Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

ESTIMATION AND VALIDATION OF STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF GUAIFENESIN IN PRESENCE OF ITS DEGRADANT PRODUCTS

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Received: 13 Dec 2012, Revised and Accepted: 04 Feb 2013

ABSTRACT

Objective: The objective of present study is to develop and validate stability indicating method to generate reliable and accurate data regardless of whether it is for acceptance, release, stability or pharmacokinetic study.

Method: The UV spectrum was scanned between 200 to 400 nm and 273 nm was selected as maximum wavelength for absorption.

Results: The proposed method for determination of Guaiphenesin in prepare tablet showed Sandell's sensitivity of 0.9419 µg/cm^2/0.001 absorbance units. Linear regression of absorbance on concentration gave the equation y = 0.0155x – 0.0136 with a regression co-efficient (R^2) of 0.991.The higher percentage recovery value indicates that there is no interference of the exipients present in the formulation. The stability study indicates that appreciable changes were observed by treating the drug with UV light, thermal stress, oxidation, acidic hydrolysis and basic hydrolysis, however there was no appreciable change with sunlight. Thus the method is useful for the determination of Guaiphenesin in bulk and pharmaceutical formulations.

Conclusion: A simple, sensitive and appreciable stability indicating UV spectrophotometric method has been developed for quantitative determination of Guaiphenesin in bulk and prepared solid dosage form (tablet). The method was fast and economical and it was also selective and sensitive for the desirable range. Results of the analysis were validated as per ICH guidelines and by recovery studies. Stability testing study includes the effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH.

Keywords: Guaiphenesin, Validation, Stability indicating method, UV spectrophotometry

INTRODUCTION

Spectrophotometric [1, 2] methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The components monitored include chiral or achiral drug, process impurities, residual solvents, exipients such as preservatives, degradation products, extractable and leachables from container and closure or manufacturing process, pesticide in drug product from plant origin, and metabolites.

The objective of present study is to develop and validate stability indicating method [3, 4, 5] to generate reliable and accurate data regardless of whether it is for acceptance, release, stability or pharmacokinetic study. Data are generated for the qualitative and quantitative testing during development and post approval of the drug products. The testing include the acceptance of raw materials, release of the drug substance and products, in process testing for quality assurance, and establishment of the expiration dating period. For the above purpose there is a need to develop simple, accurate and reliable stability indicating method for the determination of guainfenesin [6] in pharmaceutical dosage forms.

An ideal stability indicating chromatographic method should estimate the drug and also be able to resolve the drug from its degradation products. Hence an attempt has been made to develop an accurate, rapid and reproducible method for the determination of guaiphenesin in presence of its degradation products for its content analysis in pharmaceutical dosage form as per ICH [7, 8, 9] guidelines.

Literature survey reveals that spectrophotometric, HPLC and high performance thin layer chromatography (HPTLC) methods for the estimation of guaiphenesin from bulk drugs and pharmaceutical formulation have been developed. However, no stability indicating method has been reported so far for estimation of drug in pharmaceutical dosage form by spectrophotometry .This work presents a stability indicating u.v spectrophotometric method for the estimation of drugs in pharmaceutical dosage form, which can be used for its routine analysis in laboratory.

MATERIAL AND METHOD

Chemicals and Reagent

Guainfenesin was procured as a gift sample .All other solvents and reagents were purchased from S.D.Finechemicals, Mumbai, India and were of analytical grade.

Method

Instrumentation

UV – 1700(E) 23 OCE, UV 1800 Pharmaspec, Shimazdu Corporation was used to carry out the UV detection, Sonicator- Mini Ultrasonic Cleaner, Piezo-U-Sonic.

Preparation of solution

a) Preparation of stock solution of drug

Stock solution of guaifenesin was prepared by weighing 100 mg of guaifenesin and dissolved it in methanol to made the volume 100 ml. The concentration of the prepared stock solution was 1000 µg/ml.

b) Preparation of standard solution

Different aliquots were taken from stock solution and diluted with methanol to prepare a series of concentrations from 5–40 µg/ml.

Calibration Plots

Calibration standard were prepared by dissolving working standard into the solvent to yield the concentrations of 5, 10, 20, 30, 40 µg/ml and was determined for absorbance.Aliquots (0.05, 0.1, 0.2, 0.3, 0.4 ml) from standard solution of Guaiphenesin were pipetted out into the 10 ml volumetric flask and the volume was made upto10 ml with methanol. The absorbances were measured for six times each at 273 nm against reagent blank. The calibration curve was constructed by plotting absorbance v/s concentration (µg/ml). Regression coefficient was also measured.

Table 1: Calibration of proposed method

S. No.	Concentration(µg/ml)	Absorbance(n = 6)	%RSD
1	5	0.056	.064
2	10	0.149	.214
3	20	0.291	.342
4	30	0.415	.058
5	40	0.637	.049

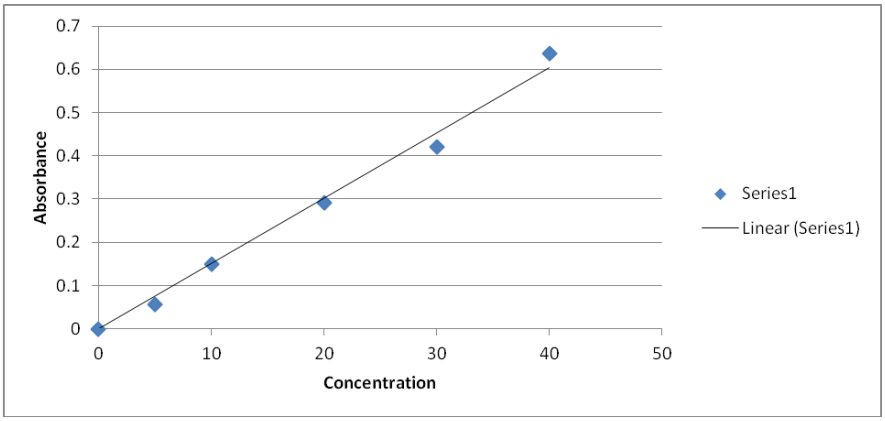

Fig. 1: Calibration curve of guaiphenesin

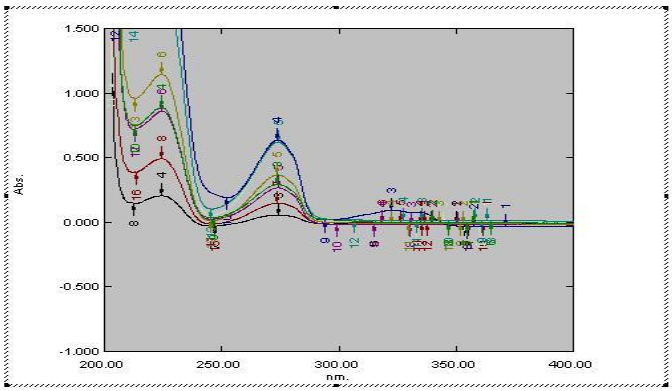

Fig. 2: Calibration spectra of the guaiphenesin

Table 2: Regression analysis of the calibration

Linearity range (µg/ml)	5-40
Detection wave length (nm)	273
Slope(m)	.015
Intercept(c)	.0136
Regression coefficient(R^2)	.991
Sandells sensitivity (µg/cm²/0.001)	0.9419
%RSD range	0.049 – 0.342
LOD(µg/ml)	48.6368
LOQ(µg/ml)	147.3845

Analysis of marketed formulation

To determine the content of guainfenesin in marketed formulation (label claim: Guaiphenesin 50mg/tablet) the contents of 20 tablets were weighed and their average weight was determined. The content of tablets was finely powdered.

Solution A: an amount equivalent to average weight of tablet contents was transferred into a 100mL volumetric flask containing 50mL methanol. It was sonicated for10min and contents were diluted to100mLwith methanol. The resulting solution was centrifuged at 3000 rpm for 5min and supernatant was collected.

Solution B: 1mL of solution A was diluted to10mL of methanol in a10mLvolumetric flask. The absorbance of the solutions was measured at 273 nm against blank. The analysis was repeated for six times.

Table 3: Analysis of the tablet formulation

S. No.	Labelled amount of drug	Amount found	% label claim	Average	%RSD
1	50	48.14	96.28		
2	50	50.19	100.38	99.61	0.5512
3	50	49.29	98.58		
4	50	52.01	104.02		
5	50	47.96	95.92		
6	50	51.26	102.52		

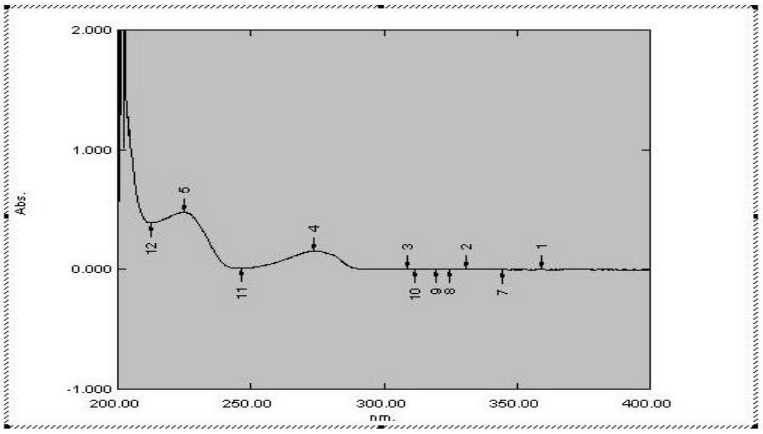

Fig. 3: UV Spectra of Guaiphenesin Formulation

Method Validation

Recovery Study

Recovery study was carried out at 80 %, 100 % and 120 % of target concentration. From the amount found, percentage recovery was calculated

Precision

Precision of the method was studied by carrying out intraday, interday analysis and expressed as Relative Standard Deviation. For this purpose 20 and 30 µg/ml solutions were prepared and the absorbances of the solutions were measured for six times at 273 nm against blank.

Table 4: Recovery study from drug solution

Amount of pure drug(µg) added to solution of formulation	Recovery from drug solution	
	Amount (µg/ml) found	% Recovery (n = 6)
16	16.4258	102.6612
20	18.9581	94.7905
24	25.3581	105.65

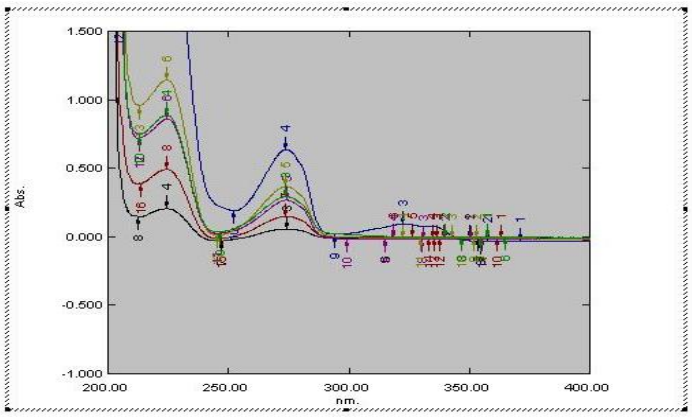

Fig. 4: UV Spectra for recovery study

Table 5: Intra and Inter day precision study

Concentration of drug(µg/ml)	Intraday absorbance (n = 6)	%RSD	Inter day absorbance (n = 6)	%RSD
20	0.287	0.149	0.298	0.74
30	0.364	0.658	0.374	0.48

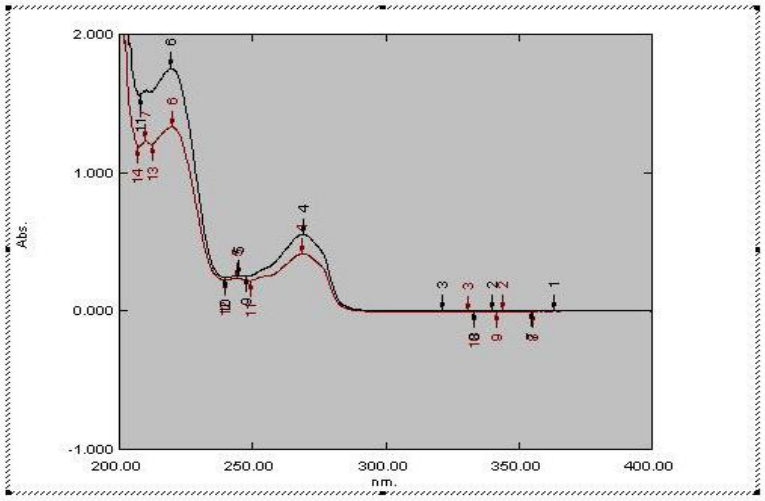

Fig. 5: UV spectra of intra and inter day precision

Limit of Detection (LOD) and Limit of Quantization (LOQ)

LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where σ = the standard deviation of the response and S = the slope of the regression equation.

Forced degradation studies

Guainfenesin was subjected to various stress condition s to affect their degradation. Thus the acid induced alkali induced, oxidative and dry heat degradation was attempted.

Acidic Hydrolysis

To 10 ml of above methanolic stock solutions of guainfenesin formulation and 10 ml of 1 M HCl, were refluxed separately for 1 hour at 80ºC on oil bath. The forced degradation in acid media was performed in the dark in order to exclude possible photo-degradation. The degradation samples were then cooled to room temperature. Suitable aliquots of resultant degradation samples were taken and neutralized for assay after suitable dilutions with solvent.

Alkaline Hydrolysis

To 10 ml of methanolic stock solutions of guainfenesin pharmaceutical formulation and 10 ml of 2 M NaOH, was added separately. These mixtures were refluxed separately for 1 hour at 80ºC on oil bath. The forced degradation in alkaline media was performed in the dark in order to exclude possible photo-degradation. The degradation samples were then cooled to room temperature. Suitable aliquots of resultant degradation samples were taken, neutralized and subjected to analysis after suitable dilutions with solvent.

Oxidative Hydrolysis

To 10 ml of above methanolic stock solutions of guainfenesin pharmaceutical formulation and, 10 ml of 3 %v/v H2O2 was added separately. These mixtures were refluxed separately for 1 hour at 80ºC on oil bath. The forced degradation in oxidative media was performed in the dark in order to exclude possible photo-degradation. The degradation samples were then cooled to room temperature. Suitable aliquots of resultant degradation samples were taken and subjected to analysis after suitable dilutions with methanol.

Dry Heat Degradation

For dry heat degradation, guainfenesin pharmaceutical formulation were placed in oven at 80ºC for 24 hours under dry heat condition in the dark and then cooled to room temperature. Degradation samples were subjected to analysis after suitable dilutions with methanol.

Photochemical Degradation

For carrying out photolysis studies the drug was treated with UV light for 6 hours at 254 nm and also in sunlight. Then test solutions were prepared and the absorbance of the solutions (20 µg/ml) were measured for six times at 273 nm against blank

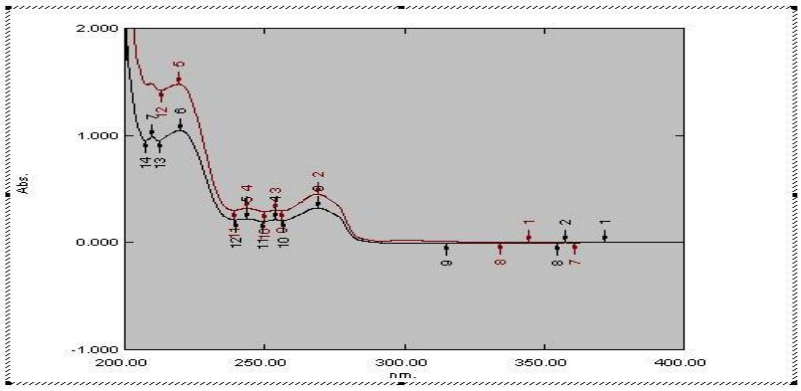

Fig. 6: UV Spectra for acid hydrolysis

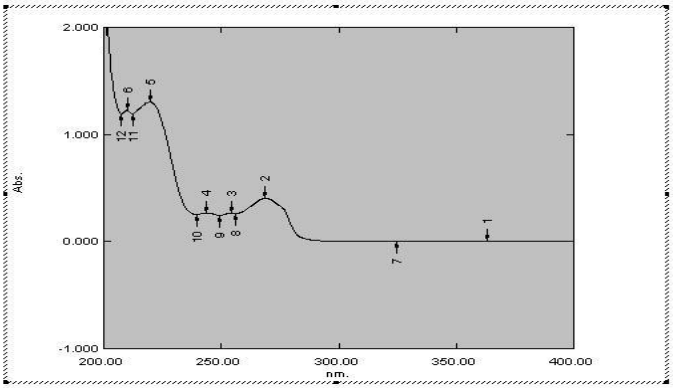

Fig. 7: UV Spectra of base hydrolysis

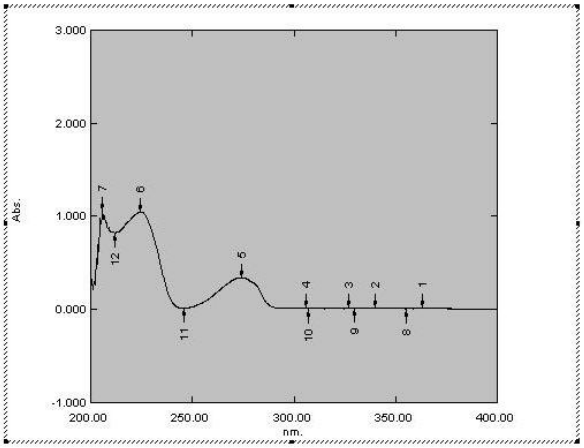

Fig. 8: UV Spectra of oxidative degradation

Table 6: Stability of the drug under stress condition

Types of reaction	Chemical Used	Temp (^{0}c)	Time (hr)	Total amount of drug used	After reaction amount of drug taken (µg/ml)	Absorbance	% Drug remaining	Amount of drug remains (mg)
Acid hydrolysis	2(M) HCl	80^0	6	50	20	0.079	29.87	14.93
Base hydrolysis	5(M)NaOH	80^0	6	50	20	0.045	18.90	9.45
Oxidation	3% H_2O_2	80^0	6	50	20	0.218	74.70	37.35

Table 7: Stability of the drug under stress condition

S. No.	Types of reaction.	Condition applied	Time (hr)	Total amt of drug used(mg)	After reaction amount of drug taken(µg/ml)	Absorbance	% Drug remains	Amount of drug remains(mg)
1.	Thermal stability	60^0c	6	50	20	0.247	84.06	42.03
2.	Photo stability	UV chamber at 254 nm	6	50	20	0.265	89.87	44.93
3.		UV chamber at 365 nm	6	50	20	0.216	74.06	37.03
4.		Sunlight	6	50	20	0.267	90.51	45.25

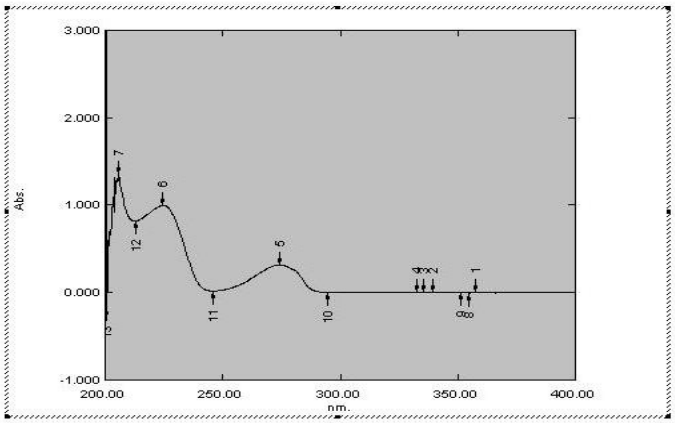

Fig. 9: UV Spectra for photochemical degradation (254nm)

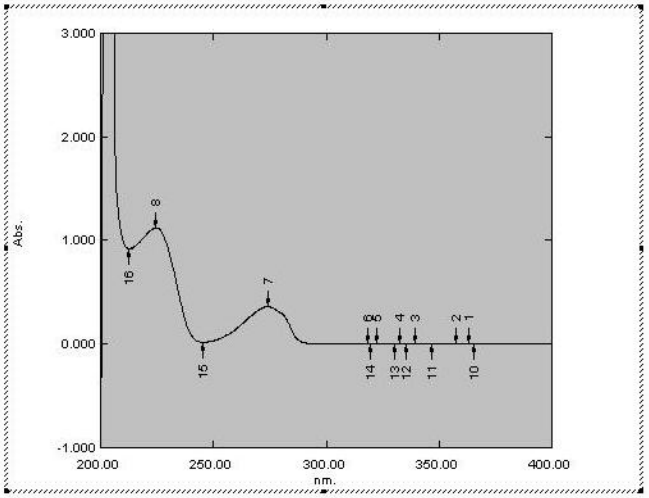

Fig. 10: UV Spectra for photochemical degradation (sunlight)

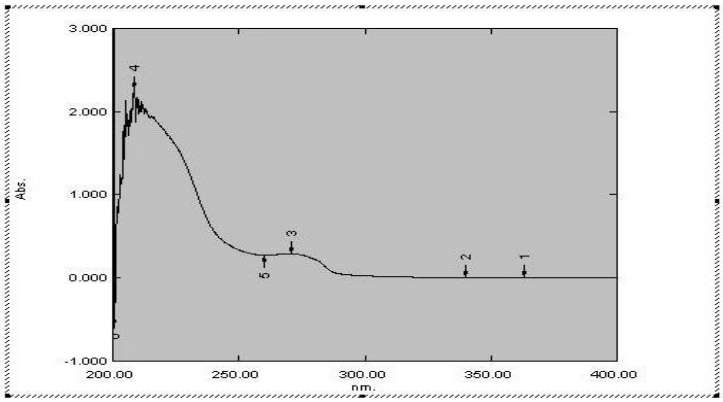

Fig. 11: UV Spectra of thermal degradation

RESULT AND DISCUSSION

The proposed method for determination of Guaiphenesin in marketed formulation (tablet) showed Sandell's sensitivity of 0.9419 µg/cm^2/0.001 absorbance units. Linear regression of absorbance on concentration gave the equation y = 0.0155x – 0.0136 with a regression co-efficient (R^2) of 0.991 and the linearity range was 5-40µg/ml.The higher percentage recovery value (105%) indicates that there is no interference of the exipients present in the formulation. The stability study indicates that appreciable changes were observed by treating the drug with UV light, thermal stress, oxidation, acidic hydrolysis and basic hydrolysis, however there was no appreciable change with sunlight. Thus the method is useful for the determination of Guaiphenesin in bulk and pharmaceutical formulations.

CONCLUSION

A simple, sensitive and appreciable stability indicating UV spectrophotometric method has been developed for quantitative determination of Guaiphenesin in bulk and prepared solid dosage form (tablet). The UV spectrum was scanned between 200 to 400 nm and 273 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 5 – 40 µg/ml. Accuracy (94.79 – 105.65) and the method were successfully applied to the pharmaceutical dosage form containing

the Guaiphenesin drug without any interference by the exipients. The method was fast and economical and it was also selective and sensitive for the desirable range. Results of the analysis were validated as per ICH guidelines and by recovery studies. Stability testing study includes the effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH

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