Stability Indicating Liquid Chromatographic Method for Estimation of Xylometazoline Hydrochloride in Pharmaceutical Dosage Form

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Abstract

A simple, specific, precise, and accurate RP-HPLC method has been developed and validated for Xylometazoline Hydrochloride. The mobile phase has been used for separation consisting of buffer (Acetate buffer in water, pH adjusted to 5 with triethyl amine)-Acetonitrile (30: 70, v/v) using phenomenax C_{18} column with flow rate 1.0 ml/min. Detection wavelength was 240 nm. The method has been linear for the range of 1 – 300 µg/mL with r^2 0.999. Drug has been shows 99 to 101 % recovery. ICH Q_2R_1 guideline has been used for validation of developed analytical method. Drug was exposing to forced degradation condition like hydrolysis, oxidation, thermal and photolytic. Method can well resolve all degraded product as compare to Xylometazoline. The isolate alkali degraded product was characterized by NMR, MASS and IR data. Developed method can routinely used for the estimation of Xylometazoline drug from the dosage form and also for stability sample.

Keywords: Xylometazoline, HPLC, Stability Indicating method, Acidic, Basic and Water degraded products, pharmaceutical dosage form.

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Introduction

2-(4-tert-butyl-1, 2-dimethylbenzyl)-2-imidazoline (Fig. 1) is chemical name of Xylometazoline (XYL). It is a white to almost white crystalline powder. It is soluble in methanol, ethanol and water. XYL have alpha adrenergic activity and used as a sympathomimetic agent.

It is used for the treatment of eye as conjunctive decongestant (1). Literature review shows that number of methods reported for the estimation of XYL, GC (1,2), HPTLC (3), LC-MS (4), Chemometric analysis (5), HPLC (6-10), AAS (11). Different UV spectroscopic method had been reported like derivative (12), Colorimetic (13-16) and fluorimetry (17). ICH guideline has been used for validation of developed method (18).

Experimental

Apparatus: A Shimadzu HPLC, Model: LC-10ATvp (Shimadzu) with rheodyne injector, UV-Visible detector, Model: SPD-10AVP (Shimadzu) and class VP software. HPLC Column, C_{18} (size-250 x 4.60 mm, I.D-5 µ) (Phenomenex). Nylon filters 0.45 µm. Previously calibrated pH meter (Thermo electro corporation). Drug was weighed on balance, Model ALC 210.4 (Acculab). Sonicator used was Ultra Sonicator (Fast Clean Ultrasonic Cleaner).

Reagents and Materials: Pharmaceutical grade of XYL reference standards were kindly supplied as gift samples by Torrent Research Center, Ahmadabad, Gujarat, India. HPLC grade Acetonitrile was purchased form S.D. Fine Chemicals Ltd. (Mumbai, Gujarat, India). The water for HPLC was prepared by triple glass distillation unit and filtered through a nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbia, Gujarat, India). AR grade Sodium hydroxide, hydrochloric acid and 30 % Hydrogen peroxide was purchased from Qualigens Fine Chemicals (Glaxo Ltd., Ahembdabad, Gujarat, India).

Chromatographic conditions: The mobile phase consisted of buffer (Acetate buffer in water, pH adjusted to 5 with triethyl amine)-acetonitrile (30: 70, v/v) was pumped at a flow rate of 1 mL/min. The mobile phase was filtered through a nylon 0.45 µm. The elution was monitored at 240 nm, and the injection volume was 20 µL and phenomenex C_{18} column was used at ambient temperature.

Preparation of XYL standard stock solutions: XYL (100 mg) was weighed accurately and transferred to 100 ml volumetric flask. It was dissolved in 10 ml acetonitrile and diluted up to mark with acetonitrile to obtain final concentration of 1000 µg/ml.

Preparation of Sample solutions: 20 nasal drop formulation (NORTAX 0.1% w/v) have mixed and pipette out solution equivalent to 10 mg of XYL in to a 100 ml volumetric flask containing 10 ml ACN, sonicated for 15 min and further diluted to 100 ml with ACN. The resulting solution was filtered through

whatman filter paper no.41. 20 µl of this solution was injected into HPLC column and the peak area was measured at 240 nm.

System Suitability Test: System suitability test of the chromatographic system was performed before each validation run using five replicate injections of a standard solution. Theoretical plates, tailing factor and resolution were determined.

Procedure for Stress studies: Stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation and photolysis, as mentioned in ICH Q1A (R2)[18]. The blank solution subjected to same stress conditions same manner as the drugs. Hydrolytic decomposition of XYL was carried out in water at neutral pH 7 at reflux condition for 4 h., for acidic condition 1N HCl for reflux for 4 h. and in alkaline condition 1 N NaOH reflux for 4 h. For oxidation stress studies was performed in 30% H_2O_2 at room temperature for 48 h. A photolytic study was done in photo stability chamber by exposing the solid drug for 48 hrs. Studies were also conducted on solid drug, which was heated at 80ºC temperature in stability oven for 48 hrs. Approximately 25 mg drug was weighed accurately and transferred to 25 ml volumetric flask. The drug was dissolved in 5 ml of ACN then diluted with the solvent selected for degradation. Similarly solid-state stability study was done by exposing 25 mg of drug to 80ºC in a stability oven and 25 mg drug to a photo stability chamber. Samples were collected for analysis at two stages, at 0 min (as soon as sample was prepared), after 24 hrs and after 48 hrs of exposure to degradation condition for room temperature and for sample under reflux condition aliquots were withdrawn after 0, 10, 30 min to 1 hr interval up to 4 hrs. Samples were prepared by taking 2 ml of degraded solution in 10 ml volumetric flask and made up to 10 ml with CAN (200ug/ml) and 20µl of that was injected in HPLC column.

Method validation

From the stock solution (1000 µg/ml), aliquots of 0.1, 0.5, 1, 1.5, 2, 2.5, and 3 ml were transferred to a series of 10 ml volumetric flasks. The volume was adjusted up to the mark with ACN to get final concentrations of 10, 50, 100, 150, 200, 250, and 300 µg/ml of solution. 20 µl of all these solutions were injected separately into HPLC column and the peak area of each solution was measured at selected wavelength. The calibration curve was constructed by plotting peak area vs. concentration (µg/ml) over the concentration range and regression equation was calculated.

Accuracy (% Recovery): The accuracy of the methods was determined by calculating recoveries by the standard addition method. Known amounts of standard solutions 50, 100 and 150 µg/ml for the HPLC method. The amounts were estimated by the regression equation of the calibration curve.

Method Precision (Repeatability): The precision of the instruments was checked by repeatedly injecting (n = 5) standard solutions of 150 µg/ml for the HPLC method.

Intermediate Precision (Reproducibility): The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 5 times on the same day and on 5 different day for 3 different concentrations of 60, 120 and 240 µg/ml for the HPLC method. The results are reported in terms of standard deviation (SD).

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guideline[12].

LOD = 3.3 x (X/ S) LOQ=10 x (X/S)

Where X = the standard deviation of the response and S = the standard deviation of y-intercept of regression lines.

Analysis of XYL in tablet dosage form: Nasal drops containing 0.1 % W/V of XYL were selected for the study. The response of dosage forms was measured at 240 nm. The amount of XYL in sample solution was determined by fitting the peak area into the regression equation of HPLC.

Result and Discussion

HPLC Method: To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for XYL was obtained with a mobile phase consisting of buffer (Acetate buffer in water, pH adjusted to 5 with triethyl amine)-acetonitrile (30: 70, v/v) to obtain better reproducibility and repeatability. Quantification was achieved with UV detection at 240 nm based on peak area. A complete resolution of the peaks with clear baseline separation was obtained (Fig. 2).

System Suitability Test: The system suitability test of the chromatographic system was performed before validation run. The system suitability test is an integrated part of the analytical method and it ascertains the suitability and effectiveness of the operating system. The approximate results were reported in Table 1.

Degradation Behaviour

Hydrolytic conditions: The drug showed 93% degradation in 1 N HCl in reflux condition. Major degradation product peak at Rt **4.025** with consider as

Impurity A (Fig. 3). The drug showed 100 % degradation in 1 N NaOH after 4 hrs reflux. Major degradation peak observed at 4.105 which consider as Impurity A (Fig. 4) There was 48% degradation take place in hydrolysis of XYL in neutral conditions (Fig. 5).

Oxidative studies: In oxidative condition drug was exposure to 30% H2O2 for room temperature for 48 hrs. Drug showed 70% degradation towards oxidative stress (Fig. 6).

Photolytic studies: XYL shows 9% degradation when drug expose to UV light in stability chamber for 48 hrs (Fig. 7).

Solid – state studies: There was 4% degradation of solid XYL on exposure to drug heat at 80°C for 48 hrs, which indicated that the drug was stable to normal room temperature (Fig. 8).

Validation of the Proposed Method: XYL shows linear response in the range of 1 – 300 µg/ml for HPLC (Table 1). XYL can recovered from its dosage form with 99.87-100.12% by HPLC (Table 1). The % C.V. values were found to be 0.39-0.73% and 0.45-0.95% for intraday and interday. For intermediate precision the % C.V. values were found to be 0.36%, reveal that the proposed methods are robust. LOD and LOQ were found to be 0.031 µg/ml and 0.10 µg/ml.

Assay of the Nasal Drops: The proposed validated methods were successfully applied to determine XYL in their Nasal Drops dosage form (NORTAX 0.1% w/v) (Table 3).

Table 1: Summary of Validation parameters by HPLC method

r^2 – Correlation coefficient n – number of repetition

Sr. No	Parameters	HPLC
1	Detection wavelength (nm)	240
2	Retention time (min)	6.145
3	Linearity range	1–300 µg/ml
4	Regression equation	Y = 25775 x conc.+
5	Correlation coefficient (r^2)	0.9973
6	Intercept	984063
7	Slope	25775
8	Assay	99.61 – 102.24%
	Precision	
	Intra day % CV (n = 5)	0.39-0.73%
	Inter day % CV (n = 5)	0.45-0.95%
	Reproducibility of measurements %CV	
		0.36%
9	% Recovery	99.87 – 100.12%
10	Limit of detection	0.03157 µg/ml
11	Limit of quantification	0.1052 µg/ml
12	**System Suitability Parameters**	
a.	Retention time (minutes)	6.145
b.	Theoretical plates	11055.41
c.	Resolution	1.094
d.	Asymmetry	0.64
e.	Width at ½ peak height	0.87
f.	Tailing factor	1.05
g.	Capacity Factor	1.90

Table 2: Percentage degradation of XYL by force degradation study by HPLC method

All solution put at room temperature in dark accept thermal and photolytic degradation.

Sr. No	Parameters (Stress condition /duration/state)	% of undergrad XYL	% of individual Degradation products		Total % Deg.
			A	Unspecified	
1	Neutral/H_2Oat pH 7/4 h/ sol./Ref.	82	18	-	18
2	Acidic/1 N HCl/4 h/ sol./Ref.	07	83	17	93
3	Alkali/1 N NaOH/4 h/ sol./Ref.	00	80	20	100
4	Oxidative/30% H_2O_2/48 h/ sol.	30	-	70	70

5	Photo/uv254 and Vis/366 nm/48	91	-	-	9
6	Thermal/80^{0}C/48 h/solid	96	-	-	4

Table 3: Estimation of XYL in Nasal Drops by HPLC

*Average of five reading S.D- Standard deviation

Tablet Formulation	Labeled Claim (mg/tablet)	HPLC	
		Amount Found* (mg/tablet)	%Assay ± S.D.
XYLDOC (Lupin)	10	10.22	102.24±.95

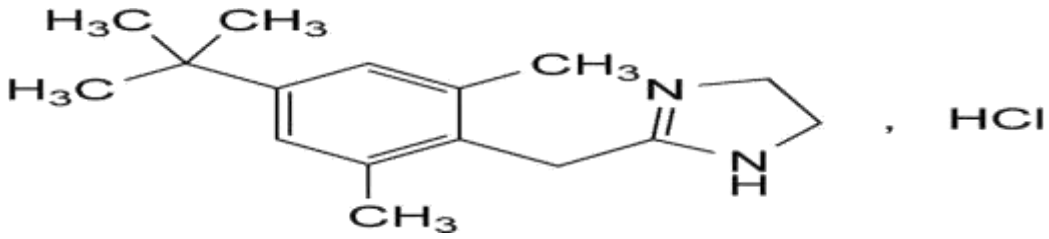

Fig. 1: Chemical structure of XYL

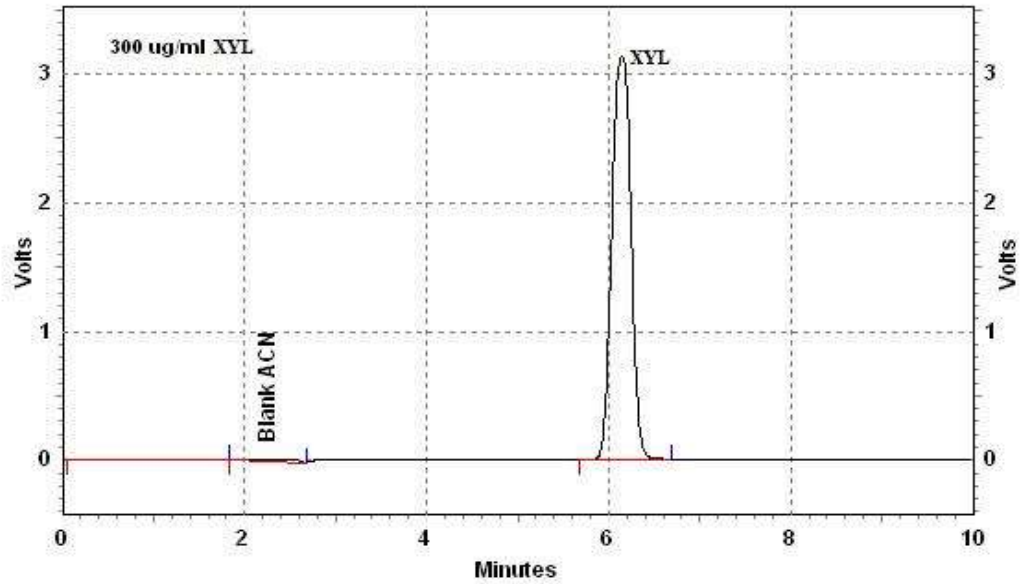

Fig. 2: Single Peak of XYL by HPLC method

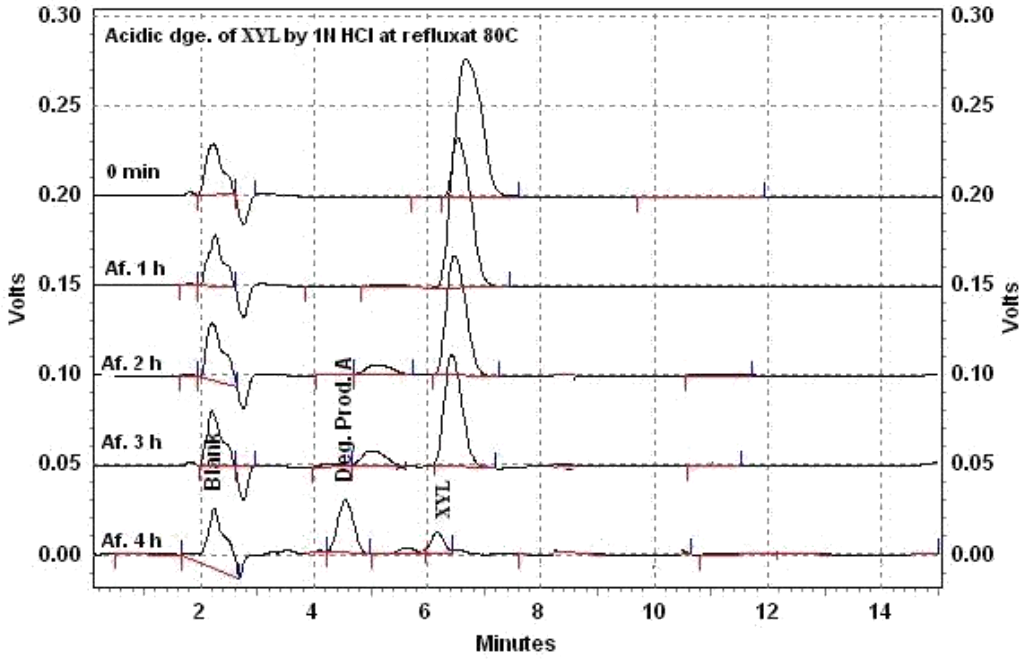

Fig. 3: Chromatograms of XYL in 1 N HCL at 0 min, after 1 hrs, 2 hrs and 3 hrs. after 4 hrs at reflux at 80 ^{0}C

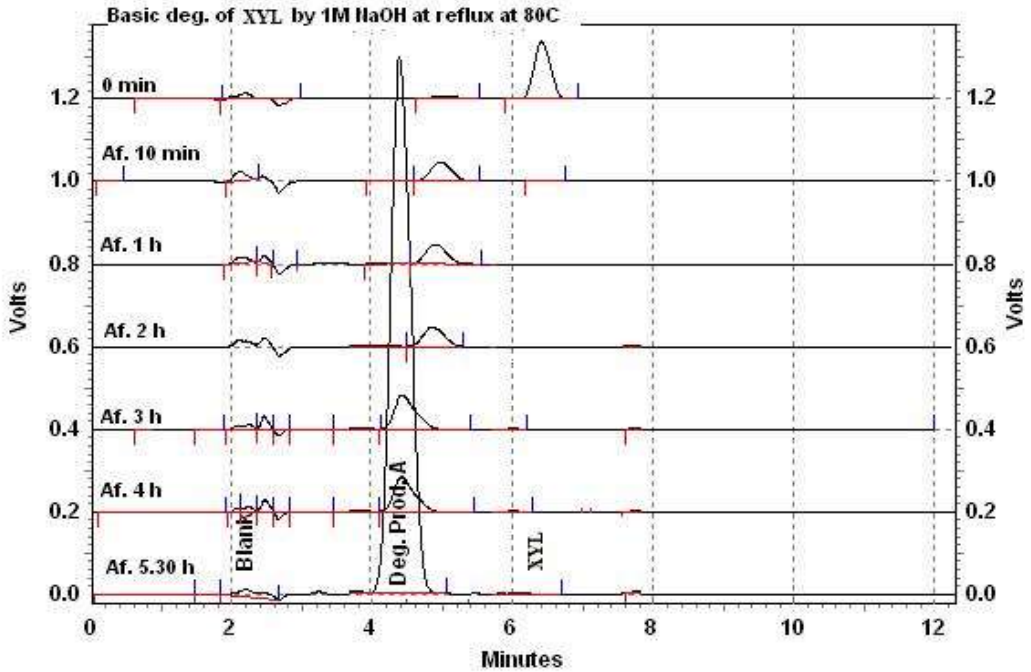

Fig. 4: Chromatograms of XYL in 1N NaOH at 0 min, 10 min after 1 hr, 2 hr, 3 hr, 4 hr and after 5.30 hrs reflux at 80^0 C

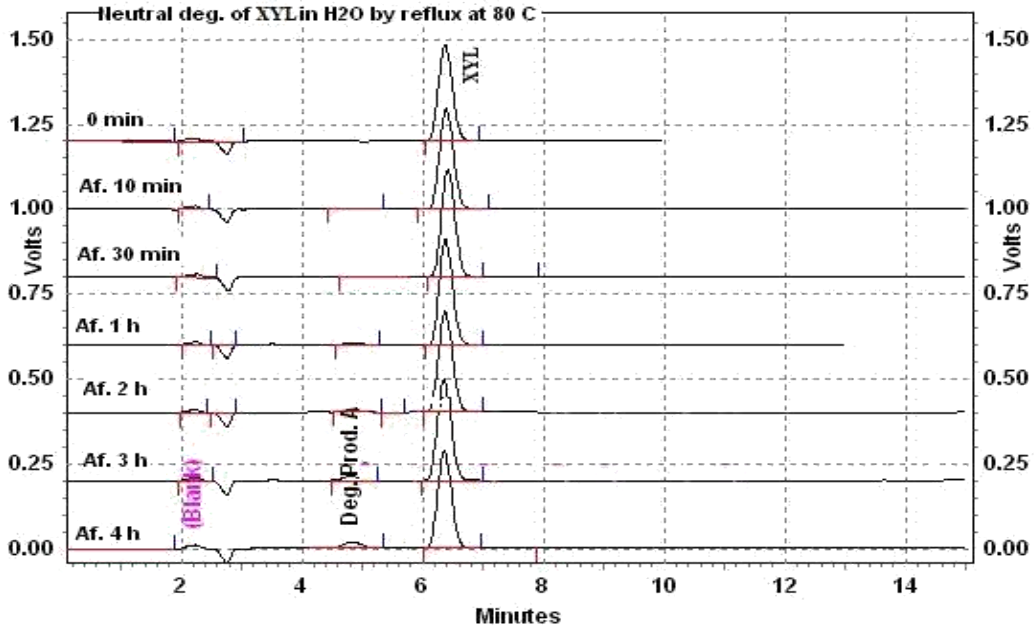

Fig. 5: Chromatograms of 100 ug/ml XYL in Neutral condition standard, 0 min, 10 min, 30 min, 60 min, 120 min, 180 min and 240 min (4hr) reflux at 80^{o} C

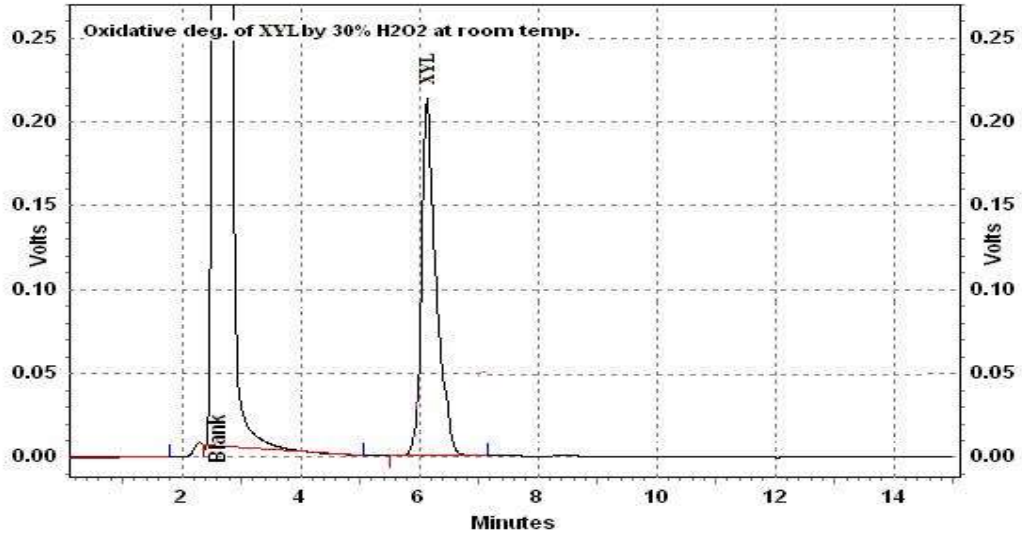

Fig. 6: Chromatograms of oxidation of XYL in 30 % H2O2 after 48 hrs

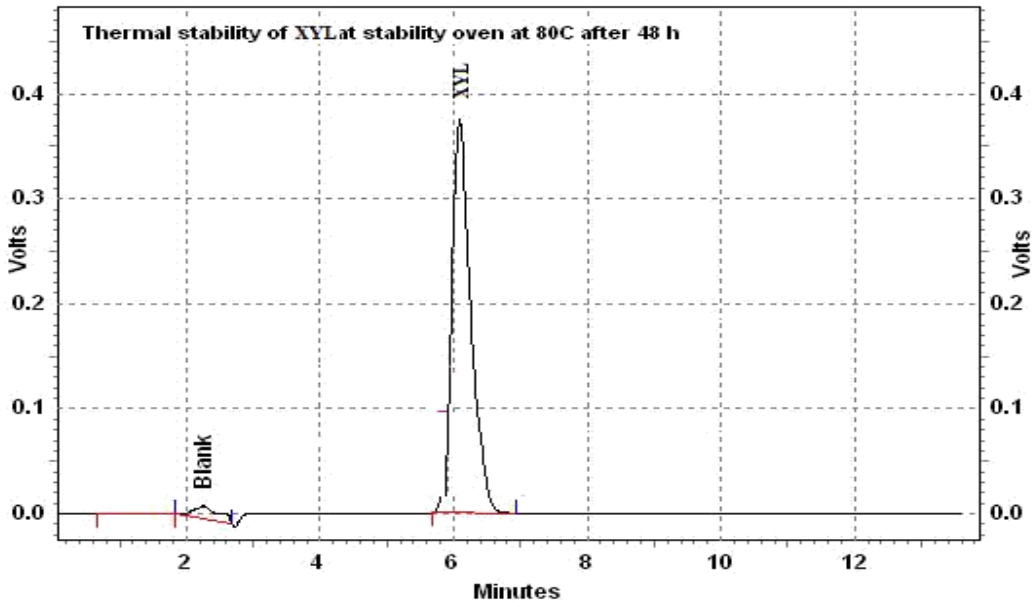

Fig. 7: Chromatograms of thermal degradation of XYL after 48 hrs

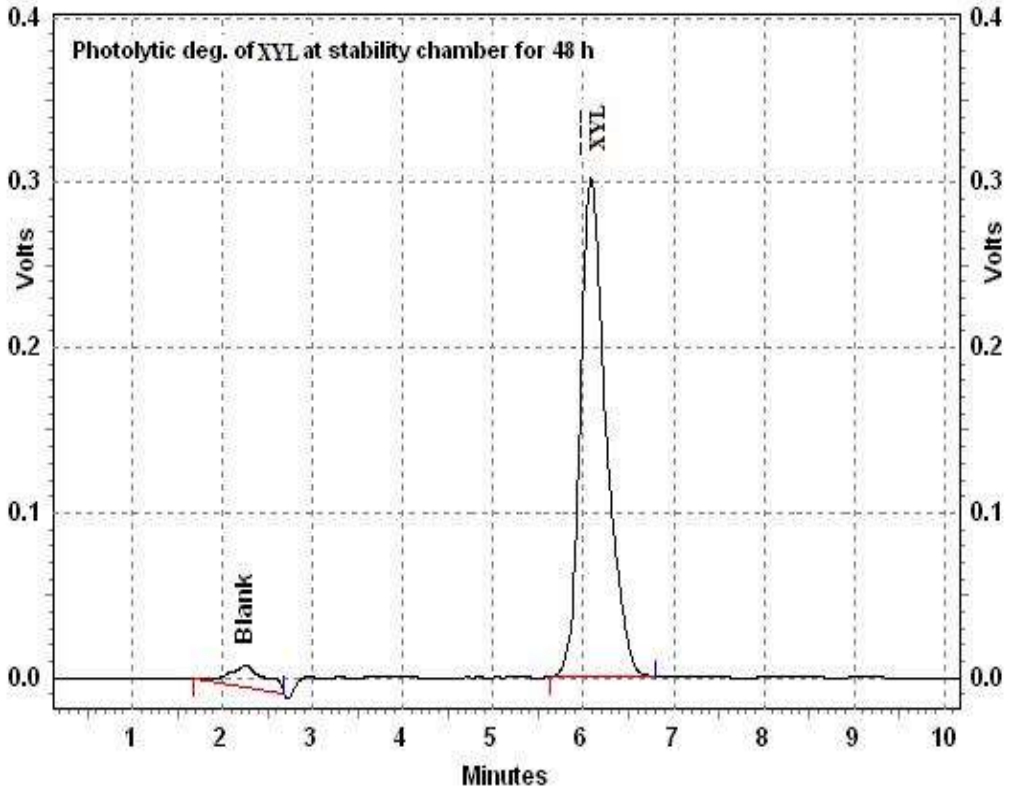

Fig. 8: Chromatograms of photolytic degradation XYL after 48 hrs

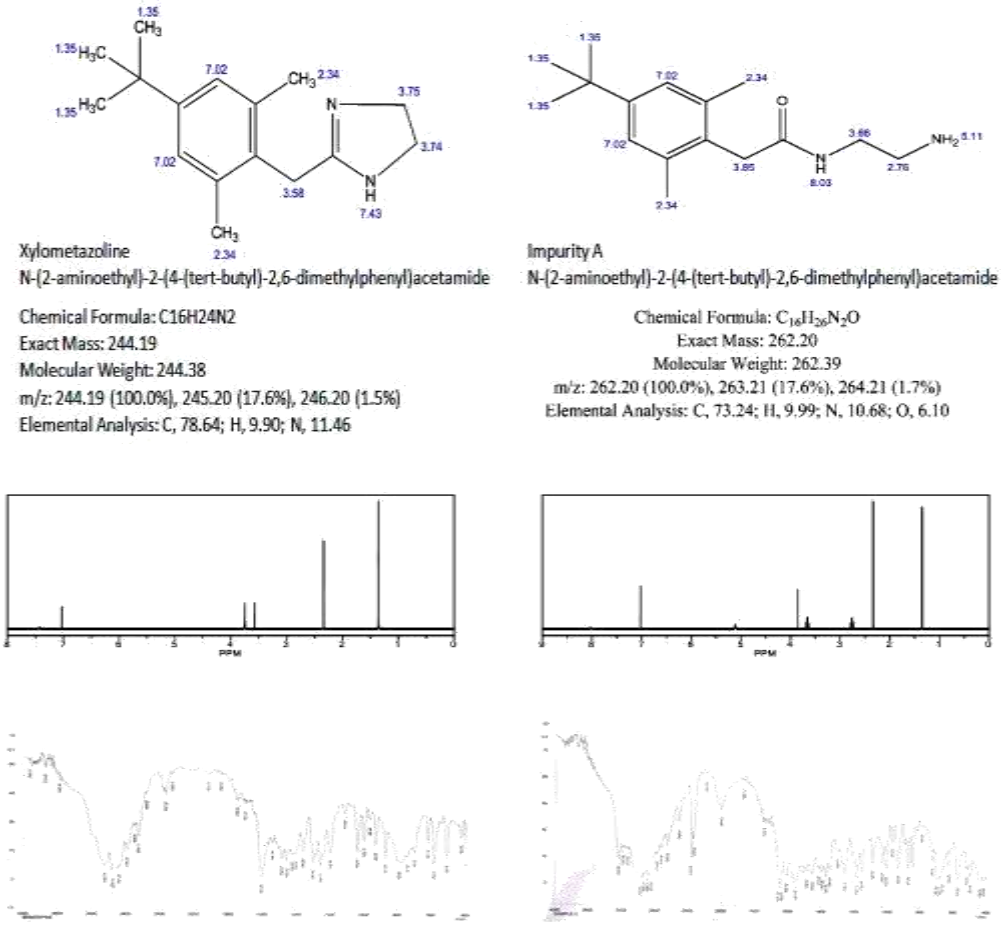

Fig. 9: Structural characterization of Xylometazoline and Impurity A by NMR, MS and IR data

Conclusions

Stability study of XYL indicates its typically undergoes hydrolysis reaction and amidine hydrolysis take place in which **2,6-dimetyl-4-tert-buty-phenylacetyl ethylenediamine** form which consider as **Impurity A**. Hydrolysis is strongly influence by temperature and alkali hydrolysis give 100% degradation of XYL (Fig. 4) in impurity A which is influence by temperature. Percentage degradation and system suitability parameters were reported in table 2. Impurity A is isolated and characterized by NMR, Mass and IR data (Fig. 9).

In this study, it was possible to develop a selective and validated stability indicating HPLC assay method for XYL at Rt 6.145 min on a C_{18} column, which could separate the drug and its degradation products formed under a variety of stress conditions. XYL was found to be unstable in acidic, alkali, neutral and oxidative conditions, whereas it was comparatively much stable thermal and photolytic conditions.

Reference

1. K. Florey, Analytical Profile of Drug Substances 14 (1985).
2. A. Sioufi, F. Leroux, and J. P. Dubois, J. Chromatogr., Biomed. Appl. 79 (1989) 81.
3. M. Massaccesi, Pharm. Acta Helv. 62 (1987) 302.
4. D. Agbaba, Z. Milojevic, S. Eric, M. Aleksic, G. Markovic, and M. Solujic, J. Planar Chromatogr. Mod. TLC. 14 (2001) 322.
5. G. A. Khan, R. Lindberg, R. Grabic, and J. Fick, J. Pharm. Biomed. Anal. 66 (2012) 24.
6. A. Detroyer, V. Schoonjans, F. Questier, Y. Vander Heyden, A. P. Borosy, Q. Guo, and D. L. Massart, J. Chromatogr. 897 (2000) 23.
7. K. Vucicevic, G. Popovic, K. Nikolic, I. Vovk, and D. Agbaba, J. Liq. Chromatogr. Relat. Technol. 32 (2009) 656.
8. Z. Milojevic, D. Agbaba, S. Eric, D. Boberic-Borojevic, P. Ristic, and M. Solujic, J. Chromatogr. 949 (2002) 79.
9. W. F. Ni, and O. Jin, Yaowu Fenxi Zazhi. 16 (1996) 392.
10. D. De Orsi, L. Gagliardi, G. Cavazzutti, M. G. Mediati, and D. Tonelli, J. Liq. Chromatogr. 18 (1995) 3233.
11. J. E. Kountourellis, and A. Raptouli, Anal. Lett. 21 (1988) 1361.
12. S. Khalil, Mikrochim. Acta 130 (1999) 181.
13. M. S. Mahrous, M. E. Abdel-Hamid, H. G. Dabees, and Y. A. Beltagy, J. Pharm. Belg. 47 (1992) 135.
14. D. G. Sankar, C. S. P. Sastry, and M. N. Reddy, Indian Drugs 26 (1989) 348.
15. M. Abdel Salam, A. S. Issa, and M. S. Mahrous, Anal. Lett. 19 (1986) 2207.
16. Y. Anjaneyulu, K. Chandra Sekhar, V. Anjaneyulu, and R. N. Sarma, Indian Drugs 22 (1985) 655.
17. R. T. Sane, B. R. Shinde, A. K. Parikh, and S. P. Tikekar, Indian Drugs 21 (1984) 257.

18. ICH, Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization, IFPMA, Geneva, 2003.