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Second derivative ultraviolet spectrophotometry and HPTLC for the simultaneous determination of vitamin C and dipyrone

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Two accurate, precise and sensitive thin layer chromatographic (TLC) and second derivative UV-spectrophotometric procedures are described for the simultaneous determination of ascorbic acid and dipyrone in pure form and in pharmaceutical dosage forms. The TLC method involved direct application of methanolic solutions of tested samples on silica gel TLC plates using water : methanol (95 : 5 v/v) as developing system. The developed plates were then directly scanned at 260 nm using a TLC scanner. The second method depends on second derivative UV-spectrophotometry with zero crossing technique of measurement. Second derivative amplitudes at 280 and 272 nm were selected for the determination of ascorbic acid and dipyrone, respectively. Both methods show good linearity, precision and reproducibility. They are simple and do not require manipulation prior to analysis. The proposed methods have been successfully applied to the determination of the drugs in various pharmaceutical dosage forms such as tablets and ampoules.

1. Introduction

Ascorbic acid (Vitamin C) and dipyrone (metamizole) are sometimes combined in pharmaceutical dosage forms to relief pain and fever and to increase in general resistance of the body against certain microbial diseases such as influenza and common cold.

Several techniques have been reported for the analysis of ascorbic acid. Besides, the iodometric methods described in the pharmacopoeias [1, 2], other methods are reported including titrimetry [3], colorimetry [4], polarography [5, 6], gas chromatography [7], enzymatic analysis [8, 9], spectrophotometry [10, 11] and HPLC [10, 12]. Dipyrone has been determined by titrimetric [13], UV-spectrophotometric [14–18], colorimetric [12, 20, 21], polarographic [22, 23], TLC [24, 25], and HPLC methods [10, 26, 27]. Only one derivative spectrophotometric method was described for the simultaneous determination of both drugs. El-Sadek et al. [28] used two different media (HCl and NaOH) for first derivative UV-spectrophotometric measurements of ascorbic acid and dipyrone, respectively.

In the present work, two methods based on TLC and second derivative UV-spectrophotometry, using one medium for measurement, are described for the simultaneous determination of ascorbic acid and dipyrone in dosage forms as tablets and ampoules.

2. Investigations, results and discussion

2.1. Thin layer chromatography

Simultaneous determination of ascorbic acid and dipyrone by TLC was achieved using a developing system composed of water and methanol in the ratio of 95 : 5 v/v. A satisfactory separation was obtained permitting simple and fast scanning of the two components on the plates directly under an UV-densitometer at 260 nm. The R_f values for ascorbic acid and dipyrone were 0.92 and 0.65, respectively.

Several wavelengths were tried for the simultaneous detection of ascorbic acid and dipyrone at equal concentrations. Although the UV-response of ascorbic acid is higher than that of dipyrone, as shown from the integrated areas (Table 1), 260 nm was found to be the optimum detection wavelength of both drugs at equal concentrations.

Linear relationships between the integrated peak areas and drug concentrations were observed (Table 2). The linear relationship remained valid up to concentrations as high as about 5 $\mu g \cdot ml^{-1}$ for both drugs. Least squares regression analysis was carried out for the slope, the intercept and the correlation coefficient (r). The relative standard deviation (RSD%) calculated for the separate determination of each drug was 0.02–0.36% indicating good precision and reproducibility of the TLC method.

The sensitivity limit of the TLC method was examined and found to be 1.25 $\mu g \cdot ml^{-1}$ for each of the drugs tested.

The precision of the TLC procedure was confirmed by analyzing five replicate samples each containing 3 $\mu g \cdot ml^{-1}$ of ascorbic acid and dipyrone. At this concentration level, RSD% was 1.8–2.3% indicating good precision and reproducibility. Also the precision of the D_2 spectrophotometric method was confirmed as mentioned above by analyzing five replicate samples each containing 10 $\mu g \cdot ml^{-1}$ of ascorbic acid and dipyrone (Table 1).

2.2. Derivative spectrophotometry

The absorption (zero-order) UV spectra of ascorbic acid and dipyrone in the 200–300 nm wavelength region are given in Fig. 1.

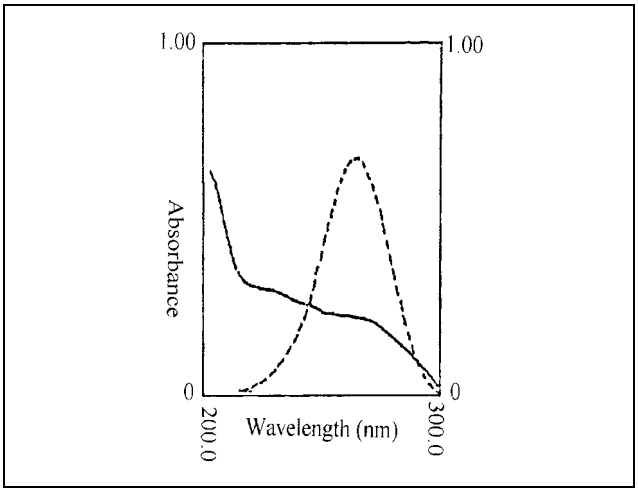

Fig. 1: Zero-order absorption of 10 $\mu g \cdot ml^{-1}$ aqueous solutions of dipyrone (——) and 10 $\mu g \cdot ml^{-1}$ of ascorbic acid (- - - -)

Table 1: Precision of the TLC and D_2 spectrophotometric methods for the determination of ascorbic acid and dipyrone

TLC				D_2			
Ascorbic acid (3 μg · ml^{-1})		Dipyrone (3 μg · ml^{-1})		Ascorbic acid (10 μg · ml^{-1})		Dipyrone (10 μg · ml^{-1})	
Peak area	Conc. found (μg · ml^{-1})	Peak area	Conc. found (μg · ml^{-1})	D_2 (280)	Conc. found (μg · ml^{-1})	D_2 (272)	Conc. found (μg · ml^{-1})
4000	2.944	2144	2.927	0.0202	9.95	0.0040	10.00
4250	3.063	2261	3.033	0.0205	10.10	0.0041	10.25
4300	3.086	2250	3.024	0.0203	10.00	0.0039	9.75
4280	3.077	2190	2.970	0.0204	10.05	0.0040	10.00
4420	3.143	2300	3.069	0.0204	10.05	0.0041	10.25
Mean	3.0626		3.005		10.025		10.050
RSD%*	2.4		1.8		0.6		2.1

* Percent relative standard deviation

The spectra of both drugs show extensive overlapping bands in the region 220–280 nm. Because of this extensive overlap of both drugs, conventional UV-spectrophotometry cannot be used for individual quantitation of such binary mixture. When second UV-derivative spectra are recorded, sharp bands of great amplitudes are produced permitting selective determination of both drugs (Fig. 2).

The choice of the optimum wavelength is based on the fact that the contribution of each component to the overall derivative signals is zero at the wavelength at which the other component has the maximum absorption. Therefore, the second derivative peak amplitudes (D_2) at 280 nm (zero-crossing of dipyrone) and the trough amplitudes (D_2) at 272 nm (zero-crossing of ascorbic acid) have been chosen for simultaneous determination of ascorbic acid and dipyrone in this binary mixture respectively. Fig. 3 shows the second-derivative spectra of ascorbic acid and dipyrone at different concentrations showing the isodifferential point for each drug.

For quantitative applications, linear calibration curves were obtained with correlation coefficients better than 0.999 (Table 2). Linearity between the D_2-absorbance and concentration remained valid to concentrations up to about 15 μg · ml^{-1} and 70 μg · ml^{-1} for ascorbic acid and dipyrone, respectively. The D_2 spectrophotometric procedure

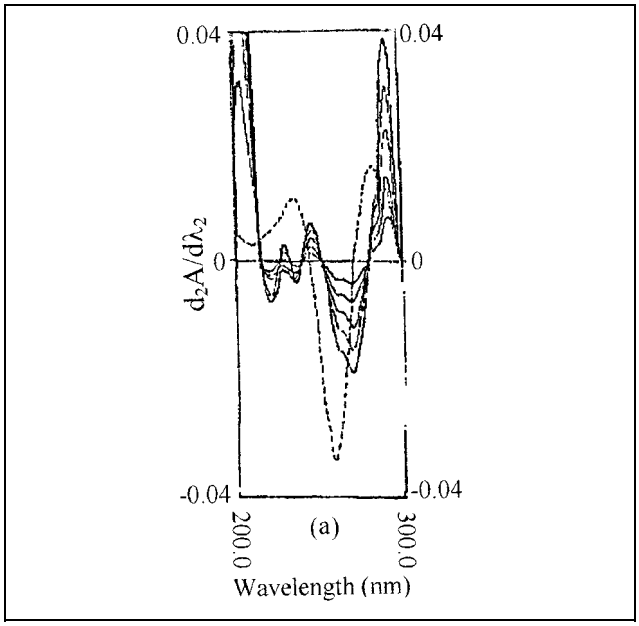

Fig. 3a: Second-order absorption spectra (D_2) of several concentrations of dipyrone (——) in the range 10–50 μg · ml^{-1} and ascorbic acid (- - - -) at a concentrations of 8 μg · ml^{-1}

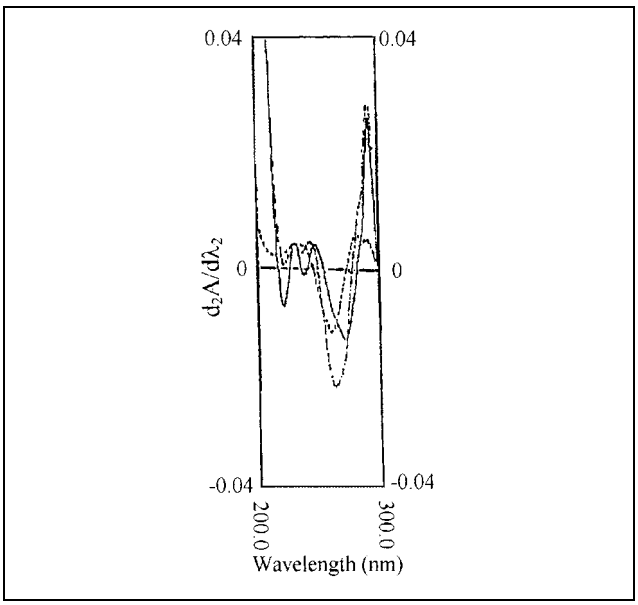

Fig. 2: Second-order spectra D_2 of 30 μg · ml^{-1} solution of dipyrone (——) and 4 μg · ml^{-1} ascorbic acid (- - - -) and mixture of 30 μg · ml^{-1} solution of dipyrone and 4 μg · ml^{-1} ascorbic acid solution (- · - ·)

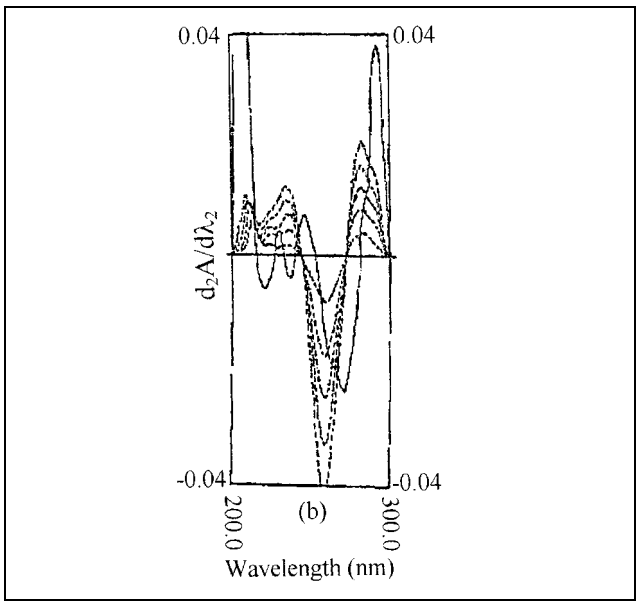

Fig. 3b.: Second-order absorption spectra of ascorbic acid (- - - -) in the range 2–10 μg · ml^{-1} and of 50 μg · ml^{-1} of dipyrone (——)

Table 2: Equations for calibration curves (n = 10) and correlation coefficients for the determination of ascorbic acid and dipyrone by TLC and D_2-spectrophotometry

Drug	Method	Equation *	Correlation coefficient **	Conc. range $\mu g \cdot ml^{-1}$	RSD% ***
Ascorbic Acid	TLC	$Y = -2212 + 2110X$	$0.9996 + 0.00019$	1.5–3.5	0.02
	D_2	$Y = 0.0003 + 0.0020X$	$0.9987 + 0.0013$	2–10	0.13
Dipyrone	TLC	$Y = -1064 + 1096X$	$0.9945 + 0.0035$	1.5–3.5	0.34
	D_2	$Y = 0.000 + 0.0004X$	$0.9938 + 0.0031$	10–50	0.32

* The equation is defined as $Y = a + bx$ where y is the integrated peak area (TLC) or peak absorbance at 280 nm for ascorbic acid or 272 nm for dipyrone (D_2 -method) and × is the concentration range ($\mu g \cdot ml^{-1}$)
** + standard deviation
*** percent relative standard deviation
D_2 : second derivative UV spectrophotometric method

was sensitive enough to detect concentrations as low as 0.5 $\mu g \cdot ml^{-1}$ and 5 $\mu g \cdot ml^{-1}$ for ascorbic acid and dipyrone, respectively. The good precision of the D_2 procedure was indicated by a RSD of 0.13–0.32%.

The precision of the D_2 procedure was confirmed by analyzing five replicate mixture samples each containing 10 $\mu g \cdot ml^{-1}$ of ascorbic acid and dipyrone (Table 1). RSD% was 0.6–2.1% indicating good precision and reproducibility.

2.3. *Analysis of pharmaceutical formulations*

The validity of the proposed methods for analysis of dosage forms were tested by analyzing Cevagen® tablets labeled to contain 0.5 g of both ascorbic acid and dipyrone and Cevagen® ampoules labeled to contain 1.0 g of both drugs. The results of analysis are given in Table 3. The results are accurate and precise as indicated by the percentage recovery that ranges from 98.3–98.7% and from 98.4–98.5 (TLC) and from 98.4–98.8 and from 98.3 to 99.0 (D_2-spectrophotometry) for ampoules and tablets, respectively.

Table 3: Determination of ascorbic acid and dipyrone in pharmaceutical formulations by TLC and second derivative UV-spectrophotometric procedures

	Ascorbic acid +		Dipyrone +	
Method	Tablets * Recovery%+	Ampoules ** Recovery%	Tablets * Recovery%	Ampoules ** Recovery%
TLC	98.5 ± 0.7	98.7 ± 1.2	98.4 ± 0.7	98.3 ± 1.0
D_2	99.0 ± 0.4	98.8 ± 0.8	98.3 ± 0.9	98.4 ± 1.0

* Cevagen tablet (Memphis chemical company, Cairo, Egypt), labeled to contain 0.5 g ascorbic acid and 0.5 g of dipyrone
** Cevagen ampoule (Memphis Chemical Company, Cairo, Egypt), labeled to contain 1.0 g of ascorbic acid and 1.0 g of dipyrone
+ Average of five determinations

It is noteworthy that due to the higher absorptivity of ascorbic acid compared to dipyrone, equal concentrations of both drugs give D_2 amplitudes at the selected zero-crossing wavelengths in the ratio of about 5 : 1. Consequently, four times amount of dipyrone was added in analyzing the above mentioned dosage forms to make nearly equal absorbance intensity (amplitudes) of ascorbic acid and dipyrone in the analyzed mixtures.

3. Experimental

3.1. *Materials and methods*

Ascorbic acid and dipyrone reference substances and their pharmaceutical formulations (tablets and ampoules) were kindly donated by Memphis Chemical Company, Cairo, Egypt. These two substances were analytical grade and were used without further purification. Other chemicals and reagents were of analytical reagent grade. The TLC plates were silica gel (60 F_{254}, 20 × 10 cm layer thickness) purchased from Merck Company (E. Merck Darmstadt, Germany). Prior to use, the plates were activated by incubation at 105 °C for 30 min.

Quantitation of TLC plates was carried out using a Shimadzu CS-920 TLC scanner (Shimadzu, Japan). Slit width was 1.2 mm and integrated peak area was recorded.

Spectrophotometric measurements were carried out using Shimadzu UV-240 recording spectrophotometer in 1 cm matched quartz cells equipped with a Shimadzu derivative accessory unit operating in the second-derivative (D_2) mode. The measurement parameters were: spectral slit width 2 nm, scan speed 20 $nm \cdot sec^{-1}$, wavelength range 200–300 nm and ordinate minimum and maximum settings ± 0.04.

3.2. *Procedure for thin layer chromatography*

3.2.1. *Calibration*

Accurately weighed amounts (200 mg) of ascorbic acid and dipyrone were separately transferred into a 100 ml volumetric flask and diluted to volume with 100 ml methanol to get working standard solutions of 2 $mg \cdot ml^{-1}$. Mixtures of ascorbic acid and dipyrone in the concentration range 150 to 350 $\mu g \cdot ml^{-1}$ of each were prepared by transferring several aliquots of ascorbic acid and dipyrone standard solutions into 1.5 ml disposable plastic microcentrifuge vials and adjusting the volume to 1 ml with methanol. From these solution mixtures, 5 µl samples equivalent to 1.5–3.5 µg were applied to the TLC plates. Each mixture was applied in the TLC plate in triplicate. The plates were developed in appropriate TLC covered tanks using a solvent system comprising water and methanol in the ratio 95 : 5 v/v and then quantified at 260 nm using TLC scanner. The integrated peak area for each concentration of ascorbic acid or dipyrone was measured and plotted versus its corresponding concentration to obtain the calibration curve (Table 2).

3.2.2. *Analysis of tablets and ampoules*

Twenty tablets were powdered and thoroughly mixed. An accurately weighed amount of the tablet powder equivalent to 200 mg of ascorbic acid and 200 mg of dipyrone was transferred into a 100 ml volumetric flask, mixed with 50 ml methanol and sonicated for 15 min. The mixture was diluted to volume with methanol and filtered using Millipore filter (0.45 µm) to remove any insoluble excipients usually formulated with the tablet such as disintegrates, lubricants etc that may interfere with the spots of either drugs. An aliquot of 350 µl of the filtered solution was suitably diluted and treated according to the TLC method.

From the contents of an ampoule, a 50 µl aliquot equivalent to a 10 mg ascorbic acid and 10 mg dipyrone was transferred into a 10 ml volumetric flask and diluted to volume with methanol to get a concentration of 1 $mg \cdot ml^{-1}$. From this solution 700 µl aliquots were suitably diluted and treated according to the TLC method.

The amount of ascorbic acid and dipyrone in each formulation was then calculated with reference to the linear regression equation of the calibration graph of the TLC method.

3.3. *Procedure for derivative spectrophotometry*

3.3.1. *Calibration*

From the working standard solution of ascorbic acid (2 $mg \cdot ml^{-1}$) prepared as above, several aliquots (10–50 µl) were separately transferred into 10 ml volumetric flasks and diluted to volume with water to obtain several concentration of ascorbic acid in the range of 2–10 $\mu g \cdot ml^{-1}$. Similarly, from the working standard solution of dipyrone (2 $mg \cdot ml^{-1}$), several aliquots (50–250 µl) were separately transferred into 10 ml volumetric flasks and diluted to volume with water to obtain several concentrations of dipyrone in the range 10–50 $\mu g \cdot ml^{-1}$.

The D_2 spectrum for each mixture was recorded against water and the peak and trough amplitudes at 280 and 272 nm were measured for ascorbic acid and dipyrone, respectively.

3.3.2. Analysis of tablets and ampoules

From the filtered solution of tablets (2 mg · ml^{-1}), 50 µl aliquots were suitably diluted and treated according to the D_2 spectrophotometric procedure after the addition of 200 µl aliquots of the working standard solution of dipyrone (2 mg· ml^{-1}).
Similarly, from the diluted ampoules (1 mg · ml^{-1}), 100 µl aliquots were mixed with 200 µl aliquots of dipyrone solution (2 mg · ml^{-1}) and treated according to the D_2 spectrophotometric procedure.

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