ARTICLE

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Shortcoming of UV spectrophotometric and RP-HPLC methods to perform as stability indicating assay method for the quantification of eugenol

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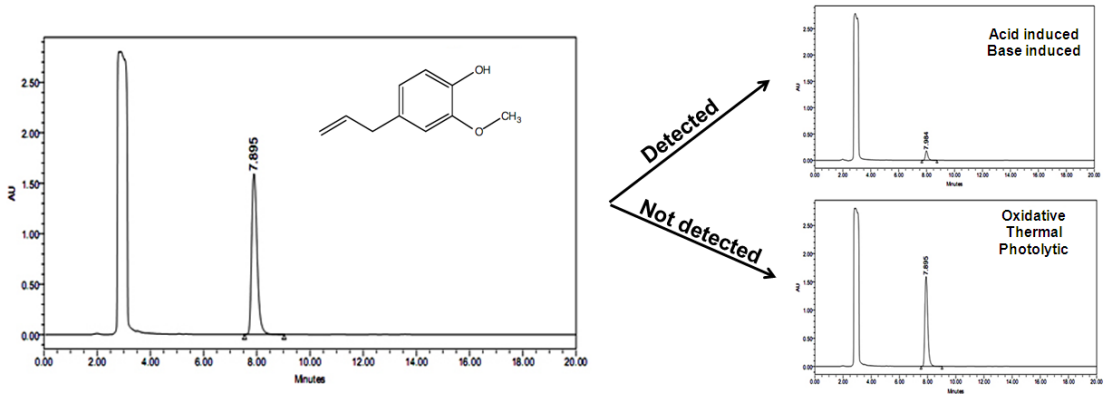
Graphical Abstract



Abstract: Availability of a stability indicating assay method for carrying out accelerated stability studies of drug products is essential. In a search of a suitable stability indicating assay method for the quantification of eugenol UV spectrophotometric and high pressure liquid chromatography (HPLC) methods were tried. Forced degradation studies included use of eugenol samples from acid induced, base induced, oxidative degradation, thermal and photolytic degradations. UV spectrophotometry using methanol as a solvent and RP-HPLC method using photo diode array detector was studied. The UV spectrophotometric and high pressure liquid chromatography (HPLC) methods failed to demonstrate itself as a stability indicating method, as shown by the results of forced degradation studies. From the study results we concluded that UV spectrophotometric and HPLC methods could not be used for the stability studies of eugenol.

Keywords: *Forced degradation studies, acid induced degradation, base induced degradation, oxidative degradation, thermal degradation, photolytic degradation.*

INTRODUCTION

Development of a stability indicating assay method for eugenol is warranted due to the development of novel drug delivery systems of eugenol for enhancement of its therapeutic activity and for targeted drug delivery.[1-6] Forced degradation studies are usually carried out by acid induced, base induced, oxidative degradation, thermal and photolytic degradations.

Stress testing could be carried out to assess the stability characteristics of drug substances and drug products. The testing should include the effect of temperature, humidity, oxidation, photolysis, and acid and base hydrolytic conditions.[7] Stability testing is a mandatory requirement for the approval of drug products. Availability of a stability indicating assay method is a prerequisite for carrying out accelerated stability studies to ascertain chemical stability of the active ingredient against accelerated stress conditions. Assessment of shelf life is only possible if we have a stability indicating assay method for the drug in question. The method should be specific enough to identify the degradation products from the drug. UV and HPLC methods are commonly used assay methods for dosage forms. Thus in a search of a suitable stability indicating assay method for the quantification of eugenol, UV spectrophotometric and high pressure liquid chromatography (HPLC) methods were tested.

MATERIALS AND METHODS

Materials

Eugenol was purchased from Central Drug House, Delhi, India, and methanol was purchased from S D Fine-Chem ltd, Mumbai, India. Reagent grade I water (Millipore, Molsheim, France) was used for the study. HPLC grade water and acetonitrile were purchased from Merck, Mumbai, India.

Preparation of forced degradation samples

The samples for the forced degradation studies were prepared according to reported procedures.[8,9] UV spectrophotometry in methanol and HPLC were tested sequentially for the development of a stability indicating assay method.

Acid induced degradation: Eugenol solution (1 mg mL^{-1}) in methanol was added to 10 mL each of methanol and 0.1 M HCl and the mixture was refluxed at 60°C for six hours. The solution was then cooled to room temperature and then neutralized to pH 7 by the addition of 0.1 M NaOH. The sample was then diluted to 100 mL with methanol to get a eugenol concentration of 100 µg mL^{-1}.

Base induced degradation: A eugenol solution (1 mg mL^{-1}) in methanol was added to 10 mL each of methanol and 0.1 M NaOH and the mixture was refluxed at 60°C for six hours. The solution was then cooled to room temperature and then neutralized to pH 7 by addition of 0.1 M HCl. The sample was then diluted to 100 mL with methanol to get a eugenol concentration of 100 µg mL^{-1}.

Oxidative degradation: **A** eugenol solution (1 mg mL^{-1}) in methanol was added to 10 mL of 30% H_2O_2 solution. Then the mixture was refluxed at 60°C for six hours. The solution was then cooled to room temperature and then diluted to 100 mL with methanol to get a concentration of 100 µg mL^{-1}.

Thermal degradation: 50 mg of eugenol was stored at 80°C for 48 hours. The sample was then dissolved in methanol, and the volume was adjusted to 50 mL to give a solution of a final eugenol concentration of 1000 µg mL^{-1}.

Photolytic degradation: 50 mg of eugenol was exposed to short and long wavelength UV light (254 and 366 nm, respectively) for 48 hours. Then it was dissolved in methanol and the volume was adjusted to 50 mL to get a solution of final concentration equivalent to 1000 µg mL^{-1} of eugenol.

UV spectrophotometric method

100mL of a stock solution of Eugenol (1 mgmL-1) in methanol was prepared in a 100 mL volumetric flask. The dilutions of this stock solution were made by diluting the required aliquot with methanol to obtain a standard solution in the range of 5- 50 µg mL^{-1}. The absorbance of the resultant solutions was determined at the λ_{max} of 282 nm using a Shimadzu UV – 1601 (Shimadzu Corp, Kyoto, Japan) spectrophotometer.[10] For the forced degradation studies, the samples prepared by the forced degradation of eugenol were diluted to theoretical concentration of 25 µg mL^{-1} using methanol. The forced degradation samples prepared without eugenol and the same dilutions were used as blanks for their corresponding samples during UV spectrophotometry.

High pressure liquid chromatography (HPLC)

The HPLC method for the determination of eugenol was carried out on a Waters Alliance e2695 separating module (Waters Co., MA, USA) using a photo diode array detector (Waters 2998) with autosampler and column oven.[11]

Mobile phase

Acetonitrile and water in the ratio 1:1 (v/v) was chosen as the mobile phase.[11]

Chromatographic system

Compounds were separated on a C18 reverse phase column (250 × 4.6 mm, particle size 5 µm; Merck, Darmstadt, Germany) maintained at room temperature. The flow rate was maintained at 1 mL min^{-1}. The run time was set to 20 min and the retention time observed was 7.968 ± 0.042 min. Detection was carried out at 280 nm. Twenty microlitres of the samples, prepared by the forced degradation of eugenol, were injected into the system after filtering through a 0.45-µm nylon filter.

RESULTS AND DISCUSSION

UV spectrophotometric method

The aim of the present work was to evaluate whether UV absorbance at 282 nm can be used for the quantification of eugenol during accelerated stability studies. Hence we assumed that the absorbance at 282 nm is exclusively due to eugenol. If the UV absorbance is due to degradation products, then the method is not suitable for accelerated stability studies.

The present study was an extension of our previous studies.[10] In those studies we have presented the full UV spectrum of eugenol in methanol and calibration curve of eugenol in methanol.

Table 1 displays the data of forced degradation studies of eugenol by UV spectrophotometry. The data showed the inability of the method in all the samples, and except for acid degradation the method indicated no degradation. In the case of acid induced degradation, the method could detect only a degradation of 7.09%.

Identification and characterization of degradation products is applicable only if the proposed method is able to detect degradation. In the present study, UV absorbance at 282 nm was unable to detect drug degradation and therefore the study of degradation products was not warranted.

Table 1 Data of forced degradation studies of eugenol by UV spectrophotometry (n=3)

Forced degradation	**Remaining eugenol concentration (µg mL^{-1}) (Mean ± SD)**	**Recovery (%)**	**Degradation (%)**
Acid induced	23.23±1.47	92.91	7.09
Base induced	27.28±1.32	109.12	Nil
Oxidative	25.78±0.94	103.12	Nil
Thermal	26.27±1.49	105.08	Nil
Photolytic	26.16±1.22	104.65	Nil

High pressure liquid chromatography (HPLC)

The present HPLC study was an extension of previous reported studies in which we have presented the full information regarding calibration of the HPLC method([11]).

The results of the forced degradation studies are displayed in Table 2 and the chromatograms are shown in Figure 1.

Table 2 Data of forced degradation studies by HPLC method (n=3)

Forced degradation	**Remaining eugenol concentration (µg mL^{-1}) (Mean ± SD)**	**Recovery (%)**	**Degradation (%)**
Acid induced	92.77±1.16	92.77	7.23
Base induced	84.87±2.33	84.87	15.13
Oxidative	102.09±2.22	102.09	Nil
Thermal	102.035±31.56	102.04	Nil
Photolytic	102.415±24.19	102.42	Nil

The results demonstrated the inability of the method, as in three samples, viz. oxidative, thermal and photolytic, the method indicated no degradation.

In the case of acid induced, base induced and oxidative treatments, the theoretical final concentration of eugenol in the samples was 100 µg mL^{-1}, whereas for thermal and photolytic degradations it was 1000 µg mL^{-1}.

Visually, acid induced and base induced chromatograms appear similar as the concentration values were close (92.77±1.16 and 84.87±2.33 µg mL^{-1} respectively**)** to the value of 102.09±2.22 µg mL^{-1} obtained for oxidative treatment.

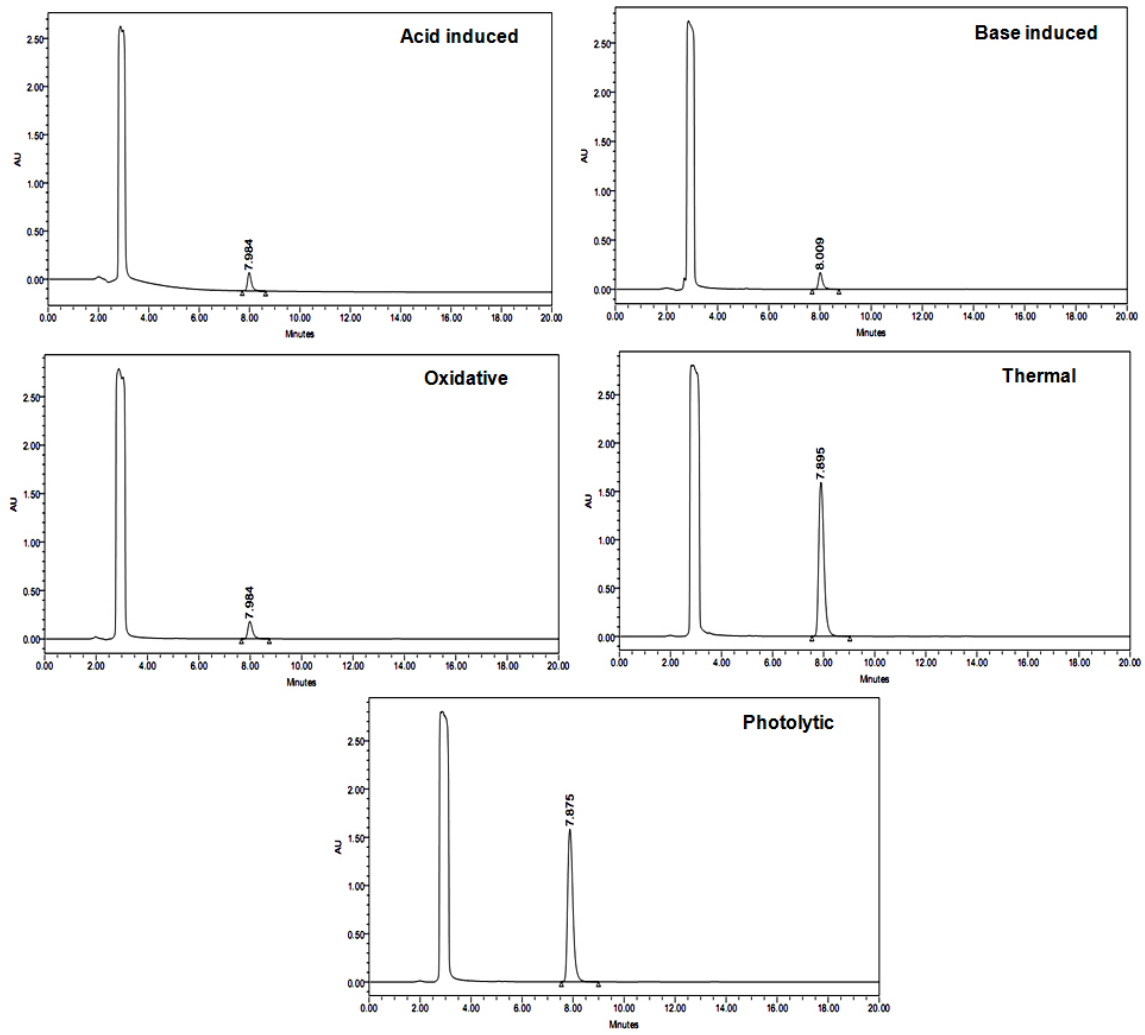


Figure 1. HPLC chromatograms of the forced degradation samples

In the case of acid and base induced degradation samples the method could detect a limited degradation within 20% only. In addition to the low degradation, as indicated by the high recovery of eugenol, the HPLC chromatograms did not show any additional peaks of degradation. This further suggested the inability of the HPLC method as a stability indicating assay method. For eugenol, in our previous study with HPTLC method for the same samples we have detected degradation, except in the case of thermal induced forced degradation (eugenol is highly stable against thermal degradation).[9]

CONCLUSIONS

The UV spectrophotometric and high pressure liquid chromatography (HPLC) methods failed to demonstrate itself as a stability indicating method as shown by the results of forced degradation studies. Thus we concluded that UV spectrophotometric and HPLC methods could not be used for the accelerated stability studies of eugenol loaded nanocarriers.

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REFERENCES

1. Kriegel, C.; Kit, K.M.; McClements, D.J.; Weiss, J. *J. Appl. Polym. Sci.*, **2010,** *118*, 2859–2868.
2. Pokharkar. V.B.; Shekhawat, P.B.; Dhapte, V.V.; Mandpe, L.P. *Int. J. Pharm. Pharm. Sci.,* **2011**, *3*, 138-143.
3. Gomes, C.; Moreira, R.G.; Castell-Perez, E. *J. Food Sci.* **2011**, *76*, N16-N24.

4. Chen, F.; Shi, Z.; Neoh, K.G.; Kang, E.T. *Biotechnol. Bioeng*., **2009**, *104*, 30-39.

5. Jadhav, B.K.; Khandelwal, K.R.; Ketkar, A.R.; Pisal, S.S. *Drug Dev. Ind. Pharm*., **2004,** *30*, 195-203.

6. Pramod, K.; Ansari, S.H.; Ali, J. *Nat. Prod. Commun.* **2010,** *5*, 1999-2006.

7. ICH Harmonised tripartite guideline, 2003. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf [Accessed 10 September, 2011].

8. Hamrapurkar, P.; Patil, P.; Phale, M.; Gandhi. M, Pawar S. *Pharm. Methods,* **2011**, *2*, 15-20.

9. Pramod, K.; Ilyas, U.K.; Singh, M.; Kamal, Y.T.; Ahmad, S.; Ansari S.H.; Ali, J. *ACTA Chromatographica*. **2014**, doi: 10.1556/AChrom.27.2015.3.12.

10. Pramod, K.; Ansari, S.H.; Ali, J. *Asian J. Pharm. Ana.*, **2013**, *3*, 58-61.

11. Pramod, K.; Ilyas, U.K.; Kamal, Y.T.; Ahmad, S.; Ansari, S.H.; Ali, J. *Journal of Analytical Science and Technology,* **2013**, *4*, doi:10.1186/2093-3371-4-16.