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Second derivative UV spectrophotometric determination of hydrochlorothiazide and hydrochlorothiazide–amiloride combination in tablets

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Summary

Assay procedures based on second-order derivative UV spectrometry (D_2) have been developed for the determination of hydrochlorothiazide and amiloride, alone or in combination, in tablets. Hydrochlorothiazide (2–12 μg/ml) and amiloride (2–7 μg/ml) can be determined separately by measuring the amplitudes of maximum–minimum $D_{2(278-264nm)}$ and $D_{2(381-365nm)}$, respectively. Combinations of amiloride (2–7 μg/ml) and hydrochlorothiazide (20–70 μg/ml) in ratios ranging from 0.08 to 0.3 can be determined using the simultaneous equations method with measurements of the amplitudes of maximum–minimum $D_{2(381-365nm)}$ and $D_{2(334-312nm)}$. The linearity of the calibration curves was excellent ($r > 0.9993$), the precision (RSD) better than 1.3% and relative errors less than 1.5%. No spectral interferences from tablet excipients were found. Applications are given for the assay of commercial tablets and content uniformity test. The procedures proved to be suitable for a rapid and reliable quality control.

Ultraviolet derivative spectrometry has recently proven useful for determining drugs in multicomponent formulation and biological fluids in the presence of interferences originating from either the coexisting drugs or the sample matrix. UV derivative spectrometry enhances the qualitative features and thus increases the fingerprinting utility of UV spectrometry for selective identification and quantification of organic compounds (O'Haver and Green, 1976; O'Haver, 1979). Drugs with poorly developed maxima or with spectral interferences by excipients and other active ingredients can be accurately determined by UV derivative spectrometry (Davidson and Elsheikh, 1982; Gill et al., 1982; Fell et al., 1981; Martinez and Gimenez, 1981; Bermego et al., 1985; Corany et al., 1984; Abdel-Hamid et al., 1984; Nobile et al., 1987; Bedair et al., 1986; Poulou and Macheras, 1986).

In this paper an application of UV derivative (D_2) spectrometry to permit a simple, accurate and rapid assay of hydrochlorothiazide (Hy) and hydrochlorothiazide–amiloride (Hy-Am) combination in tablets is described. The determination of Hy alone is based on the measurement of the maximum–minimum amplitude of the D_2 spec-

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trum at 278–264 nm, while the combination of Hy-Am is determined by using the method of the simultaneous equations and measuring maximum–minimum amplitudes at 381–365 and 334–312 nm.

A double-beam UV-vis spectrophotometer (Perkin Elmer Lambda 7) with the capability of derivative mode was used. The optimized operating conditions for recording the D_2 spectra, using 1.000 cm quartz cells, were: scan speed 240 nm/min; response time 5 s; spectral slit width 2 nm; abscissa format 20 or 50 nm/cm.

Methanol, used as the solvent, was of analytical reagent grade. Hy and Am were obtained from local manufacturers and their purity was tested using HPLC. Several excipients, used for the interference study were obtained from commercial sources.

Working standard solutions of Hy (2–12 μg/ml), Am (2–7 μg/ml) and Hy-Am mixtures, with a ratio of Am/Hy in the range of 0.08–0.3 (keeping Am in the range 2–7 μg/ml and Hy in the range of 20–70 μg/ml) were prepared daily from stock solutions of Hy (1000 μg/ml) and Am (100 μg/ml) in methanol.

For the determination of Hy, the second-order

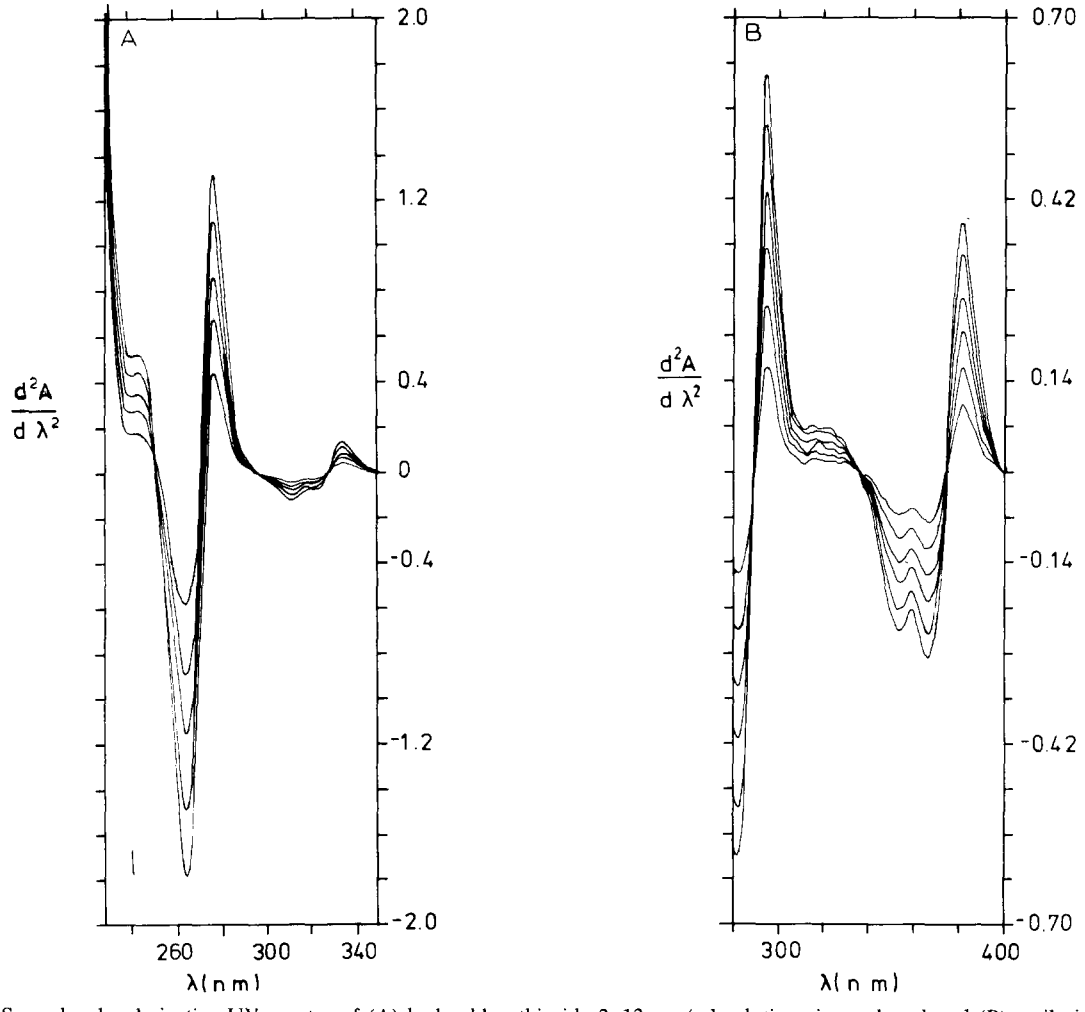

Fig. 1. Second-order derivative UV spectra of (A) hydrochlorothiazide 2–12 μg/ml solutions in methanol and (B) amiloride 2–7 μg/ml solutions in methanol.

(D_2) UV spectra of Hy working standard solutions, containing 2–12 μg/ml of Hy, were recorded over the 230–300 nm range against a methanol blank. The calibration curve was then constructed by plotting the graphically measured (mm) amplitudes of maximum–minimum $D_{2(278-264nm)}$ vs the corresponding Hy concentrations (μg/ml).

For the simultaneous determination of Hy and Am, two calibration curves were constructed as follows. The D_2 UV spectra of Am working standard solutions, containing 2–7 μg/ml of Am, and mixed Am-Hy working standard solutions were recorded over the 270–400 nm range against a methanol blank. A first calibration curve, for the estimation of Am, was constructed by plotting the measured (mm) amplitudes of maximum–minimum $D_{2(381-365nm)}$ vs the corresponding Am concentrations (μg/ml). A second calibration curve, for the estimation of Am/Hy concentration ratio was constructed by plotting the $D_{2(381-365nm)}/D_{2(334-312nm)}$ vs the respective Am/Hy concentration ratios of the mixed working standard solutions.

The procedures for the determination of drugs in tablets containing either Hy or a combination of Hy-Am were as follows. A portion of the fine and homogenized powder of the tablets, which were sampled and treated according to the USP XXI procedure, equivalent to about 5 mg of Am and 50 mg Hy, was accurately weighed and transferred in a 50-ml volumetric flask with 20 ml of methanol. The mixture was vigorously shaken for about 10 min, then the volume was completed to the mark with methanol and the obtained solution was filtered or centrifuged. For tablets containing only Hy, 1.00 ml of the obtained clear solution was diluted to 100 ml with methanol. The D_2 spectrum of this solution was recorded, the amplitude $D_{2(278-264nm)}$ was measured (mm) and the Hy concentration in the sample solution was obtained by interpolating the calibration curve. For tablets containing a combination of Hy-Am, 5.00 ml of the obtained clear solution was diluted to 100 ml with methanol. The $D_{2(381-365nm)}$ and $D_{2(334-312nm)}$ were measured (mm) and Am concentration and Am/Hy concentration ratio in the sample solution were obtained by interpolating the corresponding calibration curve.

The zero-order (D_0) absorption UV spectrum of Hy exhibits a profile with two absorption bands, namely a strong one with $\lambda_{max} = 271$ nm ($A^{1\%}_{1cm} =$

TABLE 1

Effect of tablet additives on zero- and second-order spectrophotometric determinations of hydrochlorothiazide

Additive	Concentration ratio (additive/Hy)	Recovery % ($n = 3$)	
		D_0	D_2
Lactose	6	103.6	98.2
Gelatin	6	105.4	100.2
Starch	6	107.4	100.9
Carbopol [a]	6	104.4	99.4
Sodium lauryl sulfate	6	99.8	99.4
Magnesium stearate	6	103.0	100.2
Carbowax [b]	6	145.9	141.8
Carbowax [b]	3	102.1	98.9
PVP K_{30} [c]	6	102.6	99.4
CAHP [d]	6	103.9	99.2
PVP [c]	6	104.5	100.2
Hydroxypropyl-methyl cellulose	6	107.8	106.2
Hydroxypropyl-methyl cellulose	3	98.9	99.4

[a] Carboxypolymethylene.
[b] Polyethylene glycol 4000.
[c] Polyvinylpyrrolidone.
[d] Cellulose acetate hydroxyphthalate.

660) and a weak one with $\lambda_{max} = 318$ nm ($A^{1\%}_{1cm} = 120$). These two bands are converted in sharp bands of the same relative amplitude when the second-order UV spectrum is recorded (Fig. 1A). Although a sensitive spectrophotometric determination of Hy is feasible, using the zero-order spectrum at 271 nm, with a measurable limit of less than 1 μg/ml, spectral interferences by the excipients are expected when this method is applied to formulation assays. In view of this, a second-order derivative UV spectrophotometric method was devised to determine Hy alone in tablets. The amplitude of maximum–minimum 278–264 nm of the second-order spectrum was found to be linear with Hy concentrations in the range 2–12 μg/ml, with a least-squares linear regression equation:

$$y(\text{mm}) = -0.532(\pm 3.05) + 13.81(\pm 0.29) \cdot C_{Hy}(\mu\text{g/ml})$$

$r = 0.9993$, $n = 6$

Eight replicate determinations carried out on a Hy standard solution of 10.0 μg/ml gave a relative standard deviation (RSD) of 1.3% and a relative error of -0.80%.

In order to examine the effect of common excipients, used in the formulation of tablets, on the zero- and second-order UV spectrophotometric determinations of Hy, recovery experiments were carried out from synthetic standard methanolic solutions containing 10.0 μg/ml of Hy and various excipients in excess. From the results shown in Table 1 it is evident that the D_2 spectrophotometric determination does not suffer from the spectral interference of the excipients and the direct determination of Hy in tablets without any isolation of the analyte being successful.

A further comparison of the proposed D_2 spectrophotometric method vs the zero-order spectrophotometric determination was made by analyzing commercial tablets containing 25.0 mg of hydrochlorothiazide (Essidrex) the exact content of which was confirmed by the USP official procedure (UV spectrophotometric method after a column chromatographic isolation of the analyte).

The results gave a mean of 24.82 ± 0.33 mg/tablet ($n = 5$), i.e. RSD = 1.3%, and relative error -0.72%. The results obtained by the D_0 spectrophotometric method, carried out for comparative purposes, gave 26.46 ± 0.23 ($n = 5$) i.e. RSD = 0.86% and relative error $+5.8\%$. Again the superiority of the D_2 method is clear.

The zero-order UV spectrum of Am exhibits a profile with two absorption bands of about the same intensity ($\lambda_{max} = 285$ nm with $A^{1\%}_{1cm} = 540$ and $\lambda_{max} = 360$ nm with $A^{1\%}_{1cm} = 640$). The second-order spectrum shows two maximum–minimum pairs at 292–282 nm and 381–365 nm (Fig. 1B). By comparing the D_2 spectra of Hy and Am (Fig. 1A and B, respectively) it is clear that the amplitude of maximum–minimum $D_{2(381-365nm)}$ of Am is not affected by the presence of Hy allowing the selective determination of Am in Am-Hy mixtures. The amplitude of maximum–minimum 381–365 nm was found to be linearly related to Am concentration in the range of 2–7 μg/ml with a least-squares linear regression equation:

$$y(\text{mm}) = -0.819(\pm 1.23) + 14.38(\pm 0.26) \cdot C_{Am}(\mu\text{g/ml})$$

$r = 0.9993$, $n = 6$

Unfortunately the D_2 spectrum of Hy is affected seriously, at both maximum–minimum pairs, by the presence of Am (Fig. 2).

Since in commercial tablets Hy is in higher quantities than Am, the less sensitive maximum–minimum at 334–312 nm was used in the determination of Hy. It was found that the ratio of the amplitudes $D_{2(381-365nm)}$ and $D_{2(334-312nm)}$ is linearly related to Am/Hy concentrations ratio in the range 0.08–0.3, the Am concentration being in the range 2–7 μg/ml while the Hy concentration was ranged from 20 to 70 μg/ml. A typical equation of the least-squares linear regression was:

$$y(\text{mm}) = -0.067(\pm 0.017) + 6.92(\pm 0.14) \cdot \left[C_{Am}/C_{Hy}\right]$$

$r = 0.9997$, $n = 12$

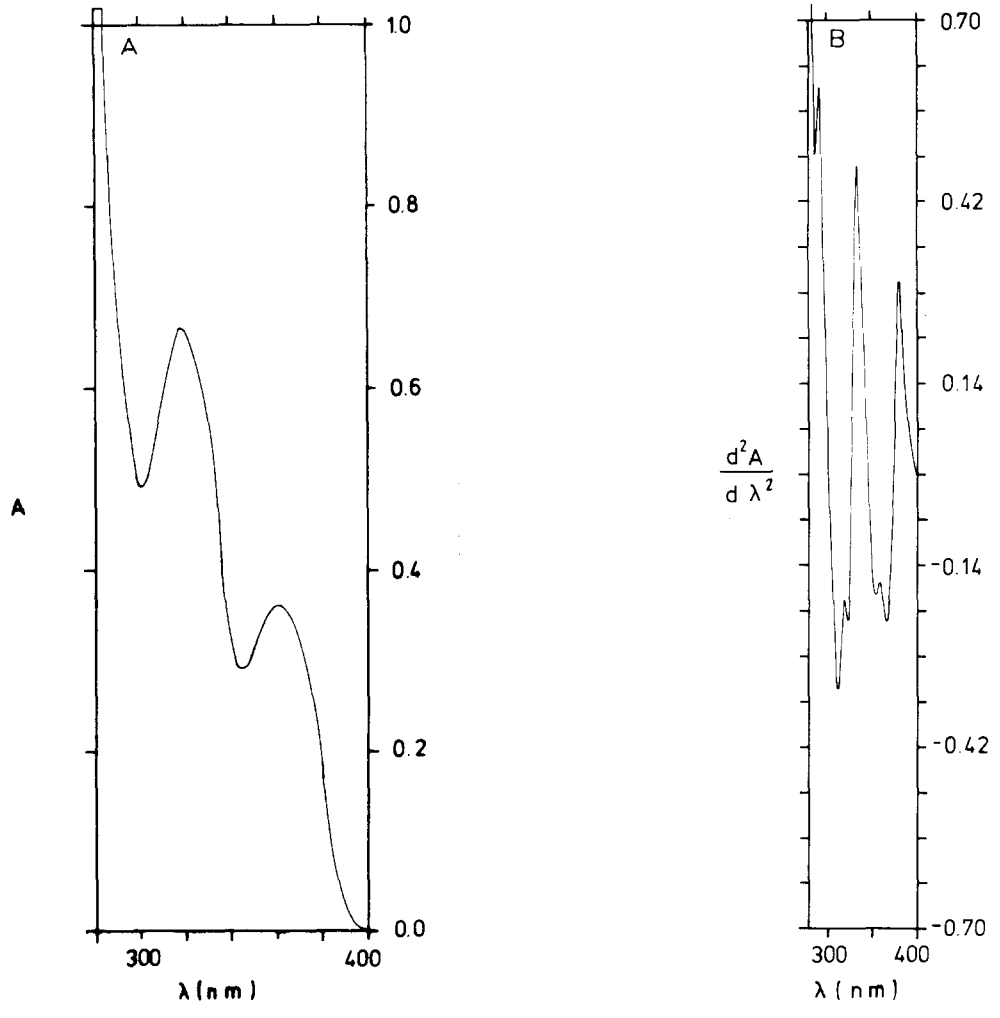

Fig. 2. Absorption (zero-order) UV spectrum (A) and second order derivative UV spectrum (B) of hydrochlorothiazide (50 μg/ml)-amiloride (5 μg/ml) mixture in methanol.

The D_2-spectrophotometric method for the simultaneous determination of Am and Hy was tested by analysing a standard synthetic methanolic solution containing 4.00 μg/ml of Am and 50.0 μg/ml of Hy. Ten replicate determinations gave 3.94 ± 0.04 μg/ml for Am (RSD = 1.0%, relative error = −1.5%) and 49.2 ± 0.5 μg/ml for Hy (RSD = 1.0%, relative error = −1.6%).

The proposed D_2-method for the simultaneous determinations of Am and Hy was further evaluated in the assay of commercial tablets of the Am-Hy combination (Moduretic, 5 mg of Am, 50 mg of Hy), and the results were compared to those obtained by the time-consuming HPLC method of the USP. A very good agreement (based on the *t*-test) between the two methods was found.

The advantages of the proposed D_2-spectrophotometric method (i.e. short analysis time, unnecessary sample pretreatment and good precision and accuracy), make the method attractive in content uniformity tests, where a great number of assays on individual tablets is required. Thirty individual Moduretic tablets (5 mg of Am, 50 mg of Hy),

were analyzed for Am. All tablets were found to be in the range of 101–105% of the stated content with an RSD = 1.3%. The analytical workload is enough minimized by using the proposed D_2-spectrophotometric method where only the recording of a D_2-spectrum of a filtered sample solution is required.

In summary, the proposed analytical procedures based on second-order derivative UV spectrometry offer the advantage of an increased resolution and decreased spectral interferences and could be used for a rapid and reliable quality control of commercial formulations containing hydrochlorothiazide and amiloride, alone or in combination.

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