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Identification and Characterization of Forced Degraded Impurities of Fenoxazoline Using Chromatographic and Spectroscopic Techniques.

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ABSTRACT

A selective specific and sensitive High Pressure Liquid Chromatography method was developed for determination of Fenoxazoline degradation impurities. The chromatographic separation was achieved on Shimadzu LC-2010 with PDA system and Kromasil C18 column using gradient elution of mobile phase.The present study is aim to degrade the product using different degradation conditions. The degraded products were subjected to LC-MS to find out the impurities. Based on the mass of impurities the structures were assigned. The proposed method was successfully employed for estimation of Fenoxazoline impurities in pharmaceutical preparations.

Keywords: HPLC – Fenoxazoline, Degradant, Impurities and assign.

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INTRODUCTION

Fenoxazoline is used as nasal decongestant [1]. It is chemically designated as 2-[(2-propan-2-ylphenoxy)methyl]-4,5-dihydro-1H-imidazole Hydrochloride. Its molecular weight is 254.76 and its empirical formula is $C_{13}H_{18}N_2O$•HCl. It is soluble in N, N methylformamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile and insoluble in ether. Fenoxazoline hydrochloride is an odorless white crystalline powder. The drug comes under the therapeutic category of Sympathomimetic.

The drug brand named Aturgyl contains generic salt Fenoxazoline Hydrochloride and is manufactured by GlaxoSmithKline. Aturgyl is mainly associated with symptoms and indications – The International Classification of Diseases (ICD) – Ro1AA12 – Fenoxazoline.

Fenoxazoline Forced degradation chromatograms are shown in Fig. 1. Fenoxazoline and its impurities chemical structure are shown in Fig.2 [I-IV]. Fenoxazoline FNZ-I undergoes hydrolysis to FNZ-II & FNZ-III and undergoes oxidation to N-oxide FNZ-IV as shown in Fig.2.

The ^{1}H-NMR and Mass spectra of final product and impurities are shown in Fig.3 and the probable degradation pathway shown in Fig.4.

So far to our present knowledge, proper HPLC method was not available in literature for identification of degradation products and unknown impurities [2] in Fenoxazoline. An assay method for determination of Fenoxazoline in pharmaceutical formulations available [3]. Attempts were made to develop a LC method for the identification of degradants in Fenoxazoline drug substance.

In order to improve the sensitivity and selectivity of the chromatographic determination of Fenoxazoline impurities, a simple reversed-phase HPLC method with UV detection at 230nm, have been developed, where all three impurities have been separated in a single analytical column with a run time of 50 minutes. In our study, Shimadzu HPLC has been successfully used for the determination of (FNZ-II), (FNZ-III) and (FNZ-IV). A reduction in separation time has been achieved, without compromising separation quality compared to other traditional Liquid Chromatography (LC) methods.

MATERIALS AND METHODS

Fenoxazoline from SDS Labs Private Limited, Navi Mumbai, India. Acetonitrile (HPLC-grade from Merck), and Ortho phosphoric acid, Heptane sulfonic acid sodium salt, Hydrochloric Acid, Hydrogen Peroxide were from Merck (Darmstadt, Germany), Sodium hydroxide AR grade from Rankem. Water was purified by a water purifier (SG water purifier) and passed through a 0.45 µm membrane filter (Durapore) before use. Standard and degradation samples were prepared in mobile phase-I in the ratio of 550:450 v/v, and then adjusted the pH 4.0 with dilute orthophosphoric acid (10%v/v).

Equipment

HPLC analysis was performed with a Shimadzu LC-2010 with PDA system consists of a Quaternary solvent manager, a sample manager, column-heating compartment, and Photodiode array detector. This system was controlled by Shimadzu LC solutions software. Kromasil C_{18}, 250 x 4.6 mm, 5 µm employed as stationary phase for chromatographic separation. The wavelength was selected at 230 nm. The gradient method was employed as the ratio of Mobile phase-I 0 to 10 minutes 75.0 %, 10 to 20 minutes 25.0 % and at this stage a linear gradient was selected of Mobile phase-I 25.0 % up to 35 minutes. After 35 minutes, the Mobile phase-I changed to 75 % within 10 minutes and further 5.0 minutes maintained to base line stabilization. The flow rate was maintained at 1.0 mL per minute and the injection volume was 20.0 µL. All samples were centrifuged by Thermo Scientific multifuged machine. The thermal and photo degradation study was conducted by using hot air oven and photo stability chamber.

Standard and Sample Preparation

Weighed accurately 25.62 mg of sample and transferred into 50 mL volumetric flask. To this 15 mL of diluent is added and sonicated to dissolve the contents, and further diluted up to the volume with same diluent and mixed well.

Weighed accurately 25.16 mg of Standard and transferred into 50 mL volumetric flask. To this 15 mL of diluent is added and sonicated to dissolve the contents, and further diluted up to the volume with same diluent and mixed well.

Forced Degradation of Fenoxazoline HCl by 0.5 N Hydrochloric acid

Weighed accurately 258.38 mg of Fenoxazoline Sample and transferred into 50 mL volumetric flask. To this 15 mL of 0.5 N hydrochloric acid is added and sonicated the contents and further diluted up to the volume with same Hydrochloric acid and mixed well. Kept this solution for 48 hours for degradation.

After 48 hours transferred 1 mL of above solution into a 10 mL volumetric flask and neutralized with same amount of 0.5 N sodium hydroxide solution, then diluted up to the mark with diluent.

Forced Degradation of Fenoxazoline HCl by 0.1 N Sodium hydroxide

Weighed accurately 51.53 mg of Fenoxazoline Sample and transferred into 10.0 mL volumetric flask. To this 5 mL of 0.1N Sodium Hydroxide is added, sonicated the contents and further diluted up to the volume with same Sodium Hydroxide and mixed well. Kept this solution for degradation.

After 48 hours transferred 1 mL degradation product into 10 mL volumetric flask and neutralized with same amount of 0.1N Hydrochloric acid, then diluted up to the volume with diluent.

Forced Degradation of Fenoxazoline by 5.0% Hydrogen peroxide

Weighed accurately 51.82 mg of Fenoxazoline sample and transferred into 10 mL volumetric flask. To this 5 mL of 5.0% hydrogen peroxide is added, sonicated the contents and further diluted up to the volume with same hydrogen peroxide and mixed well. This solution was kept for degradation.

After 48 hours transferred 1 mL of above solution into 10 mL volumetric flask and diluted up to the mark with diluent.

Forced Degradation by light

Weighed accurately about 250.0 mg of Fenoxazoline sample and kept for degradation in photostability chamber.

Transferred 25.21 mg of above degradation compound after 48 hours into a 50 mL volumetric flask and 15 mL of diluent is added, then sonicated to dissolve, finally diluted up to the mark with same diluent and mixed.

Forced Degradation by Thermal treatment

Weighed accurately about 250.0 mg of Fenoxazoline sample and transferred to petridish and kept for degradation in a calibrated oven at 100 °C for 48 hours.

Weighed accurately about 25.44 mg of above degradation product and transferred into 50.0 mL volumetric flask, added 15 mL of diluent and sonicated to dissolve. Then diluted up to the mark with diluent and mixed.

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RESULTS AND DISCUSSION

A reversed-phase chromatographic technique was used to determine Fenoxazoline and its impurities at 230nm. The presence of non-aqueous solvents in the mobile phase, such as Acetonitrile, was studied. Acetonitrile was chosen as organic modifier. Satisfactory separation was achieved when the acetonitrile concentration was 45% in mobile phase I and with 80% acetonitrile in mobile phase-II.

HPLC system has been proved to be a promising tool for separation of Fenoxazoline and its impurities. Fenoxazoline and its degradants were well separated with good peak shape and resolution. No interfering peaks were observed in blank, indicating that signal suppression or enhancement by the product matrices was negligible. As the impurities are well separated, this method can be used for routine analysis for impurities identification in Fenoxazoline drug substance. The compound was degraded in basic and oxidative conditions and it was stable under thermal, Sunlight and acidic conditions. The structure interpretation of Fenoxazoline and its impurities were as mentioned below.

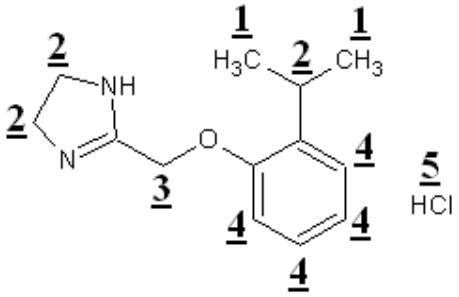
Interpretation of Fenoxazoline HCl (FNZ-I)

Molecular formula: $C_{13}H_{18}N_2O.HCl$

Formula weight: 254.75

^{1}H-NMR

The ^{1}H-NMR of Fenoxazoline HCl recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Proton assignment
1.	1.251 – 1.268 (Doublet)	-CH_3 protons (6)
2.	3.153 – 3.255 (Multiplet)	-CH proton (1) and –CH_2 protons (4) of imidazole ring
3.	4.668 (Singlet)	-CH_2 protons (2) in the vicinity of oxygen atom.
4.	6.707 – 7.210 (Multiplet)	Aromatic protons (4)
5.	9.933 (Broad singlet)	HCl proton

Mass

The structure of Fenoxazoline HCl is shown in figure. The mass spectrum was obtained using mass spectrophotometer. Molecular ion peak of Fenoxazoline HCl was observed m/z at 219.1 (M+H ion of free base).

Interpretation of Impurity-1 (4,5-dihydro-imidazole methanol) (FNZ-II):

Molecular formula: $C_4H_8N_2O$,

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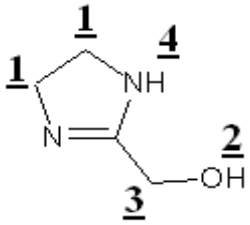
Formula weight: 100.12

^{1}H-NMR

The Impurity-1 was isolated by using preparative HPLC. The ^{1}H-NMR of Impurity-1 (4,5-dihydro-imidazole methanol) recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Proton assignment
1.	3.103 – 3.226 (Multiplet)	-CH_2 protons (4) of imidazole ring.
2.	4.159 (Broad singlet)	-OH proton (1)
3.	4.538 (Singlet)	-CH_2 protons (2) in the vicinity of oxygen atom.
4.	7.277 (Singlet)	-NH proton (1)

Mass

The structure of Impurity-1 (4,5-dihydro-imidazole methanol) was shown in figure. The mass spectrum of Base degradation of LC-MS was obtained using mass spectrophotometer. Molecular ion peak of Impurity-1 was observed m/z at 100.8 (M+H ion).

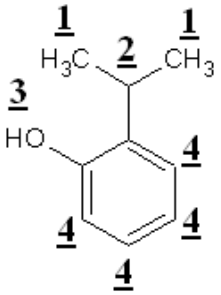
Interpretation of Impurity-2 (2-isopropyl phenol) (FNZ-III)

Molecular formula: $C_9H_{12}O$

Formula weight: 136.19

^{1}H-NMR

The Impurity-2 was isolated by using preparative HPLC. The ^{1}H-NMR of Impurity-2 (2-isopropyl phenol) recorded on 400 MHz NMR in $CDCl_3$. Observed δ values and its interpretation are as follows.



S. No.	Chemical shift δ (In ppm)	Proton assignment
1.	1.258 – 1.269 (Doublet)	-CH_3 protons (6)
2.	3.158 – 3.261 (Multiplet)	-CH proton (1)
3.	4.673 (Singlet)	-OH protons (1)
4.	6.727 – 7.214 (Multiplet)	Aromatic protons (4)

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Mass

The structure of Impurity-2 (2-isopropyl phenol) was shown in figure. The mass spectrum of Base degradation of LC-MS was obtained using mass spectrophotometer. Molecular ion peak of Impurity-2 was observed m/z at 136.9 (M+H ion).

Interpretation of Impurity-3 (Fenoxazoline-N-oxide) (FNZ-IV):

Molecular formula: $C_{13}H_{18}N_2O_2$

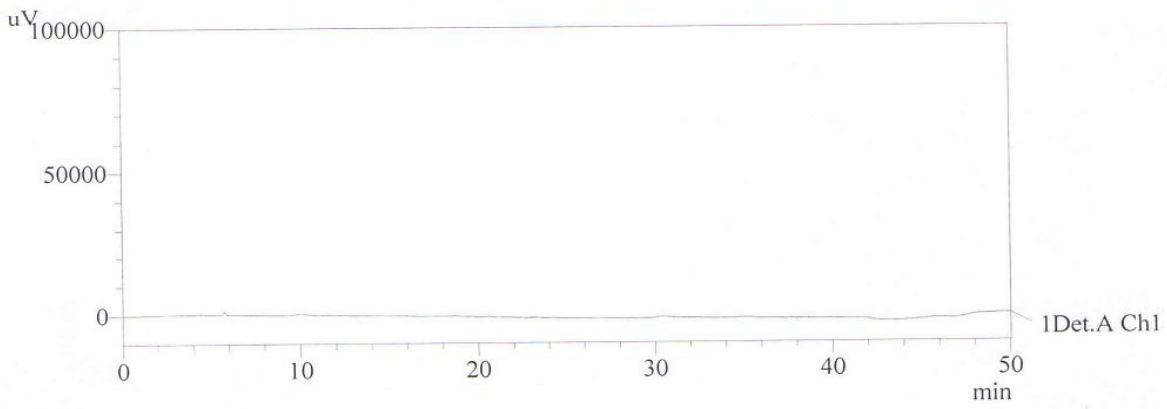
Formula weight: 234.30

Mass

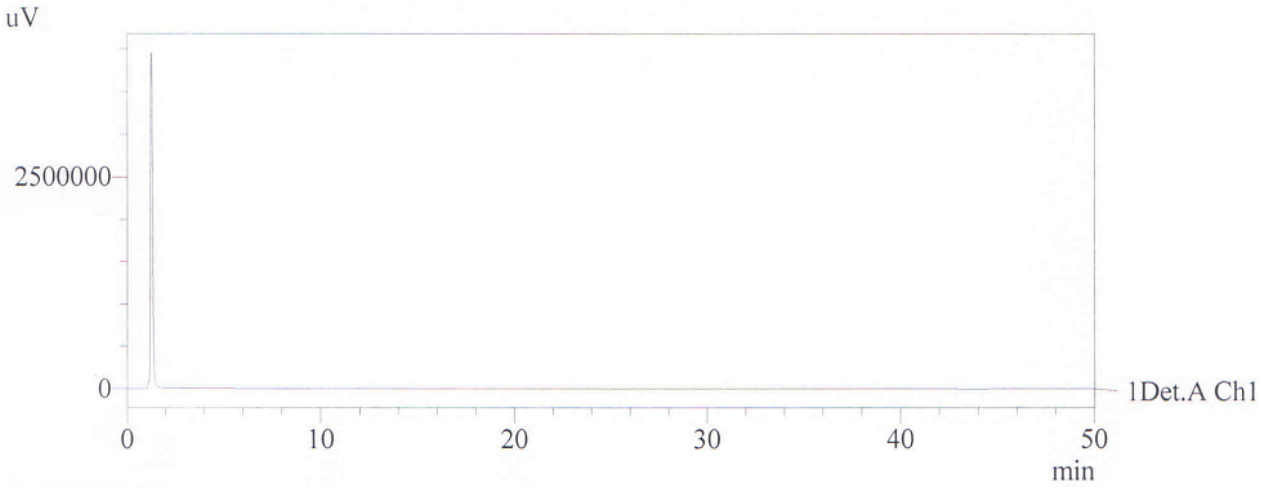
The mass spectrum of Oxidative degradation of LC-MS was obtained using mass spectrophotometer. Molecular ion peak of Impurity-3 was observed m/z at 235.1 (M+H ion).

Forced degradation chromatograms: (Fig.1)

Blank chromatogram



Blank H_2O_2 chromatogram



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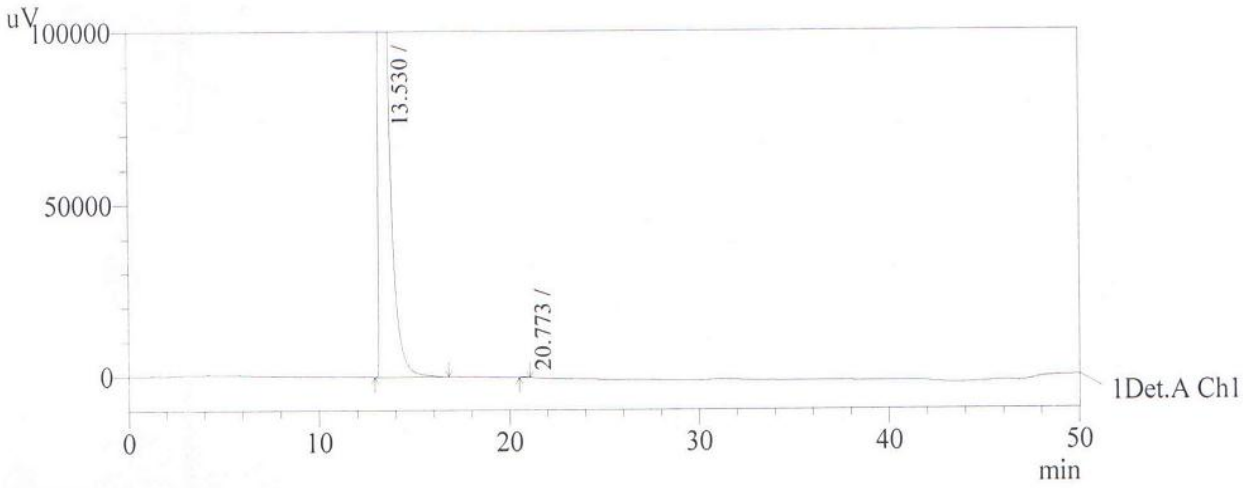
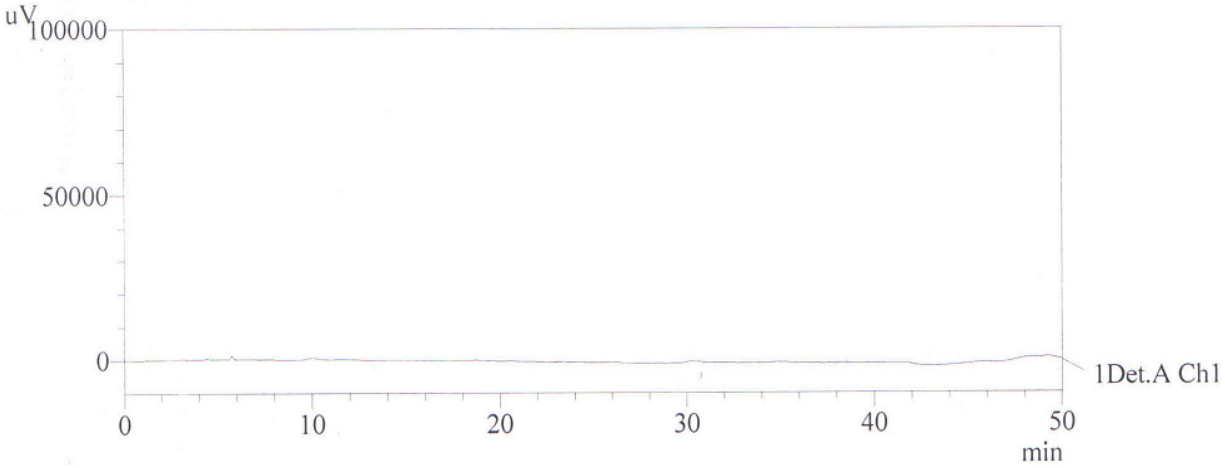
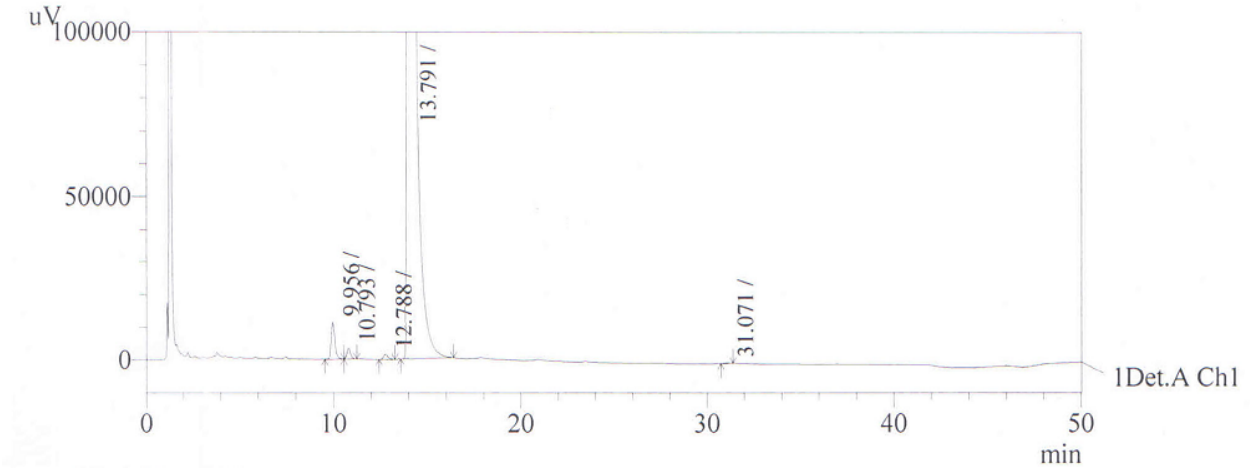
Fenoxazoline chromatogram



Blank Acid-base chromatogram



Fenoxazoline H_2O_2 (Oxidative) degradation chromatogram



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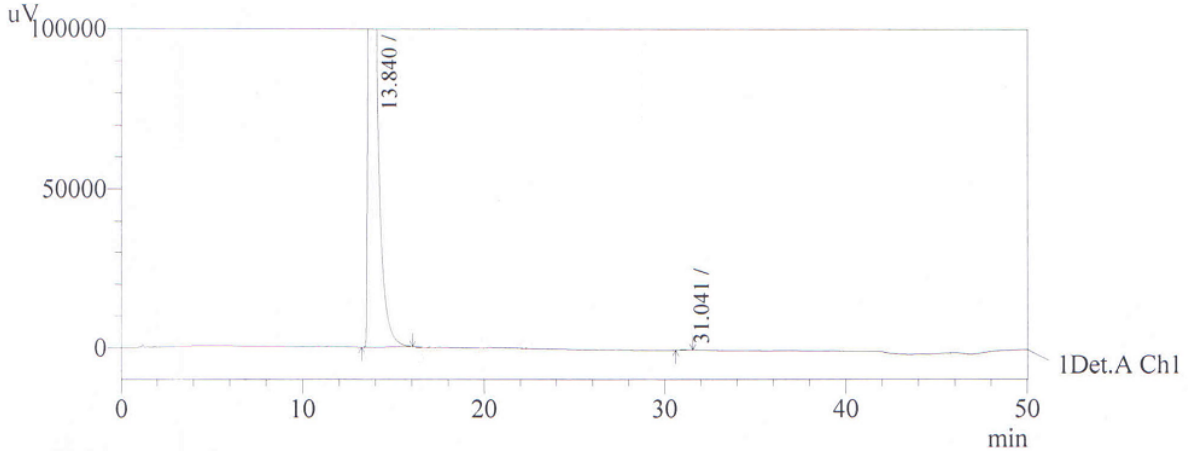
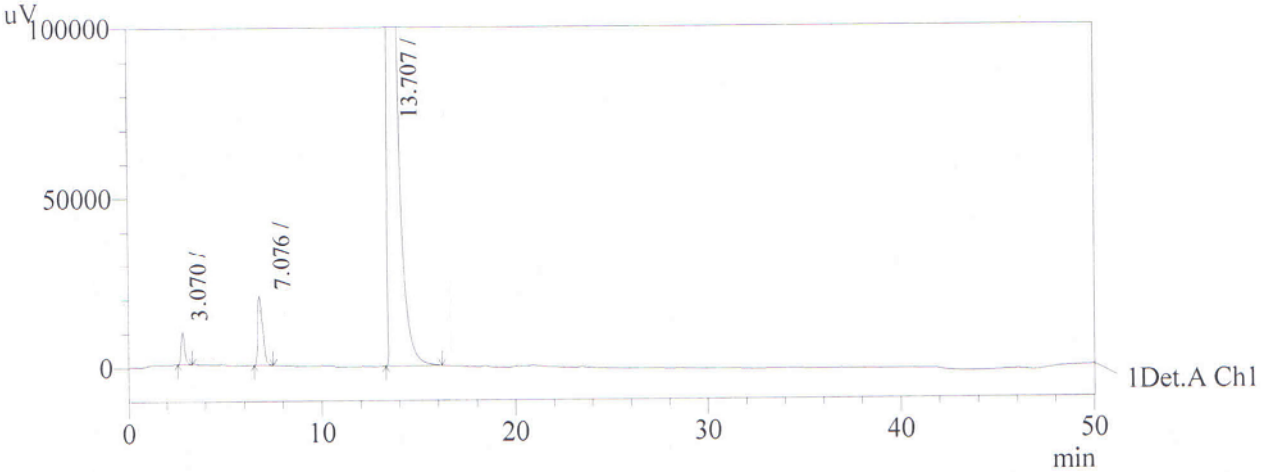
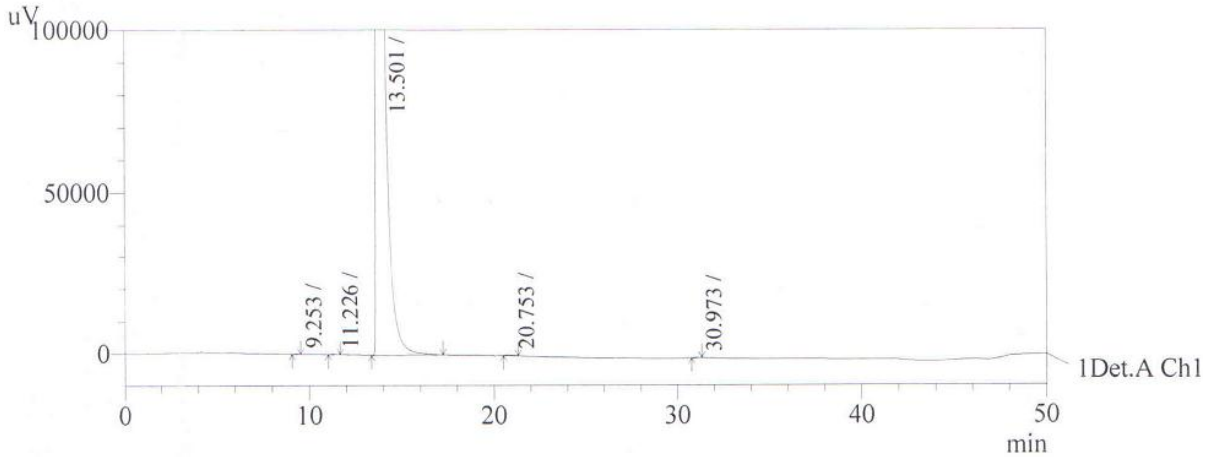
Fenoxazoline Acid degradation chromatogram



Fenoxazoline Base degradation chromatogram



Fenoxazoline Thermal degradation chromatogram



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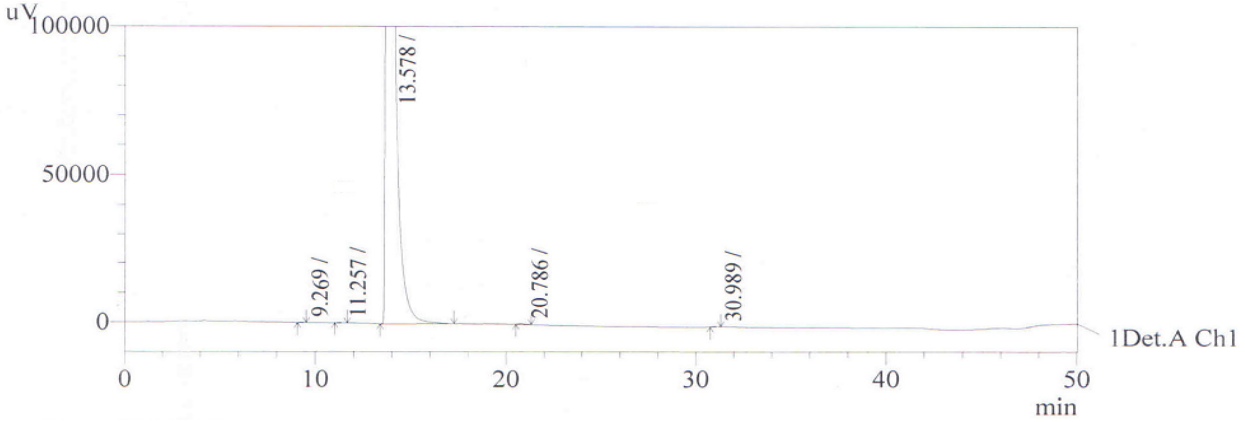
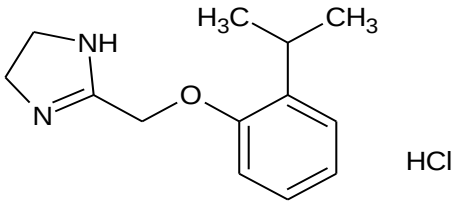
Fenoxazoline Photo degradation chromatogram

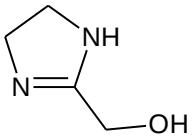


Fenoxazoline and its impurities structures (Fig.2)

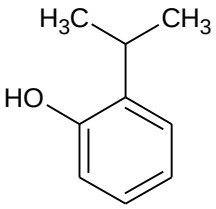
Fenoxazoline Hydrochloride (FNZ-I):



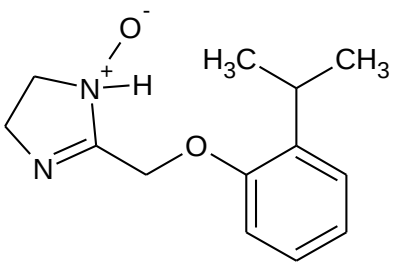
Impurity-1 (FNZ-II):



Impurity-2 (FNZ-III):



Impurity-3 (FNZ-IV):



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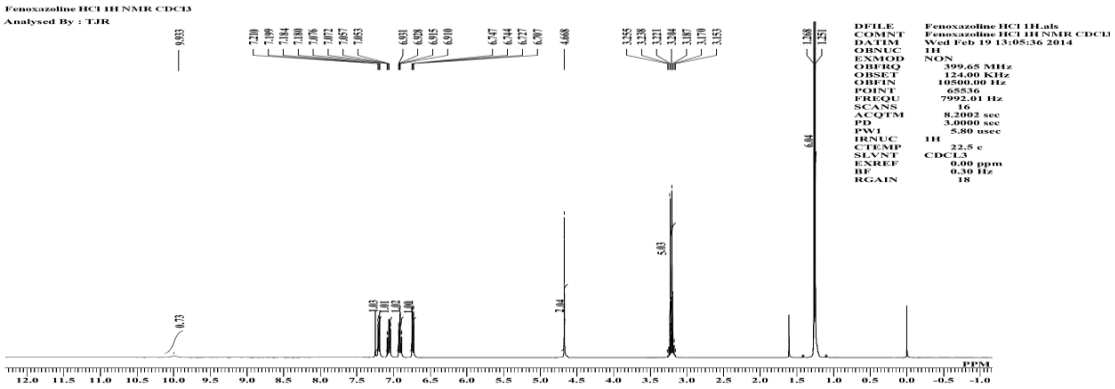
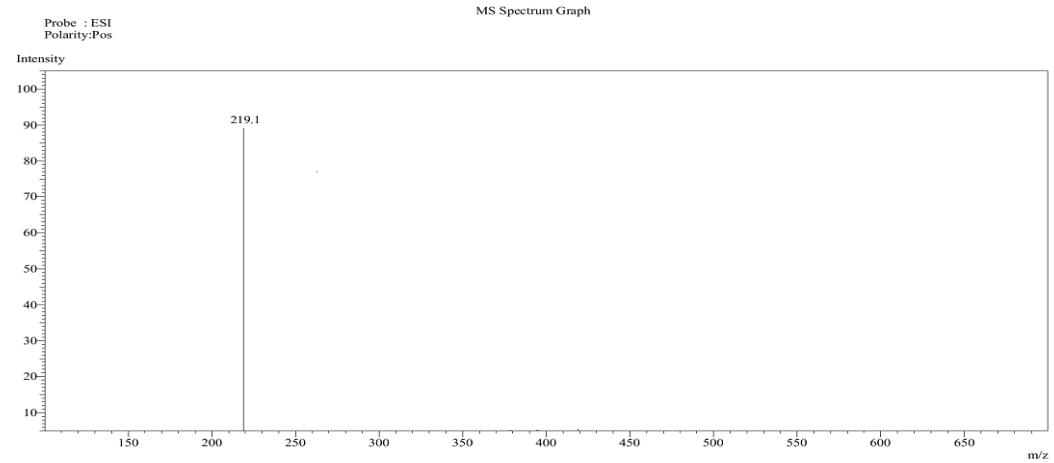
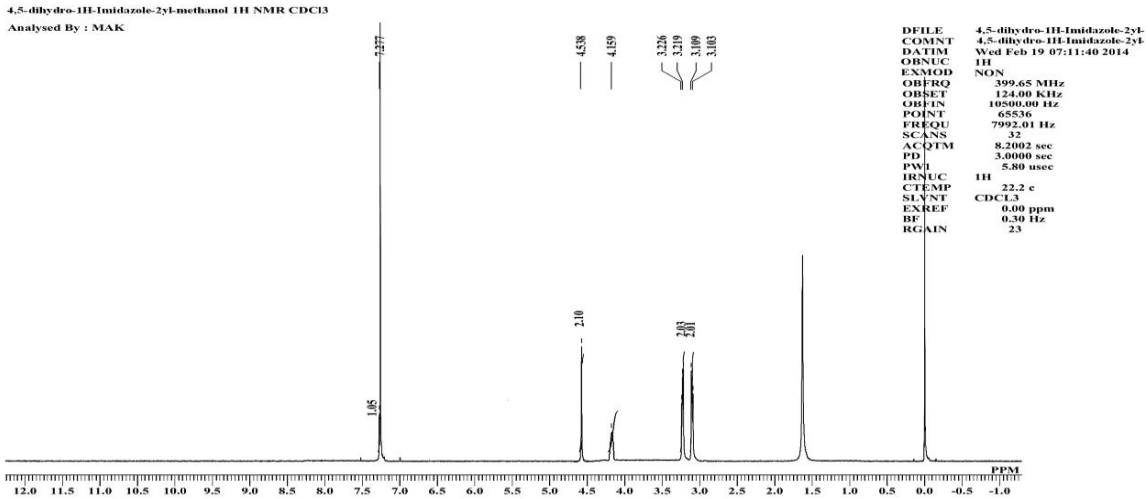
^{1}H-NMR and Mass spectra (Fig.3)

Fenoxazoline HCl (FNZ-I)





Impurity-1 (FNZ-II)



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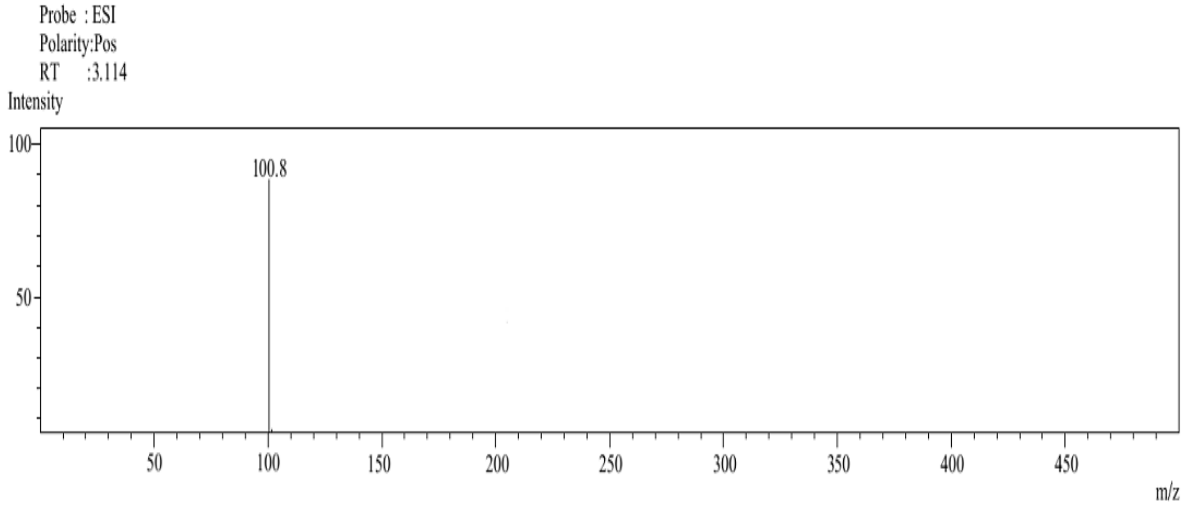
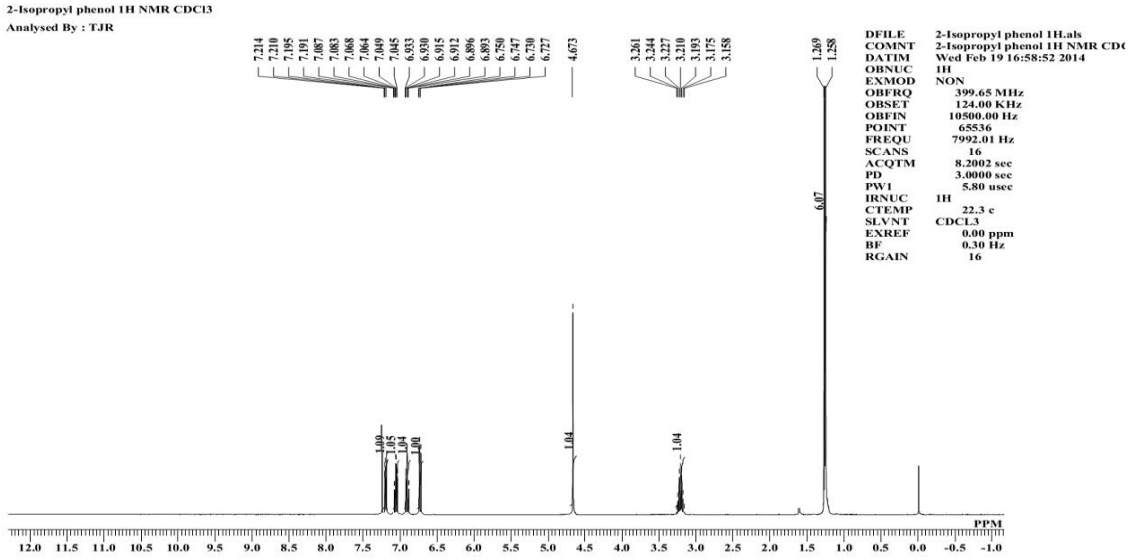
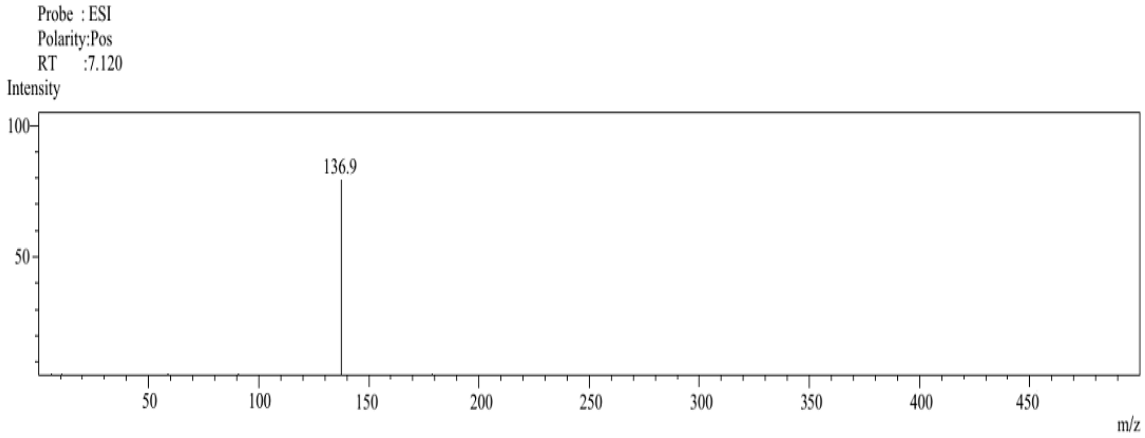


Impurity-2 (FNZ-III)





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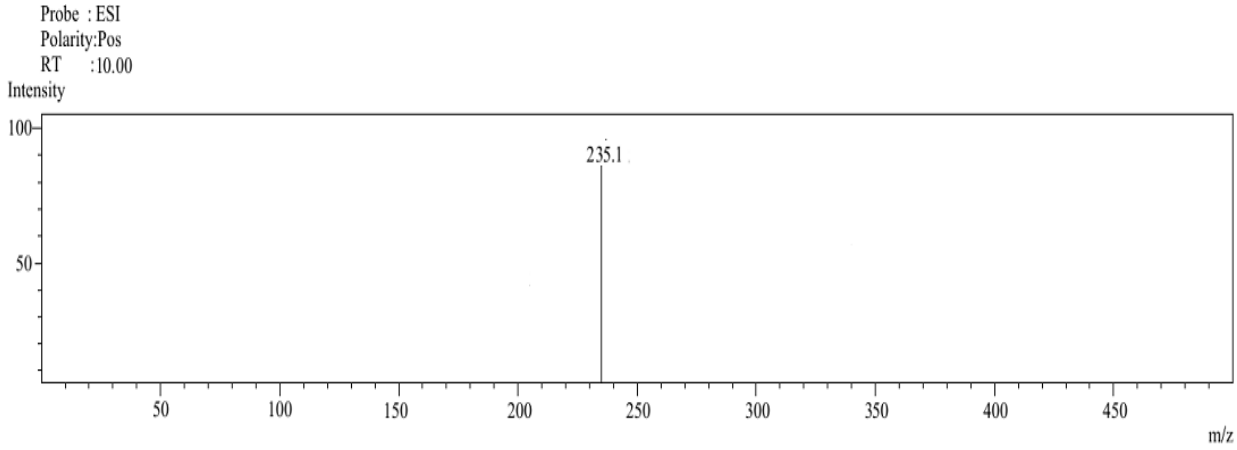
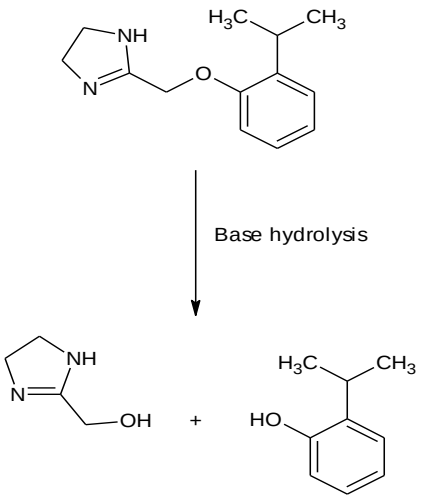
Impurity-3 (FNZ-IV)

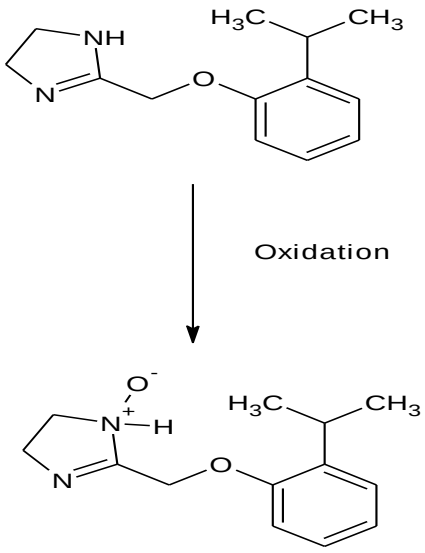


Probable Degradation pathway (Fig.4)

Base degradation (Base Hydrolysis)



Oxidative degradation (Oxidation)



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CONCLUSION

In conclusion, the compound Fenoxazoline HCl is stable under acidic, sunlight and thermal conditions. The compound Fenoxazoline HCl degrades under Basic and Oxidative conditions.

The simple HPLC method developed in this study makes it suitable for separation and estimation of impurities without interference from blank and other related substances present in the drug substance. The HPLC method developed in this study makes it suitable for quality control analysis of Fenoxazoline and its impurities.

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