# Simultaneous Determination of Ciprofloxacin Hydrochloride and Mebeverin Hydrochloride by Derivative Spectrophotometry

# Simultaneous Determination of Ciprofloxacin Hydrochloride and Mebeverin Hydrochloride by Derivative Spectrophotometry

**Maha A. Mohammmed.**
**Muna A. Kadhum**
Dept. of Chemistry/ College of Education for pur science/(Ibn Al-Haitham)/ University Of Baghdad

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## Abstract

A new Spectrophotometric method, is for individual and simultaneous determination of Ciprofloxacin hydrochloride(CIP) and Mebeverin hydrochloride(MEB) by the first and second derivative mode techniques. The first and second derivative spectra of these compounds permitted individual and simultaneous determination of CIP and MEB in concentration range of (4-28µg/mL) by measuring the amplitude of peak- to- base line and the area under peak at selected spectrum intervals. The methods showed a reasonable precision and accuracy and have been applied to determine CIP and MEB in four different pharmaceutical preparations.

**Key Words:** Derivative, Ciprofloxacin hydrochloride, Mebeverin hydrochloride, Determination, Spectrophotometry

# Introduction

Ciprofloxacin hydrochloride (CIP) is a synthetic chemotherapeutic antibiotic (1-cyclopropyl-6-fluoro-1, 4-dihydro-4- oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid), Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4 g/molfigure1, Ciprofloxacin hydrochloride is a broad-spectrum antimicrobial agents belonging to the fluoroquinolone group Its mode of action depends upon blocking bacterial DNA replication by binding itself to an enzyme called DNA gyrase, thereby preventing the enzyme ability to untwist the DNA double helix, which is required for DNA replication [1,2].
Mebeverine hydrochloride (MEB) has an empirical formula$C_{25}H_{35}NO_5$, chemical name. 4-(ethyl[1-(4-methoxyphenyl)propan-2-yl]amino)butyl-3,4 dimethoxybenzoate)and its molecular weight is 429,6[g/mol] as shown in figure 2.

MEB is a drug whose major therapeutic role is in the treatment of irritable bowel syndrome (IBS) and the associated abdominal cramping. It works by relaxing the muscles in and around the gut. It is a musculotropic antispasmodic drug without anticholinergic side-effects. The drug is also indicated for treatment of gastrointestinal spasm secondary to organic disorder Hydrochloride [3].Most methods for CIP analysis include high performance liquid chromatographic techniques [4,5], thin layer chromatography [6,7], gas chromatography [8], capillary electrophoresis [9,10] , polarography [ 11]and spectrophotometry [12,13 ] were reported. Different methods have been reported form the determination of MEB including ,spectrophotometric methods [14-15], high performance liquid chromatographic techniques [16,17]. The main goal of this work is to establish accurate, precise, rapid and reproducible stability indicating spectrophotometric method for the determination of CIP, MEB individually. And for simultaneous determination of CIP.AndMEB. In binary mixtures, this can be used for the routine quality control analysis of these drugs in raw material and pharmaceutical formulations and stability studies.

# Experimental

## Apparatus

UV-visible spectrophotometer (Shimadzu 1800) with UV-Probe Version 1.10 (Japan) connected to computer was used for the drugs estimation. Quartz cuvettes (1.00 cm) were matched and used for all absorbance measurements.

## Chemicals

Pure gift samples of (CIP) and (MEB) double distilled water were provided by the state company of drug industries and medical appliances (IRAQ_Samara), CIP (500mg) tablet and MEB (135mg) tablet from local market. All drugs were used as working standards without further purification.

# Preparation of Stock and Working Standard Solutions:

The stock solutions of CIP and MEB (100 µg/mL) were prepared by dissolving 10 mg of drugs in 100 mL water using volumetric flask. The working standard solutions of the respective drugs were prepared by several dilution using water .

# Procedure

## 1- Individual Determination of CIP and MEB

Aliquots of CIP or MEB solution containing (4-20)µg/mL for each one aliquots alone were transfered into 10 mL volumetric flasks and dilute to mark with double distilled water. The absorption spectra were recoded and showed absorption maxima at 206,276and 316 nm for CIP, and 220, 262 and 293nm for MEB.

Determination was made by measuring the first and second derivative values and area under peaks of their spectra at certain given wavelengths and wavelength regions. The concentration of CIP and MEB was determined respectively.

## Simultaneous Determination of CIP and MEB

(i) The content of series of 10 mL calibration flasks containing (8 and 14 µg/mL) for (CIP) with different concentrations (4, 8, 12, 16, and 20 µg/mL) of (MEB) were diluted with double distilled water. The absorption spectra were recorded against blank (prepared by the same manner as test solution but without CIP and MEB). The derivative values of their first andsecond spectra the concentration of CIP were masured and determined.
(ii) The content of series of 10 mL calibration flasks containing (8 µg/mL) for (MEB) with different concentrations (4, 8, 10,12and 16 µg/mL) of (CIP) were diluted with double distilled water. The absorption spectra were recorded against blank (prepared by the same manner as test solution but without MEB and CIP). The derivative values of their first and second spectra were measured and the concentration of MEB was determined.

### 3- Standard Addition Method

It was carried out by preparing several 10 mL aliquot solutions containing the same amount (40 µg) of CIP (or MEB) drug ( tablet), and different amounts of standard (0,40, 80,100,120) µg, similary for (MEB). The first and second derivative were recorded, figure 3.

### 4- Effect of interference

This research includes, a study about the effect of different interferences on the first and second derivative of spectra for CIP and MEB which are found in pharmaceutical material
A stock solution of Glucose, Lactose and Starch were prepared by dissolving (0.1g)of each additives in 10 mL volumetric flask to get a solution of 10000 µg/mL. In 10 mL calibrated flask containing (8,16) µg/mL of CIP and (12,16) µg/mL of MEB,1ml aliquots were transferred of to each additive, from which the absorption spectra were recoded.

## Preparation of Pharmaceutical Formulation

Ten tablets were weighed and crushed to fine powder.The tablet powder equivalent to 500 mg of (CIP), 135 mg of (MEB) are mixed and dissolved in 2 mL double distilled water. The resultant solution was diluted to 100 mLwith double distilled water in volumetric flask. The solution was filtrate by using Whatman filter paper no.41to avoid any suspended or undissolved material before analyses.

## Results and Discussion

### Absorption Spectra

The absorption spectra of the (CIP) and (MEB) were measured from (190-400 nm.) against double distilled water as blank. The absorption spectra of CIP and MEB and for their mixture were recorded. Fig.(4) (a) shows the absorption spectrum of CIP solution (14 µg/mL) with three absorption maxima at wavelength 206, 276 and 316 nm, while spectrum (b) shows the absorption spectrum of MEB solution (8 µg/mL) with three absorption maxima at wavelength 220,262 and 293nm .The total spectrum of mixture of ( 14 CIP /and 8 MEB µg/mL) is shown in curve( c) with (224 and 274 nm)between the absorption maxima of the two components.

### First and Second Derivative Modes

The first and second order derivative spectra of (CIP) and (MEB) and for their mixture are shown in Figure 5 and Figure 6 respectively. It was obvious that there is a large overlap of the spectra of CIP and MEB using the zero order absorption measurements. Therfore derivative spectrophotometric technique is of a particular utility in determining the concentration of single component in such mixtures with a large spectral overlapping. For this reason, derivative spectrophotometric methods have been applied .Both first and second order modes were tested and the results obtained showed that techniques could be successfully applied when the measurements are carried out under optimum concentration . The present work, graphically (peak-to-base line), technique in addition to peak area were used to deal with derivatives spectra to carry out the measurement. In the first and second derivative modes show a good proportionality to CIP and MEB concentration in their mixtures.

To select the derivative order, the first, second, third and fourth derivative spectra of CIP and MEB were also studied. The study of first and second order spectra was simple and gave results of highest accuracy and detection limits. Figurure 7 and Figure 8 show sets of first order spectra of mixtures containing different amounts of each of CIP or MEB in the presence of (14µg/mL of CIP and 8µg/mL of MEB).

The results in Figure 6 indicated that when the concentration of CIP is kept constant and the concentration of MEB varied, the peak area at the, intervals (207-233 nm ) and (239-273nm) were proportional to the concentration of MEB. Moreover, the peak-to-base line at (218nm) and (287.5nm) was found to be a function of MEB concentration. The same features were found when inspecting Figure 7 for the determination of CIP. The peak areas in the wavelengths intervals of ( 220-236nm ), (244-262nm ) and (294-320nm) and the peak amplitude measured at peak-to-baseline (251)nm were in proportion to the concentration of CIP (Table 2 ).

Figure 9 and Figure 10 have shown in further sets of second derivative of the same above mixtures. Applying the same mentioned techniques in measuring peak amplitudes (in millimeter) at peak-to-base line of the other compound, and peak areas at selected wavelengths intervals enable the measurement of MEB and CIP respectively (Table 2).

## Calibration Graph and Statistical Analysis

The analysis characteristic and most statistical data for each of the proposed methods are given in Table 2. Under the optimum conditions, liner calibration graphs were obtained in the range of (4-28µg/mL) with correlation coefficient values in the range ( 0.9981-0.9999) for different techniques.

## Accuracy and Precision

Under the optimum condition, the accuracy and precision of the proposed method (two different techniques for each of first and second order derivative modes) tested. Table 3 shows the values of relative error percent and relative standard deviation percent for two different levels of concentration of CIP and MEB.

## Application

Two of proposed methods (namely first derivative peak–to–base line at 342 nm and second derivative peak –to- base line at 326nm ) were successfully applied for direct determination of CIP in two different drugs. The results obtained are presented in Table (4) , and are in quite agreement with the spiked values. On the other hand, CIP has also been successfully determined in two different pharmaceutical preparations by two of proposed methods. The results are shown in table (4).The standard additions method was used to determine each drug in pharmaceutical Tablets. Accuracy of the proposed method was assisted by determining CIP and MEB solutions using the standard additions method for the above methods and the data obtained for pharmaceutical tablets were listed in Table (4).

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**Table No. (1) : Percent recovery for (8,16) $\mu g.mL^{-1}$ of CIP and (12,16) $\mu g.mL^{-1}$ of MEB in the presence of 1000 $\mu g.mL^{-1}$ of excipients.**

| Compound | Excipients | conc. Taken (µg/mL) | conc. Found (µg/mL) | Recovery | RE% |
|---|---|---|---|---|---|
| Ciprofloxacin hydrochloride | Lactose | 8 | 7.995 | 99.900 | -0.062 |
| | Starch | | 7.979 | 99.580 | -0.262 |
| | Glucose | | 7.999 | 99.980 | -0.012 |
| | Lactose | 16 | 15.992 | 99.890 | -0.050 |
| | Starch | | 15.998 | 99.900 | -0.012 |
| | Glucose | | 16.000 | 99.999 | 0.000 |
| Mebevrine hydrochloride | Lactose | 12 | 11.980 | 98.990 | -0.166 |
| | Starch | | 11.992 | 99.986 | -0.066 |
| | Glucose | | 11.998 | 99.969 | -0.016 |
| | Lactose | 16 | 15.997 | 99.997 | -0.018 |
| | Starch | | 15.985 | 99.959 | -0.093 |
| | Glucose | | 15.989 | 99.999 | -0.068 |

**Table No. (2) Statistical analysis of the determination of Ciprofloxacin hydrochloride and Mebeverine hydrochloride**

| Compound | Order of derivative | Mode of calculation | Wave length (nm) | Regression equation | Correlation coefficent (r) | Slope |
|---|---|---|---|---|---|---|
| Cprophloxacine Hydrochlored | First | Peak to base line | 262 | Y= 0.0026x + 0.0026 | 0.9983 | 0.0026 |
| | First | Peak to base line | 288 | Y=-0.0027x-0.0018 | 0.9984 | -0.0027 |
| | First | Peak to base line | 342 | Y=-0.0008x-0.0003 | 0.9991 | -0.0008 |
| | First | Peak area | 240-274 | Y = 0.0467x+0.044 | 0.9985 | 0.0467 |
| | First | Peak area | 276-306 | Y=-0.0401x-0.0522 | 0.9965 | -0.107 |
| | First | Peak area | 320-376 | Y=-0.0193x-0.0060 | 0.9936 | 0.0193 |
| | second | Peak to base line | 254 | Y=0.0002x+ 0.0002 | 0.9992 | 0.0002 |
| | second | Peak to base line | 276 | Y=-0.0004X-0.0006 | 0.9964 | 0.0004- |
| | second | Peak to base line | 296 | Y=0.0002X-0.0002 | 0.9995 | 0.0002 |
| | second | Peak to base line | 326 | Y=-5E-05X-4E-05 | 0.9991 | -5E-05 |
| | second | Peak area | 240-262 | Y=0.0018X+0.0005 | 0.9964 | 0.0018 |
| | second | Peak area | 264-286 | Y=-0.0049X-0.0072 | 0.9975 | 0.0049- |
| | second | Peak area | 288-308 | Y=0.0024X+0.0025 | 0.9984 | 0.0024 |
| | second | Peak area | 312-338 | Y=-0.0006X-0.0003 | 0.9992 | -0.0006 |
| Mebeverine hydrochloride | First | Peak to base line | 230 | Y=-0.002X-0.0006 | 0.9994 | -0.002 |
| | First | Peak to base line | 252 | Y=0.0007X+0.0004 | 0.9990 | 0.0007 |
| | First | Peak to base line | 274 | Y=-0.0005X-2E-05 | 0.9990 | -0.0005 |
| | First | Peak to base line | 306 | Y=-0.0005X-0.0003 | 0.9999 | -0.0005 |
| | First | Peak area | 222-242 | Y=-0.014X+0.0032 | 0.9985 | -0.014 |
| | First | Peak area | 244-262 | Y=0.0067X+0.0029 | 0.9992 | 0.0067 |
| | First | Peak area | 264-286 | Y=-0.0041X-0.0049 | 0.9982 | -0.0041 |
| | First | Peak area | 292-324 | Y=-0.0059X-0.0033 | 0.9995 | -0.729 |
| | second | Peak to base line | 238 | Y=0.0003X+4E-05 | 0.9993 | 0.0003 |
| | second | Peak to base line | 262 | Y=-0.0001X-0.0002 | 0.9995 | -0.0001 |
| | second | Peak to base line | 280 | Y=5E-05X - 1E-05 | 0.9994 | 5E-05 |
| | second | Peak to base line | 296 | Y=-4E-05X – 7E-06 | 0.9997 | -4E-05 |
| | second | Peak to base line | 314 | Y=4E-05X + 2E-05 | 0.9991 | 4E-05 |
| | second | Peak area | 230-248 | Y= 0.0023X + 4E-05 | 0.9991 | 0.0023 |
| | second | Peak area | 252-270 | Y=-0.0008X -0.0001 | 0.9989 | -0.0008 |
| | second | Peak area | 274-286 | Y=0.0002X+0.0001 | 0.9996 | 0.0002 |
| | second | Peak area | 288-304 | Y= -0.0004X-9E-05 | 0.9995 | -0.0004 |
| | second | Peak area | 304-332 | Y=0.0006X+0.0003 | 0.9993 | 0.0006 |

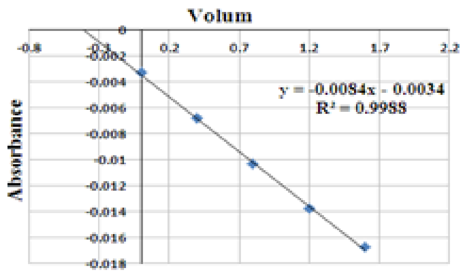
**Table No. (3) Precision and accuracy of the methods**

| Compound | Method of analysis | Taken (µg/mL) | Found (µg/mL) | RE % | RSD % |
|---|---|---|---|---|---|
| Ciprofloxacin hydrochloride | First order peak-to-base line at 342 nm | 4 | 3.970 | 0.750- | 0.436 |
| | | 12 | 12.329 | 2.741 | 1.540 |
| | | 28 | 28.185 | 0.660 | 0.378 |
| | Second order peak-to-base line at 326nm | 4 | 4.201 | 5.025 | 2.762 |
| | | 12 | 12.311 | 2.591 | 1.458 |
| | | 28 | 28.124 | 0.442 | 0.254 |
| Mebeverine hydrochloride | First order peak-to-base line at 306 nm | 6 | 6.213 | 3.550 | 1.979 |
| | | 12 | 11.998 | 0.016 - | 0.009 |
| | | 28 | 28.015 | 0.053 | 0.030 |
| | Second order peak-to-base line at 296 nm | 6 | 5.981 | -0.317 | 0.183 |
| | | 12 | 12.222 | 1.850 | 1.048 |
| | | 28 | 28.139 | 0.496 | 0.285 |

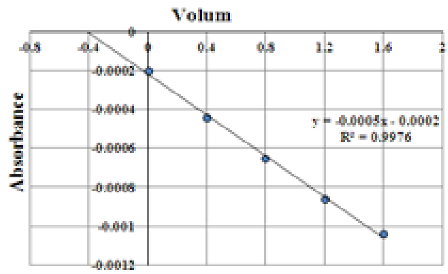
*Average of four determination

**Table No. (4) Results for analysis of Ciprofloxacin hydrochloride and Mebeverine hydrochloride in two pharmaceutical formulation sample**

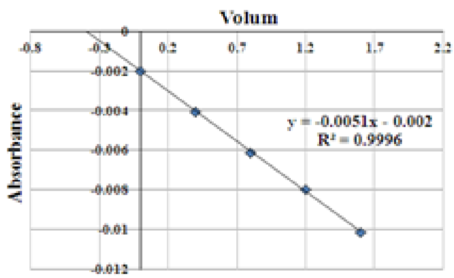
| Compound | Method of analysis | Taken (µg/mL) | Found (µg/mL) | Wave length ( nm) | Regression equation | r | RE% | RSD % n =3 |
|---|---|---|---|---|---|---|---|---|
| Ciprofloxacin hydrochloride Ras Al khaimah,U.A.E. 500 mg | First | 4 | 4.11 | 342 | Y=-0.0084X-0.0034 | 0.9993 | 2.75 | 1.892 |
| | Second | 4 | 4.10 | 326 | Y=-0.0005X-0.0002 | 0.9987 | 2.50 | 1.724 |
| Mebeverine hydrochloride Jeddah, Saudi Arabia 135mg | First | 4 | 3.91 | 306 | Y= -0.0051X+0.002 | 0.9999 | -2.25 | 1.627 |
| | Second | 4 | 3.86 | 296 | Y= -0.0004X-0.0001 | 0.9956 | -3.50 | 2.564 |



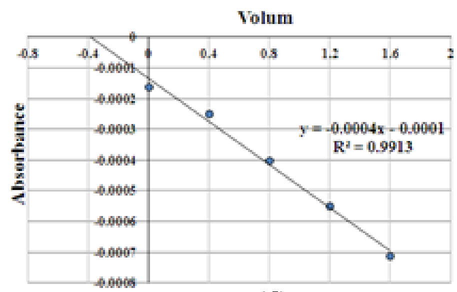

(a)




(b)




(c)




(d)

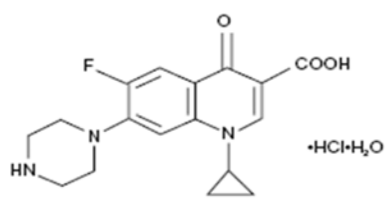



**Figure No. (1) Chemical Formula of Ciprofloxacin Hydrochloride**

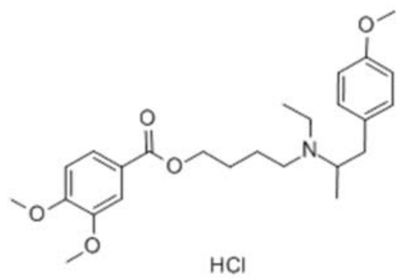



**Figure No. (2):Formula of Mebeverine hydrochloride**

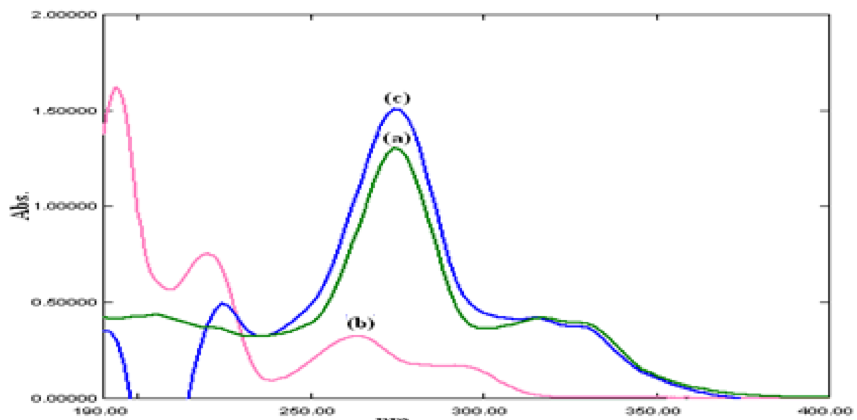



**Figure No. (3) Determination of CIP in several amount of MEB(a,b) and determination of MEB in several concentration of CIP (c,d) by standard additions method.**

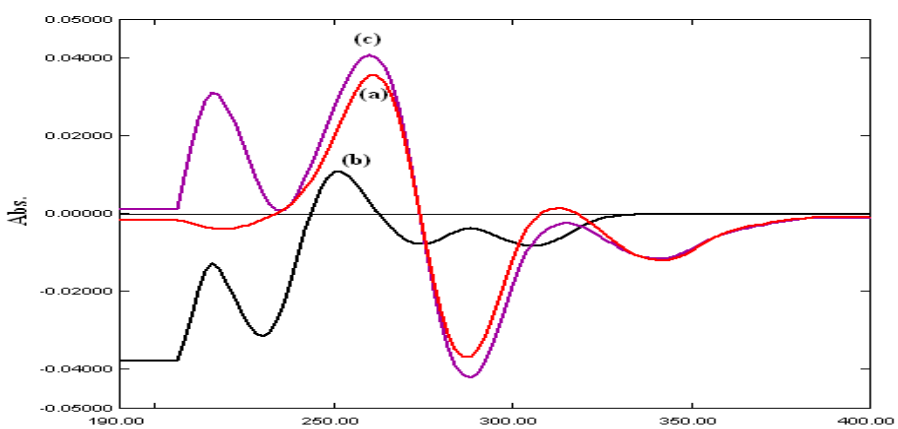



**Figure No. (4) Absorption spectra of (a) 14 µg/mL Ciprofloxacin hydrochloride , (b) 8 µg/mL Mebeverine hydrochloride (c) Ciprofloxacin hydrochloride and Mebeverine hydrochloride**

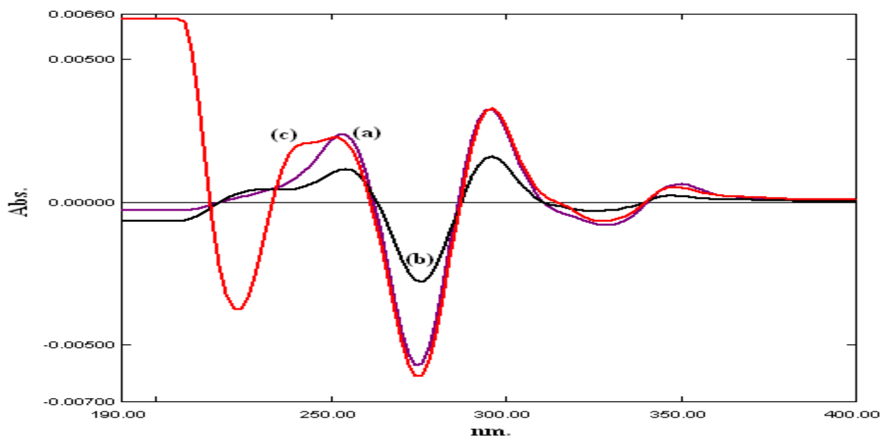



**Figure No. (5) first derivatives spectra of: (a) 14 µg/mL Ciprofloxacin hydrochloride (b) 8µg/mL Mebeverine hydrochloride (c) Ciprofloxacin hydrochloride and Mebeverine hydrochloride mixture**

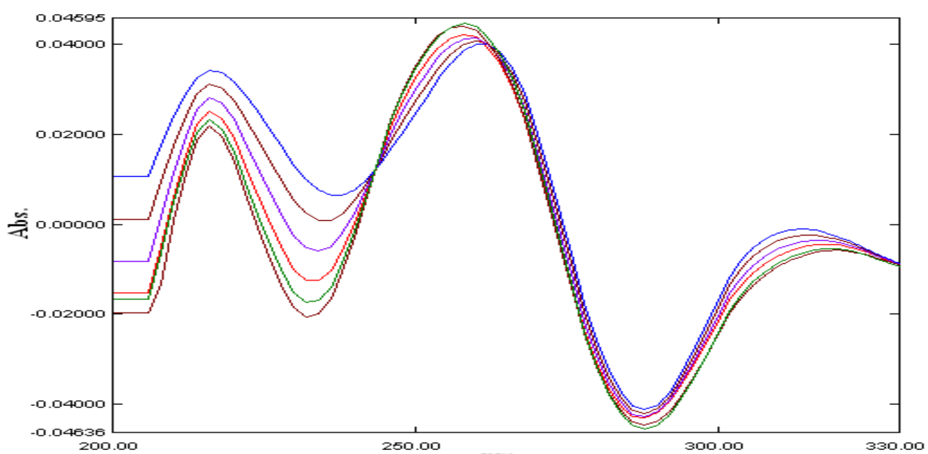



**Figure No. (6) Second derivatives spectra of: (a) 14 µg/mL Ciprofloxacin hydrochloride, (b) 8 µg/mL Mebeverine hydrochloride (c) Ciprofloxacin hydrochloride and Mebeverine hydrochlorid**

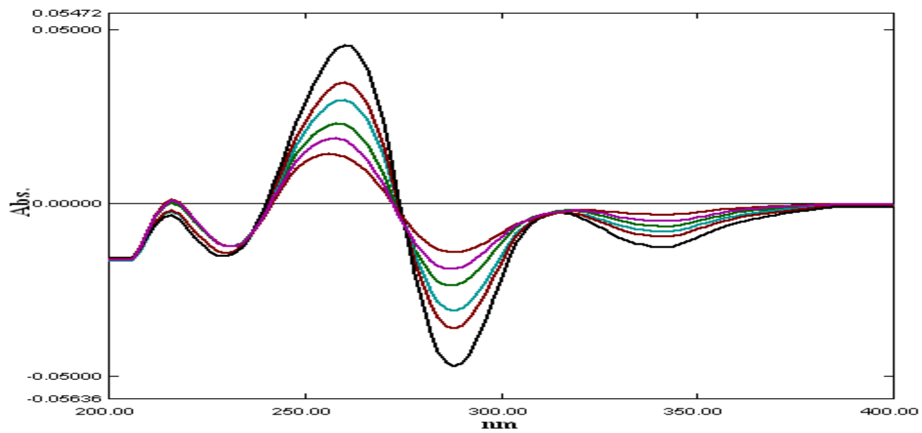



**Figure No.(7): First derivative spectra of mixtures containing(4-20 µg/mL) Mebeverinehydrochloride and 14 µg/mL of Ciprofloxacin hydrochloride**

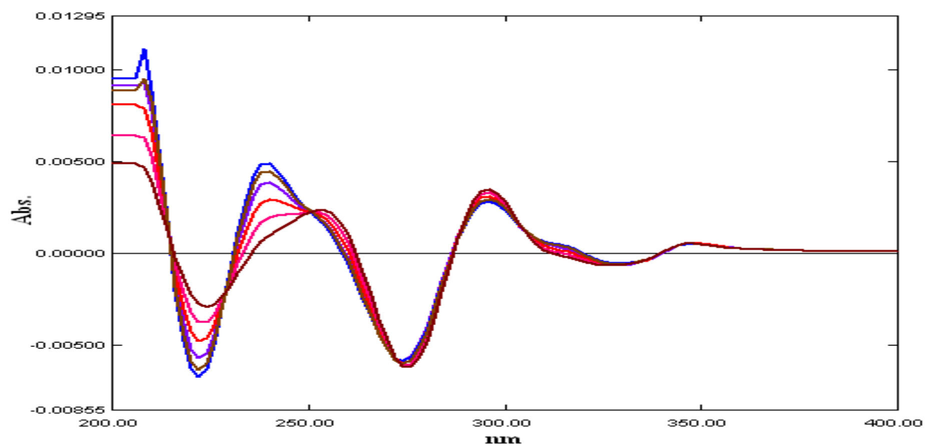



**Figure No. (8) :First derivative spectra of mixtures containing(4-20 µg/mL) Ciprofloxacin hydrochloride and 14 µg/mL of Mebeverinehydrochloride**

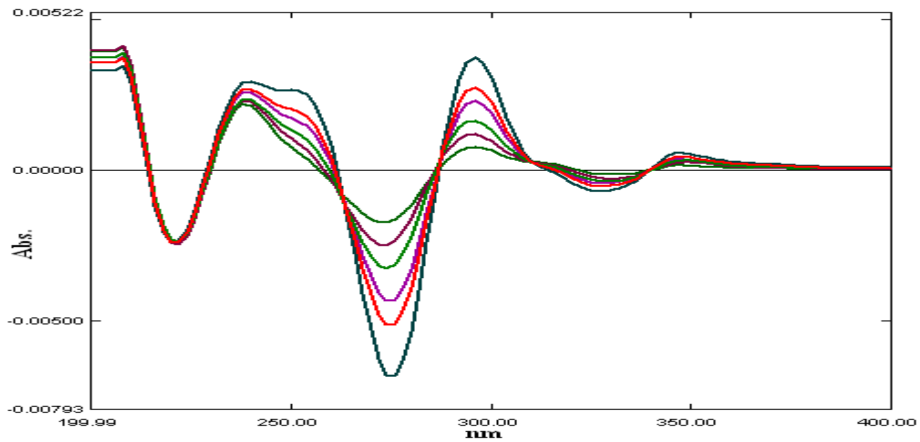



**Figure No. (9): Second derivative spectra of mixtures containing of Mebeverine hydrochloride (4-20 µg/mL) and Ciprofloxacin hydrochloride14 µg/mL**

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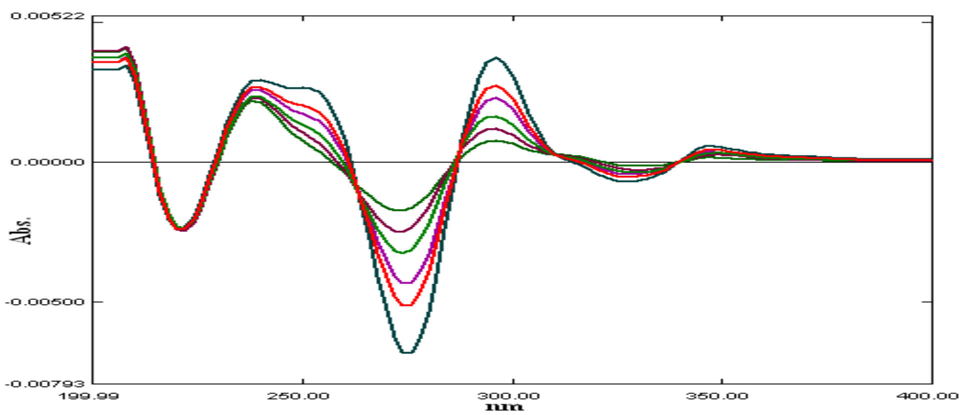


**Figure No. (10): Second derivative spectra of mixtures containingof (4-20 µg/mL) Ciprofloxacin hydrochloride and Mebeverine hydrochloride 8 µg/mL**

# التقدير الاني لعقاري السبروفلوكساسين هايدروكلوريد و المبفرين هايدروكلوريد بوساطة طيف المشتقة

**مها عبد الستار محمد نجيب**
**منى عبد الرسول كاظم**
قسم الكيمياء/كلية التربية للعلوم الصرفة(ابن الهيثم)/جامعة بغداد

**استلم البحث في:28ايار2014,قبل البحث في:15ايلول2014**

## الخلاصة

طورت طريقة طيفية جديدة لتقدير السبروفلوكساسين هيدروكلوريد والمبيفرين هيدروكلوريد بشكل منفرد وبشكل اني بالاعتماد على تقنية المشتقة الاولى والمشتقة الثانية لاطياف هذه المركبات. لقد وجد ان المشتقة الاولى والمشتقة الثانية لاطياف هذه المركبات تمكن من التقدير الاني للسبروفلوكساسين هيدروكلوريد وللمبيفرين هيدروكلوريد بمدى يتراوح بين (4- 28) مايكروغرام \مل وذلك بقياس ارتفاع القمة- خط القاعدة وكذلك من خلال قياس المساحة تحت الحزمة عند اطوال موجية محددة لكل مركب. لقد كانت النتائج التي تم الحصول عليهامن تحليل المركبات قيد الدراسة متوافقة ودقيقة بشكل مقبول, كما وامكن تطبيقها لتقدير السبروفلوكساسين هيدروكلوريد والمبيفرين هيدروكلوريد بشكل ناجح في اثنين من المستحضرات الصيدلانية

**الكلمات المفتاحية :**المشتقة , سبروفلوكساسين هايدروكلوريد, مبفرين هايدروكلوريد , تقدير , المطياف