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Simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and high-performance liquid chromatography

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Abstract

Rapid, precise, accurate and specific ratio spectra derivative spectrophotometry and high-performance liquid chromatographic procedures were described for the simultaneous determination of hydrochlorothiazide and amiloride hydrochloride in combined pharmaceutical dosage forms. For the first method, ratio spectra derivative spectrophotometry, the signals were measured at 285.7 nm for hydrochlorothiazide and at 302.5 nm for amiloride hydrochloride in the mixture, in the first derivative of the ratio spectra. The second method is based on high-performance liquid chromatography (HPLC) on LiChrosorb RP-C_{18} column (5 μm, 20 cm × 4.6 mm) using 0.025 M orthophosphoric acid (adjusted to pH 3.0 with triethylamine (TEA)), acetonitrile (84:16 v/v) as a mobile phase at a flow rate of 1.2 ml min^{-1}. Detection was carried out using a UV detector at 278.0 nm. Commercial sugar-coated and laboratory-prepared mixtures containing both drugs in different proportions were assayed using the developed methods.

Keywords: Hydrochlorothiazide; Amiloride hydrochloride determination; High-performance liquid chromatography; Ratio spectra derivative spectrophotometry; Pharmaceutical formulations

1. Introduction

The binary mixtures of hydrochlorothiazide–amiloride hydrochloride are widely used in diuretic and antihypertensive pharmaceutical formulations.

The literature reports many analytical methods for the quantitative determination of hydrochlorothiazide, and amiloride hydrochloride in their combinations with other drugs including spectrophotometry [1–23], high-performance liquid chromatography (HPLC) [24–31] and polarography [32] in pharmaceutical preparations either separately or in combination with other drugs so far.

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This paper presents ratio spectra derivative spectrophotometry and HPLC assays of hydrochlorothiazide and amiloride hydrochloride in two component mixtures without a previous separation step. The utility of the developed methods to determine the contents of both drugs in pharmaceutical formulation was demonstrated.

2. Experimental

2.1. Apparatus

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM PC computer loaded with a Lexmark printer.

The HPLC system consisted of a JASCO model PU-980 pump with a 7725 Rheodyne value injector 20 μl fixed loop, equipped with a JASCO UV-975 UV/VIS detector. The detector was set at 278.0 nm (0.02 a.u.f.s.) and peak areas were integrated automatically by computer using Borwin software programme.

2.2. Chemicals

Chromatographic grade, double distilled water, acetonitrile (Merck-13358) and analytical reagent grade triethylamine (TEA) (Merck-808352), orthophosphoric acid (BDH) were used.

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (MODURETiC® sugar-coated tablet Fako Pharm. Ind.-Turkey, batch no. ER 34) was assayed. Its declared content was as follows: amiloride hydrochloride, 5.00 mg; hydrochlorothiazide, 50.00 mg/sugar-coated tablet.

3. Procedure for ratio spectra derivative spectrophotometry

3.1. Calibration

Standard solutions of 1 mg ml^{-1} of hydrochlorothiazide and amiloride hydrochloride were prepared in methanol:0.1 M HCl (1:1).These solutions were used in the preparation of calibration graphs and for spectra.

3.2. Analysis of sugar-coated tablets

A total of 20 tablets (MODURETiC®) were accurately weighed and powdered in a mortar. Quantities of the powdered sugar-coated tablets equivalent to 50.00 mg hydrochlorothiazide and 5.00 mg amiloride hydrochloride (one tablet) were accurately weighed, taken and dissolved in methanol:0.1M HCl (1:1) in 100 ml calibrated flasks. After 30 min of mechanically shaking, the solution was filtrated in a 100 ml calibrated flask through Whatman no. 42 filter paper. The residue was washed three times with 10 ml of solvent then the volume was completed to 100 ml with methanol:0.1 M HCl (1:1) (Solution A). The solution was diluted 1:20 with the same solvent. The method was applied to the solutions prepared.

4. Procedure for HPLC

4.1. Chromatographic conditions

Routine analysis was carried out isocratically on a RP-LiChrosorb C_{18} column (5 μm, 20 cm × 4.6 mm) using 0.025 M orthophosphoric acid (adjusted to pH 3.0 with TEA): acetonitrile (84:16 v/v) as a mobile phase at a flow rate of 1.2 ml min^{-1}.

All solvents were filtered through 0.45 μm millipore filter to use and degassed in an ultrasonic bath.

4.2. Calibration

An external standard method was used for quantitative determinations. Calibration graphs were prepared from authentic samples of hydrochlorothiazide and amiloride hydrochloride in the mobile phase. Triplicate 20 μl injections were made for each solution and the peak area ratio of each drug was plotted against the corresponding concentration to obtain the calibration graph.

Table 1
Analytical data for the calibration graphs ($n = 5$) for the determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and HPLC

		Regression equation				
Drug	Method	Lin. Ran. (μg ml^{-1})	Slope	Intercept	Cor. coef. r	RSD (%)
Hydrochlorothiazide	Ratio spectra derivative	2.0–28.0	0.152	0.013	0.9989	0.890
	HPLC	1.0–38.0	0.066	−0.008	0.9996	0.105
Amiloride hydrochloride	Ratio spectra derivative	2.0–28.0	0.148	0.098	0.9997	1.650
	HPLC	1.5–37.0	0.057	0.109	0.9992	0.067

4.3. Analysis of sugar-coated tablets

A total of 20 tablets (MODURETiC®) were accurately weighed and powdered in a mortar, an amount equivalent to one tablet, was taken and dissolved in mobile phase in 100 ml calibrated flasks. After 30 min of mechanically shaking, the solution was filtrated in a 100 ml calibrated flask through Whatman no. 42 filter paper. The residue was washed three times with 10 ml of solvent then the volume was completed to 100 ml with mobile phase (Solution A). This solution was diluted 1:20 with same solvent. A total of 20.0 μl volume of the final solution was injected into the chromatograph.

5. Results and discussion

5.1. Ratio spectra derivative spectrophotometry

Hydrochlorothiazide and amiloride hydrochloride are soluble in methanol:0.1 M HCl (1:1), and their solutions were found to be stable for 3 days at least.

The absorption spectra of the solutions prepared at different concentrations of hydrochlorothiazide were recorded in range of 230.3–321.7 nm and stored on the IBM PC. The stored spectra of the binary mixtures, hydrochlorothiazide and amiloride hydrochloride were divided by a standard spectrum of 20.0 μg ml^{-1} amiloride hydrochloride. The ratio spectra were smoothed with $\Delta\lambda = 4$ nm intervals (Fig. 1a), and their first derivatives were traced with $\Delta\lambda = 4$ nm intervals (Fig. 1b). In the binary mixtures, the concentration of hydrochlorothiazide was determined by measuring the amplitudes at 285.7 nm corresponding to maximum. In the similar manner, the absorption spectra of the solutions prepared in different concentrations of amiloride hydrochloride were recorded in range of 206.5–319.9 nm and stored on the IBM PC. The stored spectra of the binary mixtures, hydrochlorothiazide and amiloride hydrochloride were divided by a standard spectrum of 16.0 μg ml^{-1} hydrochlorothiazide. Fig. 2(b) shows first derivative of the ratio spectra which was plotted with the intervals of $\Delta\lambda = 4$ nm from the ratio spectra (Fig. 2a). The contents of amiloride hydrochloride in binary mixture were determined by measuring the signals at 302.5 nm corresponding to maximum wavelength, in the range 206.5–319.9 (Fig. 2a). Various mixture compositions of hydrochlorothiazide and amiloride hydrochloride were prepared and tested between 2.0–28.0 μg ml^{-1} for hydrochlorothiazide and 2.0–28.0 μg ml^{-1} for amiloride hydrochloride (Table 1). In this method, the synthetic mixtures were prepared by adding known amounts of hydrochlorothiazide–amiloride hydrochloride. Recoveries and the relative standard deviations of method were found as 100.03 and 0.45% for hydrochlorothiazide and 99.32 and 1.75% for amiloride hydrochloride in their binary mixture (Table 2).

Table 1 shows the regression coefficients and the linearity ranges of the calibration graphs for active ingredients at the suitable wavelengths for

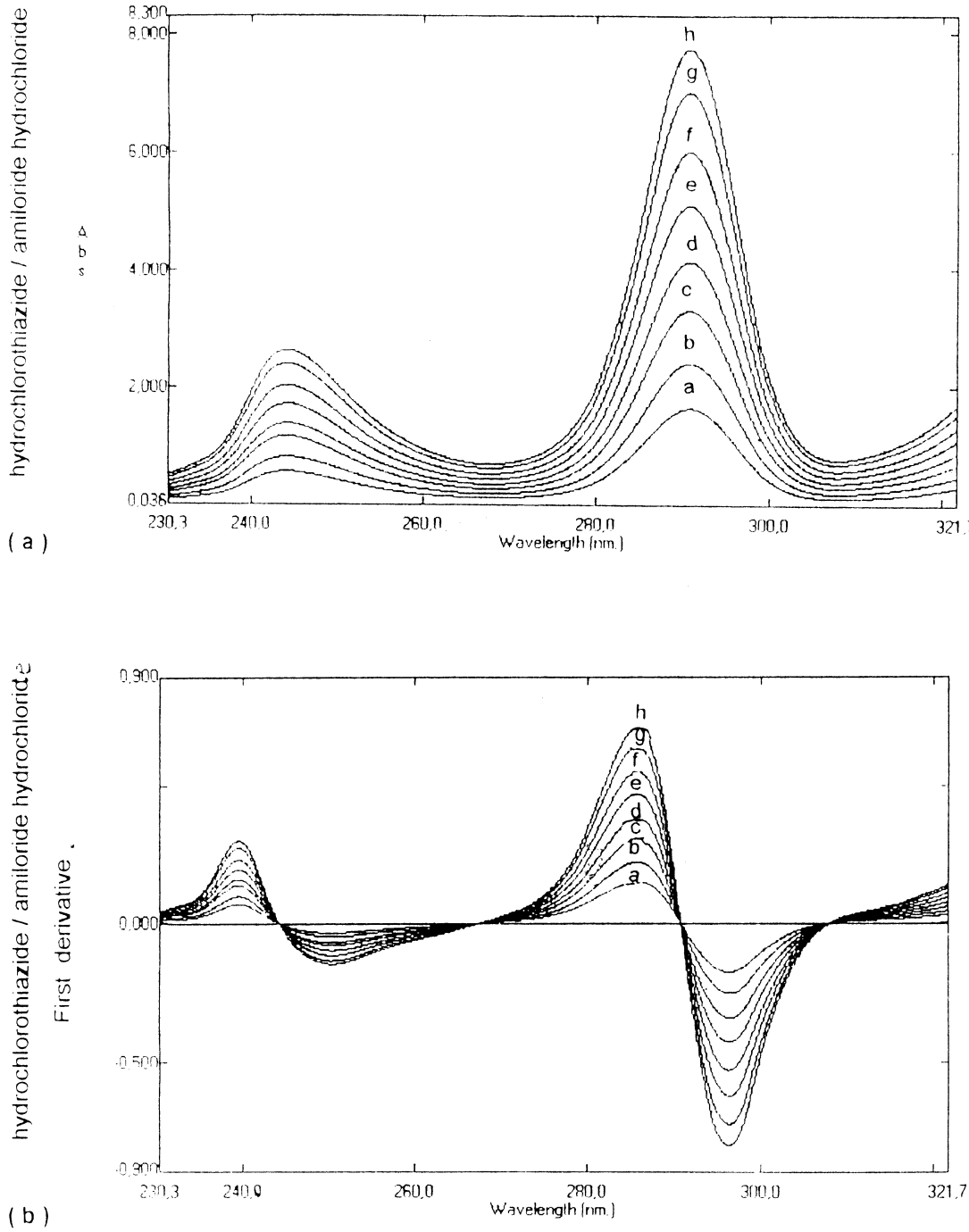

Fig. 1. (1a) Ratio spectra of hydrochlorothiazide of (a) 2.0 μg ml^{-1}, (b) 4.0 μg ml^{-1},c) 8.0 μg ml^{-1}, (d) 12.0 μg ml^{-1}, (e) 16.0 μg ml^{-1}, (f) 20.0 μg ml^{-1}, (g) 24.0 μg ml^{-1} and (h) 28.0 μg ml^{-1} when 20.0 μg ml^{-1} amiloride hydrochloride used as divisor in methanol:0.1M HCl (1:1) ($\Delta\lambda = 4$ nm). (1b) First derivative of the ratio spectra of hydrochlorothiazide of (a) 2.0 μg ml^{-1}, (b) 4.0 μg ml^{-1}, (c) 8.0 μg ml^{-1}, (d) 12.0 μg ml^{-1}, (e) 16.0 μg ml^{-1}, (f) 20.0 μg ml^{-1}, (g) 24.0 μg ml^{-1} and (h) 28.0 μg ml^{-1} when 20.0 μg ml^{-1} amiloride hydrochloride used as divisor in methanol:0.1M HCl (1:1) ($\Delta\lambda = 4$ nm).

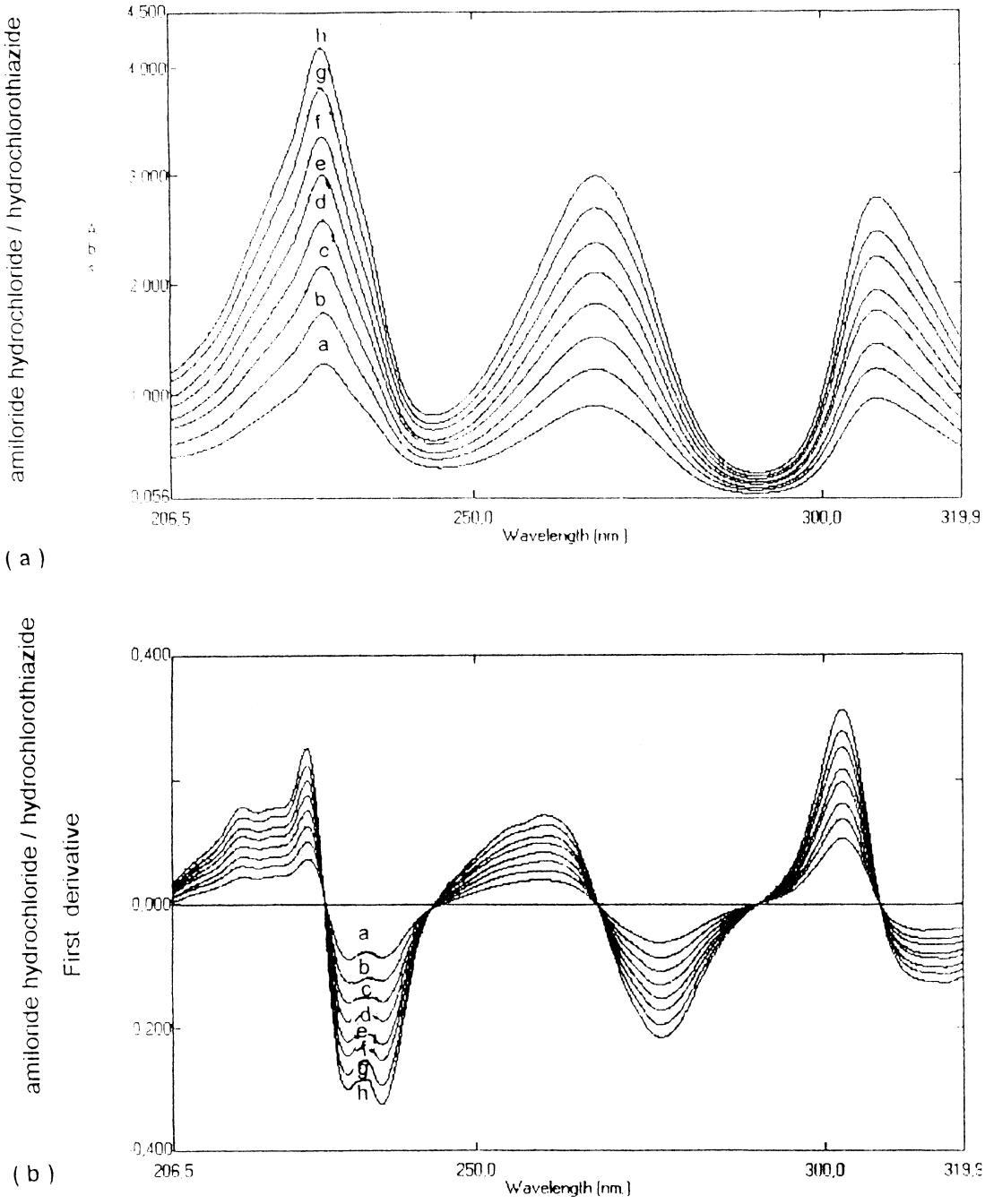

Fig. 2. (2a) Ratio spectra of amiloride hydrochloride of (a) 2.0 μg ml^{-1}, (b) 4.0 μg ml^{-1}, (c) 8.0 μg ml^{-1}, (d) 12.0 μg ml^{-1}, (e) 16.0 μg ml^{-1}, (f) 20.0 μg ml^{-1}, (g) 24.0 μg ml^{-1} and (h) 28.0 μg ml^{-1} when 16.0 μg ml^{-1} hydrochlorothiazide used as divisor in methanol:0.1 M HCl (1:1) ($\Delta\lambda = 4$ nm). (2b) First derivative of the ratio spectra of amiloride hydrochloride of (a) 2.0 μg ml^{-1}, (b) 4.0 μg ml^{-1}, (c) 8.0 μg ml^{-1}, (d) 12.0 μg ml^{-1}, (e)16.0 μg ml^{-1}, (f) 20.0 μg ml^{-1}, (g) 24.0 μg ml^{-1} and (h) 28.0 μg ml^{-1} when 16.0 μg ml^{-1} hydrochlorothiazide used as divisor in methanol:0.1 M HCl (1:1) ($\Delta\lambda = 4$ nm).

Table 2
Determination of hydrochlorothiazide and amiloride hydrochloride in laboratory-prepared mixtures by ratio spectra derivative spectrophotometry

	Hydrochlorothiazide			Amiloride hydrochloride		
Mixture numbe	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	50.00	49.99	99.98	3.00	2.95	98.33
2	50.00	49.94	99.88	4.00	3.90	97.50
3	50.00	50.30	100.60	5.00	4.98	99.60
4	50.00	50.15	100.30	6.00	5.96	99.33
5	50.00	49.70	99.40	7.00	6.95	99.28
			$x = 100.03$			
			RSD = 0.45%			
6	30.00	29.89	99.63	5.00	4.89	97.80
7	40.00	39.96	99.90	5.00	4.98	99.60
8	50.00	49.90	99.80	5.00	5.01	100.20
9	60.00	59.50	99.17	5.00	4.87	97.40
10	70.00	68.99	98.09	5.00	5.08	101.60
$n = 10$						$x = 99.32$
						RSD = 1.75%

the determinations of hydrochlorothiazide and amiloride hydrochloride.

The main instrumental parameter conditions were optimised for a reliable determination of the subject compounds. Some divisor concentrations were tested in the determinations for selecting the standard solution as divisor at an appropriate concentration, which is very important factor in practice. The standard solutions of 20.0 μg ml^{-1} amiloride hydrochloride were prepared to determine hydrochlorothiazide and the standard solutions of 16.0 μg ml^{-1} hydrochlorothiazide were prepared to determine amiloride hydrochloride in their binary mixtures which were found suitable for ratio spectra derivative spectrophotometry analysis. The influence of the $\Delta\lambda$ for the first derivative spectra and the smoothing function for the ratio spectra were tested and found very appropriate to use the values of $\Delta\lambda = 4$ nm for the first and $\Delta\lambda = 4$ nm for the second, in the determination of the two compounds.

6. Chromatography

In order to effect the simultaneous elution of the two component peaks under isocratic conditions, the mobile phase composition was optimised. A satisfactory separation was obtained with a mobile phase consisting of 0.025 M orthophosphoric acid (adjusted to pH 3.0 with TEA): acetonitrile (84:16 v/v) as a mobile phase at a flow rate of 1.2 ml min^{-1}. Under the described chromatographic conditions, the analyte peaks were well defined, resolved and almost free from tailing. At a flow rate of 1.2 ml min^{-1}, the retention times for hydrochlorothiazide and amiloride hydrochloride were 5.06, and 2.88 min, respectively (Fig. 3). Initial studies were performed while the effluent was monitored at 278.0 nm; the detector response (hydrochlorothiazide:amiloride hydrochloride) was found to be in the ratio 10:1 for equal concentrations of the drug. The optimum wavelength for detection was 278.0 nm at which much better detector responses for both drugs were obtained (Fig. 3). The proposed method allows the determination of both drugs in sugar-coated tablets (labelled to contain 50.0 mg, hydrochlorothiazide and 5.0 mg of amiloride hydrochloride per sugar-coated tablet) using the same dilution and the same injection volume and with reasonable responses for the two well resolved peaks. For quantitative applications linear calibration graphs were obtained with cor-

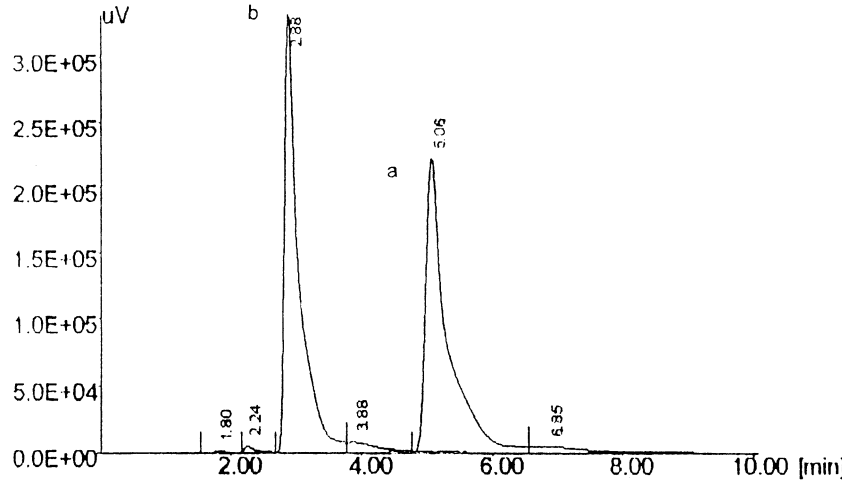

Fig. 3. HPLC trace of a 20 μl injection containing (a) 50.0 μg ml^{-1} of hydrochlorothiazide (5.06 min);and (b) 5.0 μg ml^{-1} of amiloride hydrochloride (2.88 min) in laboratory-prepared mixtures.

Table 3
Determination of hydrochlorothiazide and amiloride hydrochloride in laboratory-prepared mixtures by HPLC

Mixture number	Hydrochlorothiazide			Amiloride hydrochloride		
	Added μg	Found μg	Recovery %	Added μg	Found μg	Recovery %
1	50.00	49.99	99.98	3.00	2.98	99.33
2	50.00	49.99	99.98	4.00	3.98	99.50
3	50.00	50.01	100.02	5.00	4.99	99.80
4	50.00	49.98	99.96	6.00	6.01	100.16
5	50.00	50.01	100.02	7.00	6.98	99.71
			$x = 99.99$			
			RSD = 0.02%			
6	30.00	29.95	99.85	5.00	4.93	98.60
7	40.00	40.02	100.05	5.00	4.98	99.60
8	50.00	49.98	99.96	5.00	5.01	100.20
9	60.00	59.59	99.32	5.00	5.01	100.20
10	70.00	69.96	99.94	5.00	5.04	100.80
$n = 10$						$x = 99.88$
						RSD = 0.83%

relation coefficients better than 0.9990 (Table 1). The good precision of the HPLC procedure was indicated by the relative standard deviation (0.067–0.105%). Results of HPLC analysis of laboratory-prepared mixtures with different proportions of the drug is given in Table 3.

6.1. Analysis of pharmaceutical formulations

The validity of the propose7d methods for pharmaceutical preparations and the effect of possible interferences were studied by assaying MODURETiC® sugar-coated tablet (labelled to

Table 4
Assay results in commercial product (mg)[a]

	Ratio spectra derivative spectrophotometry		HPLC	
	Hydrochlorothiazide	Amiloride hydrochloride	Hydrochlorothiazide	Amiloride hydrochloride
Mean[b]	49.6	4.98	50.1	5.03
SD	0.95	0.15	1.63	0.25
RSD	0.99	0.18	1.58	0.27

[a] SD, standard deviation, RSD, relative standard deviation.
[b] Results obtained are the average of ten experiments for each.

contain 50.0 mg of hydrochlorothiazide and 5.0 mg of amiloride hydrochloride) and laboratory-prepared sugar-coated tablet. The latter contained 50.0 mg hydrochlorothiazide and 5.0 mg of amiloride hydrochloride together with common additives and excipients, e.g. lactose, starch, talc and magnesium stearate. The results are given in Table 3. The results are accurate and precise, as indicated by the recovery (99.99–99.88%) and the relative standard deviation (0.02–0.83%). A good coincidence was observed for the assay results of the pharmaceutical formulation by application of the two methods in this paper (Table 4).

7. Conclusions

Ratio spectra derivative spectrophotometry and HPLC are suitable techniques for the reliable analysis of commercial formulations containing combinations of hydrochlorothiazide and amiloride hydrochloride. The most striking features of the ratio spectra derivative spectrophotometry are its simplicity, sensitivity and rapidity, which renders suitable for routine analysis in control laboratories. The RP-HPLC method gives a good resolution between hydrochlorothiazide and amiloride hydrochloride within a short analysis time (<10 min.). High percentage of recovery shows that the method is free from the interferences of the excipients used in the formulations. The RP-HPLC method was shown to be a versatile reference method and may offer advantages over the derivative method for the selective determination of the drug in the presence of their degradation products or in a variety of matrices.

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