Simultaneous Determination of Paracetamol and Tramadol in Pharmaceutical Tablets by Derivative UV-Vis Absorption Spectrophotometry

Ahmad M. El-Zinati and Monzir S. Abdel-Latif*

Department of Chemistry, Islamic University of Gaza, P.O. Box 108, Gaza, Palestine

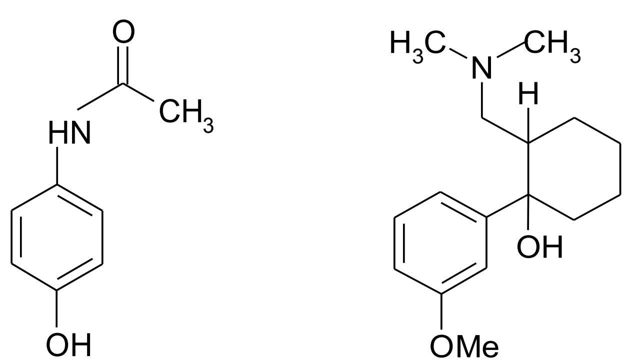
Abstract: A comparative study of the use of first derivative zero order crossing spectra for the resolution of Paracetamol and Tramadol hydrochloride in mixtures has been achieved showing the success of the first derivative method in resolving and quantifying both compounds. Using the first derivative, rather than the second derivative, results in improved signal to noise ratio. The absorption spectra of prepared mixtures were scanned in the range of 200-500 nm. The linear concentration ranges were 25-112 and 6-48 µg mL^{-1} for paracetamol and Tramadol hydrochloride, respectively. The method has been successfully used for prediction of concentrations of both compounds in mixtures with good selectivity, high sensitivity and extremely low relative error. Statistical comparison was performed using t-test at 95% confidence level. There was no significant statistical difference between the results obtained by the first derivative method and the accepted values for both compounds. Also, the percentage errors were very low which adds to the merits of our work in terms of both sensitivity and accuracy.

Keywords: First derivative spectroscopy, paracetamol, simultaneous determination, tramadol, UV spectra, zero order crossing.

INTRODUCTION

Tramadol -HCl (±cis-2-[(dimethyl amino) methyl]-1- (m-methoxyphenyl) cyclohexanol hydrochloride) and paracetamol (N-(4-hydroxyphenyl) acetamide) have been extensively used as antipyretic and analgesic drugs [1] (Scheme **1**).

(**a**) (**b**)



Scheme 1. Structure of Paracetamol (**a**) and Tramadol (**b**).

Paracetamol (PR) or acetaminophen (the name of the drug in US, Scheme **1**) is the most famous drug in treatment of pain and fever. It is used as antipyretic, analgesic and anti-inflammatory drug, due to inhibiting prostaglandin synthesis cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [1]. However, Paracetamol does not cause cancer like phenacetin [2]. Also, it has no effect on respiration. Although there are lots of drugs that work like paracetamol, it is still the most important, because it is cheap, effective, has no side effects and most important, safe. Can be used alone to treat little to moderate pain, but if we combine it with anti-inflammatory steroid drugs or opioid it can treat intense pain [3]. Even then it is safe, but the overuse of it can lead to hepatic toxem. It can also lead to serious condition if it is taken it with alcohol. It is considered as the primary reason for toxemia in USA, UK and the New Zealand [4-7].

Paracetamol was invented by Harmon Northrop Morse by reduction of para-nitro phenol with tin and glacial acetic acid [8]. However, it had not been used until 1893 in clinical treatment. The chemists produced paracetamol as white crystalline compound after it was found in urine of people that uptake phenacetin [9].

Although paracetamol has been used for more than a century but until now the mechanism of action of paracetamol has not been discovered yet, because a number of characteristics are commons between paracetamol and aspirin in effect on prostaglandin compound that cause the inflammation. However, it doesn't affect on thrombocytes compound that cause coagulation like aspirin. Two mechanisms were suggested for how paracetamol works but these are not proved yet [10-13].

Tramadol hydrochloride (TR) is a compound contains two enantiomers both of them achieve analgesic activity *via* different mechanisms. Tramadol efficiency has been observed to be improved by combining it with non-opioid analgesics [14].

Determination of Paracetamol has been performed using various methods like reversed-phase high performance liquid chromatographic (RP-HPLC) with caffeine [1], in Pharmaceutical Mixture Using HPLC and GC–MS [15], by first-order derivative spectrophotometery in combination with ambroxol hydrochloride, levocetirizine dihydrochloride,

*Address correspondence to this author at the Department of Chemistry, Islamic University of Gaza, P.O. Box 108, Gaza, Palestine;
E-mail: mlatif@iugaza.edu.ps

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and phenylephrine hydrochloride [16]. By RP-HPLC in combination with Chlorzoxazone, and Nimesulide [17]. By RP-HPLC in combination with metoclopramide hydrochloride in tablet dosage form [18]. By colorimetric analysis [19]. Analysis, with codeine phosphate, using a RP-HPLC system with manual injector has also been reported [20]. Solid–phase extraction, using deuterated internal standards, and gas chromatography-mass spectrometry has also been used [21].

Several methods have also been used for the determination of tramadol, high performance thin layer chromatography [22], simple spectrophotometric [23], tramadol and its metabolites in blood were identified in drugs-related deaths [24], and in combination with morphine as a percentage of opioid analgesic items [25]. Spectrophotometric determination of tramadol in pharmaceutical dosage forms was also achieved [26]. Liquid chromatography (LC) was used to determine tramadol in combination with Aceclofenac in a commercial tablet [27]. Ultrafiltration and LC-MS/MS in combination of O-desmehtyltramadol and N-desmethyltramadol in plasma [28], by Meta-analysis [29], and by GC-MS in human urine [30].

Several analytical procedures have been reported for the determination of the combined two compounds. A meta-analysis for single dose oral in acute postoperative pain was conducted [31]. A RP-HPLC method in pharmaceutical dosage form [32], a spectrophotometric method in Tablet formulation in methanol and distilled water [33].By first derivative method [34], using HPLC-UV and GC-MS with a mobile phase consisting of phosphate buffer at pH 6.3 and acetonitrile [35], using just RP-HPLC by the mobile phase consisting of acetonitrilec - 0.26 % triethylamine [36], using LC-ESI-MS [37], by thin layer chromatographic densitometric method (LC) [38] and the same combination using RP-HPLC isocratic mobile phase consisting of 0.1 %v/v trifluoroacetic acid: acetonitrile [39], by simple UV-spectrophotometric method [40], by second derivative spectrophotometry [41], as well as a recent epidemiological analytical study on tramadol poisoning [42].

DERIVATIVE SPECTRA ZERO-CROSSING TECHNIQUE

Assume a mixture of 2 components; x and y

$$A_m = A_x + A_y$$

At some λ_1 we have:

$$A_{m1} = \varepsilon_{x1}C_x + \varepsilon_{y1}C_y$$

Now, divide the absorbance of the mixture by the absorbance of standard x ($\varepsilon_{x1}C_x^o$)

$$(A_{m1}/\varepsilon_{x1}C_x^o) = (\varepsilon_{x1}C_x/\varepsilon_{x1}C_x^o) + (\varepsilon_{y1}C_y/\varepsilon_{x1}C_x^o)$$

This results in cancellation of ε_x

$$(A_{m1}/\varepsilon_{x1}C_x^o) = (C_x/C_x^o) + (\varepsilon_{y1}C_y/\varepsilon_{x1}C_x^o)$$

Now take the derivative of the resulting equation with respect to λ.

Evidently, $d/d\lambda(C_x/C_x^o) = 0$ as it is independent on wavelength. The resulting equation should read:

$$d/d\lambda(A_{m1}/\varepsilon_{x1}C_x^o) = 0 + d/d\lambda(\varepsilon_{y1}C_y/\varepsilon_{x1}C_x^o)$$

Now the resulting spectrum is related to the concentration of component y, where component x was totally excluded. If we are to calculate the concentration of x, we start our manipulation by dividing on absorbance of standard y.

EXPERIMENTAL

Materials and Methodology

All chemicals were of analytical grade and were used without further purification. Paracetamol 500 mg per tablet (Beit Jala Pharmaceutical CO.), ethanol, tramadol hydrochlorides 50 mg per tablet (Pharmacare PLC.), solvent is always absolute ethanol (Chempal) and distilled water in a (1:9) ratio, were used respectively. A UV-Vis spectrophotometer (GENYESYS 10 UV-Vis made in US), filter paper (made in China by HANGOW WHATMAN). A 1.0 cm quartz cuvettes were used throughout this work.

Preparation of Stock Solutions

25 mg of paracetamol and 50 mg tramadol hydrochloride were weighed and dissolved individually in a 100 ml measuring flask. Then the solutions were filtered using a conventional filter paper. From this, appropriate dilutions of the solutions were made to prepare 125 µg ml^{-1} and 60 µg ml^{-1} of paracetamol and tramadol hydrochloride stock solutions, respectively. Finally, 8 mixing solutions of both drugs were prepared using direct dilution. Table **1** presents a summary of the prepared mixtures.

Table 1. Symbols and composition of mixtures used in this work.

Mixture Symbol	Paracetamol (ml)	Tramadol (ml)
Am_2	2	8
Am_3	3	7
Am_4	4	6
Am_5	5	5
Am_6	6	4
Am_7	7	3
Am_8	8	2
Am_9	9	1

RESULTS AND DISCUSSION

Selection of Wavelength

The UV-Vis spectra of both drugs were collected in the range of 200-500 nm against a blank. Then the absorbance spectra of the eight mixture solutions were also collected in the same range, in agreement with previous studies (4-7).

Scanning for Wavelength

All solutions have been scanned in the range from 200-500 nm against a blank. Resulting spectra are shown in Figs. (**1**, **2**).

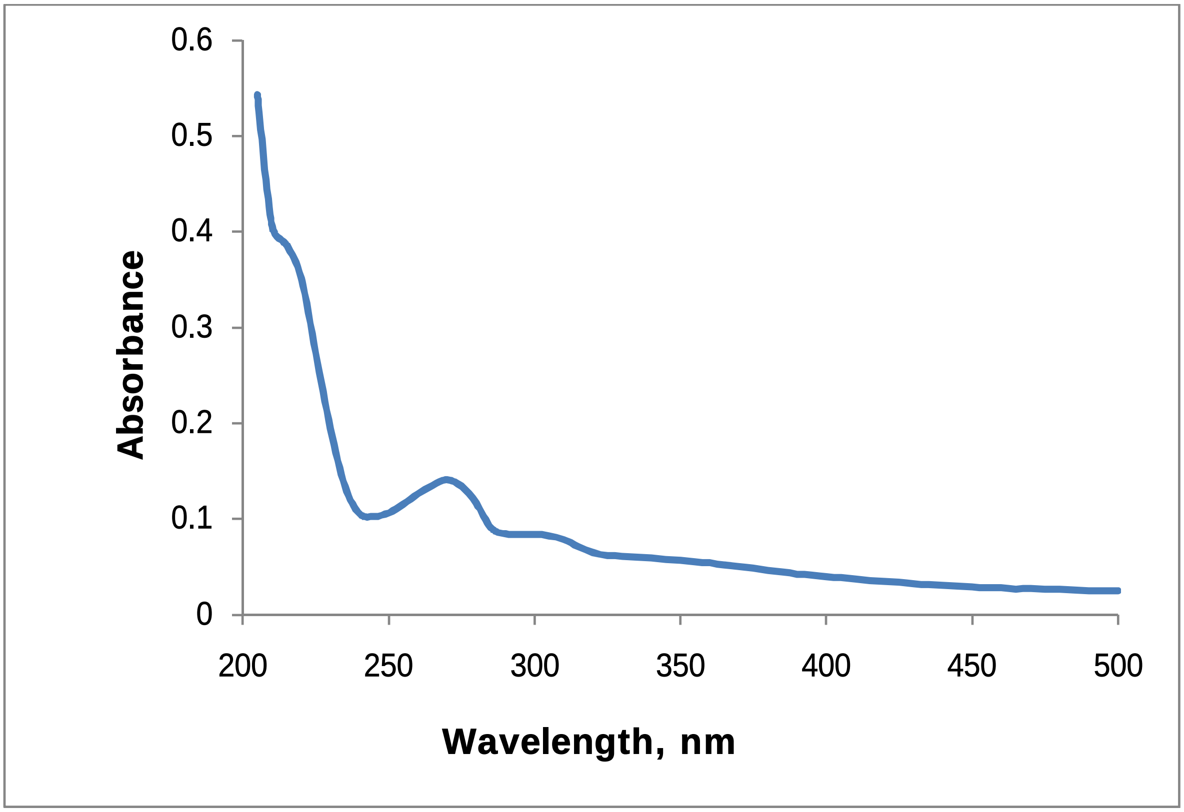

Fig. (1). Spectrum for tramadol-HCl.

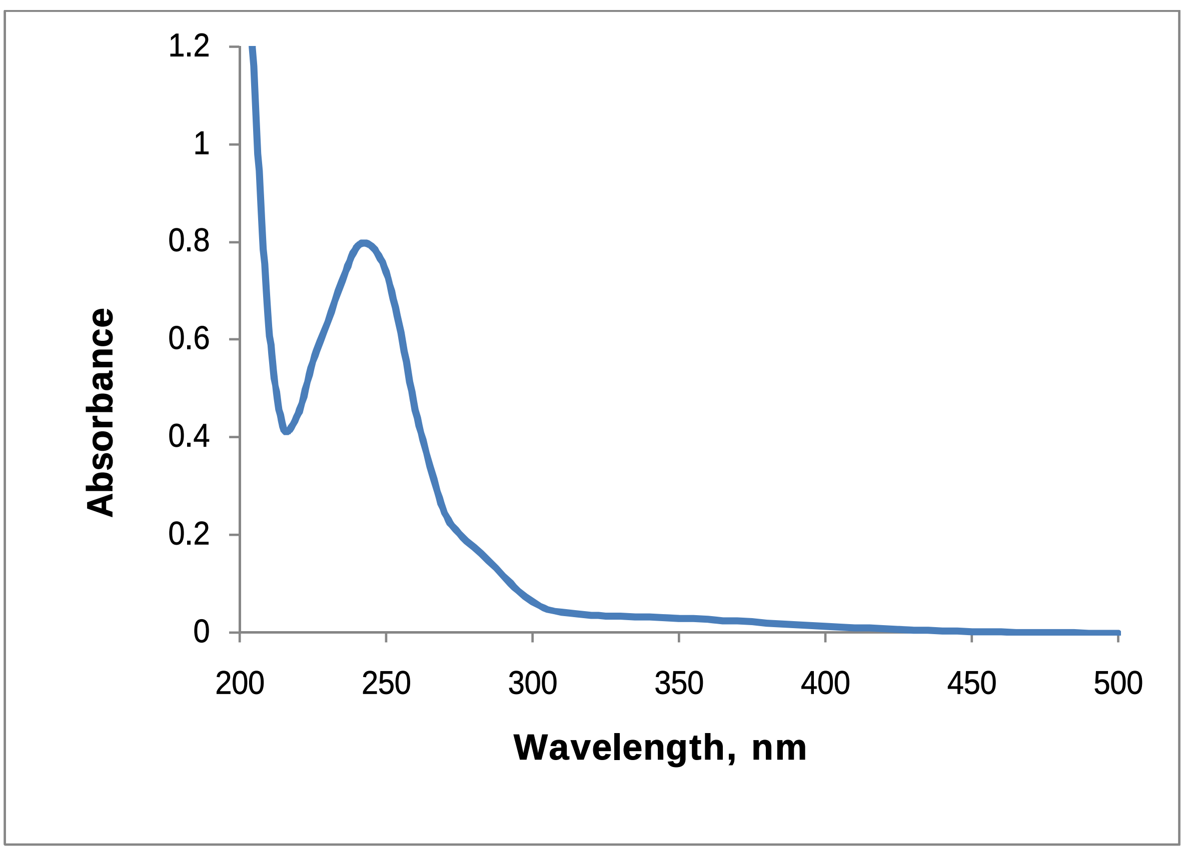

Fig. (2). Spectrum for paracetamol.

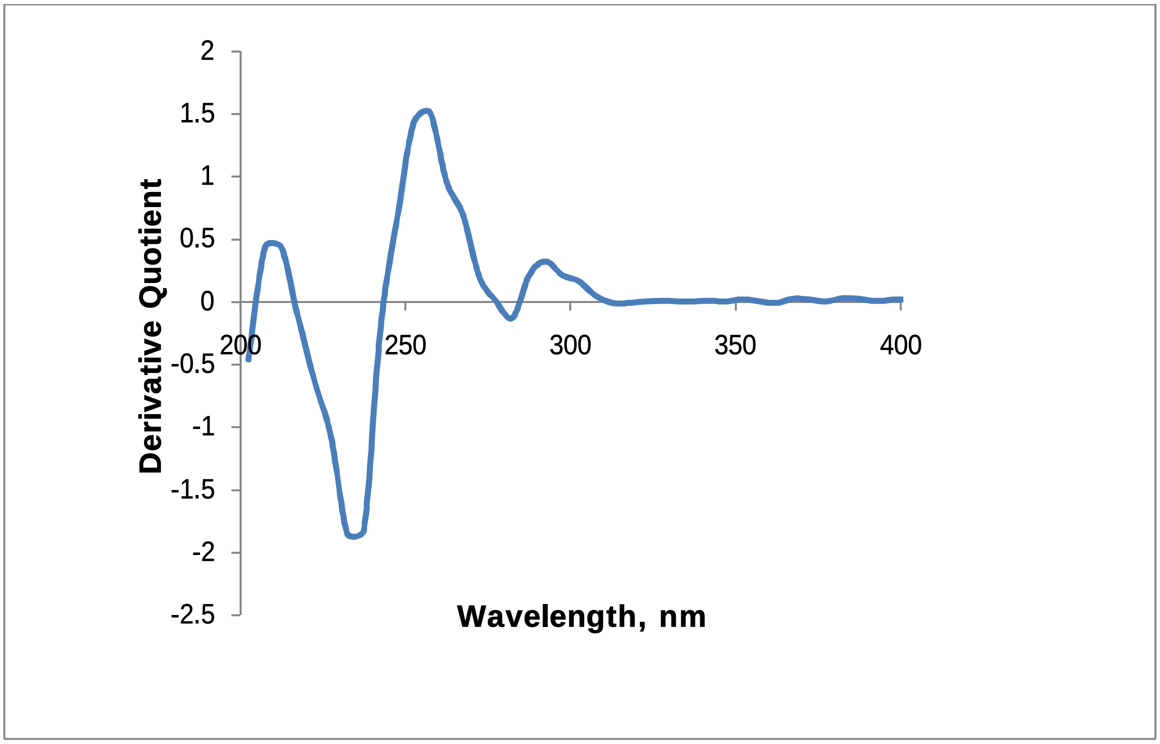

Fig. (3). Derivative zero-crossing spectrum for determination of paracetamol.

In accordance with previously reported results, paracetamol has a characteristic absorption peak at about 249 nm, while tramadol has an absorption peak at about 271 nm. These wavelengths will be used after application of the derivative zero crossing technique for the determination of both drugs in mixtures.

The absorbance of each mixture was divided by the absorbance of standard tramadol, in order to determine paracetamol in the mixture. Then the derivative of the result was taken, giving a derivative spectrum like the one shown in Fig. (**3**).

The same procedure was applied for the determination of tramadol, where the absorbance of each mixure was divided by the absorbance of standard paracetamol, then the derivative was taken and evaluated for the determination of tramadol. Fig. (**4**) illustrates the result.

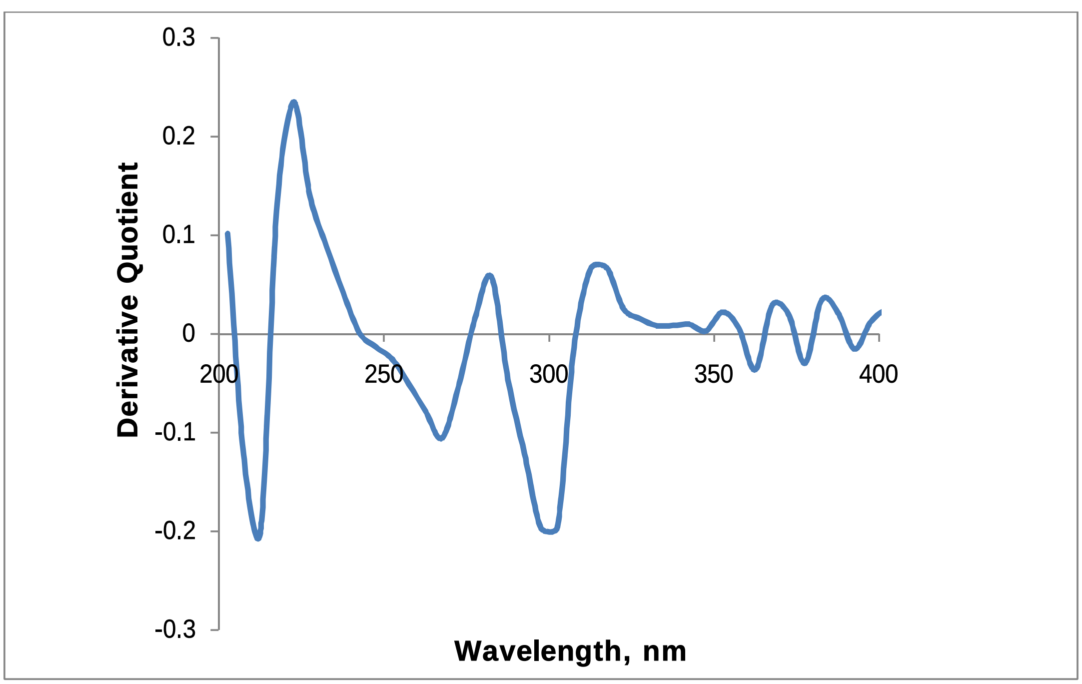

Fig. (4). Derivative zero-crossing spectrum for determination of tramadol.

Taking the distance of the peaks from bottom to top around 249 and 271 nm gives values necessary to quantify both paracetamol and tramadol, respectively, in mixtures. Plotting these values for paracetamol resulted in a linear calibration plot, where $r^2 = 0.9987$. The resulting calibration plot for tramadol was also linear with excellent correlation coefficient ($r^2 = 0.9986$).

The obtained results were further evaluated for determination of unknown mixtures of paracetamol and tramadol, excellent results were always obtained regardless of the ratio of paracetamol to tramadol in the mixture, showing a clear advantage over the simpler first derivative method as reported by Shukla *et al.* [38]. The relative error was always less than 2.0%. The obtained results were compared with the true results for several samples, the standard deviation of the difference and the t-test suggest that the reported method can be used effectively where actually no significant statistical difference can be observed. Results of these findings are summarized in Table **2**.

Table 2. Tests of Significance for paracetamol.

True Value	Exp. Value	Recovery (%)	RE	S_d	$T_{95\%}$
25.0	25.11	100.4	0.44	0.97	0.06
37.5	37.78	100.7	0.747		
50.0	50.11	100.2	0.22		
62.5	61.45	98.3	1.68		
75.0	75.05	100.1	0.07		
87.5	89.11	101.8	1.0		
100.0	98.45	98.4	2.8		
112.5	113.11	100.5	1.0		

CONCLUSION

A simple and highly reliable derivative zero-crossing method is proved successful for the determination of paracetamol and tramadol in pharmaceutical tablets. The method uses very little, cheap, and environmentally friendly reagents. The method does not suffer from disadvantages of the direct first derivative method. Correlation coefficient and relative error were very good. Statistical comparison was performed using t-test at 95% confidence level where there was no significant difference between the results of the proposed method and the true values.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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