COMPARATIVE STUDY OF DIFFERENT SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF TRAMADOL AND PARACETAMOL IN PHARMACEUTICAL FORMULATIONS**

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UV spectroscopy of tramadol (TRA) and paracetamol (PAR) shows substantial spectral overlap, which is a challenge for their simultaneous determination without preliminary separation. Three smart spectrophotometric methods based on the ratio spectra developed from the overlapping UV spectra of their binary mixture can be applied for a quantitative estimation of both drugs. The first derivative (DR^1) of the ratio spectra was computed, and then the amplitudes were measured at 268.7 and 237.4 nm for TRA and PAR, respectively. The mean centered ratio (MCR) of the spectra was estimated by measuring the MCR spectra at 279 and 241.5 nm for TRA and PAR, respectively. Finally, the dual wavelength method (DW) was applied by measuring the difference in absorbance at 224.1 and 268.5 nm for TRA determination and at 248 and 285.4 nm for PAR determination without any interference. All the above-mentioned spectrophotometric methods can be used to estimate TRA in the linear range of 10–110 μg/mL. Furthermore, PAR can be estimated in the linear range of 1–25 μg/mL. These methods were successfully applied to the analysis of the combined dosage form and bulk powder of TRA and PAR. The methods were validated according to the International Conference on Harmonization (ICH) guidelines, and the obtained results were statistically compared with those obtained by previously reported methods. No significant difference with respect to accuracy and precision was observed.

Keywords: *tramadol, paracetamol, ratio spectra, mean centering, dual wavelength.*

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ РАЗЛИЧНЫХ СПЕКТРОФОТОМЕТРИЧЕСКИХ МЕТОДОВ ОДНОВРЕМЕННОГО ОПРЕДЕЛЕНИЯ ТРАМАДОЛА И ПАРАЦЕТАМОЛА В ФАРМАЦЕВТИЧЕСКИХ ПРЕПАРАТАХ

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УФ-спектроскопия трамадола (TRA) и парацетамола (PAR) показывает значительное спектральное перекрытие, что является проблемой для их одновременного определения этих препаратов без предварительного разделения. Для количественной оценки обоих препаратов могут быть применены три спектрофотометрических метода, основанных на соотношении перекрывающихся УФ-спектров их бинарной смеси. Вычислена первая производная (DR^1) отношения спектров, измерены амплитуды при 268.7 и 237.4 нм для TRA и PAR. Среднее центрированное отношение (MCR) спектров оценено путем измерения спектров MCR при 279 и 241.5 нм для TRA и PAR. Метод двух длин

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волн (DW) применен путем измерения разницы в поглощении при 224.1 и 268.5 нм для определения TRA и при 248 и 285.4 нм для определения PAR без каких-либо помех. Вышеперечисленные спектрофотометрические методы могут быть использованы для оценки TRA в линейном диапазоне 10–110 мкг/мл. PAR может быть оценен в диапазоне 1–25 мкг/мл. Методы успешно применены для анализа комбинированной лекарственной формы и нерасфасованных порошков TRA и PAR, а также валидированы в соответствии с принципами Международной конференции по гармонизации (ICH). Результаты статистически сопоставлены с данными, полученными с помощью ранее описанных методов. Существенной разницы в точности и воспроизводимости не наблюдалось.

Ключевые слова: *трамадол, парацетамол, спектры соотношения, среднее центрирование, двойная длина волны.*

Introduction. Tramadol (TRA) abuse has significantly increased in recent decades. A study conducted in Egypt found that 20% of Egyptian male students reported drug abuse of some kind. Among secondary male students, 5.05% reported cannabis use, 0.84% abused opiates, and 2.72, 1.79, and 2.62% reported tranquilizer, stimulant, and hypnotic abuse, respectively [1]. TRA [(±-trans-2-(dimethylaminomethyl)-1-(m-methoxyphenyl)cyclohexanol hydrochloride] is a synthetic centrally acting opioid pain medication used to treat moderate to severe pain. It is often combined with paracetamol (PAR) to further improve its analgesic properties [2]. PAR [N-(4-hydroxyphenyl)acetamide] is an acylated aromatic amide that acts as an analgesic and antipyretic. It is the main ingredient in various cold and flu medications in addition to prescription analgesics [3].

Different methods for the simultaneous determination of TRA and PAR in dosage forms using liquid chromatography with tandem mass spectrometry (LC-MS/MS) [4, 5] and high-performance liquid chromatography (HPLC) [2, 6–8] techniques have been described in the literature. Additionally, various spectrophotometric methods for the determination of TRA and PAR in their combined formulations have been reported. Some of these methods require time-consuming data-processing steps to achieve the required accuracy for TRA and PAR determination. Moreover, few of these papers describe the simultaneous determination of both TRA and PAR in either a combined dosage form or a bulk powder [3, 8–11].

The aim of this work was to develop simple spectrophotometric methods that are able to resolve the substantial spectral overlap of TRA and PAR, and to apply these spectrophotometric methods for the quantitative analysis of tablets. The proposed methods were validated according to the requirements of the International Conference on Harmonization (ICH) [12] and statistically compared with reported spectrophotometric methods [13].

Experimental. A Shimadzu double–beam UV-Visible spectrophotometer, model UV-1800 (Japan), which had two matched 1-cm quartz cuvettes and was connected to a computer equipped with UV-probe software version 2.3, was used to record all the spectrophotometric measurements.

Pure standard TRA was obtained from Amriya Pharmaceutical Industries (Alexandria, Egypt) and pure PAR was obtained from Alexandria Co. for Pharmaceuticals & Chemical Industries (Alexandria, Egypt). Methanol of spectroscopic grade was purchased from El-Nasr Pharmaceutical Chemicals Co., Abu-Zaabal, Cairo, Egypt. Zaldiar® tablets were manufactured by Grunenthal, Stoleberg, Germany, and are labelled as containing 37.5 mg TRA and 325 mg PAR.

Stock standard solutions of TRA and PAR having a concentration of 10 and 1 mg/mL, respectively, were prepared in methanol by dissolving 1000 mg of TRA and 100 mg PAR in a 100-mL volumetric flask. Working standard solutions of TRA and PAR for spectrophotometric methods were prepared by dilution of the stock solution to obtain a concentration of 1 mg/mL and 100 μg/mL, respectively.

Aliquots from the TRA and PAR standard working solutions (1 mg/mL TRA and 100 μg/mL PAR), equivalent to 10–110 μg/mL for TRA and 1–25 μg/mL for PAR, were accurately transferred into two separate series of 10-mL volumetric flasks and then completed to the volume with methanol. The UV spectra of the prepared series of standard solutions were acquired from 200 to 400 nm against methanol as a blank. Although the informative part of either TRA or PAR is in the range of 200–310 nm, an expanded range (200–400 nm) was used to scan both drugs to detect spectral characteristics, overlap and predict the best methods for mixture resolution.

The zero-order absorption spectra (D^0) of both TRA (50 μg/mL) and PAR (10 μg/mL) showed substantial overlap that prevented the quantification of each drug in the presence of the other, as shown in Fig. 1. Three different spectrophotometric methods were applied for the determination of both TRA and PAR.

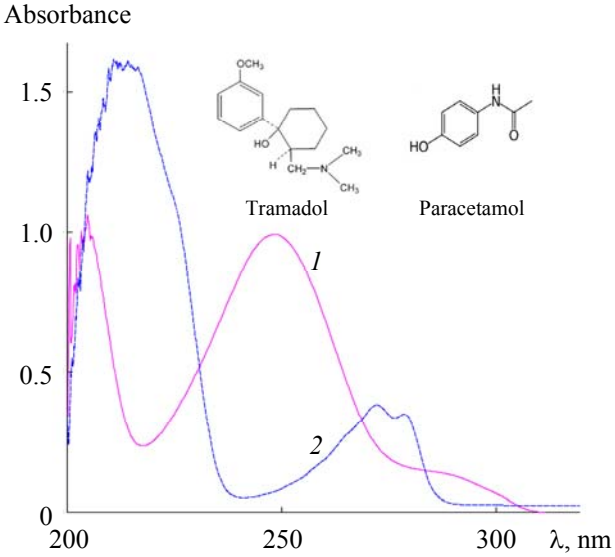

Fig. 1. Zero-order absorption spectra (D^0) of 50 μg/mL TRA (1) and 10 μg/mL PAR (2).

First derivative ratio method (DR^1). The ratio spectra were obtained by dividing the zero-order absorption spectra of one substance by the spectra of the other. Then the first derivative of the ratio spectra was computed, which allowed for the resolution of the overlapping spectra.

Mean centred ratio (MCR) of spectra. A pre-processing operation was used in this method, which was a standard transformation of the data that was applied in the multivariate analysis. This technique is known as mean centering of the ratio spectra. By applying this technique, the signal-to-noise ratio was enhanced owing to the elimination of derivative steps.

Dual wavelength (DW) method. This method is based on the selection of two wavelengths where the interfering component shows the same absorbance, while the component of interest shows a substantial difference in absorbance. Therefore, the absorbance difference at the two points on the spectra will be directly proportional to the component of interest and independent of the interfering component.

Laboratory-prepared mixtures were prepared by adding different ratios of the previously prepared solutions of TRA and PAR to a 10-mL volumetric flask and then analyzing the mixtures by using the above-mentioned methods. The final concentration of each drug in each mixture was calculated from the corresponding regression equation.

Ten tablets of Zaldiar® were accurately weighed and finely powdered. An accurately weighed amount of the powder equivalent to 325 mg PAR and 37.5 mg TRA was transferred to a 100-mL volumetric flask, mixed with methanol and then sonicated for 20 min. For PAR determination, an aliquot of 1.5 mL was transferred to a 50-mL volumetric flask and then completed to the mark with methanol. Then a second dilution was performed by transferring aliquots of 1 and 1.5 mL into two separate 10-mL volumetric flasks and completed to the mark using methanol.

For TRA determination, aliquots of 1 mL and 1.5 mL were transferred from the previously prepared solution in a 50 mL volumetric flask into two separate 10-mL volumetric flasks. Then a volume of 0.49 and 0.69 mL of standard TRA solution (1 mg/mL) was added to the two 10-mL volumetric flasks, respectively, and the volume was completed to the mark using methanol.

Then, this application was analysed by using the previously mentioned spectrophotometric methods. The validity of the methods was assessed by the standard addition technique, which was performed by adding different known concentrations of pure TRA and PAR solutions to the previously prepared application. The recovery of the added standards was then calculated by applying the proposed methods.

Results and discussion. Some of the main advantages of UV spectrophotometry are its quick analysis ability and ease of use. UV spectrophotometry is used extensively in many applications as an accurate tool for the precise quantification of various substances. It is also considered as an important method in pharmaceutical analysis for the identification of various substances in bulk and pharmaceutical formulations.

Method development and optimization. The challenge is that most active drugs and substances absorb light in the UV region, in addition to exhibiting overlapping spectra, which makes their simultaneous determination very difficult.

The main objective of this work is to develop validated spectrophotometric methods that can determine TRA and PAR in their binary mixtures, namely, either their bulk powder or pharmaceutical dosage form, with acceptable sensitivity and selectivity.

First derivative ratio (DR^1). We optimized our developed DR^1 method for the determination of TRA and PAR by testing the influence of different variables, such as the concentration of the divisor in the standard solution, the scanning speed, the smoothing function, and the wavelength increment on which the derivative was obtained (Δλ). The best peak resolution, signal-to-noise ratio, and sensitivity of the methods were obtained by employing a fast scanning speed, a scaling factor of 1, and a Δλ of 4 nm. The effect of the divisor concentration was also evaluated. Different concentrations of TRA (10, 20, 50, and 70 μg/mL) and PAR (5, 10, 20, and 30 μg/mL) were used to determine PAR and TRA, respectively.

For the determination of TRA and PAR, the best average percentage recovery was obtained by using divisor concentrations of 20 and 30 μg/mL for PAR and TRA, respectively, in their bulk powder and synthetic mixture forms, as shown in Fig. 2a. The choice of the wavelengths for measurement was carefully studied by taking into account a good linearity and recovery percentage. The selection of 268.7 nm (TRA) and 237.4 (PAR) over the other existing maxima or minima was attributed to the excellent linearity and recovery. The first derivative of the ratio spectra was obtained, along with good linearity of the calibration curves constructed by relating the peak amplitudes at 268.7 and 237.4 nm to the corresponding TRA and PAR concentrations, respectively, as shown in Fig. 2b.

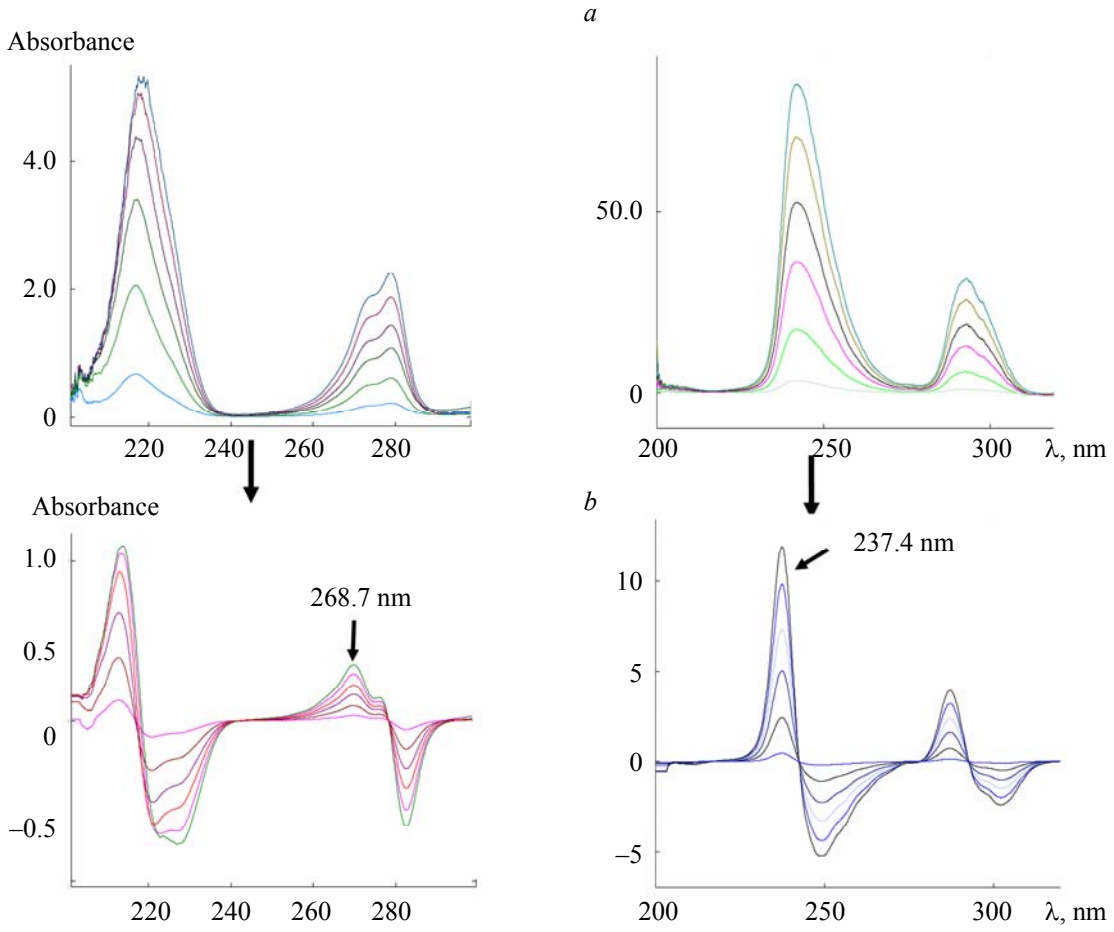

Fig. 2. (a) Ratio spectra of TRA (10–110 μg/mL) using 20 μg/mL of PAR as a divisor and PAR (1–25 μg/mL) using 30 μg/mL of TRA as a divisor; (b) first derivative of ratio spectra of TRA (10–110 μg/mL) using 20 μg/mL of PAR as a divisor and PAR (1–25 μg/mL) using 30 μg/mL of TRA as a divisor.

Mean centering ratio (MCR) of spectra. We developed and applied a method on the basis of the mean centering of ratio spectra to enhance the selectivity and resolve the spectral overlap between TRA and PAR. By applying this method, the signal-to-noise ratio was enhanced by eliminating the derivative step. In this method, the ratio spectra of the whole measured spectra were computed, after which a constant was eliminated by mean centering (averaging) of the ratio spectra.

For the determination of TRA and PAR, the best average percentage recoveries were obtained when we used divisor concentrations of 20 and 30 μg/mL for PAR and TRA, respectively, in their bulk powder and synthetic mixture forms, as shown in Fig. 3. We studied the linearity and construction of the calibration curves by relating the mean-centered values at 279 and 241.5 nm to the corresponding TRA and PAR concentrations, respectively.

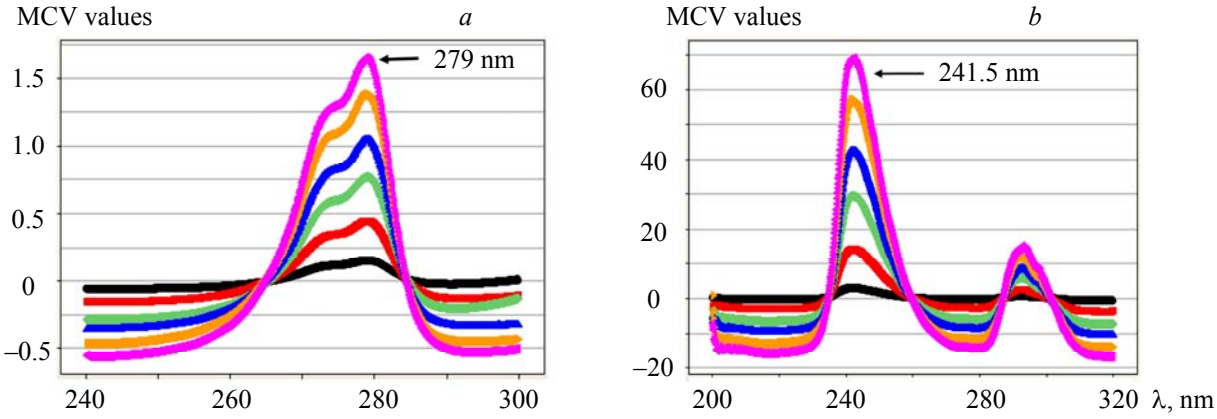

Fig. 3. Mean centered ratio spectra of (a) TRA (10–110 μg/mL) using 20 μg/mL of PAR as a divisor and (b) PAR (1–25 μg/mL) using 30 μg/mL of TRA as a divisor.

Dual wavelength (DW) method. The principle of the DW method is based on the selection of two wavelengths at which the interfering component shows the same absorbance, while the component of interest shows a significant difference in absorbance. Therefore, the absorbance difference at these two points on the spectra will be directly proportional to the component of interest and independent of the interfering component. We evaluated different sets of wavelengths, and for the determination of TRA, the absorbance values were the same at 224.1 and 268.5 nm; therefore, we selected these two wavelengths. For the determination of PAR, we selected 248 and 285.4 nm since TRA had the same absorbance values at these two wavelengths. This is illustrated in Fig. 4a by drawing connecting lines between 224.1 and 268.5 nm (pink spectrum) (TRA determination) showing equal absorbance values and another connecting line between 248 and 285.4 nm (blue spectrum) (PAR determination) also showing equal absorbance values. We constructed calibration curves by plotting the difference in the absorbance of TRA at 224.1 and 268.5 nm and the difference in the absorbance of PAR at 248 and 285.4 nm, as shown in Fig. 4b.

The proposed spectrophotometric methods were validated in compliance with the ICH guidelines [12].

The linearity of the developed methods was evaluated by analyzing six concentrations of both TRA and PAR. Each concentration measurement was repeated three times. The analysis was carried out as per the experimental conditions discussed above. The range and equation of linear regression for each of the suggested methods were computed, as shown in Table 1.

The accuracy of the results was evaluated by applying the developed methods for the estimation of various samples of TRA and PAR. The concentrations were calculated from the corresponding regression equations computed for each method. From these equations, the mean percentage recoveries were calculated, as illustrated in Table 1. The accuracy of the suggested methods was also tested by the application of the standard addition technique. This technique was carried out by adding known quantities of pure TRA and PAR to solutions containing known concentrations of the pharmaceutical preparation. The resulting mixtures were tested, and the results obtained were computed and are summarized in Table 2. Good recoveries with the standard addition technique ensured an acceptable accuracy of the proposed methods.

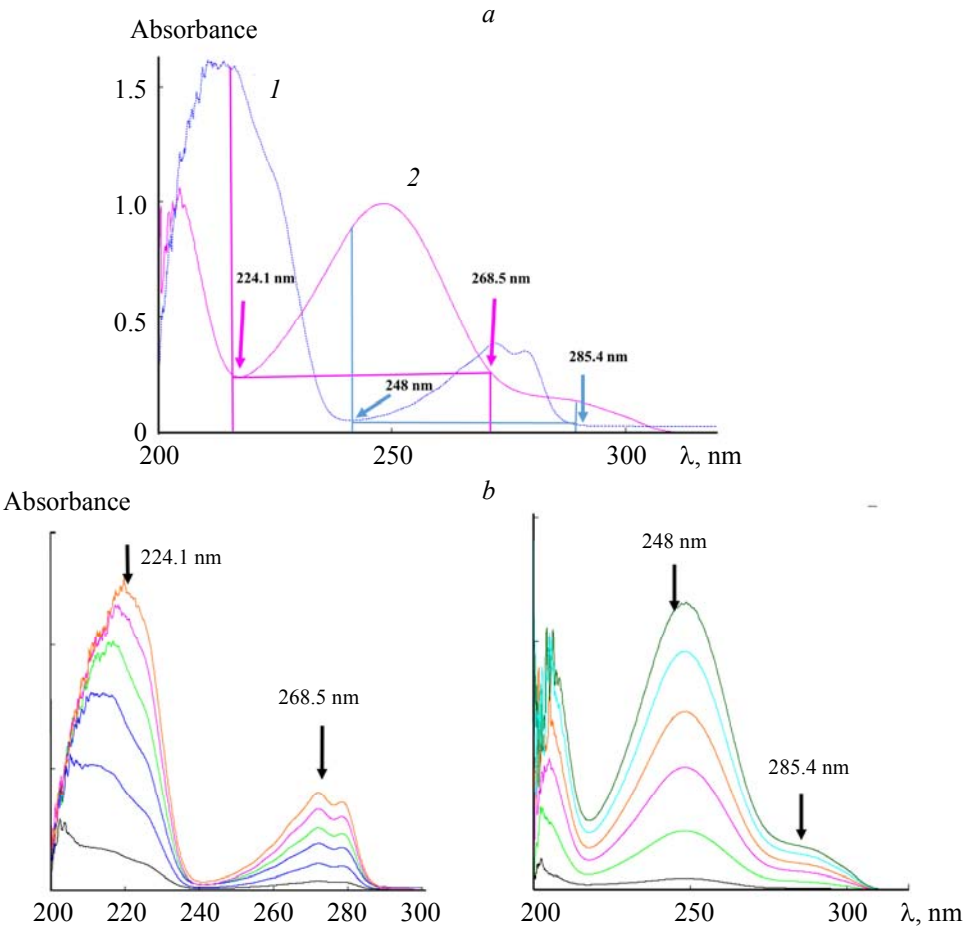

Fig. 4. (a) Zero-order absorption spectra of 50 μg/mL of TRA (1) and 10 μg/mL of PAR (2) showing the selected wavelengths for the dual wavelength method; (b) zero-order absorption spectra of (1) TRA (10–110 μg/mL) and (2) PAR (1–25 μg/mL).

TABLE 1. Assay Parameters and Method Validation Obtained by Applying the Proposed Spectrophotometric Methods

Parameter	PAR			TRA		
	DR[1]	MCR	DW	DR[1]	MCR	DW
Accuracy	100.27 ± 0.817	100.41 ± 0.263	100.26 ± 0.149	100.38 ± 0.768	100.34 ± 0.259	100.15 ± 0.311
Precision						
Repeatability	0.626	0.292	0.073	0.372	0.219	0.144
Intermediate precision	0.563	0.356	0.175	0.359	0.330	0.223
Linearity						
Slope	0.481	2.747	0.078	0.123	0.015	0.016
Intercept	0.073	1.052	0.221	0.101	–0.002	0.002
Correlation Co-efficient (R)	0.9991	0.9993	0.9991	0.9996	0.9995	0.9996
Range (μg/mL)	1.0 – 25.0	1.0 – 25.0	1.0 – 25.0	10.0 – 110.0	10.0 – 110.0	10.0 – 110.0
Wavelength (λ)	237.40	241.50	248.00, 285.40	268.7	279.00	224.10, 268.50

TABLE 2. Determination of Paracetamol and Tramadol in Laboratory Prepared Mixtures, Pharmaceutical Dosage Form and Application of Standard Addition Technique

Method	Laboratory prepared mixture		Pharmaceutical dosage form		Standard addition technique	
	Mean ± SD					
	PAR	TRA	PAR	TRA	PAR	TRA
D[1]	100.43 ± 0.630	100.57 ± 0.506	99.78 ± 0.467	98.35 ± 0.315	100.28 ± 0.866	98.24 ± 0.144
MCR	100.64 ± 0.289	100.81 ± 0.209	99.92 ± 0.374	99.24 ± 0.411	98.51 ± 0.330	100.60 ± 0.850
DW	100.63 ± 0.271	100.26 ± 0.241	100.32 ± 0.321	100.05 ± 0.380	99.38 ± 1.318	100.38 ± 0.876

Three concentrations of TRA (20, 60, 100 μg/mL) and PAR (3, 12, 22 μg/mL) were tested three times on the same day using the adopted methods. The relative standard deviation (RSD) values were computed, as shown in Table 1. The above-mentioned procedures were repeated on three different days for the three chosen concentrations. The RSD values were computed, as presented in Table 1.

The adopted methods were tested by assaying different laboratory-prepared mixtures of TRA and PAR within their linearity ranges. Acceptable results were obtained and are summarized in Table 2.

The proposed methods for the estimation of TRA and PAR in their pharmaceutical formulation (Zaldiar® tablets) were successfully developed. The studied drugs were quantified with high accuracy and were in good agreement with the amount labelled on the dosage form, as shown in Table 2.

The results produced by the application of the proposed methods for the determination of TRA and PAR were statistically compared with those obtained by a reported spectrophotometric method [13]. The calculated *t* and *F* values were lower than those obtained from theory, which showed that there was no significant difference between the suggested methods and the reported method with respect to precision and accuracy, as presented in Table 3.

TABLE 3. Statistical Comparison between Results Obtained from the Proposed Methods and the Reported Method for Analysis of Paracetamol and Tramadol Hydrochloride

Parameter / Method	D[1]		MCR		DW		Reported method	
	PAR	TRA	PAR	TRA	PAR	TRA	PAR	TRA
Mean	100.45	100.74	100.39	100.32	100.19	100.13	100.64	100.47
SD	0.467	0.374	0.321	0.315	0.041	0.380	0.797	0.415
Variance	0.218	0.14	0.1	0.099	0.001	0.144	0.635	0.172
N	3	3	3	3	3	3	3	3
Student's *t*-test	0.34 (3.182)	0.831 (2.776)	0.486 (3.182)	0.480 (2.776)	1.298 (4.302)	1.044 (2.776)	–	–
F-test	2.9 (19)	1.22 (19)	6.157 (19)	1.725 (19)	8.253 (19)	1.191 (19)	–	–

*The values in the parenthesis are the corresponding theoretical values of *t* and *F* at ($P = 0.05$).

Conclusions. The proposed methods are simple and accurate and are based on easy manipulations of the obtained absorption spectra of the studied drugs. Compared with other tedious and time-consuming spectrophotometric methods, the proposed methods do not require sophisticated software for data manipulation. We validated our proposed spectrophotometric methods according to the ICH guidelines with respect to linearity, range, accuracy, and specificity. In addition, compared with a previously reported method, our proposed methods show better sensitivity for the determination of TRA and PAR.

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