ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
16 (1997) 21–30

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Analysis of nifedipine–acebutolol hydrochloride binary combination in tablets using UV-derivative spectroscopy, capillary gas chromatography and high performance liquid chromatography

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Received 25 June 1996; received in revised form 30 September 1996

Abstract

Three methods are described for the simultaneous determination of nifedipine and acebutolol hydrochloride in combined pharmaceutical tablets. The first method depends on first-derivative ultraviolet spectrophotometry, with peak-to-base and zero-crossing measurements methods. The first derivative amplitudes at 400 and 352 nm were selected for the assay of nifedipine and acebutolol hydrochloride, respectively. Calibration graphs follow Beer's law in the range of 4–12 and 44–132 μg ml^{-1} and the linearity was satisfactory ($r = 0.999$) for nifedipine and acebutolol hydrochloride, respectively. The second method was based on the separation of nifedipine from acebutolol hydrochloride, with an internal standard thymolphthalein, using capillary gas–liquid chromatography with a programmable temperature change. The third method was based on high performance liquid chromatographic separation of the two drugs on a reversed-phase, C_{18}, column using a mobile phase of methanol–water (55:45, pH 4.5) with a programmable flow rate of 1 ml min^{-1} for 4 min which changed to 2 ml min^{-1} for the rest of the run. The detection was done at 260 nm using oxprenolol hydrochloride as an internal standard. Both chromatographic methods showed good linearity, precision and reproducibility. No spectral or chromatographic interference from the tablet excipients were found. The proposed methods were successfully applied to the assay of commercial tablets and a content uniformity test. The procedures were rapid, simple, nondestructive and suitable for quality control application.

Keywords: Acebutolol hydrochloride; First-derivative spectroscopy; Gas–liquid chromatography; High performance liquid chromatography; Nifedipine; Pharmaceutical tablets

1. Introduction

Nifedipine, (NP), [1,4-dihydro-2,6-dimethyl-4-(2-nitro-phenyl)-3,5-pyridine carboxylic dimethyl ester] is a widely used antihypertensive and antianginal drug belong to a group of compounds known as calcium channel antagonists. Acebutolol hydrochloride (AC), 1-(2-acetyl-4-*n*-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane is a beta-blocker which normalises the

0731-7085/97/$17.00
PII S0731-7085(96)01961-9

blood pressure and prevents the occurrence of hypertensive crisis. The combination of both drugs, in tablet form, increases their antihypertensive effects.

Both drugs are official in the USP XXIII [1], while NP is only official in BP 1993 [2]. Their combination is not yet official in any pharmacopoeia. The USP XXIII methods for analysis of bulk and tablets of NP and AC are high performance liquid chromatography (HPLC). The BP 1993 described a non-aqueous titration procedure for the determination of NP in bulk. Several analytical methods have been reported for assaying NP in its dosage forms and these include HPLC [3–8], gas–liquid chromatography (GLC) [9–12] and spectrophotometric methods [13–18].

HPLC [19–21], thin layer chromatography (TLC) [22] and spectrophotometric [23–30] methods have been described for AC determination in pharmaceutical tablets.

No method has been reported for their simultaneous determination in two component mixtures. The analytical profiles of NP [31] and AC [32] including their stability and some of the analytical methods used for their determination have been published.

The aim of this work was to demonstrate the capability of the first-derivative (^{1}D) method to resolve and overcome the problem of overlapping spectral bands and allow the simultaneous determination of NP and AC without need for prior separation. At the same time, the chromatographic procedures (GLC and HPLC) were functional as reference methods. The study compares the three methods for the simultaneous assay of NP and AC. The utility of the developed methods to determine the content of both drugs in commercial tablets was also demonstrated.

2. Experimental

2.1. Materials

Authentic NP (Siegfried, Switzerland) and AC (Rhone Poulenc, Paris, France) were supplied as free gifts from Pharco Pharmaceutical (Alexandria, Egypt) and Alexandria Pharmaceutical (Alexandria, Egypt), and used without further purification. The purity of both drugs, as assessed by the USP XXIII methods, were 99.5 and 99.2% for NP and AC, respectively. Samples from the photo-decomposition products of NP were purchased from the USP pharmacopoeial office. Thymolphthalein and oxprenolol hydrochloride were used as internal standards in the GLC and HPLC methods, respectively. Both internal standards were inhouse standards and their purities certified to be 99.8 and 99.0%, respectively. The common

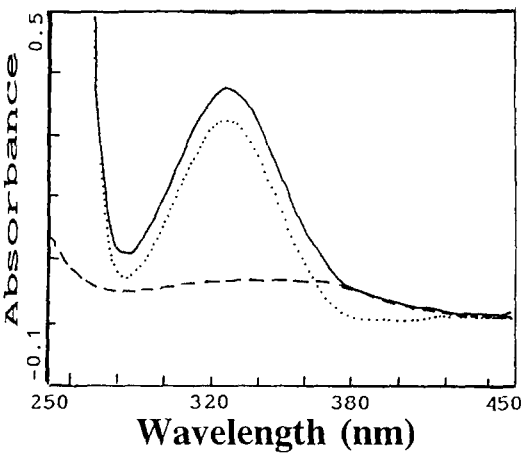

Fig. 1. Absorption (zero-order) UV spectra of 4 μg ml^{-1} NP (—-), 44 μg ml^{-1} AC (.....) and their binary mixture (——) in methanol.

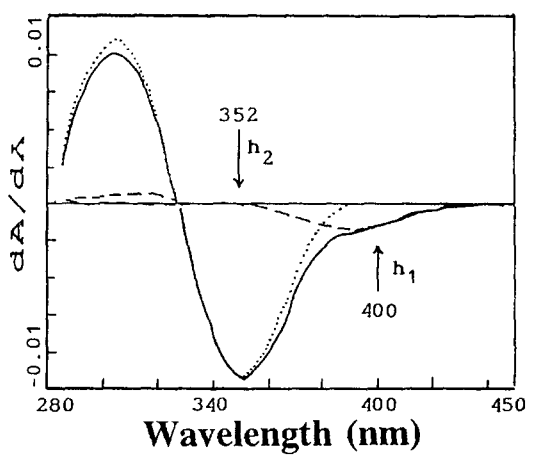

Fig. 2. First-derivative spectra of 4 μg ml^{-1} NP (—-), 44 μg ml^{-1} AC (.....) and their binary mixture (——) in methanol.

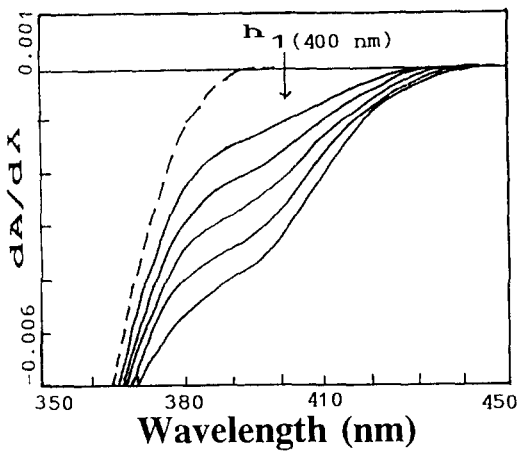

Fig. 3. First-order derivative spectra of mixture containing 44 μg ml^{-1} AC plus an increasing amount of NP ranging from 4 to 12 μg ml^{-1} (——) and the first-order derivative spectrum of 44 μg ml^{-1} AC (—-) in methanol.

excipients used in tablet manufacturing [33] were obtained from a local commercial sources. Methanol (Romel Chem, UK) was HPLC grade and water was doubly distilled from an all glass apparatus. Tablets of Tredalat (BN EU-305) (Bayer Leverkusen, Germany), labelled to contain 10 mg NP and 110.8 mg AC (100 mg of acebutolol base) per tablet, were used.

2.2. Apparatus and chromatographic conditions

Spectrophotometric analysis was performed on a Hewlett-Packard Diode-Array spectrophotometer model HP/8451A using a 1 cm quartz cell and bandpass of 2 nm. The instrument settings were: derivative mode ^{1}D (dA/dλ) with seven smoothing points and a wavelength range of 450–250 nm.

A Hewlett-Packard gas chromatograph model HP/5890 equipped with a flame ionization detector (FID) was used. The capillary column was a cross linked methylsilicone gum, 12 m × 0.33 mm i.d. (HP-1, Hewlett-Packard). The carrier gas, nitrogen, flowed at 2 ml min^{-1} and nitrogen make-up gas at 30 ml min^{-1}. The injection mode was split on a split/splitless fitting with a split ratio of 1:50. The column temperature was programmed from 150 to 300°C in steps of 20°C min^{-1} and kept at 300°C for 3 min. The injection port and the detector temperatures were set at 225 and 325°C, respectively. The peak areas integrations were performed using an HP 3392A integrator. The integrator conditions were set as follows; attenuation, 4; chart speed, 0.5 cm min^{-1}; threshold, 3; peak width, 0.04; and area rejection, 2000. The injection volume was 1 μl.

A Hewlett-Packard high performance liquid chromatograph, model HP/1090, equipped with a UV-diode-array detector, binary DR5 solvent delivery system and interfaced with an HP/85-B personal computer, was used. The peak areas integrations were performed using an HP 3392A integrator. The samples (10 μl) were injected automatically using an autoinjection system. A 25 cm × 4.6 mm i.d., reverse-phase column (Lichrosorb RP-C_{18}, Merck, Darmstadt, Germany), packed with 10 μm particles, was used. The flow rate was programmed to be 1 ml min^{-1} and changed to 2 ml min^{-1} after 4 min from the beginning of each run.

All the determinations were performed at ambient temperature. The detection was done at 260 nm with a bandwidth of 10 nm. The automatic integrator conditions was set to be: chart speed, 0.3 cm min^{-1}; threshold, 3; area rejection, 2000; and peak width, 0.04.

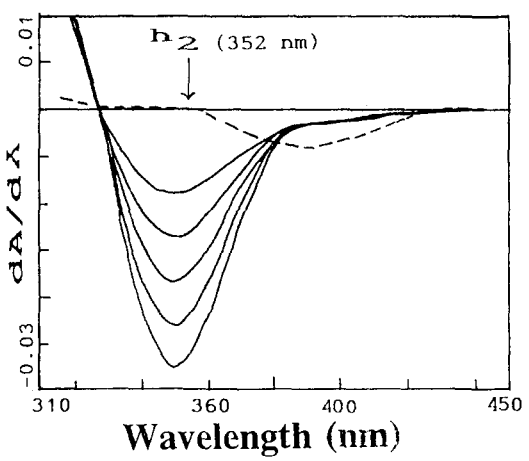

Fig. 4. First-derivative spectra of mixtures containing 4 μg ml^{-1} NP plus an increasing amount of AC ranging from 44 to 132 μg ml^{-1} (——) and the first-order derivative spectrum of 12 μg ml^{-1} NP (—-) in methanol.

Table 1
Comparative analytical data for the calibration graphs of NP and AC

Analytical method	Intercept[b] $a \pm (tS_a)$	Slope[a] $b \pm (tS_b)$	Correlation coefficient	Linearity	S_O^{2c}
For NP					
$^1D_{400}$	-1.00×10^{-2}	2.76×10^{-1}	0.9998	8.71	2.32×10^{-2}
	(0.65)	(0.08)			
GLC	4.00×10^{-4}	1.74×10^{-2}	0.9999	0.12	6.67×10^{-8}
	(8.62×10^{-4})	(6.49×10^{-5})			
HPLC	-2.70×10^{-3}	1.60×10^{-1}	0.9999	0.22	8.17×10^{-5}
	(3.02×10^{-3})	(1.14×10^{-3})			
For AC					
$^1D_{352}$	1.00×10^{-2}	3.52×10^{-2}	0.9999	0.69	2.85×10^{-4}
	(0.07)	(7.72×10^{-4})			
GLC	2.00×10^{-3}	4.04×10^{-3}	0.9999	0.56	1.01×10^{-5}
	(1.06×10^{-2})	(7.27×10^{-5})			
HPLC	-4.20×10^{-3}	4.47×10^{-2}	0.9999	0.78	2.38×10^{-3}
	(1.60×10^{-1})	(1.11×10^{-3})			

[a] Confidence intervals for the slopes values ($P<0.05$).
[b] Confidence intervals for the intercepts values ($P<0.05$).
[c] Variance of the regression equation.

2.3. Standard solutions and calibration graphs for spectrophotometric measurements

All sample preparation was carried out in a darkened room under yellow sodium light to prevent NP photo-decomposition. The standard solutions were stored in volumetric flasks wrapped in aluminium foil. Stock solutions were prepared by dissolving NP and AC in methanol to obtain concentrations of 0.1 and 1.1 mg ml^{-1}, from both respectively. The standard solutions were prepared by dilution of stock solutions in methanol to reach concentration ranges of 4–12 and 44–132 μg ml^{-1} for NP and AC, respectively. Working standard solution of NP and AC mixtures in methanol (containing 4 μg ml^{-1} of NP and increasing concentrations of AC ranging from 44–132, and 44 μg ml^{-1} of AC with increasing concentration of NP ranging from 4–12 μg ml^{-1}) were prepared from stock solutions of NP and AC in methanol.

2.3.1. UV measurements

The first-order derivative spectra (^1D) of the methanolic working standard solutions containing the varying amount of each drug and those containing a mixture of both drugs were scanned in the range 450–250 nm against methanol as a blank. The values of the ^1D amplitudes at 400 nm (peak-to-base) were measured for the determination of NP in the presence of AC. The ^1D spectra of AC and their mixtures with NP were also recorded between 450–250 nm and the ^1D amplitudes values at 352 nm (zero-crossing for NP) were used for the determination of AC in the presence of NP.

2.4. Standard solutions and calibration graphs for the chromatographic procedures

2.4.1. For GLC

Standard solutions of NP and AC containing concentration ranges 4–20 and 44–220 μg ml^{-1}, respectively, with a fixed concentration of 100 μg ml^{-1} of thymolphthalein (internal standard) were prepared in methanol. Triplicate 1 μl injections were made for each solution.

2.4.2. For HPLC

Standard solution of NP and AC containing concentration ranges 16–88 and 40–220 μg ml^{-1}, respectively and a fixed concentration of 32 μg ml^{-1} of oxprenolol hydrochloride (internal standard) were prepared in the mobile phase. Tripliate 10 μl injections were made for each solution.

Table 2
Concentration ranges, detection limits and relative sensitivity for the proposed methods applied to the determination of NP and AC

Analytical method	Concentration[a] ranges	Detection[a] limit	Relative[b] sensitivity
For NP			
$^1D_{400}$	4–12	1.46	9.12
GLC	4–20	0.04	0.25
HPLC	8–40	0.16	1.00
For AC			
$^1D_{352}$	44–132	1.32	0.44
GLC	44–220	2.27	0.75
HPLC	44–220	3.01	1.00

[a] Concentration range and detection limit in μg ml^{-1}.
[b] Calculated relative to the HPLC method.

In both the GLC and HPLC method, the peak area ratios of drug to the internal standard were plotted against the corresponding concentrations to obtain the calibration graphs.

2.5. Sample preparation

Ten tablets containing NP and AC as active ingredients were weighed and finely powdered. Portions of the powder equivalent to about 10 mg of NP were weighed accurately and dissolved in 50 ml volumetric flasks using methanol. The flasks were made up to volume with methanol. For the derivative procedure, the suspensions were filtered through a methanolic wetted filter paper and then further diluted to suit the calibration graphs for the derivative measurements. The GLC and HPLC samples were filtered through a 0.45 μm membrane filter after the dilution, to suit the calibration graphs and addition of the internal standards. For the content uniformity test, the same procedure was followed (using one tablet as sample), except that the mixture was sonicated for 10 min before filtration.

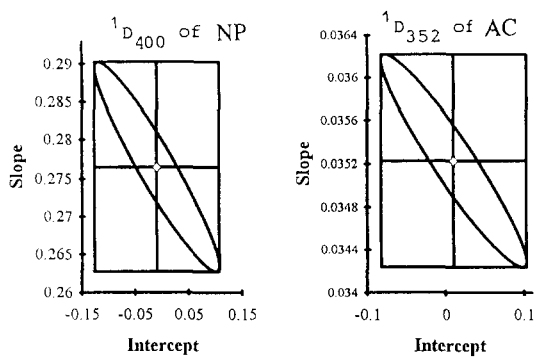

Fig. 5. Joint confidence regions at the $P = 0.05$ level of significane for slopes and intercepts of NP and AC by the first-derivative method.

3. Results and discussion

3.1. Derivative UV-spectrophotometry

The absorption spectra of NP (4 μg ml^{-1}), AC (44 μg ml^{-1}) and a mixture of both, in methanol, are reproduced in Fig. 1. The spectra clearly display considerable overlap; hence, the traditional Vierodet's method and its modification for assaying binary mixtures seems to be impossible. The first order derivative spectra (^1D) present spectral features which can be used for the simultaneous determination of NP and AC (Fig. 2).

For quantitative work, the amplitude of the derivative peak can be measured in various ways. In this study, the amplitudes (denoted h_1 and h_2 in Fig. 2) have been measured with respect to a derivative of zero, which is the true derivative amplitude. Using the amplitudes of the peak with respect to a derivative of zero of the corresponding first-derivative spectrum (h_1 in Fig. 2), NP was quantified at 400 nm (peak-to-base, where the $dA/d\lambda$ value of AC was zero); similarly, AC was quantified using the amplitude of the derivative spectrum (h_2 in Fig. 2) at 352 nm (NP zero-cross-

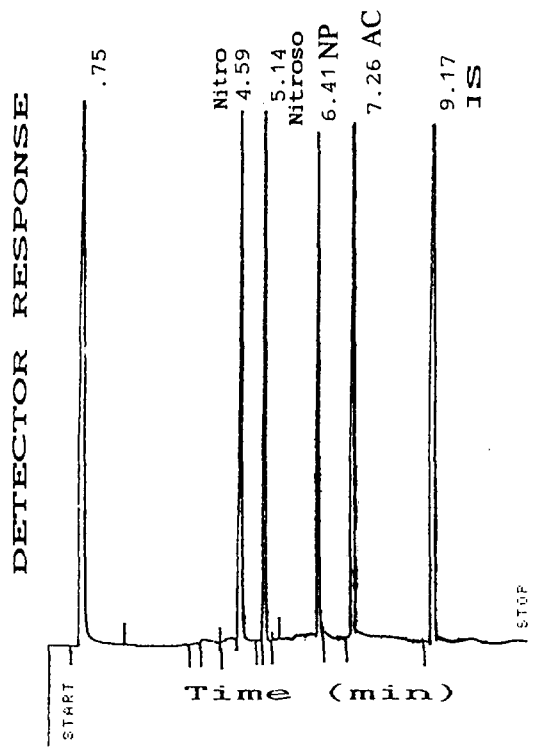

Fig. 6. GLC trace of NP and AC using thymolphthalein as internal standard (IS).

ing where the $dA/d\lambda$ value was zero). The measurement of the absolute value of the total derivative spectrum taken at the above wavelengths afforded the best linear response of the analyte to concentration.

In Fig. 3, a typical set of the first-derivative spectra of laboratory mixtures of 44 μg ml^{-1} of AC and increasing concentrations of NP (ranging from 4 to 12 μg ml^{-1}) was shown. The first-derivative spectra of mixtures of 4 μg ml^{-1} of NP plus an increasing concentration of AC (from 44 to 132 μg ml^{-1}) are shown in Fig. 4. Owing to the high ratio of AC:NP (10:1) in the tablets, two different derivative ordinates were used to achieve maximum derivative responses. The highest at 400 nm (peak-to-base, nil contribution from AC) and 352 nm (zero-crossing of NP) were proportional to the NP and AC concentrations, respectively. Moreover, the values of heights (h_1 and h_2) were not affected by the presence of NP and AC over the full range of concentrations investigated in the first derivative mode.

3.2. Linearity of the derivative procedure

Under the experimental conditions described above, linear regression equations (intercepts and slopes) for mixtures of NP and AC were established. These are given in Table 1, together with the correlation coefficients, linearities and variances. The concentration ranges, detection limits and relative sensitivities are summarized in Table 2. The high values of the correlation coefficients and the values of the intercepts on the coordinates, which were close to zero, indicate the good linearity of the calibrations. Because the values for the correlation coefficients were not sufficient to evaluate the linearity of the calibration graphs the linearity was evaluated by calculation of the R.S.D. of the slope($S_{b\ rel}$%) [34]. Also, the small degree of scatter of the experimental data points around the lines of regression was confirmed by the small values of the variances. For more confirmation, the Student's t-test was performed to determine whether the experimental intercept (a) of the above mentioned regression lines were sig-

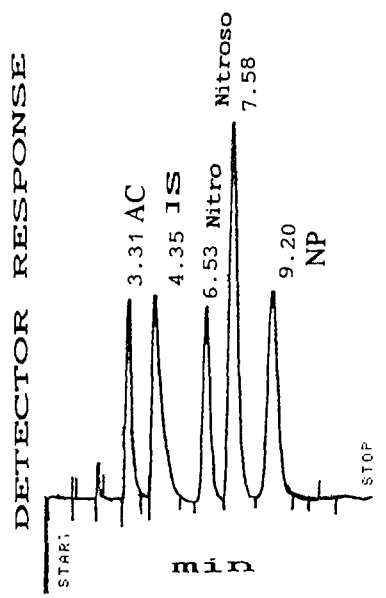

Fig. 7. HPLC trace of NP and AC using oxpernolol hydrochloride as internal standard (IS).

Table 3
Precision and accuracy for the determination of NP and AC by the proposed methods

Drug	Added (μg/ml)	Found (μg/ml), mean ± S.D.[a]	R.S.D.%	E_r (%)[c]
Derivative method				
NP	8.0	8.03 ± 0.11	1.37	0.37
AC	88.0	87.95 ± 0.23	0.26	−0.06
GLC method				
NP	10.0	9.98 ± 0.12	1.20	−0.20
AC	110.0	110.01 ± 0.19	0.17	0.04
HPLC method				
NP	60.0	59.86 ± 0.17	0.28	−0.23
AC	150.0	149.83 ± 0.55	0.37	−0.11

[a] For five determinations.
[c] Percentage relative error.

nificantly different from the theoretical zero value [35]. The values calculated for t were 0.05 for NP and 0.44 for AC (these values do not exceed the 95% criterion of $t_p = 3.182$ for five samples) so the intercepts are not significantly different from zero. However, this procedure ignores the strong correlation existing between slopes and intercepts. In a more rigorous approach, the 95% joint confidence regions [36] were drawn for the slopes and intercepts of the regression equations shown in Table 1. These regions were bounded by an ellipse having the point of best fit as its center (Fig. 5). It can be seen that the points with an intercept of zero fell well within the ellipses, confirming the conclusion that there was no significant deviation from zero.

3.3. Chromatographic methods (GLC and HPLC)

The GLC and HPLC methods were developed to provide a specific procedure suitable for rapid quality control analysis of binary mixtures containing NP and AC, and as reference methods for the derivative procedure. Typical chromatograms obtained from the GLC and HPLC are shown in Figs. 6 and 7, respectively. In The GLC method, a cross-linked methylsilicone gum, capillary column (HP-1), was used. The column gave an efficient separation including good peak shapes, with temperature programming from 150 to 300°C in steps of 20°C per min. Nifedipine was known to be thermally unstable [37,38] so the gas chromatographic analysis conditions, especially the injector temperature, had to be carefully investigated. The injector port temperature and the speed of injection represent the major source of NP decomposition to its nitro derivative. Therefore, fast injection and keeping the injection port at a temperature not higher than 225°C prevented the thermal decomposition of NP to its nitro derivative.

In HPLC, the method involved the use of an RP-C_{18} column and a mobile phase consisting of methanol–water (55:45, v/v, pH 4.5). The mobile phase was chosen after several trials with other solvent combinations. The chromatographic system described allowed complete base line separation with good a resolution factor between the adjacent peaks.

The linearity of the detectors (UV and FID) responses for NP and AC were determined by plotting peak area ratios of the drug to the internal standard versus concentrations. The analytical data for the calibration graphs are listed in Tables 1 and 2. Nifedipine is a photosensitive substance which decomposes upon exposure to daylight or UV radiation to give two oxidation products [13] so in the design of both chromatographic methods, the separation of the oxidation products from NP was taken into consideration (Figs. 6 and 7).

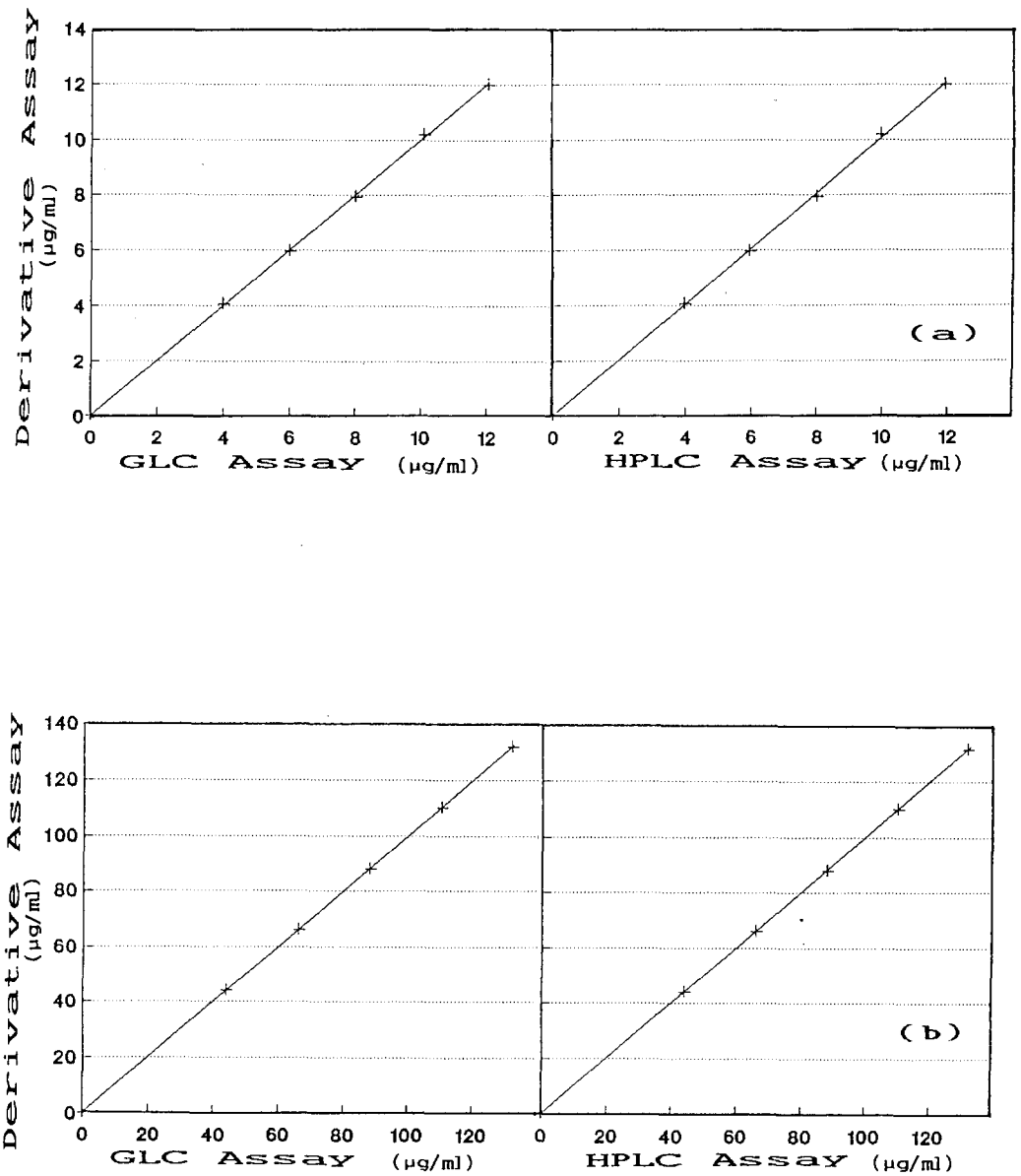

Fig. 8. (a) Comparison of the NP concentration measuread by ^{1}D, GLC and HPLC. (b) Comparison of the NP and AC concentrations measured by ^{1}D GLC and HPLC.

3.4. Accuracy and precision of the proposed methods

In order to test the precision (R.S.D.%) and accuracy (Er%) of the proposed methods, five successive determinations of mixtures of NP and AC were carried out. The data reported in Table 3 show that the precision and accuracy were very satisfactory.

3.5. Comparison of the results obtained by derivative and chromatographic procedures

For full comparison between the derivative and the chromatographic procedures, five standard mixtures containing NP and AC were prepared and analysed using the three proposed procedures. Fig. 8a and b depicts the correlation between the obtained results from the derivative and the chromatographic procedures. There is a linear correlation between the derivative and each of the chromatographic procedures over the concentration range analysed. The fitted curves can be expressed by the following equations:

$$Y_{NP} = 0.9970X_{NP} + 0.0498 \quad (n = 5; r = 0.9997).$$

$$Y_{NP} = 1.0057Z_{NP} + 4.66 \times 10^{-3} \quad (n = 5; r = 0.9996).$$

$$Y_{AC} = 0.9981X_{AC} + 0.1175 \quad (n = 5; r = 0.9999).$$

$$Y_{AC} = 0.9998Z_{AC} + 0.0335 \quad (n = 5; r = 0.9999).$$

Table 4
Determination of NP–AC combination in commercial tablets by the three proposed methods

Analytical method		
GLC	Derivative found mean ± SD[a]	HPLC
For NP content		
98.63 ± 0.25	98.47 ± 0.36	98.67 ± 0.20
$t = 0.82$	2.31[b]	1.09
$F = 2.07$	6.39[b]	3.24
For AC content		
101.43 ± 0.39	101.29 ± 0.33	101.77 ± 0.18
$t = 0.61$		2.86
$F = 1.40$		3.36
Recovery[c]		
For NP:		
99.75 ± 0.40	99.93 ± 0.15	99.90 ± 0.17
For AC:		
100.16 ± 0.53	100.04 ± 0.19	100.08 ± 0.25

[a] For five determinations; percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of 50% of the nominal content ($n = 5$).

where Y are the derivative assay values and X and Z the GLC and HPLC values, respectively. By the examination of the four equations, the slopes and intercepts were found to be close to the unity and zero values, respectively. Therefore, the differences observed between the derivative method and the two chromatographic procedures results only from the variability of measurements.

3.6. Application to a commercial formulation tablet

The methods were applied to the determination of NP and AC in tablets of Tredalat (Bayer, Leverkusen, Germany) which comprise the binary mixture (10 mg NP and 110.8 mg AC). Five replicate determinations were made. Satisfactory results (Table 4) were obtained for the recovery of both drugs and were in a good agreement with the label claims. The recovery for the three procedures was tested by adding a known amount (standard addition) of NP and AC to the commercial tablets. No significant differences were found between the results obtained by the three procedures for the same batch, at the 95% confidence level (Student's t- and F-ratio tests). As the dosage form of NP and AC is not pharmacopoeial yet, the values given by the derivative procedure were compared with both the chromatographic procedures (GLC and HPLC). The statistical evaluation indicated that there was no significant difference between the methods used.

4. Conclusions

The derivative (^{1}D), the GLC and HPLC procedures were shown to be reproducible and sensitive in the analysis of NP and AC in a simple binary mixture. The derivative procedure has been validated with respect to simple binary mixtures of NP and AC and may be applicable only to such simple mixtures in the absence of any degradation products. The other techniques (GLC and HPLC) can not be excluded as they also gave good results and avoided interference from the decomposition products of NP. The results confirm that for these mixtures derivative spectrophotometry offers ac-

curacy and precision with the added advantages of low cost, speed and simplicity. Therefore, the proposed derivative procedure is likely to be very suitable for the analysis of the two drugs in binary tablet preparations.

Acknowledgements

The author is grateful to Pharco Pharmaceutical (Alexandria, Egypt) for granting permission to use their laboratory facilities for this study. The author also wishes to thank Amany Moustafa for typing the manuscript.

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