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FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ASCORBIC ACID AND ANALGIN IN COMBINATION.

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Abstract :-

A spectrophotometric method is presented for the determination of Ascorbic Acid and Analgin in presence of each other using a first derivative spectrophotometry. By measuring the absolute value of the first-order contribution of both drugs in presence of each other. The concentration of both drugs can be calculated without interference from each other. The method was proved using synthetic mixtures of Ascorbic Acid and Analgin in presence of each other.

Key words :-

First derivative, Ascorbic Acid, Analgin.

Introduction :-

Derivative UV absorption spectroscopy has been used to quantitate several compounds of biological interest by reducing many of the spectral interferences encountered in conventional spectrophotometric methods (1-5)

Pharmaceutical applications of derivative spectroscopy are few (6) and for the most part have been limited to enhancing the spectral features of a drug to facilitate its identification (7,8) or quantitation (9), several papers (6,10,11) have reported the successful application of this technique to the determination of single component pharmaceutical dosage forms containing excipients that interfere with their spectrophotometric analysis. Only a few reports (6,12,13) have dealt with the application of derivative spectroscopy to the simultaneous determination of more than one active ingredient in multicomponent pharmaceutical dosage forms. This paper presents a first - derivative spectroscopic method, which is capable for simultaneously determining Ascorbic Acid and Analgin in CEVAGINE ampoules. Also, this paper is a simple, rapid and accurate method for determining Ascorbic Acid and Analgin in combination (5,12).

Experimental

Instrument :-

A Shimadzu recording spectrophotometer UV 260 was used with 1-cm quartz cuvettes. Suitable settings were : scan speed 40 nm min chart speed 60 nm min, and slit width 2 nm.

Materials :-

Analytical standard grade of Ascorbic Acid (Memphis co; Egypt) Analgin (Memphis Co; Egypt) CEVAGINE ampoules (Memphis Co; Egypt) labelled to contain 1.0 g Ascorbic Acid and 1.0 g Analgin per ampoule (5ml.), 0.1M HCl solution, 0.1M NaOH solution. Other reagents used were of analytical grade.

Calibration graphs :

Stock solutions were prepared containing 500 mg Ascorbic Acid in 100 ml distilled water and 500 mg Analgin in 100 ml distilled water. For each drug 1/2 ml from the first stock solution in 50 ml distilled water. For each drug, pipette (0.002-0.008 mg) of these solutions into a 25 ml measuring flasks.

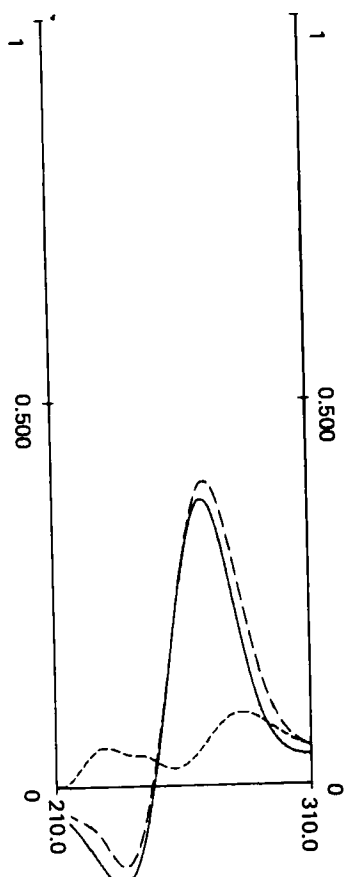
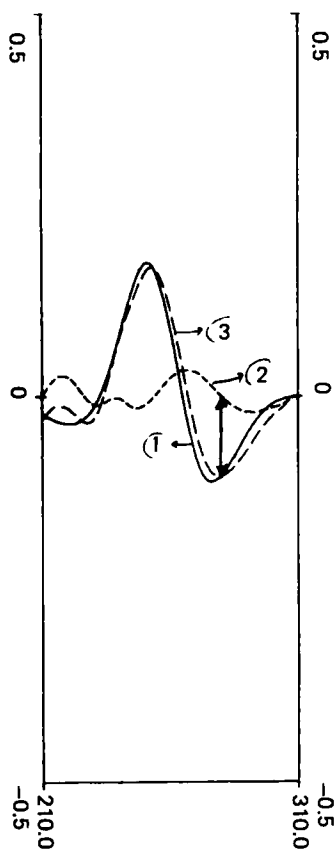
For Ascorbic Acid, complete to 25 ml volume one with distilled water and other with 0.1 M HCl, and for Analgin complete to 25 ml volume with 0.1 M NaOH solution. The first derivative D1 of the UV spectra was measured at 275 nm for Ascorbic Acid using the same concentration of Ascorbic Acid in the sample in a 0.1 M HCl as blank Fig. 1b, and at 287 nm for Analgin using 0.1 M NaOH as blank Fig. 2b.

Preparation of CEVAGINE ampoules :-

Stock solution of CEVAGINE was prepared containing 500 mg Ascorbic Acid and 500 mg Analgin per 100 ml distilled water (2.5 ml from cevagin ampoule) and 1/2 ml from the prepared solution in 50 ml distilled water. And as described under calibration graph per 25 ml distilled water, also per 25 ml 0.1 M HCl and per 25 ml 0.1 M NaOH. The procedure was then continued as described under calibration graph.

Results and Discussion :-

Figures (1a and 2a) show the zero order UV absorption curves of Ascorbic Acid in distilled water as a sample where Ascorbic Acid in 0.1 M HCl as a blank, and Analgin in 0.1 M NaOH as a sample where 0.1 M NaOH as a blank and the mixture form. In this way the strongly overlapping absorbance at zero-order spectra in the zone between (210-310 nm) seems to be resolved in the first derivative band (Fig. 1b and 2b), thus

**Fig (1a)****Fig (1b)**

Blank : Authentic drugs in 0.1 M HCl

Sample : Authentic drugs in distilled H₂O

- 1- (——) Ascorbic Acid
- 2- (-----) Analgin
- 3- (.....) Mixture form

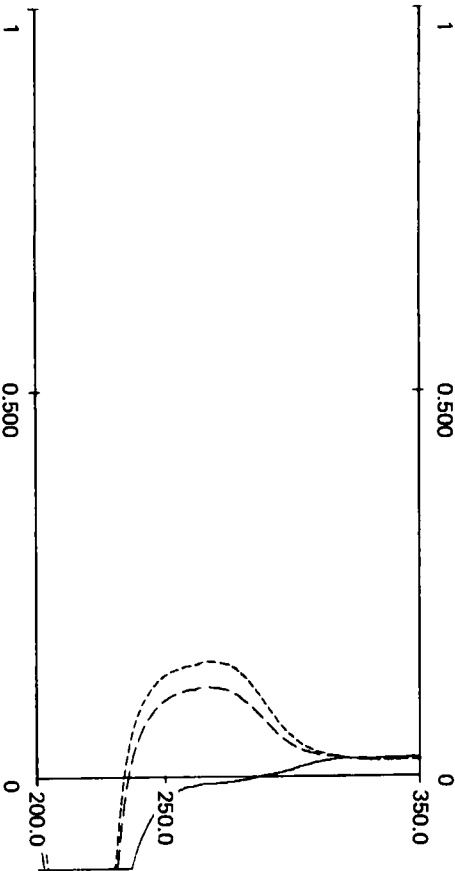


Fig. (2a)

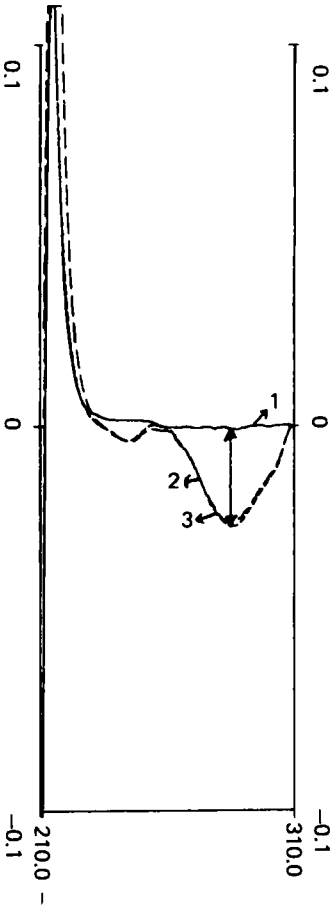


Fig. (2b)

Blank : 0.1 M NaOH

Sample : Authentic drugs in 0.1 M NaOH

- 1- (____) Ascorbic Acid
- 2- (-----) Analgin
- 3- (-----) Mixture form

allowing in principle a direct quantitative determination for Ascorbic Acid and Analgin in mixture form without interference. By measuring D1 value at 275 nm Ascorbic Acid possesses a maximum D1 value (Ascorbic acid in distilled water as sample) (Ascorbic Acid in 0.1 M HCl as blank), where the absorbance due to Analgin is practically negligible. Also, Analgin possesses maximum D1 at 287 nm (Analgin in 0.1 M NaOH as sample), (0.1 M NaOH as blank), while that of Ascorbic Acid at the same wave length is approximately equal to zero.

The plots of D1 values at 275 nm for Ascorbic Acid and 287 nm for Analgin against concentration (c) show a linear relationship within the range (0.012-0.032 mg) for Ascorbic Acid and (0.012-0.032 mg) for Analgin. The linear equations are found to be :-

for Ascorbic Acid

$$C = 0.1414 D1 + 0.0002 \quad (r = 0.9965)$$

and for Analgin

$$C = 1.0163 D1 + 0.0026 \quad (r=0.9980)$$

Where D1 = (dA/dλ), C is concentration in mg/100 ml and r - regression coefficient.

The proposed method is applied for determination of Ascorbic Acid and Analgin in prepared mixture containing the same ratio of the drugs that found in pharmaceutical preparation (Table 1).

Also pharmaceutical preparations containing these drugs (CEVAGINE AMPOULES) is analyzed using the proposed method (Table2). Statistical analysis of the results shows that mean recovery 99 % for Ascorbic Acid and 100.4 % for Analgin in table 1. With relative standard deviation which equals to 0.1 and 0.2 respectively.

Table (1)
Determination of Ascorbic Acid and Analgin in Authentic mixture using the proposed method.

| Ascorbic Acid | | | | Analgin | |
|-----------------------------|-----------------|----------------|------------|-----------------|---------------------------|
| EXP. No. | Taken mg /100ml | found mg/100ml | % recovery | Taken mg /100ml | found mg/100ml % recovery |
| 1 | 0.012 | 0.0116 | 96.8 | 0.012 | 0.0125 102 |
| 2 | 0.016 | 0.015 | 98.5 | 0.016 | 0.0158 98.9 |
| 3 | 0.020 | 0.0199 | 99.98 | 0.020 | 0.0205 102 |
| 4 | 0.024 | 0.0238 | 99.16 | 0.024 | 0.0237 98.75 |
| 5 | 0.028 | 0.0279 | 99.60 | 0.028 | 0.0280 100 |
| 6 | 0.032 | 0.0318 | 99.40 | 0.032 | 0.0322 100.8 |
| Mean Recovery (\bar{x}) | | | 99 | 100.4 | |
| V | | | 1.316 | 2.082 | |
| S.D | | | 1.147 | 1.442 | |
| S.E | | | 0.468 | 0.588 | |
| R.S.D | | | 0.191 | 0.220 | |

Table (2)
Determination of Ascorbic Acid and Analgin in CEVAGINE ampoules using the proposed method.

| Ascorbic Acid | | | | Analgin | | |
|-----------------------------|-----------------|----------------|------------|-----------------|----------------|------------|
| EXP. No. | Taken mg /100ml | found mg/100ml | % recovery | Taken mg /100ml | found mg/100ml | % recovery |
| 1 | 0.012 | 0.012 | 100 | 0.012 | 0.0122 | 101.6 |
| 2 | 0.016 | 0.0158 | 98.8 | 0.016 | 0.0158 | 98.75 |
| 3 | 0.020 | 0.0199 | 99.8 | 0.020 | 0.0198 | 99 |
| 4 | 0.024 | 0.0241 | 100.5 | 0.024 | 0.0245 | 102 |
| 5 | 0.028 | 0.0284 | 101 | 0.028 | 0.028 | 100 |
| 6 | 0.032 | 0.0322 | 100.6 | 0.032 | 0.0325 | 101.5 |
| Mean Recovery (\bar{x}) | | | | | | 100.47 |
| V | | | | | | 2.002 |
| S.D | | | | | | 1.414 |
| S.E | | | | | | 0.537 |
| R.S.D | | | | | | 0.235 |

In table 2 mean recovery 100.11 % for Ascorbic Acid and 100.47 % for Analgin with relative standard deviation equals to 0.1 and 0.2 respectively. From the above study it can be concluded that the proposed method is simple, accurate, precise and selective depending upon intact molecule with capabilities for its application as stability for each ingredient of the mixture.

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