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Short communication

Simultaneous determination of amiloride HCl, hydrochlorothiazide and atenolol in combined formulations by derivative spectroscopy

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1. Introduction

A combination of amiloride HCl (AMH), hydrochlorothaizide (HTZ) and atenolol (ATL) in the form of a tablet or capsule preparation is widely used for moderate to severe hypertension not controlled by a single antihypertensive agent. The official monographs describe the procedure for individual assay of AMH [1], HTZ [1], ATL [2] as well as AMH and HTZ combination [1].

There are reports on the derivative spectrophotometric method for simultaneous determination of AMH and HTZ [3,4], a reversed phase HPLC method for the simultaneous determination of AMH, HTZ and ATL [5]. However, no spectrophotometric method has been reported for the quantitative determination of these drugs from their combined formulations.

In recent years, derivative spectrophotometry has been found to be a useful method in the determination of mixtures with two or more components having overlapping spectra and in eliminating interference from formulation matrix by using the zero crossing technique [6–10]. Furthermore, derivative compensation methods [11,12] have also been found to be useful in the estimation of drugs from their mixtures. In the present paper, we describe the utilization of first derivative zero crossing points for the simultaneous determination of AMH, HTZ and ATL in the presence of each other, as well as of the excipients.

2. Experimental

2.1. Standard solutions

The stock solution of pure AMH, HTZ and ATL were prepared by dissolving 5 mg of each of the pure drugs in 10 ml of methanol. Appropriate volume aliquots of HTZ and ATL were trans-

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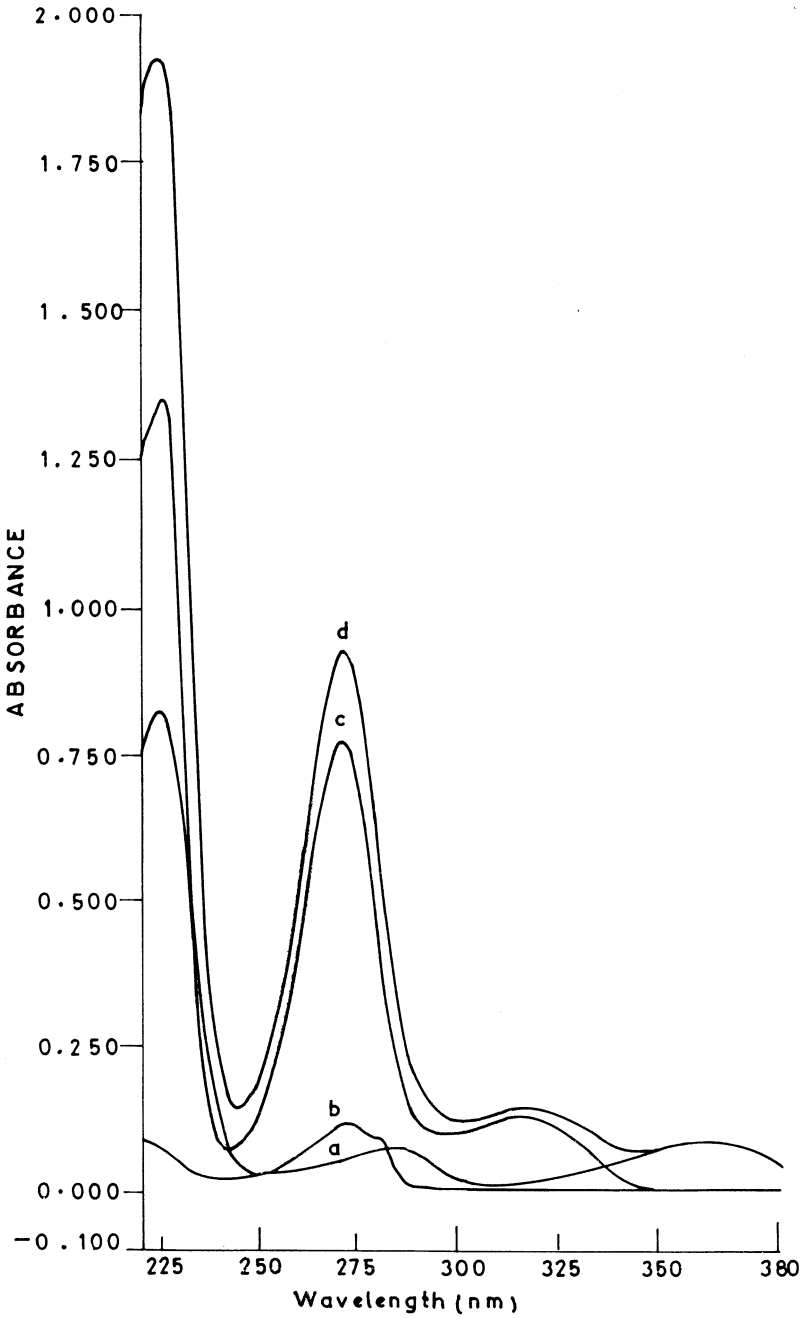

Fig. 1. Absorption spectra of: (a) amiloride HCl (1.25 μg ml^{-1}); (b) atenolol (25 μg ml^{-1}); (c) hydrochlorothiazide (12.5 μg ml^{-1}); and (d) their mixture spectrum.

ferred separately into 10 ml volumetric flasks. The volumes were made up with 0.1 N HCl to give a series of equimolar solutions containing 7.5–15.0 μg ml^{-1} of HTZ and 15–30 μg ml^{-1} of ATL. The dilutions of AMH were prepared by transferring 5 ml of the above prepared stock solution

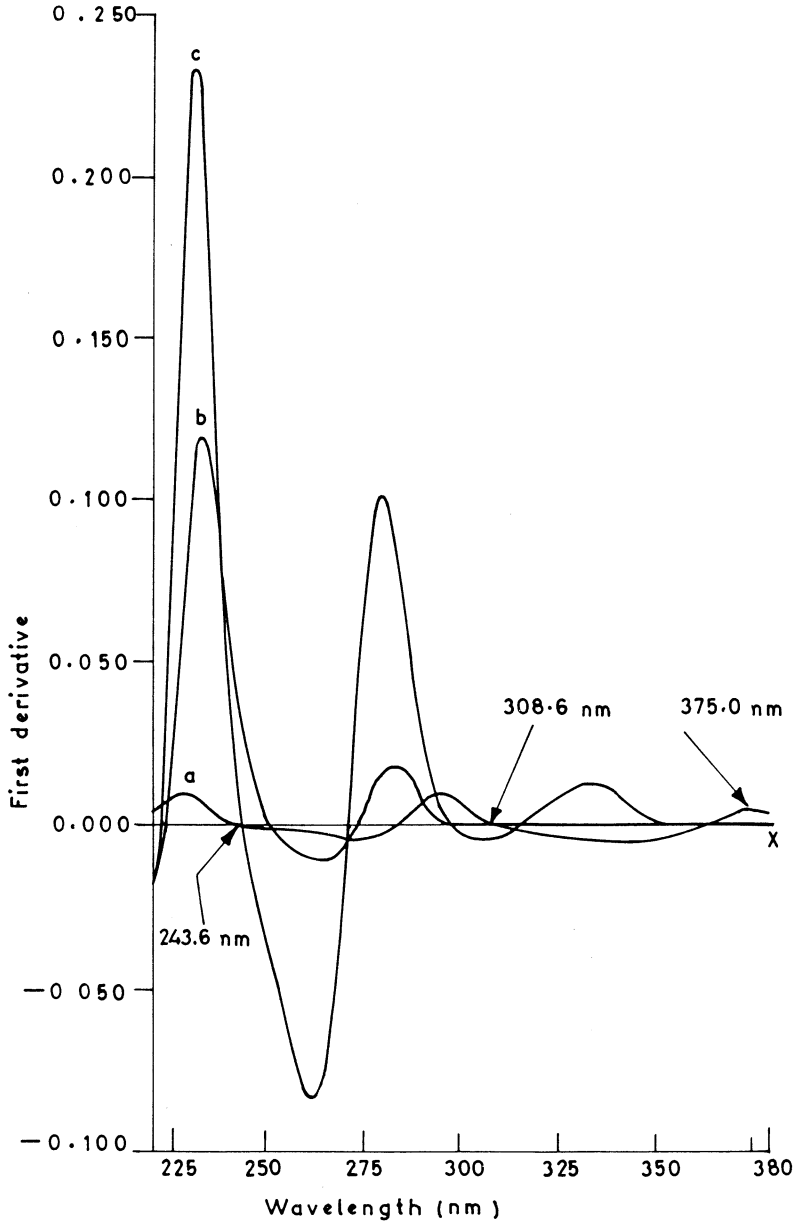

Fig. 2. First derivative spectra of: (a) amiloride HCl (1.25 μg ml^{-1}); (b) atenolol (25 μg ml^{-1}); and (c) hydrochlorothiazide (12.5 μg ml^{-1}).

into a 10 ml volumetric flask and diluting to volume with methanol to give a second stock solution. Appropriate volume aliquots of AMH were transferred from the second stock solution into 10 ml volumetric flasks. The volumes were made up with 0.1 N HCl to give a series of equimolar solutions containing 1.25–5.0 μg ml^{-1} of AMH.

Similarly, three series of 10 ml of each mixture were also prepared in 0.1 N HCl from the stock solutions. The first series contained a constant concentration of HTZ (12.5 μg ml^{-1}), ATL (25

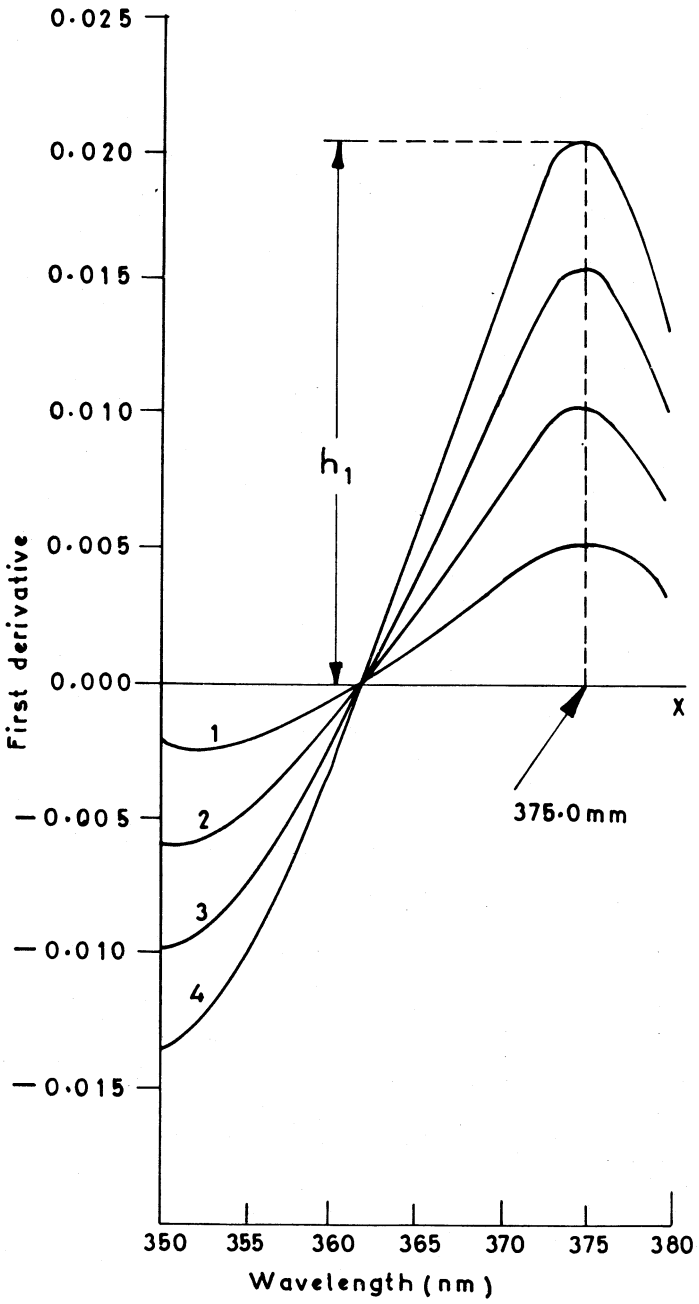

Fig. 3. First derivative mixture spectra of amiloride HCl (1.25, 2.50, 3.75 and 5.0 μg ml^{-1}; curves 1–4) with a constant concentration of atenolol (25 μg ml^{-1}) and hydrochlorothiazide (12.5 μg ml^{-1}).

μg ml^{-1}) and a varying concentration of AMH (1.25–5.0 μg ml^{-1}), while the second series contained a constant concentration of ATL (25 μg ml^{-1}), AMH (1.25 μg ml^{-1}) and a varying concentration of HTZ (7.5–15.0 μg ml^{-1}). Finally, the third series contained a constant concentra-

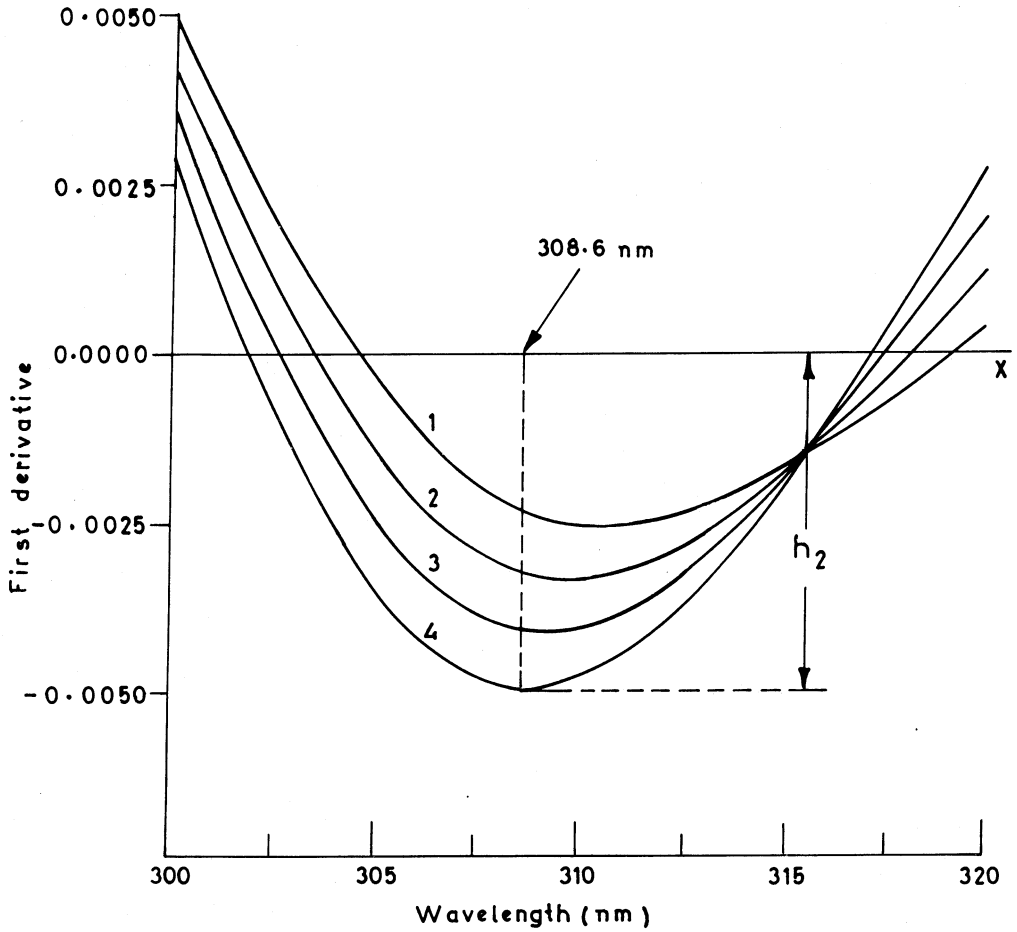

Fig. 4. First derivative mixture spectra of hydrochlorothiazide (7.5, 10.0, 12.5 and 15.0 μg ml^{-1}; curves 1–4) with a constant concentration of atenolol (25 μg ml^{-1}) and amiloride HCl (1.25 μg ml^{-1}).

tion of HTZ (12.5 μg ml^{-1}), AMH (1.25 μg ml^{-1}) and a varying concentration of ATL (15–30 μg ml^{-1}). The absorbance of the solutions were taken within 3 h of their preparation. All reagents used were of analytical grade.

2.2. Sample preparation

Twenty tablets of Beta-Biduret (Crydon, India) labelled as containing 50 mg of ATL, 25 mg of HTZ, 2.5 mg of AMH and excipients were accurately weighed and powdered and a powder weight equivalent to 25 mg of ATL (corresponding amount of HTZ 12.5 mg and AMH 1.25 mg) was dissolved in methanol by thorough mixing and diluted to volume in a 50 ml of volumetric flask. Similarly, 20 capsules of Hipres-D (Cipla, India) labelled as containing 50 mg of ATL, 25 mg of HTZ, 2.5 mg of AMH and excipients were accurately weighed, mixed and a powder weight equivalent to 25 mg of ATL (corresponding amount of HTZ 12.5 mg and AMH 1.25 mg) was dissolved in methanol by thorough mixing and diluted to volume in a 50 ml of volumetric flask.

The extracts were filtered separately through Whatman No. 1 filter paper. The first and last 5 ml of each filtrate was discarded. The sample solutions of 10 ml of each ATL were prepared in 0.1 N HCl by transferring appropriate amounts of each filtrate to obtain an equimolar solution con-

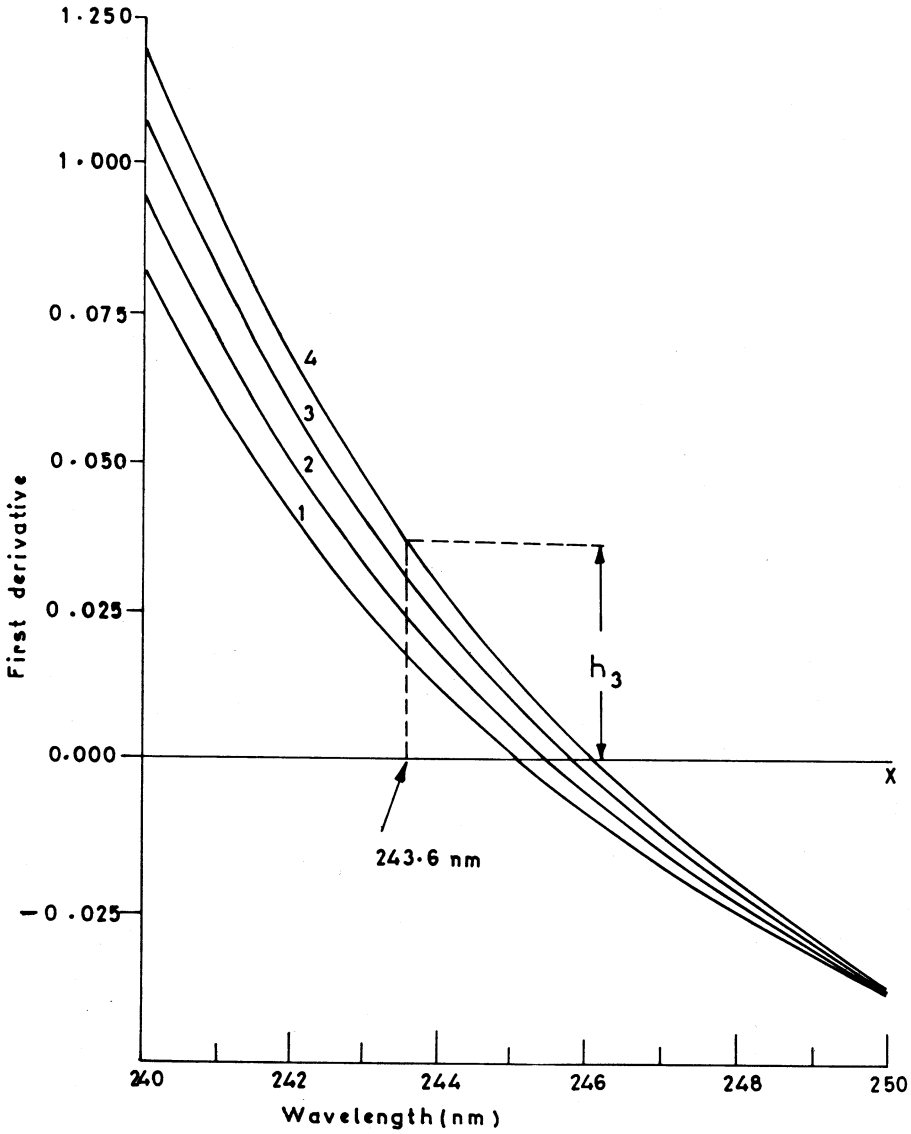

Fig. 5. First derivative mixture spectra of atenolol (15, 20, 25 and 30 μg ml^{-1}; curves 1–4) with a constant concentration of hydrochlorothiazide (12.5 μg ml^{-1}) and amiloride HCl (1.25 μg ml^{-1}).

taining approximately 25 μg ml^{-1} of ATL and corresponding amounts of HTZ (12.5 μg ml^{-1}) and AMH (1.25 μg ml^{-1}).

The stock and sample solutions were measured in the range of 220–380 nm with a Jasco 7800 UV-visible spectrophotometer against the acidic solution as blank. The suitable settings were a scan speed of 480 nm min^{-1} and chart speed of 10 nm min^{-1} were maintained. The first derivative spectra were recorded by using Savizky–Golay [13] parameter of $\Delta = 15$ points. No smoothing of spectra was found necessary. Ordinate maxima and minima were adjusted to the magnitude of derivative values and then measured at 243.6, 308.6 and 375.0 nm for ATL, HTZ and AMH, respectively.

3. Results and discussion

The zero order spectra of pure drugs was found

Table 1
Statistical analysis of the determination of amiloride HCl, hydrochlorothiazide and atenolol in a mixture by first derivative spectroscopy

Drug name	Regression equations	r	Variance (S_0^2)	Standard error		DL[b] μg ml^{-1}
				Intercept	Slope	
AMH	$D_1 = 4.00\text{E-}03.C_a\text{-}7.45\text{E-}09$ (375.0 nm)	0.9999	3.76E-09	5.72E-06	1.67E-06	0.033
HTZ	$D_1 = 3.99\text{E-}04.C_b\text{-}9.99\text{E-}04$ (308.6 nm)	0.9999	5.00E-11	6.33E-06	5.46E-07	0.038
ATL	$D_1 = 1.20\text{E-}03.C_c\text{-}1.86\text{E-}08$ (243.6 nm)	0.9999	3.76E-09	2.30E-05	9.94E-07	0.111

Number of samples, $n = 10$; b, level of significance at $P = 0.05$; C_a, C_b and C_c, concentration of drugs in μg ml^{-1}.

to be overlapping, making simultaneous determination difficult (Fig. 1). In contrast, the first derivative (D_1) spectra of each pure drug was found to show zero crossing points (ZCP; Fig. 2) and assisted in their simultaneous estimation. The first derivative wavelengths considered were 375.0 nm for AMH, 308.6 nm for HTZ and 243.6 nm for ATL. The negligible absorption of ATL, HTZ at a wavelength of 375.0 nm made it easier for the estimation of AMH and a wavelength of 308.6 nm being a ZCP for ATL, AMH did not interfere in the measurement of HTZ. In a similar manner, a wavelength of 243.6 nm was considered for the determination of ATL being a ZCP to HTZ, while AMH did not show any appreciable absorption at a selected concentration level of 1.25 μg ml^{-1} and its contribution to the absorption of the mixture was considered to be negligible. This enabled the linear concentration range for estimation from combined preparation to be chosen.

The proportionality of D_1 values and concentrations of AMH, HTZ and ATL were found by measuring D_1 values of ten pairs of pure solutions and mixtures at the selected wavelengths. The derivative curves for mixture solutions were obtained by considering a mixture of two drugs with a constant concentration as a single component and the other as the second component with varying concentration showed a linear response to analyte concentrations at the selected wavelengths (Figs. 3–5).

The linear regression equations together with correlation coefficients, variance, detection limits (DL) at $P = 0.05$ level of significance [14], standard error of slope and intercept obtained for each drug are shown in Table 1. Thus, it was evident from the values obtained that the intercept values did not differ significantly from zero in each case.

The D_1 height values of standard solutions of AMH (1.25 μg ml^{-1}, h_1), HTZ (12.5 μg ml^{-1}, h_2) and ATL (25 μg ml^{-1}, h_3) at the selected wavelengths were used in the determination of analytes from their sample preparation. These results are given in Table 2. They indicated that there was no interference from the formulation matrix and also suggested that the complex problem of quantitating drugs from mixtures having two or more components with overlapping spectra can be solved by this technique. The solutions were stable throughout the study.

Table 2
Assay results of amiloride HCl, hydrochlorothiazide and atenolol in combined formulations by first derivative spectroscopy

Sample	AMH (375.0 nm)		HTZ (308.6 nm)		ATL (243.6 nm)	
	mg per tab/caps	% Recovery[a]	mg per tab/caps	% Recovery[a]	mg per tab/caps	% Recovery[a]
Brand A (tablet)	2.502	100.08 ± 0.59	25.03	100.15 ± 0.66	49.85	99.07 ± 0.84
Brand B (capsule)	2.502	100.11 ± 0.49	25.05	100.21 ± 0.46	49.47	98.94 ± 1.18

[a]Mean and standard deviation for ten determinations.

4. Conclusions

Our work has shown that derivative spectroscopy can be successfully utilized for the simultaneous estimation of a three component drug mixture without any interference. The method is precise and reproducible. In the absence of an official monograph it can be used for their determination.

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