Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR GUAIFENESIN

Sandhya Rajkumar Gawai*[1], Kiran Gadge[1], Sandeep Sonwane[1]

[1]*Department of Quality Assurance, MET's (Bhujbal knowledge City) Institute of Pharmacy, Nasik, Maharashtra, India.

ABSTRACT

A simple, accurate and precise stability indicating RP HPLC method was develop and validated for the estimation of Guaifenesin in bulk and formulation. The drug and its degradation products formed under various forced degradation conditions were separated on Phenomenex C18 column (250 × 4.6 mm, 5µ) using mobile phase consisting of methanol: Water (60:40% v/v). The flow rate was kept constant at 1.0mL/min and eluents were detected at 230 nm. In forced degradation experiments, guaifenesin was found to degrade in Acid, Alkali, Photolytic and wet heat conditions and was found stable in H_2O_2 (Oxidative) and dry heat conditions. In calibration curve experiments, linearity was found in concentration range 2-12µg/mL with regression coefficient (R^2) 0.999. The equation obtained was y = 102800 x + 24264.

KEYWORDS

Guaifenesin, Stability indicating method, RP-HPLC and Analytical method validation.

Author for Correspondence:

Sandhya Rajkumar Gawai,

Department of Quality Assurance,

MET's (Bhujbal knowledge City) Institute of Pharmacy, Nasik, Maharashtra, India.

Email: g.sandhya118@gmail.com

INTRODUCTON

A Stability-indicating assay method can be defined as "Validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug products are specific so that the content of active ingredients and degradation products can be accurately measured without interference"[1].

Generally forced degradation/stress testing is used to generate the samples for stability-indicating assay methods. Forced degradation/stress testing is defined as "the stability testing of drug substance

and drug product under conditions exceeding those used for accelerated stability testing"[2].

Degradation can be achieved by exposing the drug, for extended period of time, to extremes of pH (HCl or NaOH solutions of different strengths), at elevated temperature, to hydrogen peroxide at room temperature, to UV light, and to dry heat (in an oven) to achieve degradation to an extent of 5-20%. Generally, trial and error experimentation is used during these experiments. This trial and error approach is generally cost, labor, and time intensive and should be substituted with some systematic approach[3,4].

Guaifenesin is 3-(2-methoxyphenoxy)-propane-1, 2-diol (Figure No.1)[22] acts as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It also stimulates the flow of respiratory tract secretions, allowing ciliary movement to carry the loosened secretions upward toward the pharynx[22]. It is a White or grey crystalline powder and having melting point 79°C to 83°C. It is freely soluble in methanol, ethanol, HCl and Sparingly soluble in water, ethyl ether[21]

As per literature review, several method were reported for the estimation of guaifenesin by UV-Vis spectrophotometery[22], HPLC and UPLC[15]. Only one stability indicating RP-HPLC method.

The aim of present work was to develop a simple, accurate and precise stability indicating RP-HPLC method for determination Guaifenesin in presence of its degradation products.

MATERIAL AND METHODS

Apparatus and Equipment's

HPLC instrumentation consisting of pump PU-2080 plus (JASCO, Tokyo, Japan), with Rheodyne manual loop injector (injection loop capacity 100µL) was used. Detection was carried out using UV- V-630 detector (JASCO, Tokyo, Japan). Data acquisition was done by Borwin chromatography software version 1.5 (JASCO, Tokyo, Japan).

Reagents and Chemicals

Pharmaceutical grade Guaifenesin was supplied as a gift sample from Meditab Specialities Pvt. Ltd. Daman Gujrat, India. Methanol and used in analysis were of HPLC grade and all other chemicals and reagents were of analytical grade and were purchased from Thomas Baker (Chemicals), Mumbai (India). Double distilled water used was freshly prepared by Double Distillation Assembly (Borosil, Mumbai, India) and further used in analysis after filtering through 0.45µ membrane filter papers purchased from Millipore (India) Pvt. Ltd., Peenya, Bangalore, India. Guaifenesin tablets were purchased from the local market, manufactured by Akums Drugs and Pharmaceuticals Ltd., Ranipur, Haridwar. India.

Chromatographic Conditions

All chromatographic separations were carried out on Phenomenex C18 column (250 × 4.6 mm, 5μ), using mobile phase comprising methanol: water 60:40% v/v. The flow rate was kept constant throughout analysis at 1.0 mL/min and eluent was detected at 230 nm by UV- detector.

Standard Preparation

(Guaifenesin 100µg/ml)

An accurately weighed quantity of 10 mg of the guaifenesin was transferred in 100mL volumetric flask, dissolved with sufficient quantity of methanol and volume was made up to the mark with methanol. This gave 100 µg/mL standard stock solution for guaifenesin. The chromatogram of standard Guaifenesin solution was shown in Figure No.2. And the average retention time was found to be 4.058 min.

METHODS VALIDATION

System suitability

A Standard solution of Guaifenesin working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from six replicate injections are within range and results were shown in Table No.1.

Linearity

Linearity is the ability of the method to elicit test results that are proportional to concentration of the analyte in the sample[6,7,4].

It was found to be in the range of 2-12µg/ml. The calibration graph was plotted, equation was obtained

y= 102800X + 24264 and the drug was found to be linear with a correlation coefficient (r2) of 0.999 were shown in Figure No.3.

Accuracy and Precision

It is the closeness of test results obtained by the method to the true value. It was determined by percent recovery of the standard API to the blank and it is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It was determined by studying repeatability, intra-day and inter-day precision of method[6,7,4]. The average recovery of the analyte of 80%, 100% and 120% solution. The amount found (mg) and %RSD was calculated and were shown in Table No.3.

Specificity

Specificity is the ability to measure unequivocally the desired analyte in the presence of components such as excipients and impurities[4]. Twenty blank tablets were weighed accurately and triturated. Quantity equivalent to the average weight of the tablet was weighed accurately and analyzed by the described method of analysis and the chromatograms were observed for the interfering peaks at the retention time of guaifenesin. The blank tablets exposed to stress testing were also analyzed using optimized chromatographic conditions and the resulting chromatograms were inspected for interfering peaks at the retention time of guaifenesin. Specificity was also indicated by the separation of guaifenesin from any potential degradation products. The method was declared specific if there were no interfering peaks at retention time of guaifenesin and guaifenesin peak was well resolved from the peaks of all potential degradation products.

Specificity was carried out as blank, placebo, standard and sample solution was injected and interference was observed.

Blank preparation

Diluent (mobile phase) was used as a blank.

Placebo preparation

10 mg of placebo powder was weighed and transferred to 100mL volumetric flask. 50mL of diluents was added and sonicated for 10min. Diluted to volume with diluent, and filtered through whatman filter paper.

Standard preparation

10 mg of guaifenesin was accurately weighed and transferred to 100mL volumetric flask. 50 mL of diluents was added and sonicated for 10 min. Diluted to volume with diluent, and filtered through whatman filter paper.

Sample preparation

20 tablets were crushed to powder. Tablet powder equivalent to 10 mg of guaifenesin was accurately weighed and transferred to 100 mL volumetric flask. 50mL of diluents was added and sonicated for 10 min. Diluted to volume with diluent, and filtered through whatmann filter paper.

From each of the above samples 0.1mL was diluted up to 10 mL with mobile phase to get a final concentration of 10µg/mL.100 µL of each solution (diluents, placebo,

Limit of Detection (LOD)

LOD is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated[6,4]. It is calculated to be 0.3589µg/mL by using the formula,

LOD= 3.3σ/S Where, σ = Standard deviation of the response, S = Slope of calibration curve.

Limit of Quantitation (LOQ)

LOQ is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied[6,4]. It was calculated to be 1.0876µg/mL by using the formula,

LOQ=10σ/S Where, σ = Standard deviation of the response, S = Slope of calibration curve.

Degradation studies

Forced degradation experiments were carried out on Guaifenesin under various conditions explained in ICH guideline Q1A (R2), *namely*, acid, alkali, wet heat, dry heat, and oxidative and photolytic conditions[6].

Acid degradation

There was no degradation found when the drug was refluxed with 1 N HCl for 1hr at 80°C. Further, the concentration of HCl was kept same and exposure time was extended to 2 hrs. When stressed sample was analyzed, there were two additional peaks at the retention time 2.292 min and 2.625min. There was no additional peak at the same retention time when zero time, stressed blank and blank sample were analyzed confirming the formation of two degradation products. Comparison of the peak area of guaifenesin in stressed condition with that of the zero time sample gave 10.75% degradation.

Alkali degradation studies

There was no degradation found when the drug was refluxed with 1N NaOH for 1 hr at 80°C. So it was decided to extend heating up to 5hrs without changing temperature. When this stressed sample was analyzed, there were two additional peaks at the retention of 1.658 min and 2.658 min. There was no additional peak at the same retention time when blank, zero and stressed blank samples analyzed were analyzed confirming the formation of two degradation products. Comparison of the peak area of guaifenesin in stressed condition with that of the zero time sample gave 20.26% degradation.

Wet heat degradation

There was degradation found when the drug was refluxed for 30 min with water at 80°C.When stressed sample was analyzed, there was one additional peak at the retention time 2.633 min. There were no additional peaks at the same retention time. When blank, zero and stressed blank samples analyzed and confirming the formation of one degradation product. Comparison of the peak area of guaifenesin in stressed condition with that of the zero time sample gave 5.21% degradation.

Oxidative degradation

Oxidative degradation of guaifenesin was studied using 30 % H_2O_2 for 30 min when the samples were analyzed no degradation found. So, it was decided to extend the exposure time of drug for 5hrs, 24hrs, and 48hrs.When the stressed sample was analysed, there were no additional peaks. Also the comparison between the peak areas of stressed sample of guaifenesin with that of zero time sample showed no difference, indicating that there was no degradation. Hence it was concluded that drug was stable under the conditions tested.

Dry heat degradation

Stability of guaifenesin in dry heat was studied by keeping it for 1 hr at 50°C. When the stressed sample was analyzed, no degradation was found and hence it was decided to extended the heating time for 3hrs, 5hrs, 24hrs, 48hrs with increased in temperature 70°C.When the stressed sample was analyzed, there was no additional peak found. Also the comparison between the peak areas of stressed sample of guaifenesin with that of zero time sample showed no difference, indicating that there was no degradation. Hence it was concluded that the drug was stable under the conditions tested.

Photolytic degradation

Degradation of methanolic drug solution

The drug was dissolved in methanol exposed to sunlight for 8hrs. When the stressed sample was analyzed, no degradation was found and hence the exposure time was extended for 24hrs and 48hrs. When stressed sample was analyzed, there was one additional peak found at the retention time of 2.667 min. There were no additional peaks at the same retention time when blank, zero and stressed blank samples analyzed and confirming the formation of one degradation product. Comparison of the peak area of guaifenesin in stressed condition with that of the zero time sample gave 6.11% degradation.

Degradation of dry drug powder in sunlight

The powdered drug was exposed to sunlight for 8hrs. When the stressed sample was analyzed, no degradation was found and hence the exposure time was extended for 24 hrs and 48hrs. When stressed sample was analyzed, there was one additional peak at the retention of 2.866 min. There were no additional peaks at the same retention time when blank, zero and stressed blank samples analyzed and confirming the formation of one degradation product. Comparison of the peak area of guaifenesin in stressed condition with that of the zero time sample gave 7.84% degradation.

Hence, it was found that guaifenesin was degraded under acid, alkali, wet heat, and photolytic

conditions while it was stable under oxidative and dry heat conditions.

Chromatographic Analysis of Forced Degraded Samples

After degradation, each sample obtained under each forced degradation condition was diluted appropriately with mobile phase to get a final concentration of 10µg/mL; the resulting solution was injected in the column under described chromatographic condition. The chromatogram obtained was studied for area of drug peak and appearance of secondary peaks.

The decrease in the area of the drug peak and the occurrence of secondary peaks was considered as indication of degradation. The % degradation was calculated by using below formula [9] and was result shown in Table No.5.

$$\% \text{ Degradation} = \frac{\text{Peak area of stressed sample}}{\text{Peak area of unstressed sample}} \times 100$$

RESULTS AND DISCUSSION

Table No.1: System suitability for Guaifenesin

S.No	Parameter	Value
1	Theoretical plates	9595
2	Retention time (min)	4.058
3	Asymmetry	1.34

Table No.2: Linearity for Guaifenesin

S.No	Conc µg/ml	Peak area (µV.sec)			Average peak area (µV.sec)	S.D. of Peak Area	% RSD
		1	2	3			
1	2	228157	225169	226248	226524.66	1513.09	0.66
2	4	435286	437459	436138	436294.33	1094.90	0.25
3	6	649122	646512	647691	647775	1307.02	0.20
4	8	852439	846142	844175	847585.33	4316.92	0.51
5	10	1042664	1044483	1043876	1043674.33	926.116	0.09
6	12	1264249	1258385	1261372	1261335.33	2932.17	0.23
Equation		y= 102800x + 24264					
R^2		0.999					

Table No.3: Accuracy and Precision studies

S.No	Amount Added	Amount Found (mg)			Within mean square	Between mean square	F Value
		Day 1	Day 2	Day 3			
1	80% 160 mg	159.4	159.3	159.7	0.107	0.347	3.22
		159.1	158.9	159.4			
		159.7	158.4	159.4			
2	Mean	159.4	158.86	159.5			
3	S.D.	0.3	0.45	0.17			
4	%R.S.D	0.18	0.28	0.10			
5	100% 120mg	201.5	200.5	201.6	0.215	0.823	3.81
		201.3	200.9	200.9			
		200.8	199.7	201.5			
6	Mean	201.2	200.36	201.33			
7	S.D.	0.36	0.611	0.37			
8	%R.S.D	0.17	0.304	0.81			
9	120% 240mg	239.6	239.7	239.7	0.105	0.439	4.67
		239.1	239.1	240.4			
		239.5	239.6	240.3			
10	Mean	239.4	239.46	240.13			
11	S.D.	0.26	0.3214	0.37			
12	%R.S.D	0.11	0.13	0.15			

Analysis of the marketed formulation

S.No	Amount per tablet(mg)	Amount Found (mg/mL)	(%) Found	Average	±SD	%RSD
1	200	211.30	105.0	104.6	0.53	0.506
2	200	210.34	104.0			
3	200	210.40	104.8			

Table No.4: Degradation Data for Guaifenesin

Summary of forced degradation studies on Guaifenesin

S.No	Stress Condition	Drug peak area at zero time sample (mcV.sec)	Drug peak area of stressed sample (mc.V.sec)	Retention time(s) of degradation products (min)	% Degradation
1	Acid 1N HCL (Refluxed for 2hrs)	1315623.57	1174292.25	2.292,2.625	10.75 %
2	Alkali 1N NaOH (Refluxed for 5hrs)	1315623.57	1049132.50	1.658,2.658	20.26%
3	Wet heat 80°C for 30min	1327470	1258439	2.633	5.21%
4	Oxidative 30%v/v H_2O_2(in direct room temperature	1327470	1380854	No Degradant	No Degradation
5	Dry heat 70°C(kept in oven for 48hrs)	1315623	1267201	No Degradant	No Degradation
6	Photolytic				
a)	drug with Methanol (exposed to direct sunlight for 48hrs)	1327470	1246428	2.667	6.11%
b)	Powder drug form (exposed to sunlight for 48 hrs)	1327470	1223280	2.860	7.84%

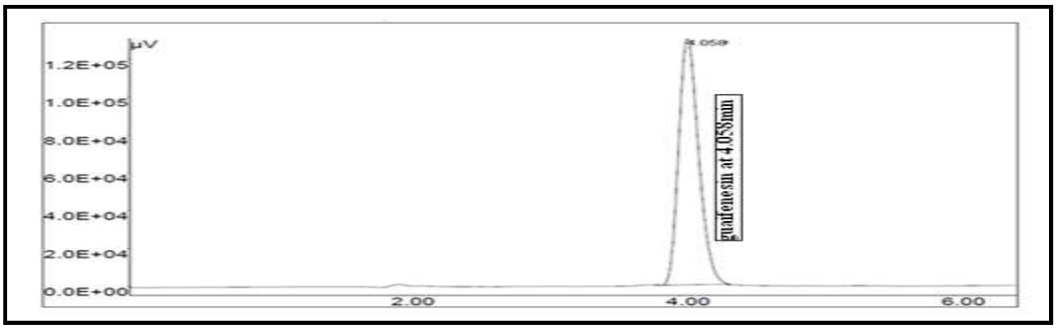


Figure No.1: System Suitability Chromatogram of Guaifenesin

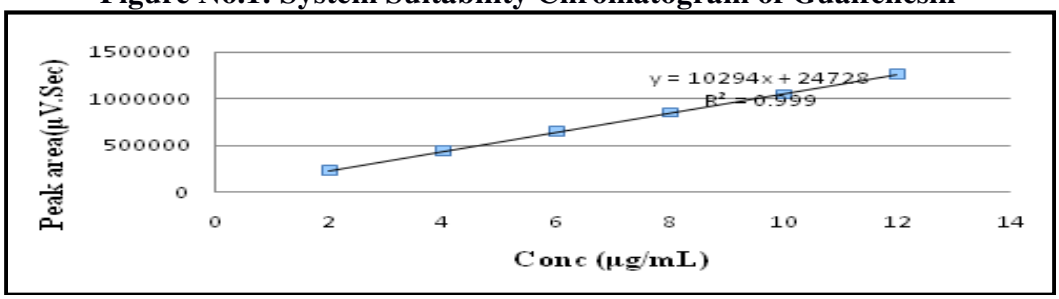


Figure No.2: Calibration Curve of Guaifenesin

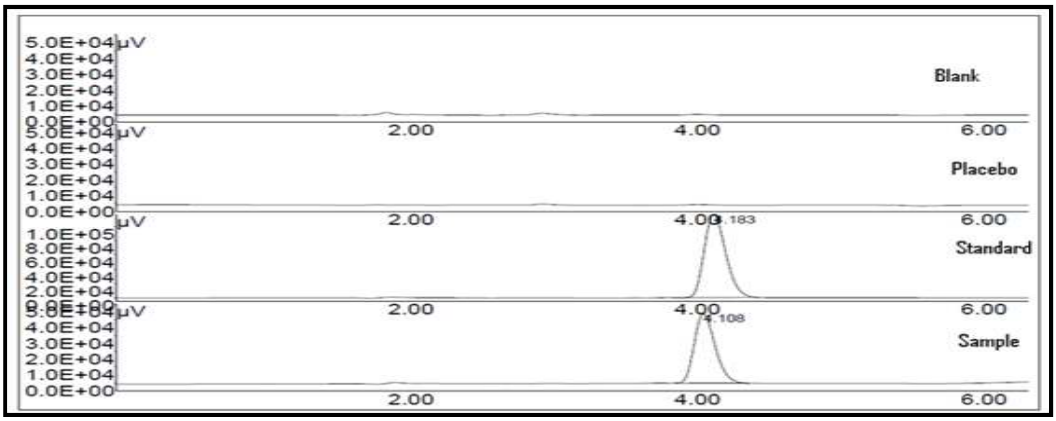
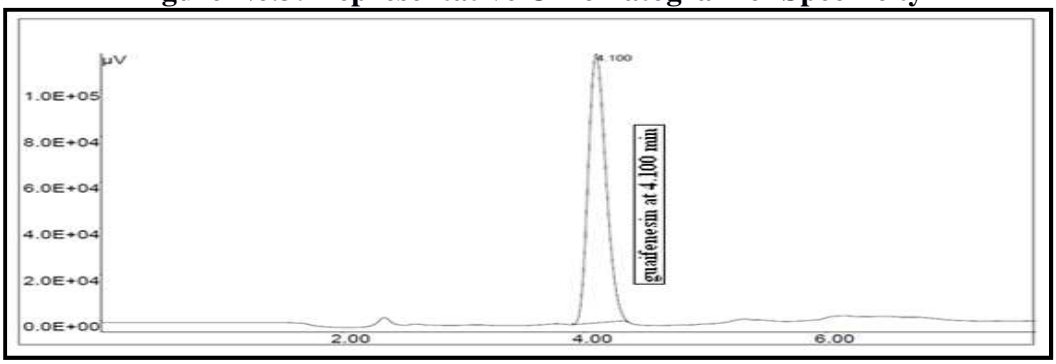


Figure No.3: Representative Chromatogram of Specificity



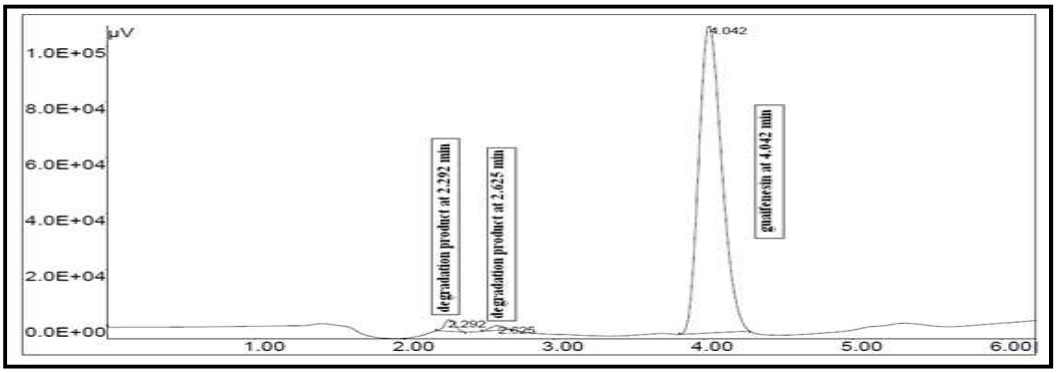


Figure No.4: Representative Chromatogram of Acid Degradation of Guaifenesin

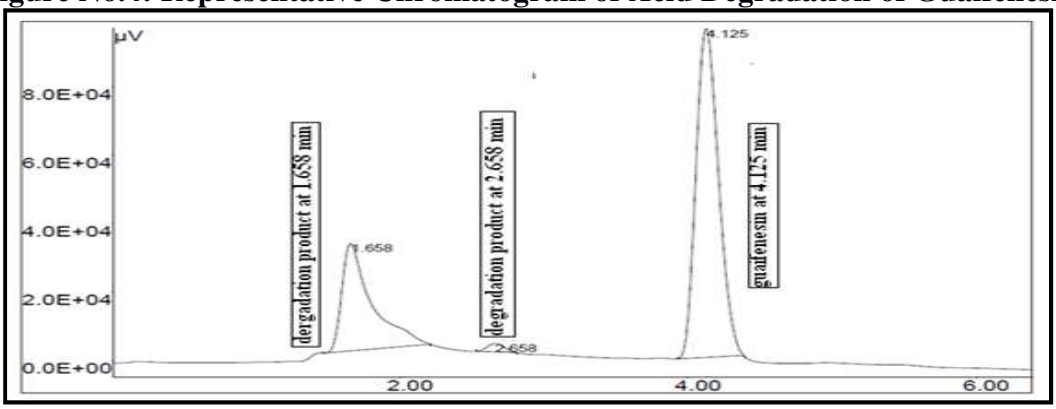


Figure No.5: Representative Chromatogram of Base Degradation of Gauifenesin

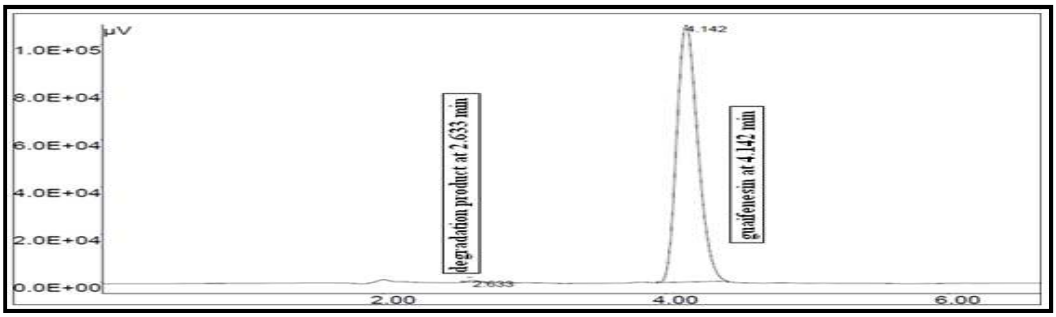


Figure No.6: Representative Chromatogram of Wet Heat Degradation of Guaifenesin

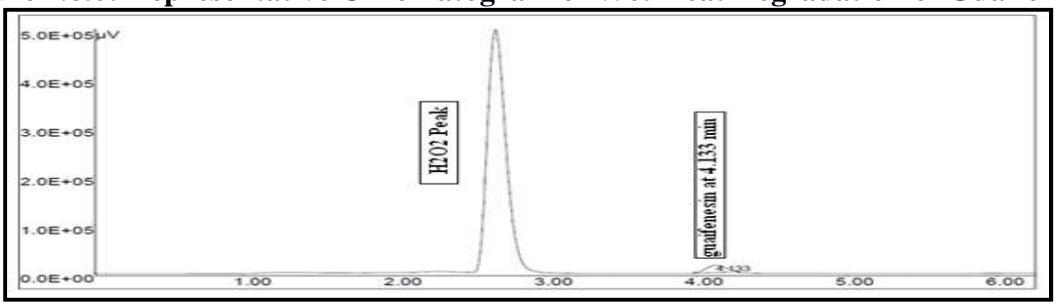


Figure No.7: Representative Chromatogram of Peroxide Degradation of Guaifenesin

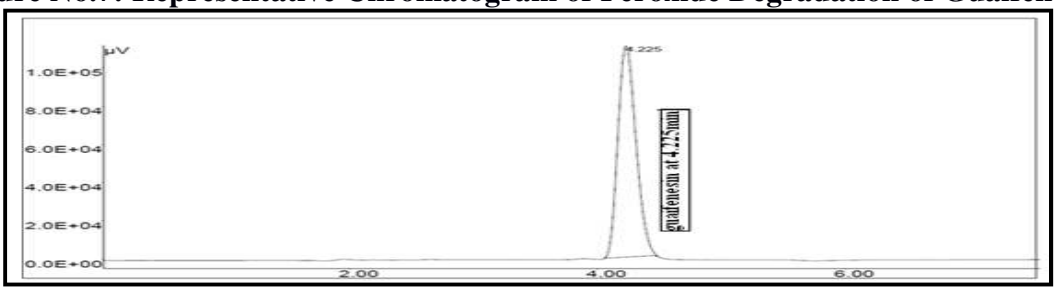
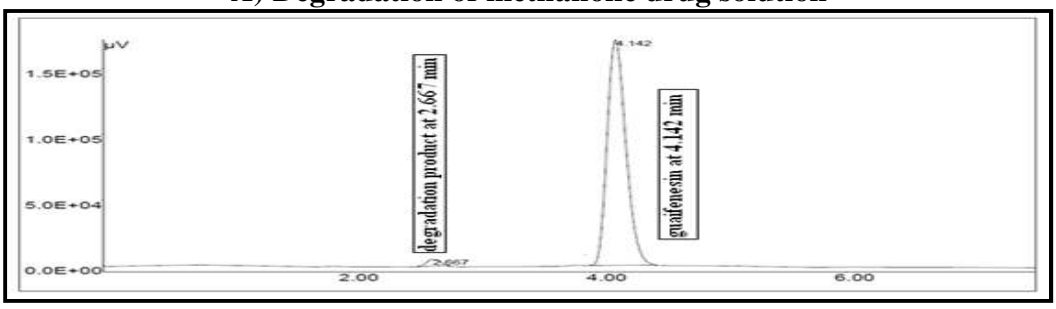


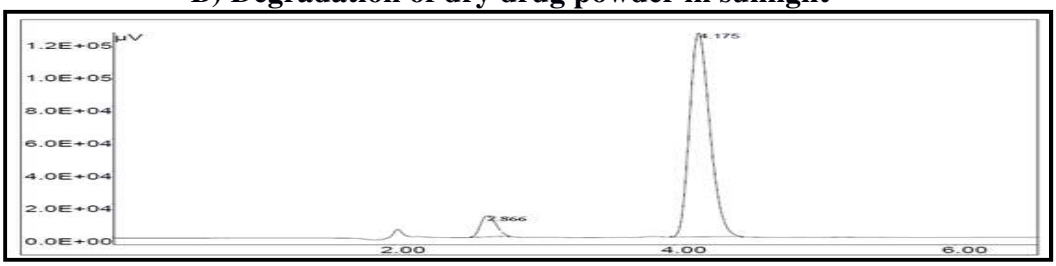
Figure No.8: Representative Chromatogram of Dry heat Degradation of Guaifenesin

Photolytic degradation

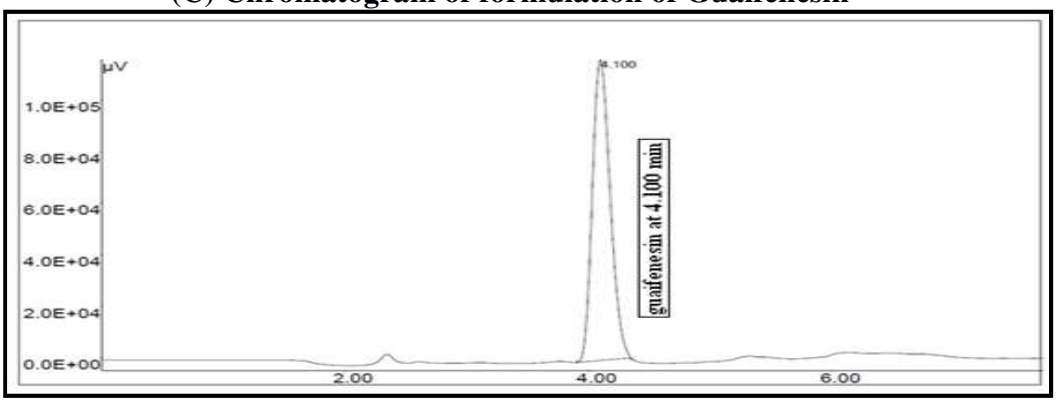
A) Degradation of methanolic drug solution



B) Degradation of dry drug powder in sunlight



(C) Chromatogram of formulation of Guaifenesin



CONCLUSION

A rapid simple, sensitive and accurate HPLC-UV method has been described for the determination of guaifenesin.

The method describe does not require expensive chemicals and solvents and does not involve complex instrumentation or complicated sample preparation.

From the forced degradation studies it can be concluded that guaifenesin undergoes acid, alkali, wet heat and photolytic degradation.

ACKNOWLEDGEMENT

The authors thankful to Department of Quality Assurance, MET Institute of Pharmacy, Nasik, Maharashtra, India for providing all laboratory facilities during research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Bakshi M and Singh S. Development of validated stability-indicating assay methods - critical review, *Journal of Pharmaceutical and Biomedical Analysis,* 28(6), 2002, 1011-1040.
2. Singh S and Bakshi M. Guidance on Conduct of Stress Testing to Determine Inherent Stability of Drugs, *Pharmaceutical Technology,* 2000.
3. ICH Harmonised Tripartite Guideline, "Stability testing of new drug substances and products Q1A (R2)," in *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use,* February 2003.
4. ICH Harmonized Triplicate Guidelines, "Validation of analytical procedures: text and methodology, Q2 (R1)," in *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use,* 2005.
5. Snyder L R, Kirkland J J and Glajch J L. Practical HPLC method development have comprehensively described an empirical approach to developing and optimizing HPLC methods, *A Wiley Inter Science Publication,* 2nd Edition, 1997, 800.
6. Klick S. *et al*. Toward a Generic Approach for Stress Testing of Drug Substances and Drug Products, Journal of *Pharmaceutical and Biomedical Analysis,* February 2005, 48-66.
7. Kovaleski J. *et al.* Impurities in generic pharmaceutical development, *Advanced Drug delivery review,* 59(1), 2007, 56-63.
8. Lee Y C. Method validation for HPLC analysis of related substances in pharmaceutical drug products, *In CHAN C C. et al.,* (eds.) *Analytical Method Validation and Instrument Performance Verification,*

Hoboken, New Jercy: John Wiley and Sons, Inc, 2004, 27-49.

9. Maged H M. Sharaf and Dwight D. Stiff. Determination of guaifenesin in human serum by capillary gas chromatography and electron capture detection *Journal of Pharmaceutical and Biomedical Analysi,* 35(4), 2004, 801-806.
10. Sethi P D. High Performance thin layer chromatography Quantitative analysis of Pharmaceutical formulation, *CBS, New Delhi,* 1st Edition, 2001, 161-196.
11. Kim H B. *Handbook of stability Testing in Pharmaceutical Development, Newyork USA,* 2009 Edition, 2008, 10-11, 389.
12. Reynolds D W *et al*. Available Guidance and Best Practices for Conducting Forced Degradation Studies, *Pharmaceutical Technology,* 2002, 48-56.
13. Ansari M, Kazemipour M. Shahriar. Simultaneous Quantitation of Theophylline and Guaifenesin in syrup by HPLC, Derivative and Derivative Ratio Spectrophotometry for Quality Control Purposes, *Iranian journal of Pharmacology and therapeutics,* 5(1), 2006, 67-72.
14. Rhodes C T and Carstensen J T. Intoductory overview, In: Drug Stability: Principle and Practices, *New York, Marcel Dekker,* 3rd Edition, 2000, 1-12.
15. Mehdi Ansari, Kazemipour M and Shahriar M. Simultaneous Quantation of Theophyline and Guaifenesin in Syrup by HPLC Derivative and Derivative ratio spectrophotometric for quality control purposes, *Iranian Journal of Pharmacology and Therapeutics,* 5(1), 2006, 67-72.
16. Melveger A J and Huynh-Ba K. Critical Regulatory Requirements for a Stability Program. In: HUYNH-BA, K. (ed.) *Handbook of Stability Testing in Pharmaceutical Development, New York, USA: Springer,* 1st Edition, 2009, 9-15, 389.
17. Lindsay S. *High Performance Liquid Chromatography Analytical Chemistry by open Learning*, *England: John Wiley and Sons,* 2nd Edition, 1992, 360.
18. Reddy Sunil, Sudhakar Babu K, Navneet Kumar, Sasi Sekhar Y V V. Development and validation of stability indicating the RP-HPLC method for the estimation of related compounds of guaifenesin in pharmaceutical dosage forms, *Pharma method,* 2(4), 2011, 229-234.
19. Gutierrez K. Pharmacotherapeutics: Clinical Reasoning in Primary Care, *W. B. Saunders Co,* 2nd Edition, 2007, 1280.
20. Pushpalatha E, Tejaswini P, Najboon bi M, Vineesha S, Madhanna M D, Anil Kumar T, Raju M. Development of UV Spectroscopic Method for the Determination of Guaifenesin in Bulk and Formulation Pushpalatha E *et al*, *IJPRA,* 5(2), 2015, 90-95.
21. Eglal A. Abdelaleem, Nada S. Abdelaleem. Stability indicating RP-HPLC method for simultaneous determination of guaifenesin and pseudoephedrine hydrochloride in the presence of syrup excepients, *Arabian Journal of Chemistry,* 10(2), 2017, S2896-S2901.

Please cite this article in press as: Sandhya Rajkumar Gawai. Development and validation of stability indicating RP-HPLC method for guaifenesin, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 6(2), 2018, 81-91.