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Significance of Propylene Carbonate as a Mobile Phase Component in Estimation of Aspirin and its Impurities using RP-HPLC

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ABSTRACT

The present paper describes the use of Solvent-X (Propylene Carbonate: Methanol, 60:40) as a mobile phase component in place of Acetonitrile (ACN). A need to replace ACN with a new environmental friendly solvent is increasingly gaining importance because it has been speculated that by 2030, all Class II solvents, one of which is ACN will be completely phased out. PC has numerous advantages over ACN with respect to boiling point, dipole moment, dielectric constant, biodegradability, clinical toxicity etc. Besides showing similar selectivity characteristics, Solvent-X has certain significant advantages over ACN. This paper describes the method applied to determine Aspirin and its impurities in the presence of degradation products. Successful separation is achieved on Intersil C_{18} Column using a mobile phase consisting of 5mM Ammonium acetate: Solvent-X (60:40 v/v) pH 3.5, with glacial acetic acid with the flow rate of 1mL/min and detected at wavelength of 237 nm. Similarly second method has been developed by replacing amount of Solvent-X with Acetonitrile. Both these methods have been validated with respect to linearity, accuracy, precision, specificity and robustness. Performances of these methods have been compared.

KEYWORDS: Propylene Carbonate; Acetonitrile; Aspirin; Validation; Force Degradation

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INTRODUCTION

Propylene carbonate (4-methyl-1, 3, dioxolan-2-one) is a five-membered alkylene carbonate manufactured most commonly by carbon dioxide insertion into the appropriate oxirane[1]. It is an odorless, colorless, aprotic solvent with a strong dipole-dipole interaction and fairly high dielectric constant[2]. Some of the important advantages of PC in comparison with ACN are listed below:

PC has a higher dielectric constant (64.4) and Polarity Index value (6.1) as compared to ACN (Dielectric constant = 36.6 and Polarity index value = 5.8)[3].The chances of accidental fires is greatly reduced while working with PC (Vapor pressure = 0.045 mm Hg at 25°C and flashpoint temperature = 135°C) as compared to ACN (Vapor pressure = 91.1 mm Hg at 25°C and flashpoint temperature 5.6°C.) [4,5]

PC is less toxic than ACN because LD_{50} value (on rats g/kg) of PC is >5.0 whereas that of ACN is in the range of 2.46-6.5[6].

PC (Log $P_{o/w}$ = -0.41) shows a lower ability to bioaccumulate than ACN (Log $P_{o/w}$ =-0.34)[7].

PC is most commonly used as a solvent for various synthetic polymers and as an electrochemical solvent. It is also used for degreasing, paint stripping and cleaning applications. However, till date PC has not been used as a mobile phase component in RP-HPLC.

On examining the properties of PC, it is found that it has numerous advantages over ACN with respect to boiling point, vapour pressure, flammability, dipole moment, dielectric constant, toxicity, biodegradability, bioaccumulation, overall environmental impact and clinical toxicity.

The benefits of PC have been mentioned, so a demonstration of its feasibility is in order. RP-HPLC methods for the simultaneous separation and estimation of drugs in three combined dosage forms have been developed and validated using both ACN and Solvent-X (PC:MeOH::60:40).

Impurities are unwanted chemical present in Active Pharmaceutical ingredient (API) or drug formulation. The control of impurities in drug is critical issues to pharmaceutical industry. The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of impurities. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now getting receiving important critical attention from regulatory authorities. The different pharmacopoeias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations.

Aspirin is also known as acetylsalicylic acid, is often used as an analgesic, antipyretic and antiflammatory. It is easily synthesized by treating salicylic acid with acid anhydride. It is a very unstable drug. The aim of this work was to develop a simple and rapid method for the estimation of the aspirin, most simple drug, and its impurities by using Solvent-X and by another method using ACN.

MATERIALS AND METHODS

Chemicals

Working standards of Aspirin were received as a gift sample from reputed company. For the assay of tablets, locally available brands were procured, viz. Ecosprin of USV (Corvette) for Aspirin.

HPLC grade ACN and MeOH were obtained from E-Merck Limited, Mumbai. HPLC grade PC of Sigma Aldrich was imported from Germany. Double distilled water was used for solution preparations throughout the project. Mobile phase was always filtered through 0.45µ membrane filter paper and degassed before use.

Preparation of standard solutions

A stock solution of aspirin and impurities (1.0mg/mL) was prepared by dissolving an appropriate amount of the substance in Solvent-X. Working solutions of different concentrations were prepared from the above stock solution and diluted with the mobile phase.

Preparation of sample solutions

Twenty tablets, each containing 75mg Aspirin were weighed and powdered equivalent to dose, transferred to a 100mL volumetric flask, and extracted with MeOH and Water (80:20). The mixture was sonicated for 30 minutes in an ultrasonic bath. The volume was adjusted to 100mL with the same solvent mixture and subsequently filtered using Whatmann filter paper No. 41. From the filtrate, 1.0mL was pipette out and the volume was made up to 10.0mL with the mobile phase to give a 10 times diluted solution.

Equipment

The LC system used for method development and validation consisted of a JASCO HPLC-900 series equipped with PU-980 intelligent pump, AS-950 intelligent auto sampler (1-100 µL) and UV-975 intelligent UV-Vis detector with 8 µL flow cell.

METHOD DEVELOPMENT AND OPTIMIZATION

Some important parameters like the pH of the mobile phase, concentration of acid or buffer solutions, percentage and type of organic modifier, etc. were tested for good chromatographic separation. The following are the chromatographic condition under which separation was achieved:

I. Chromatographic conditions for the separation and estimation of Aspirin and its impurities

Method A: Using ACN as the mobile phase component.

The separation was carried out on an Inertsil ODS 3V (250 x 4.6 mm, 5 µ) column using mobile phase containing a mixture of ACN:Buffer (60:40, v/v) as the mobile phase at a flow rate of 1 mL/min .The buffer used was 5mM ammonium acetate and pH of the mobile phase was adjusted to 3.5 with dil. Acetic acid, the wavelength monitor at 237 nm and the column temperature was at 25^{oC}. The typical RP-HPLC chromatogram of the sample is shown in Figure 1.

Method B: Using Solvent-X as a mobile phase component.

When Solvent-X was used instead of ACN, all other chromatographic conditions were kept unchanged except that the mobile phase composition used was Solvent-X: Buffer (60:40, v/v). The typical RP-HPLC chromatogram of sample is shown in Figure 2.

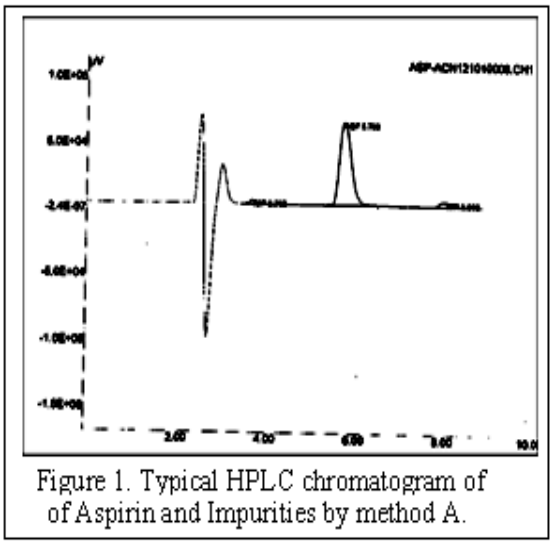


Figure 1. Typical HPLC chromatogram of of Aspirin and Impurities by method A.

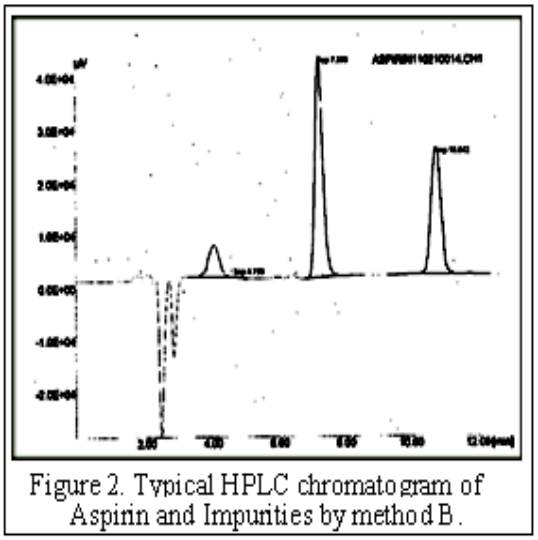


Figure 2. Typical HPLC chromatogram of Aspirin and Impurities by method B.

METHOD VALIDATION

The develop chromatographic methods A and B using ACN and Solvent-X, respectively were validated using the following parameters[8]:

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

The LOD and LOQ for aspirin, impurity 1 and impurity 2 were determined at signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentrations. A precision

study was also carried out at the LOQ level by injecting six individual preparations of aspirin, imp-1 and imp-2 and calculating the % R.S.D. of the area.

Linearity

Linearity test solutions for the assay were prepared from the aspirin stock solution at eight assay analyte concentration levels from 3.75, 4.5, 5.25, 6.0, 6.75, 7.5, 8.25 and 9.0µg/mL. The peak area versus concentration data was treated by least squares linear-regression analysis.

Linearity test solutions for the related substances method were prepared by diluting the stock solutions to the required concentrations. The solutions were at eight concentration levels from 50, 60, 70, 80, 90, 100, 110 and 120ng/mL.

Accuracy

The accuracy of the assay was evaluated in triplicates at three concentration levels, i.e. 3.75, 7.5 and 9.0 g/mL. The percentage of recoveries was calculated from the slope and Y-intercept of the calibration curve obtained from linearity.

Precision

The precision of the assay method was evaluated by carrying out six independent assays of the aspirin test sample against a qualified working standard and calculating the % R. S. D of the assay.

The precision of the related substance method was checked by injecting six individual preparations of aspirin (7.5µg/mL) spiked with 100% of imp-1 and imp-2 with respect to aspirin analyte concentration. %R. S. D. of area of each imp-1, and imp-2 was calculated.

The intermediate precision of the method was also evaluated using a different instrument in the same laboratory.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between aspirin, imp-1, and imp-2 was recorded. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, flow was changed by 0.2 units from 0.8 to 1.2 ml/min. The effect of the column temperature on resolution was studied at 20 and 30 ◦C

instead of 25 ◦C. The effect of the percent organic strength on resolution was studied by varying ACN by ±3 while other mobile phase components were held constant.

Solution stability

The solution stability of aspirin in the assay method was determined by leaving both the test solutions of the sample and the reference standard in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed after 2, 4 6, 8, 24 and 48h during the study period. The mobile phase stability was also tested by assaying the freshly prepared sample solutions against freshly prepared reference standard solution for 48 h. Mobile phase prepared was kept constant during the study period. The % R.S.D. for the assay of aspirin was calculated during the mobile phase and solution stability experiments.

The solution stability of aspirin and its impurities in the related substance method was tested by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 48 h. The content of imp-1 and imp-2 were determined after 2, 4 6, 8, 24 and 48h for the study period. The mobile phase stability was also determined for 48 h by injecting the freshly prepared sample solutions. The content of imp-1, and imp-1 were checked in the test solutions.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed LC method for aspirin carried out in the presence of its impurities namely imp-1 and imp-2. Stress studies were performed for aspirin bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted under stress conditions of heat (60◦C), acid (0.5N HCl), base (0.5N NaOH) and oxidation (3.0% H_2O_2) so as to evaluate the ability of the proposed method to separate aspirin from its degradation product. For heat study, the study period was 48 days whereas for the acid and base hydrolysis was half an hour while for oxidation was four hour. Peak purity test was carried out for the aspirin peak by using PDA detector in stress samples. Assay studies were carried out for stress samples against qualified aspirin reference standard.

Thus, all the developed methods were validated and found to meet the acceptance criteria set by the ICH.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main objective of the chromatographic method is to separate aspirin from imp-1 and imp-2. Impurities were coeluted using different stationary phases such as C18, C8, phenyl and cyano as well as different mobile phases. The chromatographic separation was achieved on an Intersil ODS C18 250mm×4.6 mm, 5µm column using mixture of aqueous 5mM ammonium acetate–acetonitrile/solvent-X (60:40, v/v) adjusted to pH 3.5 by dil. acetic acid as a mobile phase. The flow rate of the mobile phase was 1.0 ml/min, at 25 ◦C column temperature, the peak shape of the aspirin was found to be symmetrical. In optimized chromatographic conditions aspirin, imp-1 and imp-2 were separated with a resolution greater than 2, the typical retention times were about 3.8, 5.8 and 8.1 min for method A and 4.73, 7.3 and 10.8min for method B, respectively (Fig. 1 and2). The system suitability results are given in Table 1. The developed LC method was found to be specific for aspirin and its two impurities namely imp-1and imp-2 (Fig. 1 and 2).

Table 1: Chromatographic Figures of Merit for Aspirin and its Impurities

Parameter	Method (A)			Method (B)		
	Impurity 1	Aspirin	Impurity 2	Impurity 1	Aspirin	Impurity 2
Retention Time (R_t)	3.8	5.8	8.1	4.73	7.3	10.8
Capacity Factor (α)	0.23	0.89	1.64	0.14	0.75	1.59
Selectivity (K')	-	3.87	1.84	-	5.36	2.12
Resolution (Rs)	-	4.84	3.65	-	5.97	6.50
Tailing Factor (T)	1.36	1.36	1.28	1.75	1.20	1.36
Theoretical Plates (N)	5854	12190	11751	5659	13563	12365
HETP (m)	0.0043	0.0021	0.0021	0.0044	0.0018	0.002

Limit of detection and limit of quantification

The limit of detection of aspirin and all the impurities namely, imp-1 and imp-2, was achieved at 30 ng/ml for 20 µL injection volume. The limit of quantification for all three impurities namely, imp-1 and imp-2, was achieved at 50 ng/ml for a 20µL injection volume. The % R.S.D. at the LOQ concentrations for aspirin, imp-1 and imp-2 were 1.8, 1.6 and 1.9 for method A and 1.8, 1.3 and 1.6 for method B, respectively.

Linearity

The linearity calibration plot for the assay method was obtained over the calibration ranges tested and correlation coefficient obtained was greater than 0.999 for methods A and B. The results show that an excellent correlation existed between the peak area and concentration of the analyte. A linear calibration plot for the related substance method was obtained over the calibration ranges tested, for impurity imp-1 and imp-2. The correlation coefficient obtained was greater than 0.998. The above result show that an excellent correlation existed between the peak area and the concentration of imp-1 and imp-2.

Accuracy

The percentage recovery of aspirin in the bulk drug samples ranged from 98.6 to 100.7% for method A and B.

Precision

The % R.S.D. of assay of aspirin during the assay method precision study was within 0.8% for both methods A and B, the % R.S.D. for the area of imp-1 and imp-2 in the related substance method precision study was within 1.5% for both methods (A and B). The %R.S.D. of the assay results obtained in the intermediate precision study was within 0.9 % for both methods A and B, the % R.S.D. for the area of imp-1 and imp-2 were well within 3% for both method A and B, conforming good precision of the method A and method B.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and composition of organic solvent), the resolution between critical pair, i.e. imp-1, aspirin and imp-2 was greater than 2.0, illustrating the robustness of the method A and method B.

Solution stability and mobile phase stability

The % R.S.D. of the assay of aspirin during solution stability experiments were within 2%. Significant changes were observed in the content of impurities imp-2 during solution stability experiments when performed using the related substance method. The solution stability and mobile phase stability experiment data confirms that the sample solutions used during assay and the related substance determination were unstable after 24h. The Aspirin sample degraded to form imp-2 after 24h.

Results of forced degradation studies

Degradation was not observed in aspirin sample when subjected to stress conditions like heat and oxidation. Aspirin was degraded to impurity 2 under acid and base hydrolysis. Peak purity test results confirmed that the aspirin peak is homogenous and pure in all the analyzed stress samples. The assay of aspirin is unaffected in the presence of imp-2 and its degradation products confirm the stability indicating power of the method A and B.

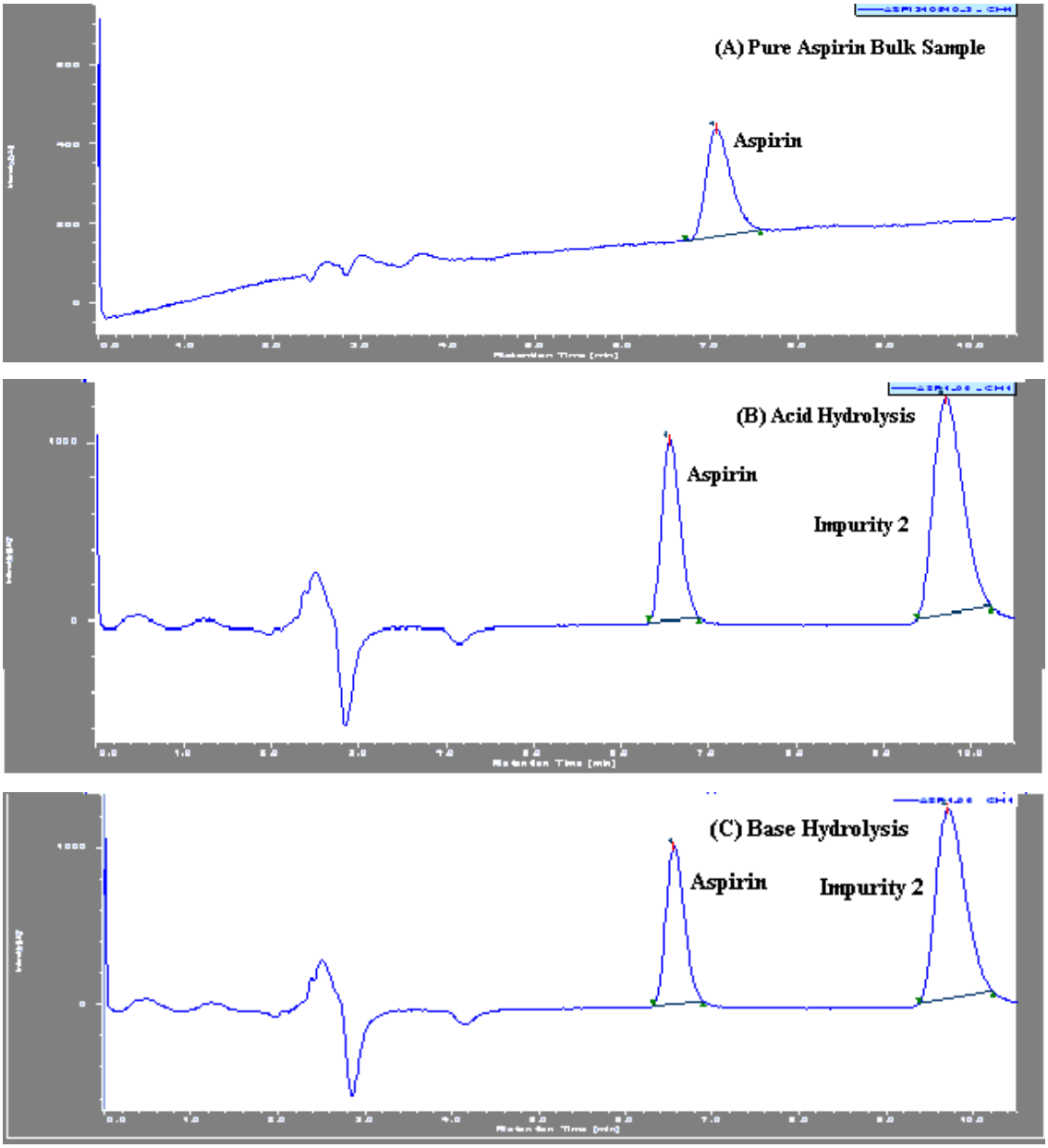
The summary of forced degradation studies is given in Table 2

Table 2: Summary of Forced Degradation Results

Stress Conditions	**Time**	**Method A**		**Method B**	
		% Assay of active substance	% Mass Balance (active + impurity)	% Assay of active substance	% Mass Balance (active + impurity)
Acid Hydrolysis (0.5N HCl)	30 min	76.62	98.40	76.97	98.56
Base Hydrolysis (0.5N NaOH)	30 min	-	98.35	-	98.45
Oxidation (3% H_2O_2)	4 hours	75.25	98.60	75.34	98.74
Thermal 60^oC	48 hours	98.55	98.55	98.61	98.61

From the above experimental work it is proved that, RP-HPLC methods that use ACN can be replicated using exactly the same amount of Solvent-X instead of ACN to give reasonably good results without any change in other chromatographic conditions for aspirin. All methods developed using Solvent-X meet the acceptance criteria set by the ICH. Hence, these methods can be employed in the industry as well as other chemical laboratories for routine analysis. None of the columns showed any deterioration

in performance on being exposed to mobile phases containing Solvent-X as a component. However, as compared to the column pressure observed when ACN based mobile phase is used, a slight increase of upto 60 kg/cm^2 was reported when Solvent-X based mobile phase was used. This may be attributed to the higher viscosity of PC which forms 60% of Solvent-X. Solvent-X showed good compatibility with the buffers used during analysis. It was also found to be UV transparent above 210 nm.



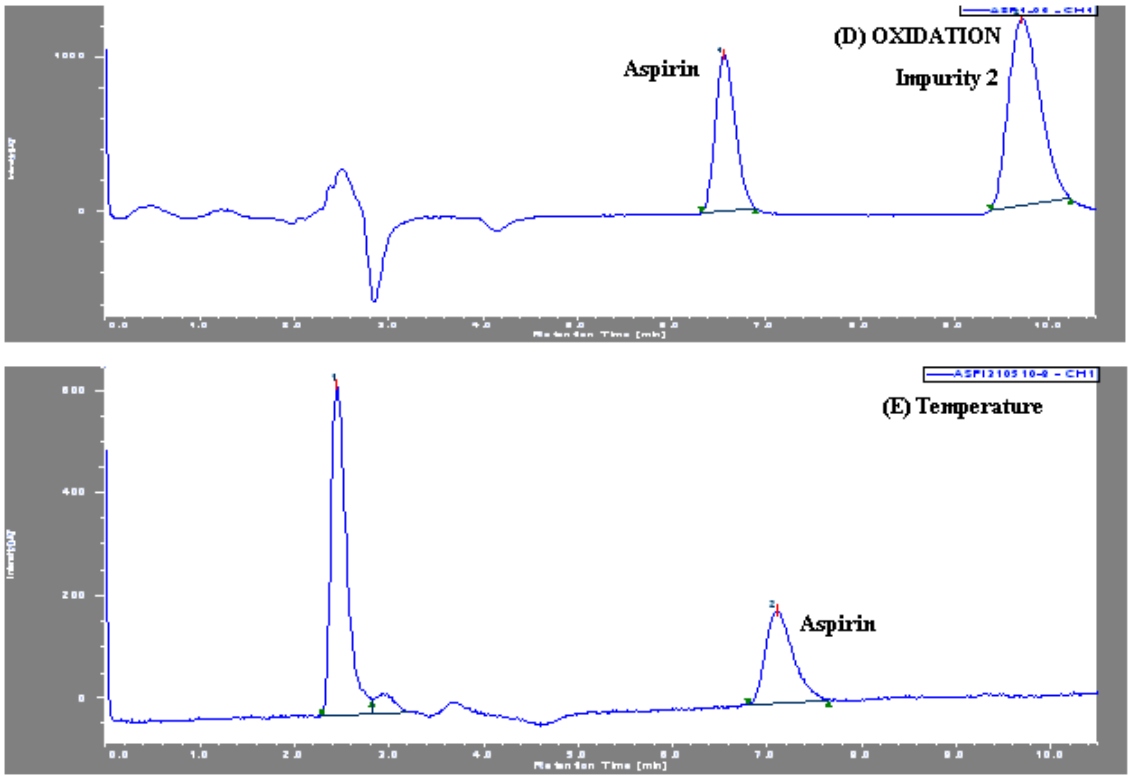


Figure 3.Typical HPLC chromatograms of aspirin under stress condition (A) pure aspirin bulk sample, (B) 0.5N HCl, (C) 0.5N NaOH, (D) 3% H_2O_2 and (E) 60 ◦C for method B.

CONCLUSION

Thus, it can be concluded that Solvent-X is an ecofriendly replacement for ACN in RP-HPLC. Due to the various advantages of PC over ACN, viz. low vapour pressure and low clinical toxicity, the cases of accidental fires and occupational hazards would be minimized if Solvent-X is used instead of ACN. Its high biodegradability would help industries in saving money spent on effluent treatment without any concern about environmental pollution. Thus, this work is expected to be of interest to scientists who are working in the field of developing cleaner and greener chemical technologies which is definitely the need of the hour.

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