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Simultaneous Spectrophotometric Determination of Tramadol and Acetaminophen in Pharmaceutical FormulationsUsing H-Point Standard Addition Method

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ABSTRACT

H-point standard addition method (HPSAM) was applied to the simultaneous spectrophotometric determination of tramadol and acetaminophen in pharmaceutical formulations. The results showed that tramadol and acetaminophen can be determined simultaneously in the range 5.0-100 and 1.0-10 μg/ml, respectively. The limits of detection (LOD) for tramadol and acetaminophen were obtained 2.0 and 0.50 μg/ml, respectively. Relative standard deviation for five replicate simultaneous determinations of tramadol and acetaminophen were calculated to be 3.2 and 3.3%, respectively. The proposed method has been successfully applied to the simultaneous determination of tramadol and acetaminophen in some synthetic, pharmaceutical formulation.

Keywords: Tramadol, Acetaminophen, Spectrophotometric, H-point standard addition method, Pharmaceutical formulation.

INTRODUCTION

Tramadolis a synthetic monoamine uptake inhibitor and centrally acting analgesic, used for treating moderate to severe pain and it appears to have actions at the μ-opioid receptor as well as the noradrenergic and serotonergic systems.[1]Acetaminophenor paracetamol is a widely used analgesic and antipyretic drug. It is well tolerated, lacks many of the side effects of aspirin, so it is commonly used for the relief of fever, headaches, and other minor aches, pains and the management of more severe pain, where it allows lower dosages of additional non-steroidal anti-inflammatory drugs to be used, thereby minimizing overall side effects.[2,3]In relation to the effectiveness in pharmaceutical formulations, the mechanisms of action of two analgesics (37.5 mg tramadol and 325 mg acetaminophen), combining these drugs as a fixed tablet, enhance the analgesic effectiveness, reduce the side effects and also increase the drug's compliance and provide effective analgesia in patients with moderate to severe acute pain and those with chronic painful conditions characterized by intermittent exacerbations of pain.[4-6]The importance of this pharmaceutical combination has been demonstrated by the World Health Organization (WHO) analgesic scale in level II pain treatment.[7]Therefore, it is necessary to develop a rapid, precise, simple and reliable method for the determination of tramadol and acetaminophen in pharmaceutical preparations.

In spite of publishing several reports about the individual determination of tramadol and acetaminophen, only a few reports have been published on the simultaneous determination of two analytes in pharmaceutical formulations, which include liquid chromatography[8-11],electrochemical[12-14],and spectrophotometry[15].

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Among the various procedures for quantitationof chemical species, spectrophotometric methodsplay a prominent role, but the selectivity of mostspectrophotometric procedures are not satisfactory,owing to the close related properties, andseparation procedures that must be introduced inmost cases. For achieving quantitative informationfrom such systems without previous chemicalseparation, several chemometric methods havebeen developed. Some of these procedures arebased on the use of multivariate data, such asregression on latent variables (principal componentregression, PCR and partial least squares,PLS) and other methods were presented which usebivariate data such as H-point standard additionmethod (HPSAM).

Bosch-Reig and CampinsFalco presented a technique called the H-point standard addition method that was based on the principle of dual-wavelength spectrophotometry and the standard addition method.[16]The greatest advantage of the HPSAM is that it can remove the presence of an interference and reagent blank.The HPSAM can determine the two components simultaneously with extensively or even coincident overlapping spectra.[17]So far, several reports have been published on the simultaneous determination of drugs in pharmaceutical formulations by using spectrophotometric H-point standard addition method (SHPSAM).[18-24]

The main goal of the present report is developing a simple, selective and accurate method for the simultaneous determination of tramadol and acetaminophen by using H-point standard addition method. Thismethod has been successfully applied forsimultaneous determination of tramadol and acetaminophenin synthetic samples.

MATERIALS AND METHODS

Apparatus

A Hitachi model 3310 UV-V spectrophotometer with 1-cm quartz cells was used for recording the absorbance spectra. All spectral measurements were performed by using a blank solution as a reference.

Chemicals

Pharmaceutical grade tramadol and acetaminophen were obtained as a gift sample from local pharmaceuticalcompanies. Tablets oftramadol hydrochloride, acetaminophen, and combined dosage forms were purchased from the local market for analysis. Stock solutions of tramadol and acetaminophen(1000 µg/ml) were prepared by dissolving appropriate volumes of each drug in methanol and diluting to the mark ina 100 ml volumetric flask. Working solutions were prepared by diluting the standard solution in water.

Recommended Procedure

Appropriate volumes oftramadol and acetaminophenwere added into a 10 ml volumetric flask. The solution was diluted to the mark. A portion of the solution was transferred into a 1 cm quartz cell to measure the absorbance at appropriate wavelengths. Synthetic samples containing different concentrations of tramadol and acetaminophenstandard solutions were prepared, and a standard addition of acetaminophen(up to 6.0 µg/ml) was made. The simultaneous determination of a tramadol and acetaminophenstandard solution with the HPSAM was performed by measuring the absorbance at 258 and 281 nm for each sample solution. These two compounds can be determined simultaneously in the concentration range 5.0-100 and 1.0-20µg/ml, respectively. The procedure was repeated for some mixtures to show the applicability of the method.

RESULTS AND DISCUSSION

Preliminary Investigations

The absorption spectra of tramadol and acetaminophenare shown in Fig. 1. As can be seen, the spectra of the two compounds overlap, and each compound interferes in the spectrophotometric determination of the otherone. Therefore, the simultaneous determination oftramadol and acetaminophenis impossible by classical spectrophotometry. However,the system is suitable for simultaneous determination of tramadol and acetaminophenusing the HPSAM.

Applying HPSAM

For the selection of the appropriate wavelengths for the application of the HPSAM, the following principles were followed. At two selected wavelengths, the analyte

signals must be linear with the concentration; the interferent signals must remain equal, even if the analytical concentrations are changed.Also,the analytical signals of the mixture composed from the analyte and the interferent should be equal to the sum of the individual signals of the two compounds. In addition, the slope difference of the two straight lines obtained at λ_1 and λ_2 must be as large as possible in order to get good accuracy and sensitivity.

By considering Fig. 1, the absorbance corresponding toacetaminophen at two selected wavelengths λ_1= 258 and λ_2= 281nm, are b_i and A_i, respectively, while the absorbance corresponding to tramadol under the same conditions are b and A';b and A' are equal. The following equations show the relation between them:

$A_{\lambda 1} = b_0 + b + M_{\lambda 1} C_{acetaminophen}$ (1)

$A_{\lambda 2} = A_0 + A' + M_{\lambda 2} C_{acetaminophen}$ (2)

where $A_{\lambda 1}$ and $A_{\lambda 2}$ are the analytical signals measured at258 and 281 nm, respectively; b_0 and A_0 ($b_0 \# A_0$) are the originalanalytical signals of acetaminophenλ_1 and λ_2, respectively; b and A'arethe analytical signals of tramadol at λ_1 and λ_2, respectively; $M_{\lambda 1}$and$M_{\lambda 2}$are the slopes of the standard addition calibration linesat 258and 281 nm, respectively. The two straight lines obtained intersect at the so-called Hpoint ($-C_H$, A_H) (Fig. 2).

At H-point, since $A_{\lambda 1} = A_{\lambda 2}$,$C_{acetaminophen} = -C_H$ from Eqs (1) and (2) it follows that:

$b_0 + b + M_{\lambda 1}(-C_H) = A_0 + A' + M_{\lambda 2}(-C_H)$ (3)

$-C_H = [(A_0 - b_0) + (A' - b)]/(M_{\lambda 1} - M_{\lambda 2})$ (4)

A' and b are equal, hence

$C_{acetaminophen} = -C_H = (A_0 - b_0)/(M_{\lambda 1} - M_{\lambda 2})$ (5)

Substitution of $C_{acetaminophen}$into Eqs1 and 2 yields A_H=b and the overall equation for the absorbance at H-point simplified to:

$A' = b = A_H = A_{tramadol}$ (6)

The concentration of tramadol was calculated from theanalytical signal from a calibration graph (Table 1).

Accuracy and Precision of the Method

Under the optimum conditions, simultaneous determination of different binary mixtures of tramadol and acetaminophen was made using the HPSAM. Regarding the tabulated results in Table 2, the accuracy of the results is satisfactory when the concentration of the tramadol and acetaminophen vary in the ranges of 5.0-100 and 1.0-20 µg/ml, respectively. To check the reproducibility of the method, five replicate experiments were performedand the relative standard deviations (RSD) were obtained for the mixtures. The results are given in Table 3. The precision of the results is satisfactory.

Application

In order to the test reliability of the proposed method, it was applied to the determination of tramadol and acetaminophenin pharmaceutical formulations. The results are presented in Table 4. The good agreement between these results and certificate values indicates the successful applicability of the proposed method for simultaneous determination of tramadol and acetaminophenin pharmaceutical formulations.

CONCLUSION

The HPSAM could be used for simultaneousspectrophotometric determination of tramadol and acetaminophen.The results of this study clearly show the potential and versatility of this method, which could be applied to evaluating tramadol and acetaminophenin pharmaceutical formulations. Finally, in comparison with chemometric methods, the H-point standard additions method (HPSAM) is very simple and mathematical software programs and additional information is not necessary.

Table 1.Characteristics of calibration graphs for the determination of tramadol and acetaminophen

Analyte	Wavelength (nm)	Slope (ml/µg)	Intercept	Correlation coefficient (n = 10)	Range (µg/ml)	Limit of detection* (µg/ml)
Tramadol	258 or 281	0.00147	0.041	0.999	5.0-100	2.0
Acetaminophen	243	0.0754	0.0507	0.998	1.0-20	0.50

*Limit of detection was defined as $C_L=3S_b/m$ where C_L, S_b and m are limit of detection, standard deviation of the blank signal and slope of the calibration graph, respectively.[25]

Table 2.Results obtained by the HPSAM for mixtures of tramadol and acetaminophen

A- C equation	r (n=6)	Presented in sample (µg/ml)		Found[a](µg/ml)	
		Tramadol	Acetaminophen	Tramadol	Acetaminophen
$A_{258} = 0.1692 + 0.0454C_i$ $A_{281} = 0.1009 + 0.0127C_i$	0.9988 0.9991	10	2.0	9.8 (98)	2.1 (105)
$A_{258} = 0.3120 + 0.0449C_i$ $A_{281} = 0.1837 + 0.0136C_i$	0.9991 0.9984	30	4.0	28.9 (96.3)	4.1 (102.5)
$A_{258} = 0.2558 + 0.0449C_i$ $A_{281} = 0.1851 + 0.0119C_i$	0.9994 0.9993	40	2.0	40.2 (101)	2.1 (105)
$A_{258} = 0.4214 + 0.0440C_i$ $A_{281} = 0.2481 + 0.0108C_i$	0.9990 0.9998	50	5.0	51.7 (103.4)	5.2 (104)
$A_{258} = 0.5321 + 0.0502C_i$ $A_{281} = 0.3231 + 0.0148C_i$	0.9985 0.9983	70	6.0	67.4 (96.3)	5.9 (98.3)
$A_{258} = 0.4449 + 0.0483C_i$ $A_{281} = 0.3366 + 0.0108C_i$	0.9988 0.9990	90	3.0	92.3 (102.6)	2.9 (96.7)
$A_{258} = 0.5834 + 0.0446C_i$ $A_{281} = 0.3156 + 0.0117C_i$	0.9989 0.9990	60	8.0	61.8 (103)	8.2 (102.5)

Values of recovery are given in parentheses

Table 3. Results of five replicate experiments for the analysis of tramadol-acetaminophen mixtures.

A-C equation	r(n=6)	Present in sample (µg/ml)		Found[a] (µg/ml)	
		Tramadol	Acetaminophen	Tramadol	Acetaminophen
$A_{258} = 0.3414 + 0.0440C_i$ $A_{281} = 0.1721 + 0.0108C_i$	0.9988 0.9991	50	5.0	51.7	5.1
$A_{258} = 0.4079 + 0.0453C_i$ $A_{281} = 0.2452 + 0.0121C_i$	0.9991 0.9984	50	5.0	49.6	4.9
$A_{258} = 0.3459 + 0.0438C_i$ $A_{281} = 0.1733 + 0.0106C_i$	0.9994 0.9993	50	5.0	52.5	5.2
$A_{258} = 0.3519 + 0.0463C_i$ $A_{281} = 0.1826 + 0.0131C_i$	0.9999 0.9998	50	5.0	50.9	5.1
$A_{258} = 0.3858 + 0.0423C_i$ $A_{281} = 0.2265 + 0.0091C_i$	0.9985 0.9983	50	5.0	48.5	4.8
Mean Standard deviation RSD(%)				50.64 1.61 3.2	5.06 0.16 3.3

Table 4.Determination of tramadol and acetaminophen in pharmaceutical formulations by proposed method.

Tablet	Certified value (mg)		Found (μg/g)		Error(%)	
	Tramadol	**Acetaminophen**	**Tramadol**	**Acetaminophen**	**Tramadol**	**Acetaminophen**
Tramadol	100	-	97.5±1.76	-	-2.5	-
Acetaminophen	-	325	-	329±2.27	-	+1.23
*Combined dosage form	37.5	325	36.1±1.12	334±1.89	-3.73	+2.77
**Combined dosage form	50	500	38.6±1.3	319±2.05	+2.93	-1.85

∗This combined formulation contain: Acetaminophen, 325 mg and tramadol, 37.5 mg (corresponding to Tramacet and Ultracet tablets).

∗∗This combined formulation contain: Acetaminophen, 500 mg and tramadol, 50 mg (corresponding to Instrel.tablet).

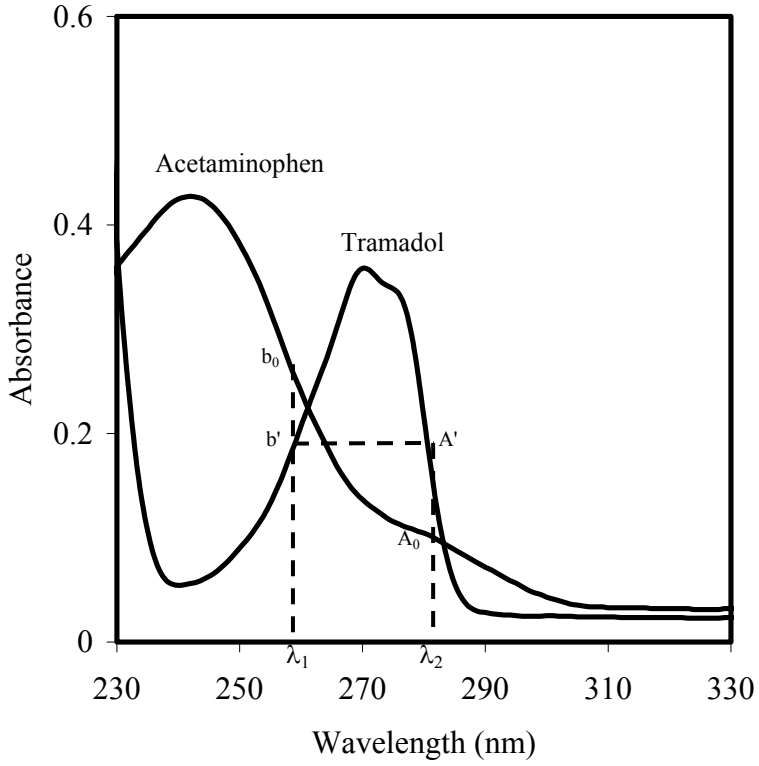

Figure 1.Absorption spectra of 5.0 μg/ml tramadol and 50 μg/ml acetaminophen.

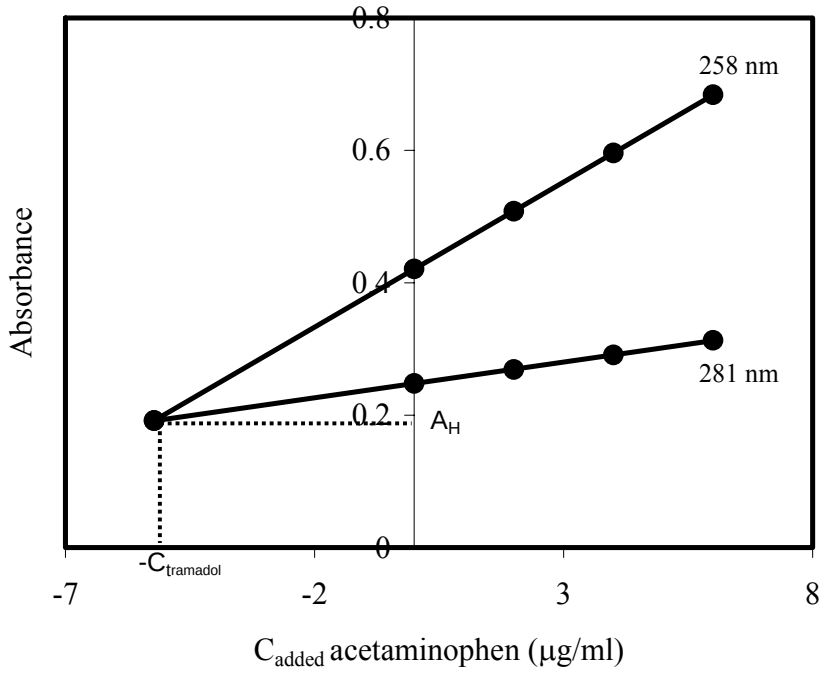

Figure 2.Plot of HPSAM for simultaneous determination of the mixture of tramadol-acetaminophen, Conditions: tramadol, 5.0 μg/ml; acetaminophen, 50 μg/ml.

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التحديد الطيفي المتزامن للتركيبة الدوائية لـ Acetaminophen و Tramadol باستخدام طريقة الإضافة القياسية للنقطة H

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ملخـص

تم تطبيق طريقة الإضافة القياسية للنقطة H على أنه التحديد الطيفي المتزامن على المستحضرات الدوائية الآتية: Acetaminophen وTramadol . وأظهرت النتائج بأنه يمكن تحديدهم بتزامن 5.0-100مل، على التوالي، وتم الحصول على حدود الكشف وهي 0.5-2.0 لـ Acetaminophen و Tramadol على التوالي. كما انه تم حساب معدل الانحراف المعياري للتحديد المتزامن المكرر 5 مرات. وتم تطبيق الطريقة المقترحة بنجاح في كلا التركيبتين الدوائيتين.

الكلمـات الدالـة: طيفية، طريقة الإضافة القياسية للنقطة H، التركيبة الدوائية.

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