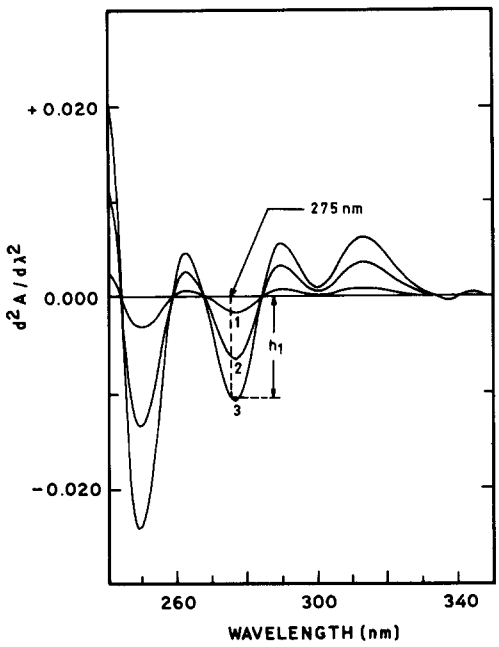

Fig. 1. Second-order derivative spectra of pure imipramine HCl in 0.1 M HCl; imipramine HCl concentration 10 μg ml^{-1}, 40 μg ml^{-1} and 70 μg ml^{-1} in curves 1, 2 and 3, respectively.

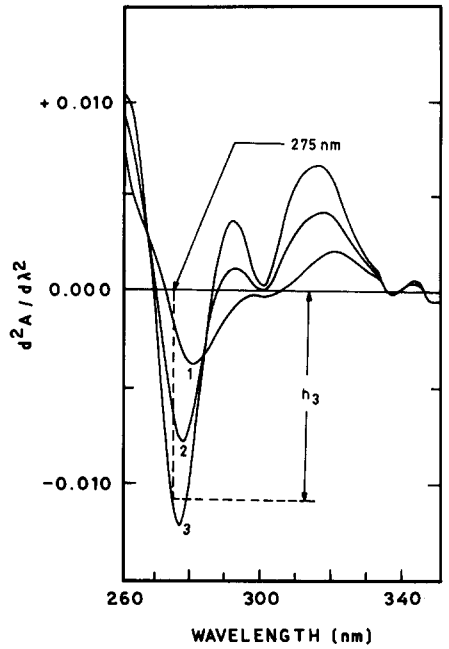

Fig. 3. Second-order derivative spectra of drug mixture in 0.1 M HCl; concentration of diazepam 5 μg ml^{-1}; concentration of imipramine HCl 10 μg ml^{-1}, 40 μg ml^{-1} and 70 μg ml^{-1} in curves 1, 2 and 3, respectively.

300 nm range, whereas titanium dioxide (insoluble in water and mineral acids) will not interfere if these solvents are used. However, the degree of interference of absorbing excipients depends on the drug-to-excipient ratios [9], and where direct determination of the drugs by the proposed method is not possible, a suitable extraction procedure to eliminate or reduce the

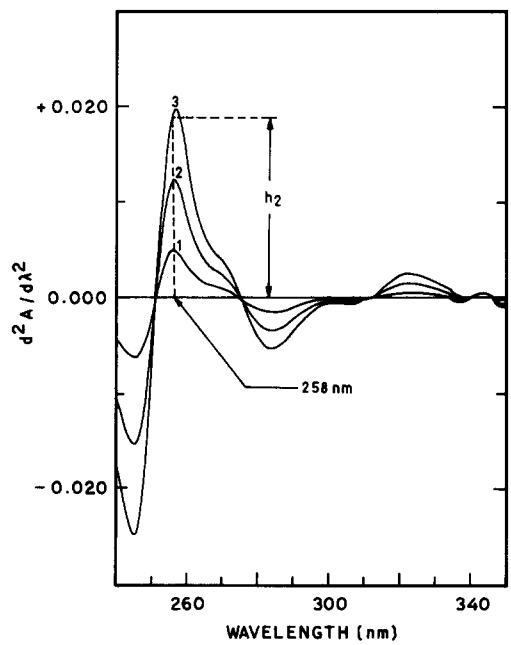

Fig. 2. Second-order derivative spectra of pure diazepam in 0.1 M HCl; diazepam concentration 2 μg ml^{-1}, 5 μg ml^{-1} and 8 μg ml^{-1} in curves 1, 2 and 3, respectively.

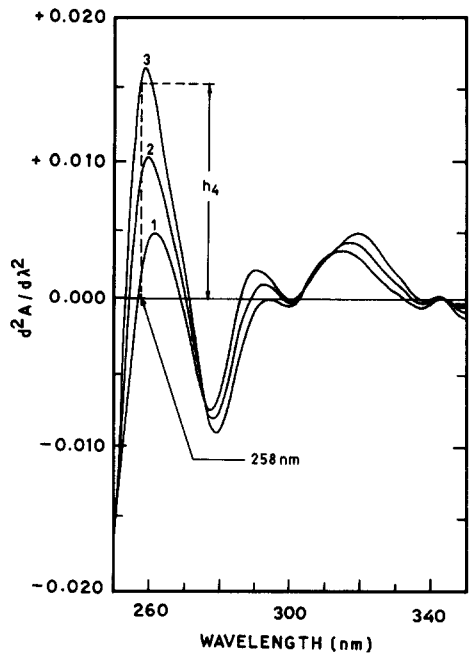

Fig. 4. Second-order derivative spectra of drug mixtures in 0.1 M HCl; concentration of imipramine HCl 40 μg ml^{-1}; concentration of diazepam 2 μg ml^{-1}, 5 μg ml^{-1} and 8 μg ml^{-1} in curves 1, 2 and 3, respectively.

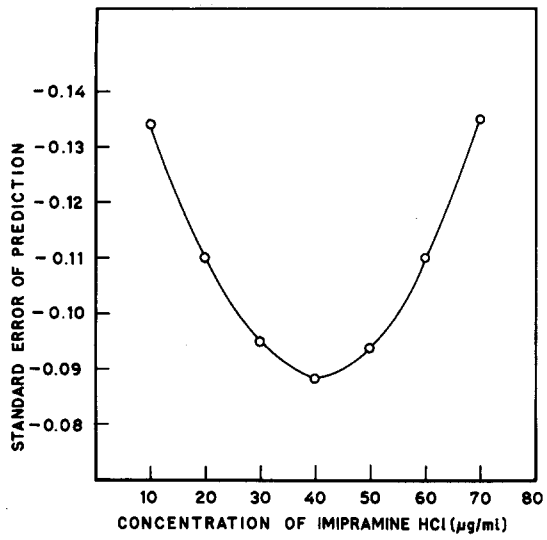

Fig. 5. Standard error of predicted values of drug mixture (Series C) solutions at 275 nm; imipramine HCl 10–70 µg ml^{-1} with 5 µg ml^{-1} of diazepam.

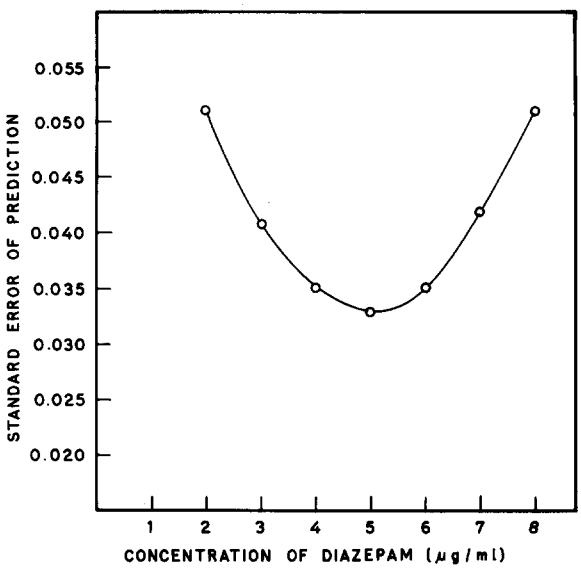

Fig. 6. Standard error of predicted values of drug mixture (Series D) solutions at 258 nm; diazepam 2–8 µg ml^{-1} with 40 µg ml^{-1} of imipramine HCl.

effect of interfering substances may be used prior to recording the derivative spectra.

The concentration of the drug solutions were chosen on the basis of the proportion of the drugs in commerical formulations as well as to achieve minimum relative error in absorption measurements [12]. The pK_a value of imipramine is 9.5 and that of diazepam is 3.3 [13]. Hence, the drug solutions in 0.1 M HCl (pH $\approx$ 1.0), which were at least two pH units away from their respective pK_a values, did not show appreciable changes in absorbance with small changes in the pH of 0.1 M HCl [14]. The stability of the standard and sample solutions (stored prior to scanning in low actinic flasks at 28–32 °C) in 0.1 M HCl were monitored spectrophotometrically (at 275 nm for imipramine HCl and 258 nm for diazepam) for 2 h, and were found to vary by the following absorbance units (AU): 0.1 M HCl solution of imipramine HCl by $\pm$0.003; 0.1 M HCl solution of diazepam by $\pm$0.005; the tablet sample solutions in 0.1 M HCl by $\pm$0.005 (at 275 nm) and by $\pm$0.004 (at 258 nm).

4. Conclusions

The quantification of two drug components in a mixture by even-order derivative spectrophotometry will depend on the fortuitous juxtaposition of the spectra, so that the maximum amplitude of spectrum of one drug lies at or near the zero value of the spectrum of the other drug. The combination of imipramine HCl with diazepam fulfills this requirement. The data in Tables 1–3 indicate the rectilinearity, precision and reproducibility of the proposed method. Other techniques such as GLC and HPLC cannot be excluded as also giving good results. However, since there are no official methods available for the simultaneous quantification of the drugs in dosage forms, and because of its low cost and ease of operation, the proposed method is likely to be very suitable for the analysis of the two drugs in tablet preparations.

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