Research Journal of Chemical Sciences _____E-ISSN 2231-606X
Vol. **6(5),** 6-10, May **(2016)** *Res. J. Chem. Sci.*

Simultaneous UV-Spectrophotometric for Validation of Acetaminophen and Guaiphenesin by AUC Method in Pharmaceutical Dosages

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Available online at: www.isca.in, www.isca.me
Received 3rd March 2016, revised 10th May 2016, accepted 17th May 2016

Abstract

A economical spectrophotometric method i.e. area under curve method for the validation of acetaminophen and guaiphenesin is proposed in this study. ICH guidelines were used for validation of dosages. In this method measurement of area at selected analytical wavelength ranges was carried out. The "Cramer's rule and Matrix method" was used for analysis .The wavelengths ranges such as 239-249nm and 268-277 nm for estimation of acetaminophen and guaiphenesin were used i.e.. The method showed linearity for acetaminophen and guaiphenesin at of 2 – 10 and 5 – 100 µg /ml respectively. The relative standard deviation was found to 0.2367 for acetaminophen and 0.2032 for guaiphenesin respectively. The ICH guidelines method was used for validation of its linearity, accuracy and precision.

Keywords: Spectrophotometry, Area under curve method, Acetaminophen, Guaiphenesin.

Introduction

Acetaminophen is chemically known as *N*-(4- hydroxyphenyl) acetamide. It prevents prostaglandin biosynthesis by blocking the cyclo-oxygenase 2-5 enzyme. Acetaminophen is an analgesic-antipyretic agent. It is effective in treatment of different types of pain i.e. pain of neuro-muscular origin to headache. Hence rapid and sensitive methods for the stimation of acetaminophen are being investigated. Guaiphenesin is, 3-(2-Methoxyphenoxy)-1,2-propanediol, is used to increase the volume and reduce the viscosity of tenacious sputum and is used as expectorant for productive cough. It has structural formula $C_{10}H_{10}O_4$ with molecular weight as 198.2. Literature reports HPLC methods[1-3] and miscellaneous[4-6] for validation of acetaminophen and guaiphenesin in pharmaceutical formulation.

Materials and Methods

Instrument and Reagents: i. Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm. ii. Standard of acetaminophen and guaiphenesin.

Standard drug solutions: Standard solution of paracetamol and guaiphenesin were standard solution of concentration 100 µg/ml was prepared in methanol as solvent.

Sample solution from pharmaceutical dosage: Tablet blend of 325 mg of acetaminophen and 200 mg of guaiphenesin was transferred in 100 ml of standard flask. It was sonicated for 10 minutes with small quantity of methanol. After sonication were further diluted with methanol. The solution was filtered. A 1 ml of above solution was further diluted to 10 ml. It gave a solution of 325 and 200 µg/ml of acetaminophen and guaiphenesin as analysis solution.

Area under curve method: Integrated values of absorbance between two selected wavelengths such as λ_1 and λ_2 were used for Area under curve method. UV probe 2.42 software was used for calculation of area under curve between λ_1 and λ_2.

For acetaminophen: A acetaminophen, standard solution of concentration 10 µg/ml was used for recording spectrum in the range of wavelength as 350 to 200 nm. After examining of the spectrum, 239- 249 nm was selected as working wavelength range for acetaminophen.

For guaiphenesin: A guaiphenesin, standard solution of concentration 10 µg/ml was used for recording spectrum in the range of wavelength as 350 nm to 200 nm. After examining of the spectrum, 268-277 nm was selected as working wavelength range for guaiphenesin.

Preparation of calibration curves: The different aliquots of varying concentration of acetaminophen and guaiphenesin were used for linearity study. Area under curve of above solutions of acetaminophen and guaiphenesin were measured at their respective selected analytical wavelength ranges. [Figure-1,2]. This area under curve (AUC) was then divide by concentration in g/lit to get X_{acet} for acetaminophen and X_{guaip} for guaiphenesin. After measuring the area under curve of acetaminophen at 239-249 nm and 268-277 nm for guaiphenesin by using UV-Probe software 2.42, the standard graphs were plotted of area against concentrations [Figure-3, 4]. Regression values are summarized in table-1.

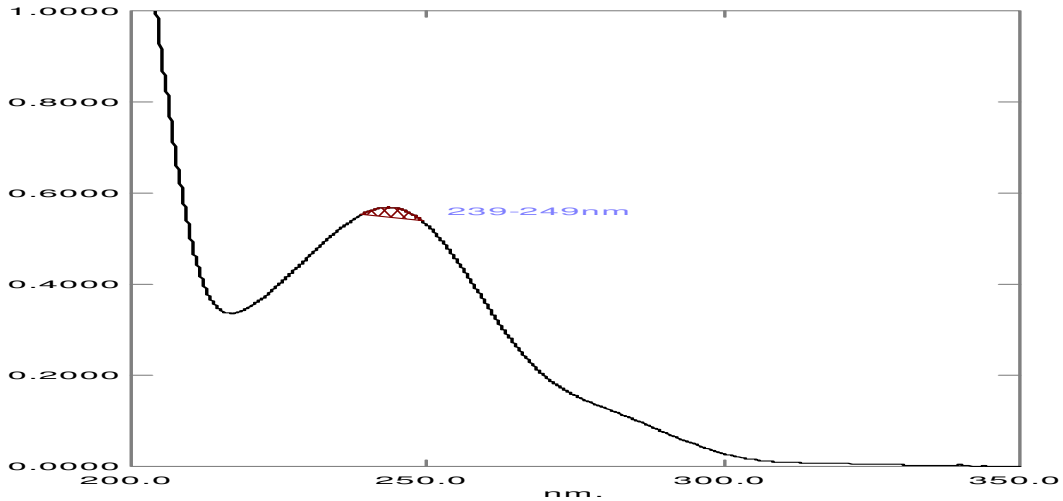

Figure-1
Zero order spectrum of acetaminophen at 239-249 nm

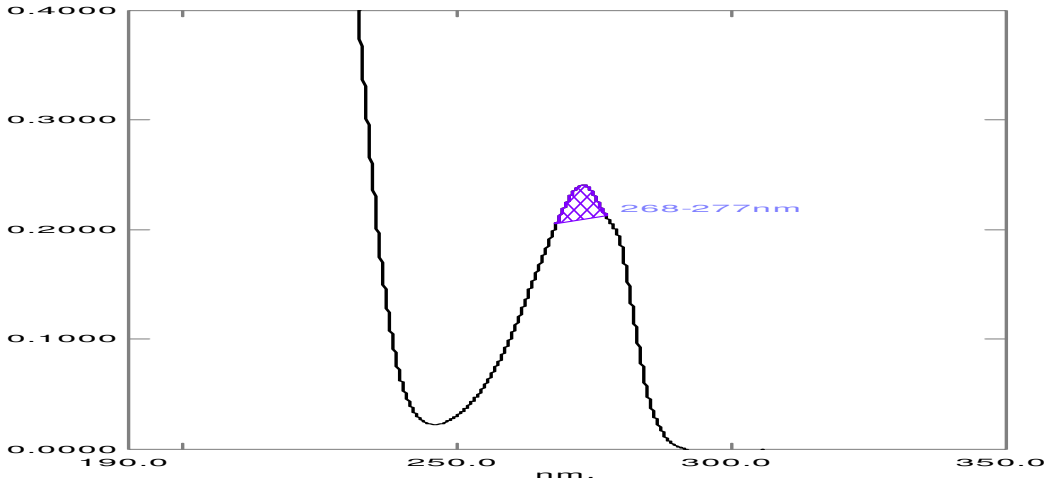

Figure-2
Zero order Spectrum of guaiphenesin at 268-277 nm

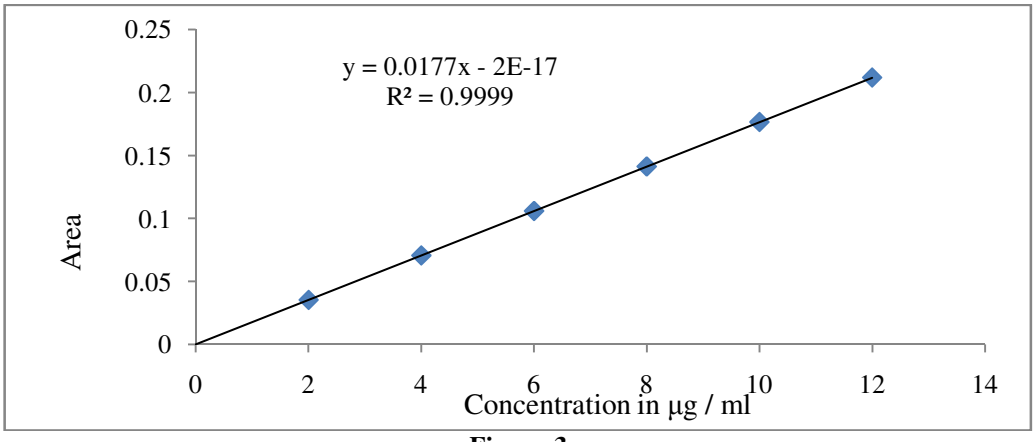

Figure-3
Linearity curve for acetaminophen (2-12 µg/ml)

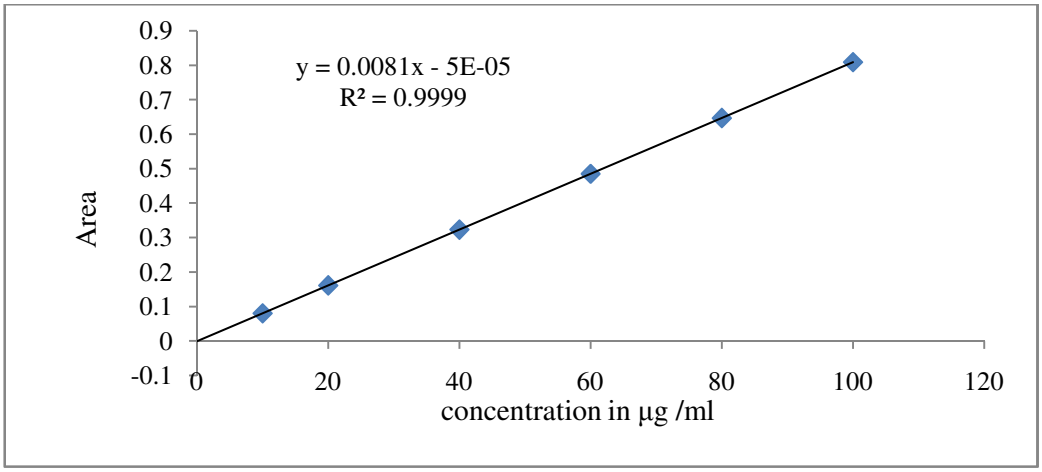

Figure-4
Linearity curve for guaiphenesin (5-100 µg/ml)

Table-1
Values of regression

Parameter	Acetaminophen	Guaiphenesin
Wavelength for area range (nm)	239-249	268-277
Limits of Beer's law in µg/ml	1-12	5-100
Correlation coefficient(r^2)	0.9999	0.9999
Slope	0.0177	0.0081
Intercept	-2E-17	-5E-05

Estimation from pharmaceutical dosage: A tablet blend to 325 and 200 mg of acetaminophen and guaiphenesin were used for analysis. It was collected in 100 ml of standard flask. It is treated with small quantity of methanol and sonicated for 10 minutes further diluted with methanol. It gave solution of 3250 and 2000 µg/ml of acetaminophen of guaiphenesin respectively

Appropriate aliquot was collected out from the sample solution and was further diluted to obtain working solution. The spectrum of sample solution containing and guaiphenesin was recorded and areas under curves were recorded in wavelength ranges of 239-249 nm and 268-277 nm. The areas under curves were analyzed by applying "Crammer's rule and "Matrix method". It is defines as "The total area under curve of mixture at particular wavelength range is equal to sum of area under curve of individual component at same wavelength range" (Figure-3).

Figure-3 Spectrum showing area under curve of mixture at 239-249 nm and 268-277 nm.

X = AUC of component between selected wavelength ranges
Concentration of that component in mg/lit

$$C_{acet} = \frac{(X^{guaip2}.AUC^{M268-277}) - (X^{guaip1}.AUC^{M239-249})}{(X^{acet1}.X^{guaip2}) - (X^{acet2}.X^{guai1})}$$

$$C_{guaip} = \frac{(X^{acet1}.AUC^{M268-277}) - (X^{acet2}.AUC^{M239-248})}{(X^{acet1}.X^{guaip2}) - (X^{acet2}.X^{guai1})}$$

Where: C_{acet} = Concentration of acetaminophen,
C_{guaip} = Concentration of guaiphenesin
X^{acet1} = Area under curve of acetaminophen at 239-248 nm
X^{acet2} = Area under curve of acetaminophen at 268-277 nm
X^{guaip1} = Area under curve of guaiphenesin at 239-248 nm
X^{guaip2} = Area under curve of guaiphenesin at 268-277 nm
AUC^{M} = Area under curve of mixture

Validation of method: The ICH guidelines were used for validation of drugs.

Accuracy: For the study of accuracy of methods by standard addition method, the three different levels such as 80%, 100% and 120% were used. The acetaminophen was found in the range of 99.93% to 100.06% and guaiphenesin was found in the range of 100.01% to 100.06 % in percentage recovery studies respectively (Table-2).

Linearity and Precision: The study was carried for different aliquots of standard solutions of acetaminophen and guaiphenesin respectively. It was found that the linearity were

1-12 with 5-100 µg/ml for acetaminophen and guaiphenesin. It is performed by analyzing tablets blend with 325 mg and 200 mg of acetaminophen and guaiphenesin. The six replicates were performed out for assay. The percentage recovery values were 100.03 and 00.01 for acetaminophen and guaiphenesin. The table 3 indicates result of precision**.**

Stability study of solution: The study of Inter-day and intraday precision were performed out by using tablet blend with 325 mg of 200 mg of acetaminophen and guaiphenesin respectively. The data is recorded in six replicates. A 1st, 3rd and 5th days were selected for inter-day precision study. The values of mean recovery are given in Table 4.

Table-2
Statistical evaluation for accuracy

% recovery	Quantity taken in µg/ml		Quantity placed in µg/ml		Quantity recovered in µg/ml		Percentage obtained		Mean of % Obtained	
	ACET	GUIP	ACET	GUIP	ACET	GUIP	ACET	GUIP	ACET	GUIP
80%	3.0	10	2.4	8	5.409	18.025	100.18	100.14	100.06	100.02
	3.0	10	2.4	8	5.394	18.021	99.89	100.12		
	3.0	10	2.4	8	5.405	17.965	100.11	99.81		
100%	3.0	10	3.0	10	5.987	20.016	99.79	100.08	99.93	100.01
	3.0	10	3.0	10	6.008	20.018	100.14	100.09		
	3.0	10	3.0	10	5.992	19.968	99.88	99.84		
120%	3.0	10	3.6	12	6.604	22.037	100.07	100.17	100.02	100.06
	3.0	10	3.6	12	6.612	22.033	100.19	100.15		
	3.0	10	3.6	12	6.588	21.969	99.82	99.86		

ACET = Acetaminophen, GUIP= Guaiphenesin

Table-3
Results of method of precisions

Sample No.	Results of % Assay	
	Acetaminophen	Guaiphenesin
1	99.56	100.18
2	100.14	100.14
3	100.08	99.81
4	100.19	99.87
5	100.16	100.24
6	100.08	99.79
% assay(Mean)	**100.03**	**100.01**
%Relative Standard Deviation	**0.2367**	**0.2032**

Table-4
Data of stability study

No.	Study	Acetaminophen	Guaiphenesin
1(A)	Intra-day study	100.17%	100.12%
	± % COV.	0.2417	0.2037
1(B)	Inter-day study	99.25%	99.19%
	± % COV.	0.2857	0.2847

The % RSD values are found to be less than 0.1. it indicates the proposed method have high degree of precision.

Results and Discussion

In UV UV-spectrophotometric, area under curve method for validation of acetaminophen and guaiphenesin was found to be simple and convenient. It can be applicable for the assay of combined formulation. The method is simple, economical as well as reproducible. It is confirmed from results obtained in tables 1 to 4. The values of standard deviation and %RSD were low. Linear regression equation method was used to study of linearity for acetaminophen and guaiphenesin at various concentrations. The correlation coefficients of these drugs were in the range of 0.9999 to 1.00. It shows good linearity of the method.

The results of the validation obtained by above method are in good agreement (Table-2). Therefore area under curve method will be easily applicable for validation of acetaminophen and guaiphenesin in pharmaceutical preparations.

Conclusion

The reproducibility of above the method suggested is very convenient and economical. It can be used for assay of acetaminophen and guaiphenesin from their dosage form.

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