ELSEVIER

International Journal of Pharmaceutics 108 (1994) 11–19

international journal of pharmaceutics

Determination of atenolol, nifedipine, aspirin and dipyridamole in tablet preparations by second-order derivative spectrophotometry

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(Received 14 June 1993; Modified version received 10 November 1993; Accepted 30 November 1993)

Abstract

The paper discusses the quantitation of atenolol, nifedipine, aspirin and dipyridamole in tablet preparations by derivative spectrophotometry. Atenolol and nifedipine in combined preparations have been quantified using the second-order derivative spectra of their solutions in 0.1 N hydrochloric acid. Aspirin and dipyridamole were quantified using the second-derivative difference spectra obtained by scanning equimolar drug solutions in 0.1 N HCl and 0.1 N NaOH. The method has been applied to pure drug mixtures as well as commercial preparations and is found to be precise and reproducible. The detection limits at 2.5% level of significance for the drugs of atenolol, nifedipine, aspirin and dipyridamole were found to be 1.53, 0.72, 1.30 and 1.46 μg/ml respectively.

Key words: Atenolol; Nifedipine; Aspirin; Dipyridamole; Commercial formulation; Simultaneous determination; Second-order derivative UV spectrophotometry

1. Introduction

The combination of atenolol and nifedipine as a tablet preparation has been recently introduced in the market for essential hypertension (Martindale, 1989). The combination of aspirin and dipyridamole as a tablet is being widely used for secondary prevention of myocardial infarction (Martindale, 1989). Some methods have been reported for the estimation of atenolol (Bryan and Copsey, 1975; Mehvar, 1989), nifedipine (Bruno and Suresh, 1988; Schmid et al., 1988; Suzuki and Fuji, 1988), aspirin (Norman and Joseph, 1967; Norman and and Marie 1968; Miles and Schenk, 1970) and dipyridamole (Berberi et al., 1991; Ganescu et al., 1991) individually as well as in combined dosage forms by GLC (Ramana Rao et al., 1990). The present work investigates the simultaneous determination of atenolol and nifedipine and aspirin and dipyridamole in combined preparations without prior separation from each other as well as formulation excipients by UV derivative spectrophotometry.

2. Experimental

2.1. Materials, reagents and apparatus

Hydrochloric acid, A.R. grade (E. Merck India Ltd), sodium hydroxide, A.R. grade (E. Merck

SSDI 0378-5173(93)E0368-Z

India Ltd), methanol, spectroscopic grade (spectrochem, India) were obtained from the indicated source: aspirin IP, dipyridamole USP, atenolol BP and nifedipine USP were obtained as gift samples.

The following commercial samples were assayed: Betatrop, atenolol 50 mg + nifedipine 20 mg (Sun Pharmaceuticals, India); Depten, atenolol 50 mg + nifedipine 20 mg (Cadila Chemicals, India); Presolar atenolol 50 mg + nifedipine 20 mg (Cipla Ltd, India); Cardiwell Plus, aspirin 50 mg + dipyridamole 75 mg (Torrent Pharmaceuticals Ltd India); Dynaspirin, aspirin 60 mg + dipyridamole 75 mg (US Vitamin Ltd, India); Thrombospirin, aspirin 100 mg + dipyridamole 75 mg (Sun Pharmaceuticals, India).

The second-order derivative spectra were recorded at a scan rate of 240 nm/min with a Jasco 7800 UV-visible double-beam scanning spectrophotometer using 1 cm matched quartz cuvettes.

2.2. Standard and sample solutions

The stock solutions of the drugs containing 1 mg/ml of the drug were prepared in methanol by dissolving the pure drugs in methanol. Appropriate volumes of aliquots from the stock solutions were used to prepare eight series of solutions.

The first series (Series A) consisted of solutions of atenolol of varying concentrations (10–30 μg/ml) prepared by pipetting out appropriate volumes of aliquots (1.0, 1.4, 1.8, 2.2, 2.6, 3.0 ml) from the stock solution of atenolol (1 mg/ml) into 100 ml volumetric flasks and making up the volume with 0.1 N HCl. The second series (series B) consisted of solutions of nifedipine of varying concentration (5–20 μg/ml) prepared in a similar fashion in 0.1 N HCl. The third series (series C) consisted of mixtures of the drugs having a constant concentration of nifedipine (10 μg/ml) and a varying concentration of atenolol (10–30 μg/ml) prepared by pipetting out the same volumes of aliquots from atenolol stock solution as in the preparation of series A solutions into 100 ml volumetric flasks, adding 1 ml of nifedipine stock solution (1 mg/ml) to each flask and making up the volume with 0.1 N HCl. Similarly, the fourth series (series D) of solutions were prepared by using appropriate volumes of aliquots from the atenolol and nifidepine stock solutions so as to give solutions containing various concentrations of nifedipine (5–20 μg/ml) along with a constant concentration of atenolol (20 μg/ml) in 0.1 N Hcl.

The quantitation of aspirin and dipyridamole was performed using the second-order derivative difference spectra. Hence, the four series of drug solutions were prepared in a slightly different manner from that of the solutions of atenolol and nifedipine. In the case of aspirin as well as dipyridamole, each series was prepared in duplicate as equimolar solutions in 0.1 N HCl and 0.1 N NaOH to record derivative difference spectra (Fell, 1985). Thus, each of the series comprised 12 solutions out of which the first six were in 0.1 N HCl and the remaining six were in 0.1 N NaOH. The first series (series E) of solutions were equimolar solutions in 0.1 N HCl and 0.1 N NaOH of varying concentrations of aspirin (20–40 μg/ml). These were prepared by pipetting out appropriate volumes of aliquots (2.0, 2.4, 2.8, 3.2, 3.6, and 4.0 ml) of the aspirin stock solution in methanol (1 mg/ml) into 12 volumetric flasks. Each volume of aliquot was pipetted out twice, i.e., in duplicate, so as to prepare equimolar solutions of each concentration in 0.1 N HCl and 0.1 N NaOH. Hence, the volume in six out of the 12 flasks was made up with 0.1 N HCl and in the rest with 0.1 N NaOH. The second series (series F) consisted of equimolar solutions of dipyridamole in 0.1 N HCl and 0.1 N NaOH of varying concentration (20–40 μg/ml) and were prepared in a similar fashion to that of series E. The third and fourth series consisted of equimolar solutions of mixtures of aspirin and dipyridamole. The third series (series G) consisted of equimolar solutions containing a varying concentration of aspirin (20–40 μg/ml) and a constant concentration of dipyridamole (30 μg/ml) and the fourth series (series H) consisted of equimolar solutions containing a varying concentration of dipyridamole (20–40 μg/ml) along with a constant concentration of aspirin (30 μg/ml) in 0.1 N HCl and 0.1 N NaOH, prepared in a similar manner to that of the mixtures of atenolol and nifedipine.

20 tablets of atenolol and nifedipine (of each brand) were finely ground and a weight of the powder equal to the average weight of the tablet was dissolved in methanol, filtered (Whatman No. 1 filter paper) and appropriate volumes of aliquots of the filtrate were used to prepare sample solutions containing approx. 25 μg/ml of atenolol (and 10 μg/ml of nifedipine) respectively. Similarly, 20 tablets of aspirin and dipyridamole (of each brand) were processed to obtain solutions containing approx. 25, 30 or 38 μg/ml of dipyridamole (and approx. 33, 24 or 25 μg/ml of aspirin) respectively.

3. Results and discussion

The technique of derivative spectrophotometry may be used for the quantitation of one analyte whose peak is obscured by a larger overlapping peak of some other analyte with minimum error (Haver, 1979). The advantage of second-order derivative spectrophotometry in eliminating the background absorption due to formulation excipients has been studied previously (Traveset et al., 1980). The first derivative of an absorption spectrum represents the gradient at all points of the spectrum and may be used to locate hidden peaks since $dA/d\lambda = 0$ at peak maximum, but the higher even-order derivatives are potentially more useful for analysis. The even-order derivatives are bipolar functions of alternating signs at the centroid (negative for second, positive for fourth, etc.) whose position coincides with that of the original peak maximum (Fell, 1985). Therefore, 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 one analyte's derivative signal goes through a value of zero. Although the derivative centroid peak width of a Guassian peak decreases to 53, 41 and 34% of the original peak width in the second, fourth and sixth orders, respectively, resulting in increased resolution of overlapping peaks, the increasingly complex satellite patterns detract from resolution enhancement in higher derivative spectra (Fell, 1985). Hence, the second-order derivative spectra of the drugs were chosen for quantitation. For quantitation of atenolol and nifedipine, the wavelengths of 227 and 219 nm were chosen, since the peak of the second-order spectrum of atenolol lies at or near the zero value of nifedipine and vice versa at these wavelengths.

For the quantitation of aspirin and dipyridamole, the second-order derivative difference spectra of the drugs recorded with the 0.1 N NaOH solutions in the sample path and the corresponding equimolar 0.1 N HCl solutions in the reference path were used (Fig. 3 and 4). This involves the successful combination of difference spectrophotometry with derivative spectrophotometry to give second-derivative difference spectra when enhanced discrimination against interfering substances and sharpened fine structural features were observed (Fell, 1985).

The technique of difference spectrophotometry has proved particularly useful in the determination of medicinal substances by eliminating specific interference from degradation products and co-formulated drugs and involves the reproducible alteration of the spectral properties of the analyte in equimolar solutions and the measurement of the absorbance difference between the two solutions provided the absorbances of the other absorbing interferents are not affected by the reagents used for the spectral property alteration. Simple aqueous acids, alkalis and buffers are most frequently used for inducing spectral alterations, since many drugs are weak acids or bases whose state of ionisation and absorptivity depend on the pH of the solution (Doyle and Fezzari, 1974).

In the present investigation, the second-order derivative difference spectra were produced from the zero-order difference spectra recorded by scanning the 0.1 N HCl and 0.1 N NaOH equimolar drug solutions in the spectrophotometer. The zero-order difference spectra of aspirin were recorded with a 0.1 N HCl solution of aspirin in the reference path and an equimolar 0.1 N NaOH solution of aspirin in the sample path. Similarly, the zero-order difference spectra of dipyridamole were recorded with a 0.1 N HCl solution of

dipyridamole in the reference path and an equimolar 0.1 N NaOH solution of dipyridamole in the sample path. These zero-order difference spectra were then converted to second-order derivative difference spectra using digital algorithms. The pK_a values of aspirin and dipyridamole are 3.5 and 6.4, respectively (Martindale, 1989). Hence, equimolar solutions of the drugs in 0.1 N HCl (pH ≈ 1) and 0.1 N NaOH (pH ≈ 13.0) which were at least 2.0 pH units away from the pK_a values produced about 90% of individual species and small changes in the pH values of 0.1 N HCl and 0.1 N NaOH did not result in large alteration in the absorbance values (Doyle and Fezzari, 1974) during the recording of the zero-order difference spectra. The peaks of the second-order derivative difference spectra of aspirin and dipyridamole lie at or near the zero value of one another at the chosen wavelengths of 272 and 310 nm and hence the heights of the spectra at these wavelengths have been used for quantitation of the drugs.

For quantitative work, the amplitude of a derivative peak can be measured in various ways. In the present investigation, the amplitudes have been measured with respect to derivative zero which is the true derivative amplitude (Fell, 1985). Using the amplitudes of the peak to derivative zero of the corresponding second-order derivative spectra (denoted by h_1 in Fig. 1) atenolol was quantified at 227 nm (where the derivative spectrum of nifedipine shows a value of zero) and nifedipine was quantified by using the amplitudes of the spectra (denoted by h_2 in Fig. 2) at 219 nm (where the derivative spectrum of atenolol has zero value), respectively.

Similarly, using the true derivative amplitudes of the derivative difference spectra (Fig. 3 and 4), aspirin was quantified by using the derivative amplitudes (denoted by h_3 in Fig. 3) at 272 nm (where the $d^2A/d\lambda^2$ value of the derivative difference spectrum of dipyridamole is zero) and dipyridamole by using the amplitudes (denoted by h_4 in Fig. 4) at 310 nm (where the $d^2A/d\lambda^2$ value of the derivative difference spectrum of aspirin is zero). The amplitudes of the derivative spectra of solutions of different concentrations of drugs (Table 1–4) were directly measured from the video

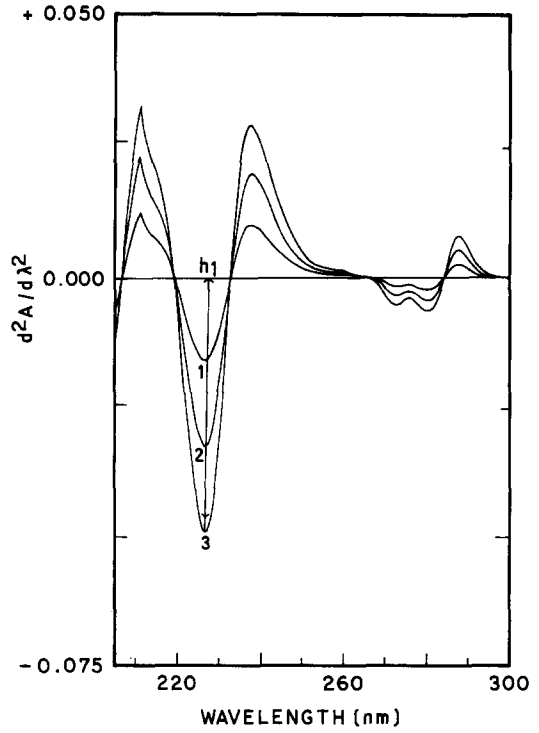

Fig. 1. Second-order derivative spectra of pure atenolol in 0.1 N HCl (atenolol concentration: 10, 20 and 30 μg/ml in curves 1, 2 and 3, respectively).

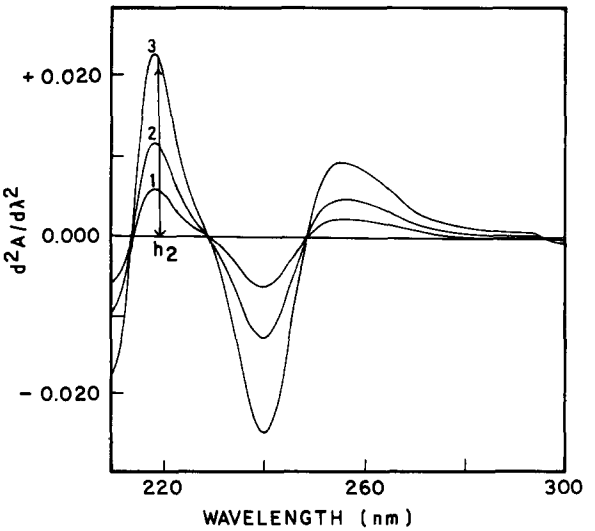

Fig. 2. Second-order derivative spectra of pure nifedipine in 0.1 N HCl (nifedipine concentration: 5, 10 and 20 μg/ml in curves 1, 2 and 3, respectively).

Table 1
Selectivity of the method for the determination of atenolol in the presence of nifedipine by second-order derivative spectrophotometry

Composition of mixture (μg/ml)		Mean value [a] of $d^2A/d\lambda^2$ (at 227 nm)	Confidence [b] limit (95%)	F test values [c]	
ATN	NIF			Crt	Cal
10	10	−0.0161 ± 0.0003	±0.0003	4.72	4.00
14	10	−0.0217 ± 0.0004	±0.0003	4.72	2.25
18	10	−0.0279 ± 0.0004	±0.0002	4.72	2.25
22	10	−0.0359 ± 0.0004	±0.0003	4.72	2.25
26	10	−0.0410 ± 0.0003	±0.0001	4.72	4.00
30	10	−0.0475 ± 0.0005	±0.0001	4.72	1.44

ATN, atenolol; NIF, nifedipine; Crt, critical value; Cal, calculated value;
[a] Mean value of 10 replicate determinations.
[b] Based on Student's t-test distribution.
[c] Based on F test for non-linearity; F critical = $F\alpha(4,9)$ values from % F table for a tail area, $\alpha = 0.0025$ (2.5% level of significance); F calculated = (S_y^2/S_s^2) where S_y is the standard error of estimate and S_s is the standard deviation of the single measurement of y.

monitor by positioning the cursor at the appropriate wavelengths for quantitation. They may also be measured from the curves obtained on chart paper as recorder output (Fig. 1–4).

The regression equations and correlation coefficients obtained from the data in Tables 1–4 at the appropriate chosen wavelengths are given in Tables 5 and 6.

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 order of 0.99 indicate the non-interference of one drug on the absorption measurement of the other at the chosen wavelengths. In addition, the values of the test for significance of evidence of correlation based on Students's t-test (Roach and McCormick, 1987) presented in Tables 5 and 6 show that the calculated t values with four 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 and clearly confirm the existence of strong positive correlation between the concentrations of the drugs in solu-

Table 2
Selectivity of the method for the determination of nifedipine in the presence of atenolol by second-order derivative spectrophotometry

Composition of mixture (μg/ml)		Mean value [a] of $d^2A/d\lambda^2$ (at 219 nm)	Confidence [b] limit (95%)	F test values [c]	
NIF	ATN			Crt	Cal
05	20	0.0054 ± 0.0002	±0.0001	4.72	1.00
08	20	0.0093 ± 0.0001	±0.0001	4.72	4.00
11	20	0.0127 ± 0.0001	±0.0001	4.72	4.00
14	20	0.0159 ± 0.0003	±0.0002	4.72	0.44
17	20	0.0190 ± 0.0002	±0.0002	4.72	1.00
20	20	0.0223 ± 0.0002	±0.0001	4.72	1.00

ATN, atenolol; NIF, nifedipine; Crt, critical value; Cal, calculated value.
[a] Mean value of 10 replicate determinations.
[b] Based on Student's t-test distribution.
[c] Based on F test for non-linearity at 2.5% level of significance.

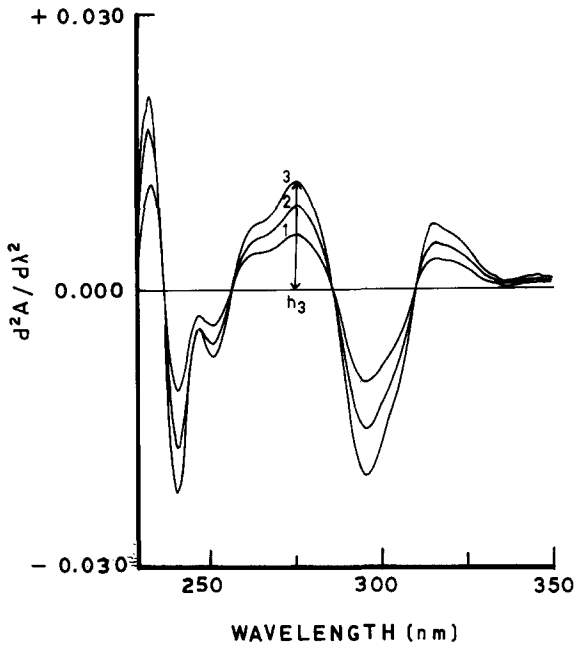

Fig. 3. Second-order derivative difference spectra of pure aspirin obtained by scanning 0.1 N NaOH solutions vs corresponding equimolar 0.1 N HCl solutions (aspirin concentration: 10, 20 and 30 μg/ml in curves 1, 2 and 3, respectively).

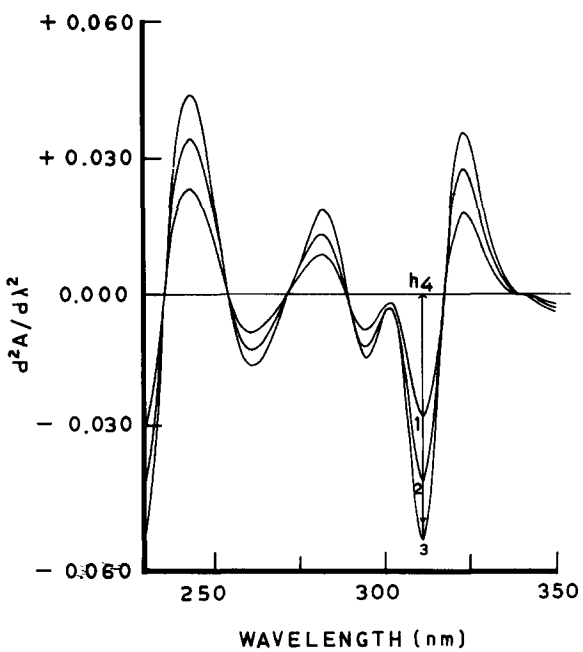

Fig. 4. Second-order derivative difference spectra of pure dipyridamole obtained by scanning 0.1 N NaOH solutions vs corresponding equimolar 0.1 N HCl solutions (dipyridamole concentration: 10, 20 and 30 μg/ml in curves 1, 2 and 3, respectively).

tion and the $d^2A/d\lambda^2$ values (i.e., heights) of the respective derivative spectra. The small standard deviation values associated with the determination of the amplitudes of the derivative spectra (Table 1–4) of the drug mixtures indicate the high level of precision associated with the determination of derivative values at the appropriate wavelengths for quantitation as well as the independence of one drug from the absorption mea-

Table 3
Selectivity of the method for the determination of aspirin in the presence of dipyridamole by second-order derivative difference spectrophotometry

Composition of mixture (μg/ml)		Mean value [a] of $d^2A/d\lambda^2$ (at 272 nm)	Confidence [b] limit (95%)	F test values [c]	
				Crt	Cal
ASP	DIP				
20	30	0.0054 ± 0.0001	±0.0001	4.72	1.00
24	30	0.0066 ± 0.0002	±0.0002	4.72	0.25
28	30	0.0078 ± 0.0002	±0.0002	4.72	0.25
32	30	0.0093 ± 0.0002	±0.0001	4.72	0.25
36	30	0.0101 ± 0.0001	±0.0001	4.72	1.00
40	30	0.0117 ± 0.0001	±0.0001	4.72	1.00

ASP, aspirin; DIP, dipyridamole; Crt, critical value; Cal, calculated value.
[a] Mean value of 10 replicate determinations.
[b] Based on Student's t-test distribution.
[c] Based on F test for non-linearity at 2.5% level of significance

Table 4
Selectivity of the method for the determination of dipyridamole in the presence of aspirin by second-order derivative difference spectrophotometry

Composition of mixture (μg/ml)		Mean value [a] of $d^2A/d\lambda^2$ (at 310 nm)	Confidence [b] limit (95%)	F test values [c]	
DIP	ASP			Crt	Cal
20	30	-0.0277 ± 0.0002	± 0.0001	4.72	4.00
24	30	-0.0325 ± 0.0003	± 0.0002	4.72	1.78
28	30	-0.0377 ± 0.0002	± 0.0002	4.72	4.00
32	30	-0.0435 ± 0.0002	± 0.0002	4.72	4.00
36	30	-0.0484 ± 0.0003	± 0.0002	4.72	1.78
40	30	-0.0545 ± 0.0002	± 0.0002	4.72	4.00

ASP, aspirin; DIP, dipyridamole; Crt, critical value; Cal, calculated value.
[a] Mean value of 10 replicate determinations.
[b] Based on Student's t-test distribution.
[c] Based on F test for non-linearity at 2.5% level of significance

surement of the other. The negligible intercepts of the equations indicate regression through or close to the origin at the chosen wavelengths.

In order to assess the rectilinearity of the calibration curves represented by the regression equations, the F test for non-linearity (Roach and McCormick, 1987) which gives a quantitative measure of the strength of evidence for non-linearity was applied to the $d^2A/d\lambda^2$ values presented in Tables 1–4 and the results are presented in the last columns of the respective tables. The values strongly indicate the existence of

Table 5
Regression analysis of atenolol and nifedipine standard solutions

Sample	Composition of sample (μg/ml)		Regression equation [a] (at 227 nm for ATN and at 219 nm for NIF)	Correlation coefficient	Test for significance [b] of evidence of correlation	
	ATN	NIF			Critical	Calculated
Series A	10–30	0	$y = -0.0016x + 0.0002$	0.9987	7.17	39.81
Series B	0	5–20	$y = 0.0011x + 0.0001$	0.9995	7.17	65.59
Series C	10–30	10	$y = -0.0015x + 0.0004$	0.9990	7.17	46.77
Series D	20	5–20	$y = 0.0011x + 0.0001$	0.9994	7.17	58.36

ATN, atenolol; NIF, nifedipine.
[a] Based on six calibration values; x, concentration of the drug (in μg/ml).
[b] Based on Student's t-test at a significance level of 0.1% and 4 degrees of freedom.

Table 6
Regression analysis of aspirin and dipyridamole standard solutions

Sample	Composition of sample (μg/ml)		Regression equation [a] (at 272 nm for ASP and at 310 nm for DIP)	Correlation coefficient	Test for significance [b] of evidence of correlation	
	ASP	DIP			Critical	Calculated
Series E	20–40	0	$y = 0.0003x - 0.0007$	0.9990	7.17	46.60
Series F	0	20–40	$y = -0.0013x + 0.0002$	0.9993	7.17	56.70
Series G	20–40	0	$y = 0.0003x - 0.0006$	0.9989	7.17	43.63
Series H	0	20–40	$y = -0.0013x - 0.0003$	0.9993	7.17	54.17

ASP, aspirin; DIP, dipyridamole.
[a] Based on six calibration values; x, concentration of the drug (in μg/ml).
[b] Based on Student's t-test at a significance level of 0.1% and 4 degrees of freedom.

Table 7
Assay results of atenolol and nifedipine in commercial formulations by second-order derivative spectrophotometry

Sample	Average weight [a] (per tablet in mg)	Label claim (mg/tablet)		Mean recovery (%) [b]		CV (%) [c]	
		ATN	NIF	ATN	NIF	ATN	NIF
Brand A	322.2	50	20	99.76 ± 0.27	99.08 ± 0.31	0.27	0.31
Brand B	342.4	50	20	98.82 ± 0.23	99.92 ± 0.36	0.23	0.36
Brand C	310.7	50	20	99.96 ± 0.27	98.68 ± 0.37	0.27	0.37

ATN, atenolol; NIF, nifedipine.
[a] Mean value of weights of 20 tablets.
[b] Mean of five determinations; assay as percentage of label claim.
[c] Coefficient of variance (%).

a linear relationship between $d^2A/d\lambda^2$ values and the drug concentrations at a significance level of 2.5%. Thus, the adopted graphical second-order derivative (obtained by using digital algorithms) fulfills the criterion of linear response with concentration for all the four drugs. The results of the assay of the commercial formulations by the proposed method are listed in Tables 7 and 8.

The concentrations of the drug solutions were chosen on the basis of the proportion of the drugs in commercial formulations as well as to have minimum relative error in absorption measurement (Connors, 1982).

The solutions of aspirin, dipyridamole and atenolol were prepared and stored under room conditions (temperature ≈ 25–28°C) in ordinary Pyrex glass volumetric flasks (class A). The solutions containing nifedipine (pure, mixtures with atenolol as well as tablet samples) were prepared under red light and stored in low actinic Pyrex volumetric flasks at room temperature until their analysis in order to avoid photodegradation (Syed Ali, 1989). The stability of the solutions was monitored spectrophotometrically for a period of 3 h and was found to vary by the following absorbance units (AU): aspirin in 0.1 N HCl by ±0.001 AU and in 0.1 N NaOH by ±0.009 AU; dipyridamole in 0.1 N HCl by ±0.001 AU and in 0.1 N NaOH by ±0.009 AU; atenolol in 0.1 N HCl by ±0.002 AU and nifedipine in 0.1 N HCl by ±0.005 AU. All the measurements for replicate determinations were recorded within a time interval of 60–90 min after preparation of the solutions in 0.1 N HCl and 0.1 N NaOH to minimise the variations in absorbance with time.

4. Conclusion

The quantitation 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

Table 8
Assay results of aspirin and dipyridamole in commercial formulations by second order derivative difference spectrophotometry

	Average weight [a] (per tablet in mg)	Label claim (mg/tablet)		Mean recovery (%) [b]		CV (%) [c]	
		ASP	DIP	ASP	DIP	ASP	DIP
Brand A	387.7	60	75	98.90 ± 0.19	100.36 ± 0.32	0.19	0.32
Brand B	354.8	60	75	99.94 ± 0.34	99.90 ± 0.25	0.34	0.25
Brand C	345.4	100	75	98.90 ± 0.28	99.00 ± 0.47	0.29	0.47

ASP, aspirin; DIP, dipyridamole.
[a] Mean value of weights of 20 tablets.
[b] Mean of five determinations; assay as percentage of label claim
[c] Coefficient of variance (%)

the spectrum of one drug lies at or near the zero value of the spectrum of the other drug. The combination of atenolol and nifedipine as well as aspirin and dipyridamole fulfills this requirement. The quantitation of aspirin and dipyridamole has been carried out using derivative difference spectra which give enhanced discrimination against interfering substances. The data in Tables 1–6 with high values of correlation coefficients and low standard deviations as well as the results of the F test for non-linearity and test for significance of evidence for correlation indicate the strong positive correlation, rectilinearity and precision of the measurements by the proposed method. In the absence of official methods for the simultaneous quantitation of atenolol and nifedipine as well as aspirin and dipyridamole, the proposed methods are found to be very suitable for the determination of the drugs in tablets.

Acknowledgements

The authors are grateful to Messrs IPCA Laboratories, India and Messrs Nicholas Piramel India Ltd, India for the gift samples of pure atenolol and nifedipine, respectively. The authors also wish to thank Messrs Torrent Pharmaceuticals Ltd, Ahmedabad, India for the gift sample of dipyridamole.

References

Berberi, M., Merlin, J.L. and Weber, B., Sensitive determination of free and plasma protein bound dipyridamole by high pressure liquid chromatography. *J. Chromatogr. Biomed. Appl.*, 565 (1991) 511–515.

Bruno, L. and Suresh, J., New spectrophotometric method based on reaction with vanillin in methanol phosphoric acid medium and folin reagent for determination of nifedipine in dosage forms. *Ind. J. Pharm. Sci.*, 50 (1988) 109–112.

Bryan, S. and Copsey, B.P., The gas chromatographic determination of atenolol in biological samples. *J. Pharm. Pharmacol.* 27 (1975) 430–433.

Connors, K.A., Absorption spectroscopy. *A Textbook of Pharmaceutical Analysis*, 3rd Edn, Wiley, New York, 1982, p. 195.

Doyle, T.D. and Fazzari, F.R., Determination of drugs in dosage forms by difference spectrophotometry. *J. Pharm. Sci.*, 63 (1974) 1921–1926.

Fell, A.F., Ultraviolet, visible and fluorescence spectrophotometry. In Mofatt, A.C. (Ed.), *Clarke's Isolation and Identification of Drugs*, 2nd Edn, The Pharmaceutical Press, London, 1985, pp. 230–231.

Ganescu, I., Preda, M. and Papa, I., Thiocyanatechromium(III) complexes in chemical analysis. 49. Determination of dipyridamole using these complexes. *Arch. Pharm.*, 324 (1991) 321–323.

Haver, C.O., Derivative and wavelength modulation spectrometry. *Anal. Chem.*, 51 (1979) 91A–93A.

Martindale, *The Extra Pharmacopoeia*, 29th Edn, The Pharmaceutical Press, London, 1989, pp. XXIV–XXV, 6, 784, 1511.

Mehvar, R., Liquid chromatographic analysis of atenolol enantiomers in human plasma and urine. *J. Pharm. Sci.*, 78 (1989) 1035–1039.

Miles, C.I. and Schenk, G.H., Fluorescence of acetylsalicylic acid in solution and its measurement in presence of salicylic acid. *Anal. Chem.*, 27 (1970) 425–429.

Norman, A.S. and Joseph, I.R., Use of isosbestic point as a base line in differential spectrophotometry. *Anal. Chem.*, 39 (1967) 414.

Norman, A.S. and Marie, K., Determination of acetylsalicylic acid, salicylamide, acetaminophen and caffeine in tablets or powders by independent methods. *J. Pharm. Sci.*, 57 (1968) 1218–1220.

Ramana Rao, G., Avadhanalu, A.B. and Giridhar, R., estimation of atenolol and nifedipine in single and combined dosage forms by GLC, 33, (1990) 113–115.

Roach, A. and McCormick, D., The elementary statistics of calibration. In Chapman, N.B. (Ed.), *Measurement, Statistics and Computation*, 2nd Edn, Wiley, New York, 1987, pp. 379–400.

Schmid, B.J., Perry, H.E. and Idle, J.R., Determination of nifidepine and the metabolites in plasma and urine by automated electron capture capillary gas chromatography. *J. Chromatogr. Biomed. Appl.*, 425 (1989) 107–119.

Suzuki, H., Fujiwara, S., Kondo, S. and Sugimato, I., Determination of nifedipine in human plasma by HPLC with electro chemical detection. *J. Chromatogr. Biomed. Appl.*, 341 (1985) 341–347.

Syed Ali, L., Nifedipine. In Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, Vol. 18, Academic Press, New York, 1989, pp. 250–251.

Traveset, V.S., Gonzalo, R. and Gelpi, E., Derivative and graphical procedures for correction of irrelevant UV spectrophotometric absorption in changeable matrices. *J. Pharm. Sci.*, 69 (1980) 629–633.