Stability-indicating RP-HPLC method for the determination of doxepin, benzyl alcohol and capsaicin in bulk & pharmaceutical dosage forms

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Abstract : An accurate, rapid economical and simple, reliable method was developed and validated for the simultaneous estimation of Doxepin, Benzyl alcohol and capsaicin using RP-HPLC. In the proposed method efficient chromatographic separation was achieved using symmetry C18 column (150mmx4.6mm, dp=3.5µm) as a stationary phase and acetonitrile:buffer (40:60v/v, 3.0 gm octane-1-sulphonic acid in 1lt water adjust pH-2.5 with OPA) as a mobile phase with a flow rate of 1.0 ml/min and UV detection at 273nm.Chromatography was carried out isocratically at ambient temperature and the run time was 12min. In this method a good linearity was observed in the range of 6.6-99 µg/ml, 2-30 µg/ml and 0.1-1.5 µg/ml with the limit of detection(s/n=5,4and7) 0.06µg/ml, 0.02µg/ml and 0.001µg/ml for doxepin, benzyl alcohol and capsaicin respectively. The retention time for capsaicin, doxepin and benzyl alcohol were 3.57, 6.10 and 9.10 minutes respectively. The proposed method was validated as per ICH Q2(R1) guidelines.

IndexTerms **- benzyl alcohol; capsaicin; high performance liquid chromatography.**

I. INTRODUCTION

Recently there is an increase in interest in various combination of doxepin, benzyl alcohol and capsaicin commercially known as Nuero cream, for various potential applications such as to control muscle pain, head lice infestation, pruritus, postherpetic neuralgia, diabetes and HIV-related neuropathy etc…

Doxepin(DXP),chemically(EIZ)-3-(dibenzo[b,e]oxepin-11(6H)-ylidene)-N,N-dimethylpropan-1- amine.It can acts as an antidepressant drug. It is used as second line treatment of chronic idiopathic urticaria (hives) [1, 2] and treat depression, anxiety disorders, itchiness and trouble sleeping. [3]

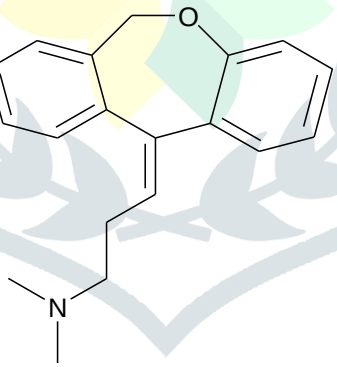


Fig. 1- Chemical structure of Doxepin

Benzyl alcohol (BZA), chemically phenyl methanol, is frequently used as a co-solvent in a variety of pharmaceutical injection formulations and an antimicrobial preservative. A variety of essential oils including jasmine, hyacinth and ylang-ylang [4] are composed chiefly of benzyl alcohol. It is found in castoreum and is gathered from the beaver plant food. [5] It is used as the preservative material. [6] It is toxic to neonates and is associated with the gasping syndrome. [7], [8]

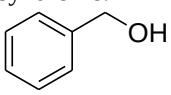


Fig. 2- Chemical structure of Benzyl alcohol

Capsaicin (CPS), chemically 8-methyl-N-vanillyl-trans-6-nonen amide, is mostly present in hot chili peppers [9, 10] and it causes a burning sensation to human skin. [11] It is used to treat foot pain, laminar ischemia and arthritis in veterinary medical practice. [12] It produces significant desensitization and analgesia and is highly selective agonist for vanilloid subtype l and as a substance -P deplete. [13-18] It has many possible clinical applications but prevented by the paucity of clinical trials. High performance liquid chromatography has been widely used to characterize the capsaicin and its analogues. [19-23]

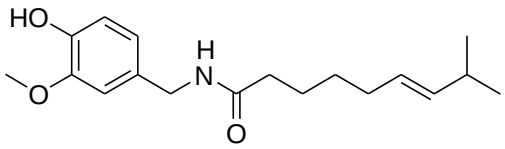


Fig. 3- Chemical structure of Capsaicin

Different chromatographic methods were studied in an attempt to optimize simple, reliable and sensitive and an accurate method for the estimation of studied compounds in bulk and pharmaceutical dosage forms. But literature search reveals that there was no HPLC method for the simultaneous estimation of these drugs has been reported so far. The purpose of the present work therefore was to development a fast, economical, sensitive and confirmation of doxepin, benzyl alcohol and capsaicin in bulk and pharmaceutical dosage forms.

EXPERIMENTAL:

Chemicals:

Acetonitrile, octane-1-sulphonic acid, ortho phosphoric acid and water (HPLC grade) were purchased from Merk (India) Ltd. Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of doxepin, benzyl alcohol and capsaicin as reference standards were procured from Glenmark pharmaceuticals private Ltd., Andheri (E), Mumbai, India(99.7-99.9% purity).

Equipment:

Water alliance-2695 chromatographic system consisting of quaternary pump, PDAdetector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions:

Chromatographic separation was carried out in isocratic mode at room temperature using a symmetry C18 (150x4.6mm, 3.5µm) column. The mixture of buffer (3.0gm Octane-1-Sulphonic acid in 1lt water sonicated to dissolve adjusted the pH-2.5 with OPA): acetonitrile 60:40v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was10µl and eluent was monitored at 273nm using PDA detector. The run time was 12min and each of the studied component was quantified by using total peak area.

Preparation of buffer P^H:

Simple, economical and proper acidic buffer was selected for the estimation of the current drugs in their combined dosage forms. 3.0gm of octane-1-sulphonic acid was weighed accurately and transferred in to 1lt beaker and made up the volume up to the mark with HPLC grade water. The P^H 2.5 was adjusted with ortho phosphoric acid.

Selection of Mobile Phase

The mobile phase was set by injecting different ratios of buffer and acetonitirle. The selected mobile phase ratio was 40:60 v/v of ACN: Buffer. The selected mobile phase has given a sharp peaks with low tailing factor i.e. < 2.0 and also plate count will be more than 3,000.

Selection of wavelength

The absorption spectra of solution of each doxepin, benzyl alcohol and capsaicin were scanned over the range 200-400nm by using photodiode spectrophotometer and the spectra were recorded. Among three drugs combined market formulation, Capsaicin having lowest label claim and shows the maximum absorbance at wavelength 273nm at which the three drugs showed good absorbance. So 273nm was selected as a detection wavelength.

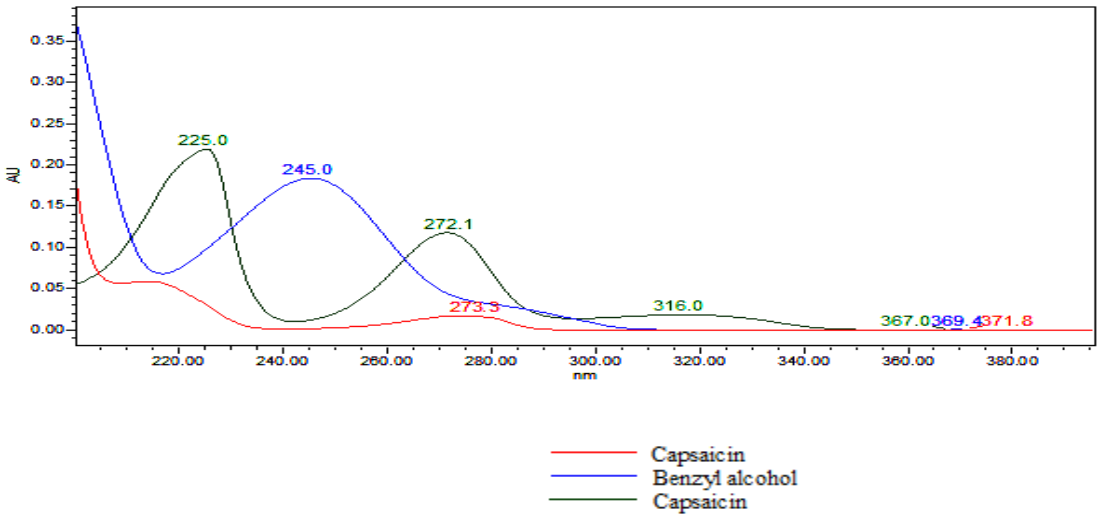


Fig. 4- Combined PDA-Spectra for Doxepin, Benzyl alcohol and Capsaicin

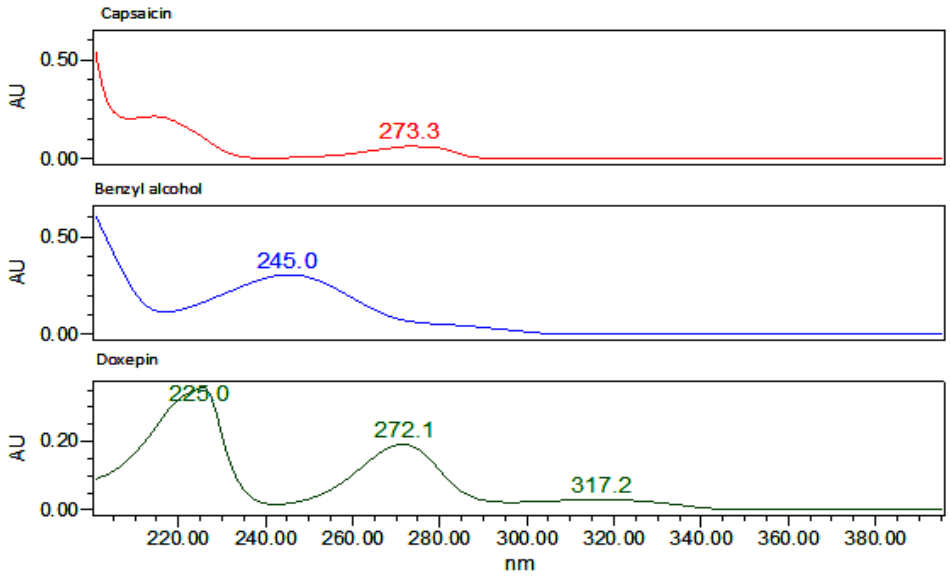


Fig. 5- Individual PDA Spectras for doxepin, benzyl alcohol and capsaicin

Preparation of Diluent:
Diluent was prepared by mixing water and methanol in the ratio of (50:50 v/v).

Preparation of standard stock solution

Standard stock solution-A: About 20 mg of benzyl alcohol and 66mg of doxepin (working standards) were accurately weighed and transferred to a 100ml volumetric flask. Then they were dissolved in 70ml diluent and sonicated for about 10min with intermittent shaking and diluted up to the mark with diluent.
Standard stock solution-B: About 10mg of capsaicin (working standard) was weighed accurately and transferred to a 100ml volumetric flask and dissolved in 70ml diluent and sonicated to dissolve and diluted to volume with the diluent. Further dilute 10ml to 100ml with diluent.

Preparation of standard solution
Each 5ml of standard stock solution-A and 5ml of standard stock solution-B were transferred into 50ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution
Accurately weighed 500mg quantity of sample and was transferred into 100 ml volumetric flask and dissolved in 70 ml of diluent and sonicated to dissolve and diluted up to the mark with the diluent. Then, 10ml of the above solution was diluted with the 50ml diluent and was filtered through 0.45µ nylon syringe filter.

Procedure for Analysis:
A steady baseline was recorded by the optimized chromatographic conditions. It was stabilized for about 30min and successive aliquots of the standard solution of the same concentration were injected and chromatogram was recorded until the reproducibility of the peak areas was satisfactory. This procedure was repeated using the sample solution so that duplicate injection of the sample solution was bracketed by injection of the standard solution. The response factor of the standard peak and sample peak was obtained and the amount of each drug in the sample was determined. This procedure was repeated six times. The concentration of each drug in the triple component dosage form was calculated using the formula,

$$\text{Concentration of drug} = \frac{\text{Response factor of the sample} \times \text{Concentration of standard}}{\text{Response factor of the standard}}$$

Validation Procedure

The analytical method was validated as per ICH Q2(R1) [24] guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), forced degradation and stability.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excepients), which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with benzyl alcohol, capsaicin and doxepin.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision

Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a different range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves. The LOD and LOQ for doxepin, benzyl alcohol and capsaicin were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

Stress degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q2(R1). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flowrate (±0.2 ml/min), wavelength (±5nm), variation in PH (±0.5), organic content in the mobile phase (±2%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

Stability:

Analytical solution was prepared and injecting into the HPLC system at periodic intervals of 0 hour to 24 hours at 6 hour intervals depending on the instrument utilization and sequence of injection.

Results and discussion

The current study was designed to develop a simple, precise, and rapid analytical RP-HPLC method, which can be used for the analysis of assay method for simultaneous estimation of doxepin, benzyl alcohol and capsaicin in bulk and pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. To optimize mobile phase, various combinations of buffer (3.0 gm octane-1-sulphonic acid in 1lt water adjust pH-2.5 with OPA): acetonitrile were tried for doxepin, benzyl alcohol and capsaicin, and the final working mobile phase is octane-1-sulphonic acid buffer and acetonitrile in composition of 60:40v/v. Mobile phase for each drug was selected based on its polarity. Detection was carried out in several wavelengths in order to obtain enough sensitivity for the three APIs in smaller proportion (doxepin, benzyl alcohol and capsaicin). At last, the wavelength 273nm, at which the three drugs showed good absorbance, was selected as a detection wavelength. The flow rate was 1.0ml/min, which is critical as it affects the peak symmetry parameters. The retention time for capsaicin, doxepin and benzyl alcohol were 3.57, 6.10 and 9.10 minutes respectively. The proposed method is validated in accordance with the ICH guidelines with all of the results within the limits. In this method a good linearity was observed in the range of 6.6-99 µg/ml, 2-30 µg/ml and 0.1-1.5µg/ml with the limit of detection (s/n=5,4and7) 0.06µg/ml, 0.02µg/ml and 0.001µg/ml for doxepin, benzyl alcohol and capsaicin respectively.

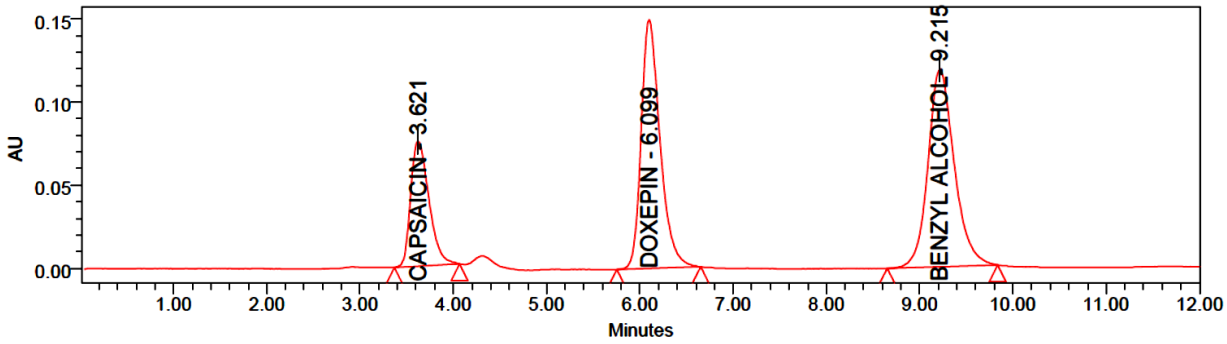


Fig. 6-Typical Chromatogram for doxepin, benzyl alcohol and capsaicin

Method validation tests

System Suitability

The HPLC system was stabilized for 60min to get a stable base line. Six replicate injections of the mixture containing 66 µg/ml of doxepin, 20 µg/ml of benzyl alcohol and 1µg/ml of capsaicin were assessed to check the system suitability. The system suitability parameters were evaluated from six replicate injections. The study concludes that the suitability of the HPLC system being used and results were summarized below.

Table 1: System suitability data

System suitability Parameter	Acceptance criteria	Drug Name		
		Doxepin	Benzyl Alcohol	Capsaicin
% RSD	NMT 2.0	0.5	0.2	0.2
USP Tailing	NMT 2.0	1.4	1.2	1.4
USP Plate count	NLT 3000	4558	5348	4254

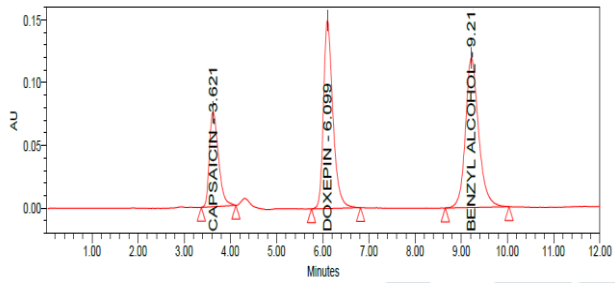


Fig 7: Chromatogram for System suitability-1

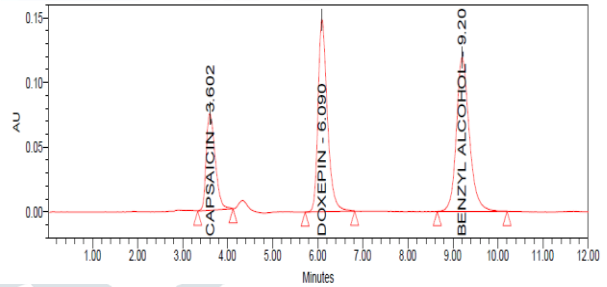


Fig 8: Chromatogram for System suitability-2

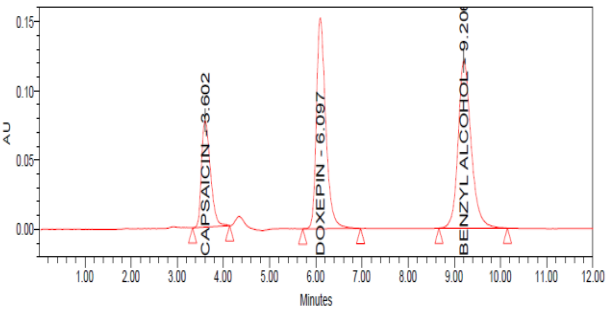


Fig 9: Chromatogram for System suitability-3

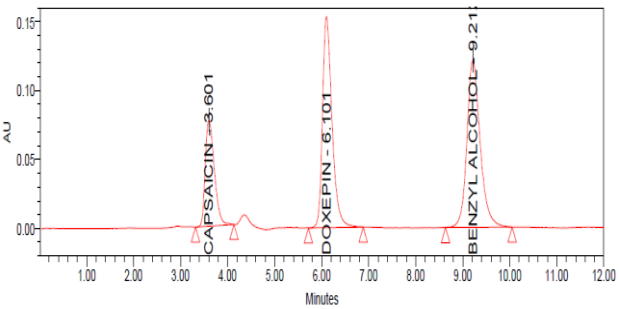


Fig 10: Chromatogram for System suitability-4

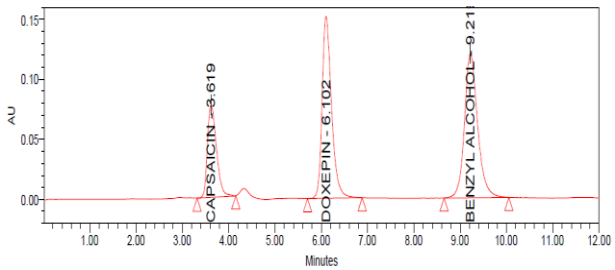


Fig 11: Chromatogram for System suitability-5

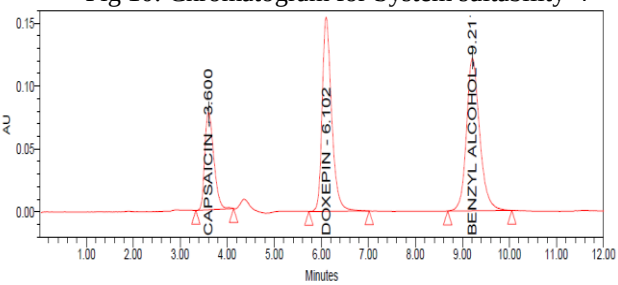


Fig 12: Chromatogram for System suitability-6

Specificity

There was no interference from blank at the retention time of doxepin, benzyl alcohol and capsaicin. Hence the method is specific.

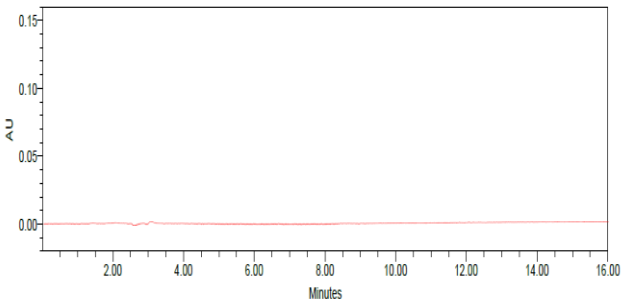


Fig 13: Chromatogram for Blank

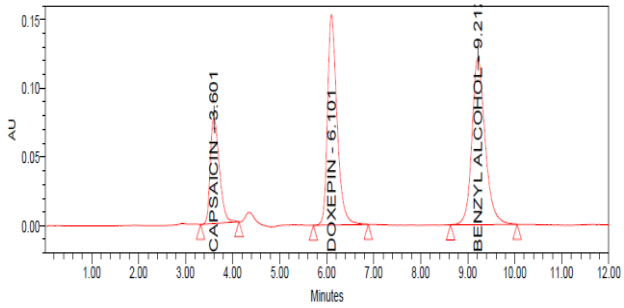


Fig 14: Typical Chromatogram of Standard solution

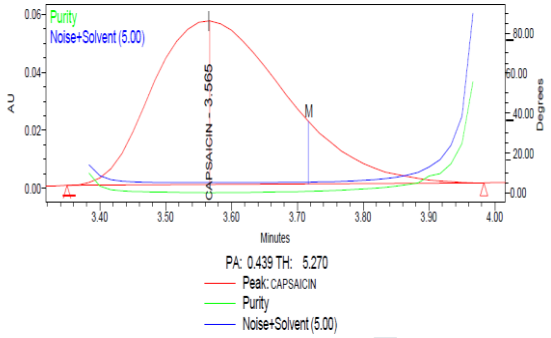


Fig 15: Peak Purity of Capasaicin Standard

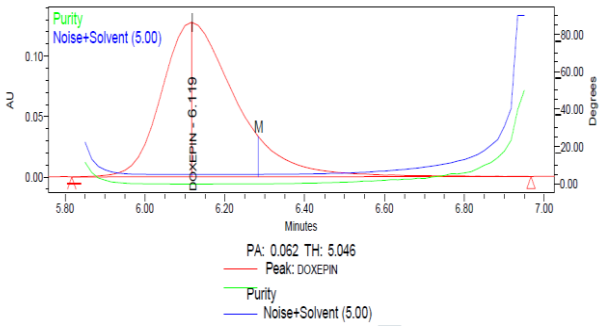


Fig 16: Peak Purity of Doxepin Standard

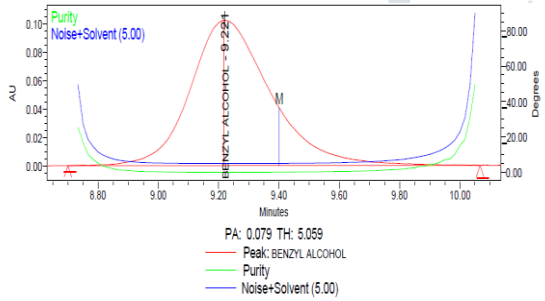


Fig 17: Peak Purity of Benzyl alcohol Standard

Placebo interference:

1ml of the placebo solution was injected into HPLC system and no peaks were observed while running the solution which proves that the method is specific. The chromatogram of the standard and sample were identical.

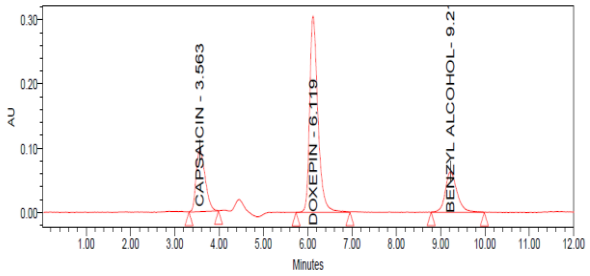


Fig 18: Typical Chromatogram of Sample

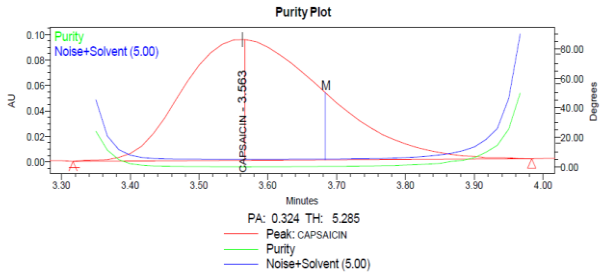


Fig 19: Peak Purity of Capasaicin Sample

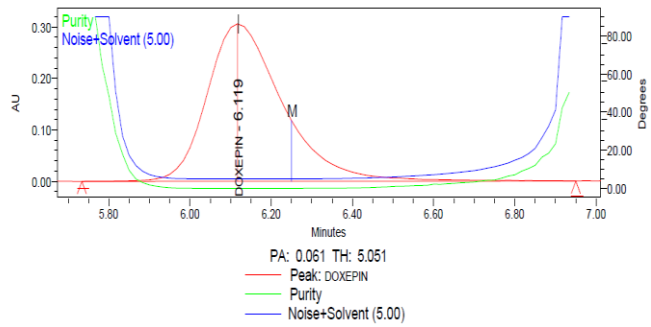


Fig 20: Peak Purity of Doxepin Sample

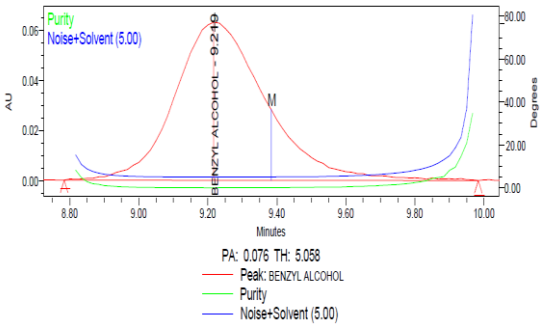


Fig 21: Peak Purity of Benzyl alcohol Sample

Linearity

Linearity was determined by plotting a calibration curve of peak area against their respective concentration. From this calibration curve it was found that the curve was linear in the range of

6.6-99 µg/ml for doxepin, 2-30 µg/ml for benzyl alcohol and 0.1-1.5 µg/ml for capsaicin. The regression equations for calibrations curve was y=32164x+8731.4(R2=0.9999) for doxepin, y=90814x+14982(R2=0.9991) for benzyl alcohol and y=90814x+14982(R2=0.9991) for capsaicin respectively.

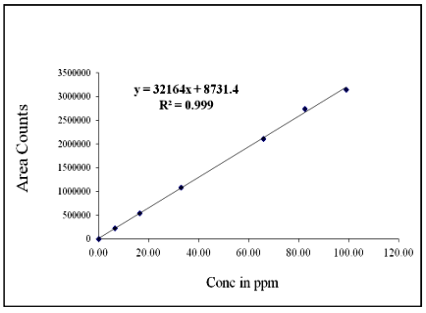


Fig22. Linearity plot for Doxepin

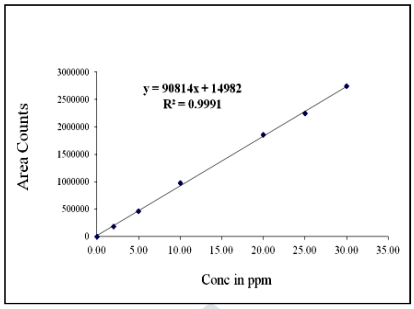


Fig. 23- Linearity plot for Benzyl alcohol

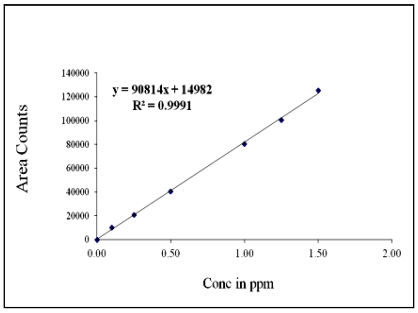


Fig. 24- Linearity plot for Capsaicin

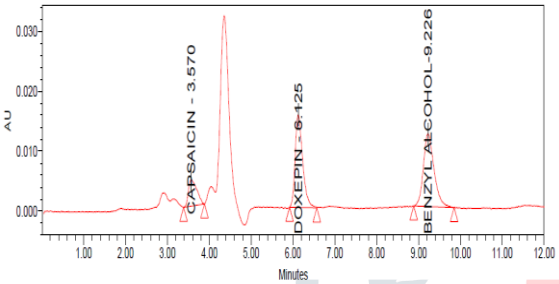


Fig 25: Chromatogram for Linearity-1

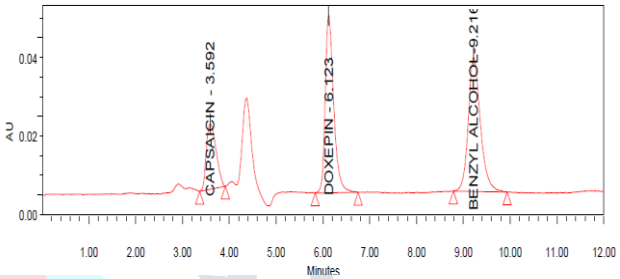


Fig 26: Chromatogram for Linearity-2

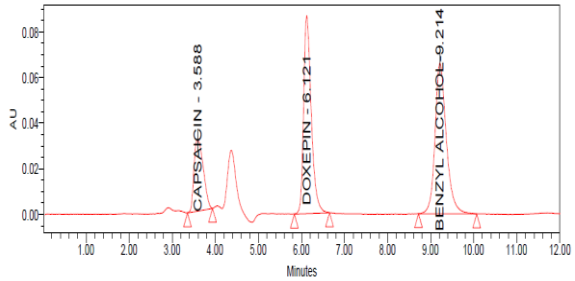


Fig 27: Chromatogram for Linearity-3

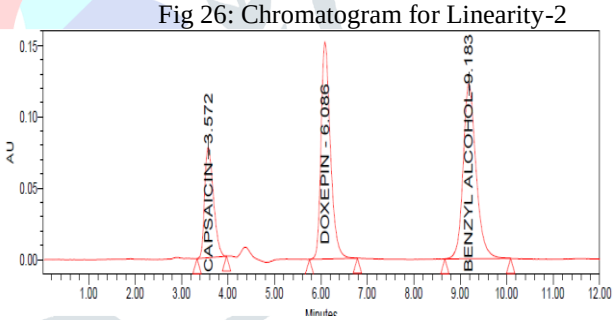


Fig 28: Chromatogram for Linearity-4

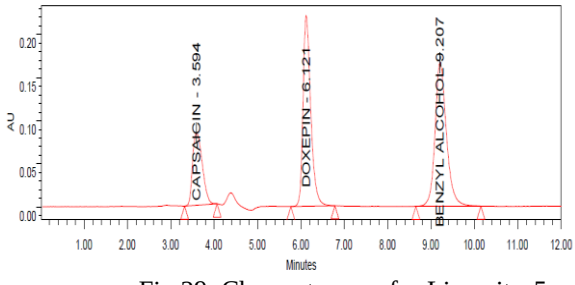


Fig 29: Chromatogram for Linearity-5

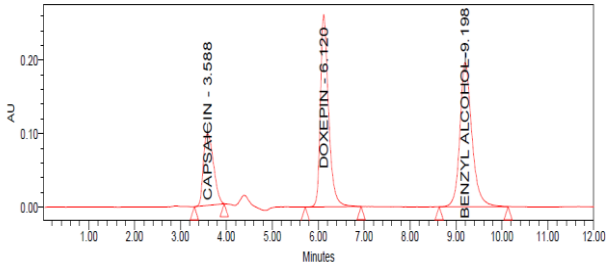


Fig 30: Chromatogram for Linearity-6

Accuracy

The accuracy of the method was performed by calculating the recovery experiments at three levels (50%, 100% and 120%). APIs with concentration 33, 66 and 99µg/ml of doxepin; 10, 20 and 30µg/ml of benzyl alcohol; and 0.5, 1 and 1.5µg/ml of capsaicin were prepared. The test solution was injected three times for each spike level and assay was performed as per the test method. The recovery results were close to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviation were calculated. Recovery values demonstrated that the method was accurate within the desired range. The results are summarized below.

Accuracy	Amount of Doxepin drug µg/ml	Recovery Solution (area) mAU	% drug Recovery
50%	33	1064058	100.1
100%	66	2160136	100.8
150%	99	3235394	100.3

Table 2 : Accuracy data for Doxepin

Accuracy	Amount of Benzyl alcohol drug µg/ml	Recovery Solution (area) mAU	% drug recovery
50%	10	1202107	100.3
100%	20	2380105	100.2
150%	30	3575349	100.1

Table 3 : Accuracy data for Benzyl alcohol

Accuracy	Amount of Capsaicin drug µg/ml	Recovery Solution (area) mAU	% drug recovery
50%	0.5	476040	100.5
100%	1.0	1027641	100.4
150%	1.5	1523767	100.7

Table 4: Accuracy data for Capsaicin

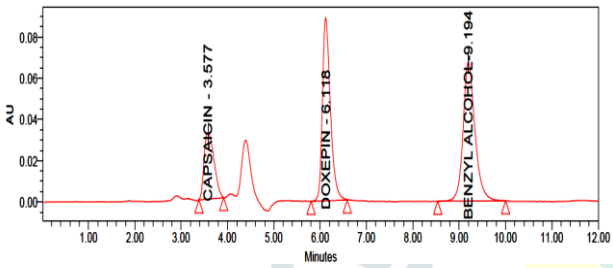


Fig.31-Chromatogram for Accuracy 50%

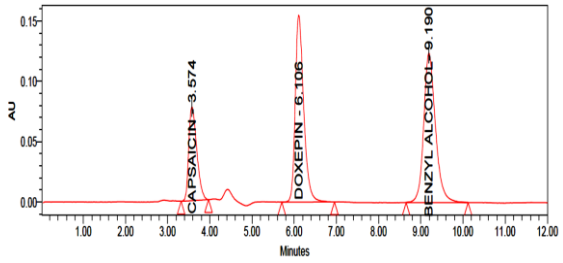


Fig.32-Chromatogram for Accuracy 100%

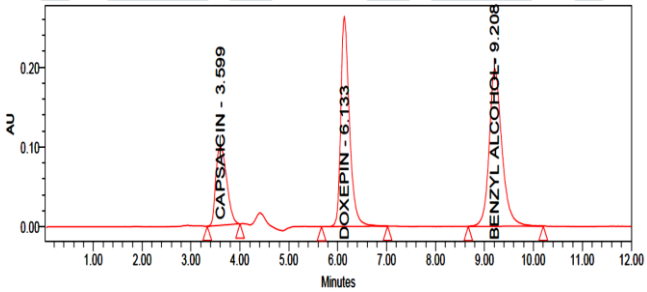


Fig.33-Chromatogram for Accuracy 150%

Precision

Precision of this method was assesed interms of intraday (repeatability) and interday (intermediate precision) variations. The intraday studies were determined by performing six repeated analysis of the sample solution of doxepin, benzyl alcohol and capsaicin on the same day under the same experimental conditions. The intermediate precision of the method was carried out in the same laboratory by studying the analysis with different analyst and different instrument. The method is highly precise as % RSD values were found to be < 2%. Good recoveries (98 – 100%) of the drug were obtained at each added concentration, indicating that the method was accurate. The results and chromatograms were furnished below.

Concentration of Doxepin drug µg/ml	Area mAU	Relative standard deviation (RSD)
66	***59463***	***0.59***
	63353	
	57591	
	61937	
	37014	
	35682	

Table 5 Method Precision data for Doxepin

Concentration of benzylalcohol drug µg/ml	Area mAU	Relative standard deviation (RSD)
20	***2389566***	***1.01***
	2386570	
	2364180	
	2392411	
	2375350	
	2370095	

Table 6 Method Precision data for Benzyl alcohol

Concentration of Capsaicin drug µg/ml	Area mAU	Relative standard deviation (RSD)
1.0	***1028900***	***0.28***
	1027076	
	1026946	
	1027992	
	1023059	
	1020626	

Table 7 Method Precision. data for Capsaicin

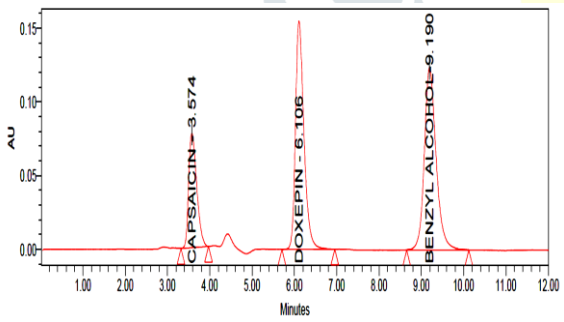


Fig.34-Chromatogram for Method Precision-1

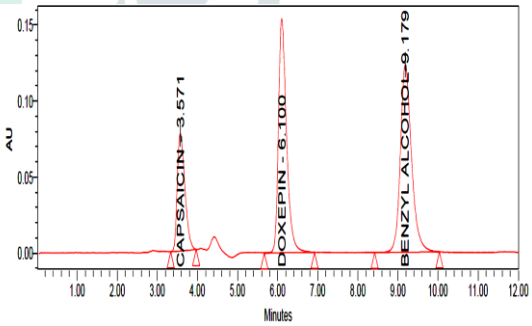


Fig.35-Chromatogram for Method Precision-2

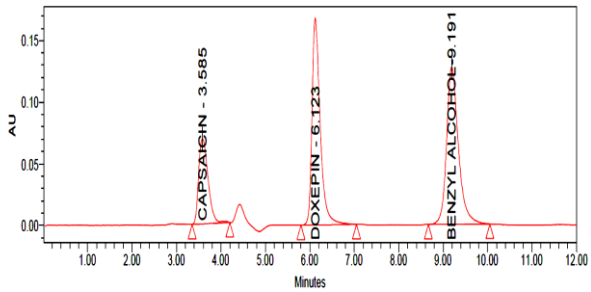


Fig.36-Chromatogram for Method Precision-3

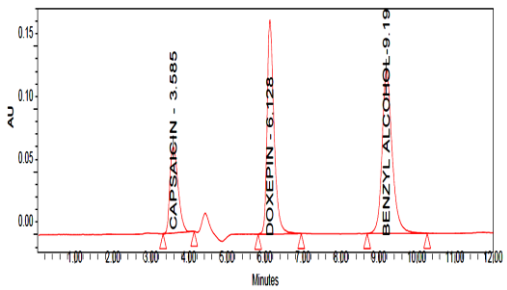
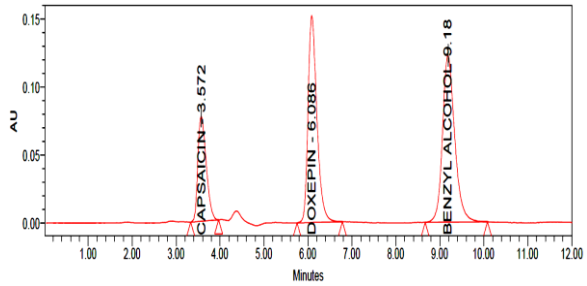


Fig.37-Chromatogram for Method Precision-4



Chromatogram for Method Precision-5

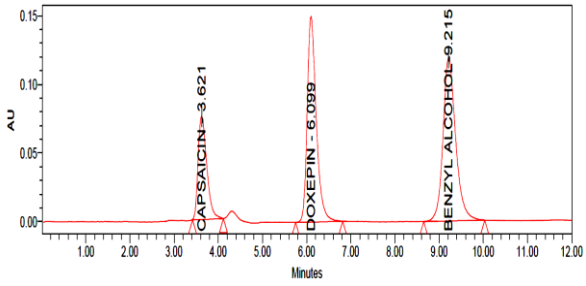


Fig.39- Chromatogram for Method Precision-6

Fig.38-

Concentration of Doxepin drug µg/ml	Area mAU	Relative standard deviation (RSD)
66	***59463***	***0.59***
	63353	
	57591	
	61937	
	37014	
	35682	

Table 8 Intermediate Precision data for doxepin

Concentration of benzylalcohol drug µg/ml	Area mAU	Relative standard deviation (RSD)
20	***2389566***	***0.48***
	2386570	
	2364180	
	2392411	
	2375350	
	2370095	

Table 9 Intermediate Precision data for benzyl alcohol

Concentration of Capsaicin drug µg/ml	Area mAU	Relative standard deviation (RSD)
1.0	***1028900***	***1.00***
	1027076	
	1005445	
	1019044	
	1023059	
	1035146	

Table 10 Intermediate Precision. data for capsaicin

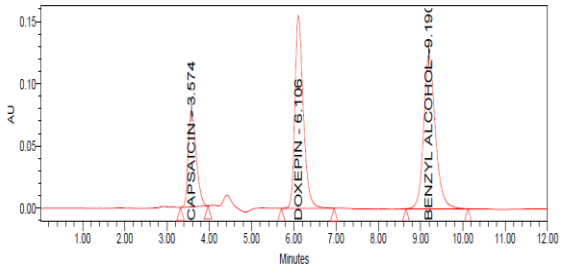


Fig.40-Chromatogram for Intermediate Precision-1

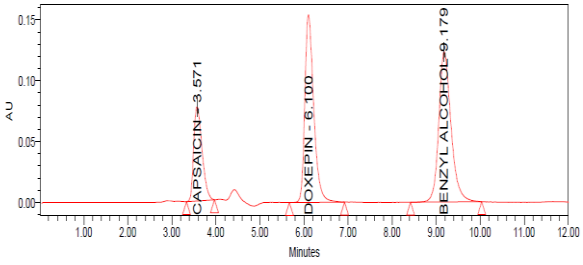


Fig.41-Chromatogram for Intermediate Precision-2

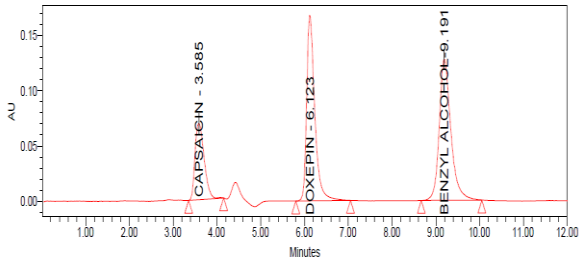


Fig.42-Chromatogram for Intermediate Precision-3

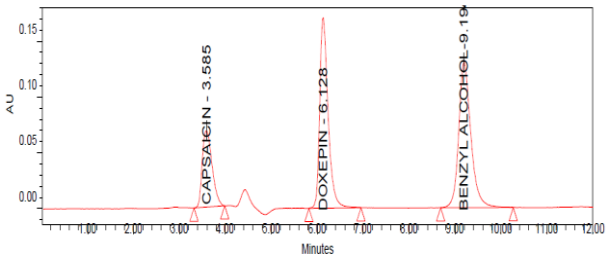


Fig.43-Chromatogram for Intermediate Precision-4

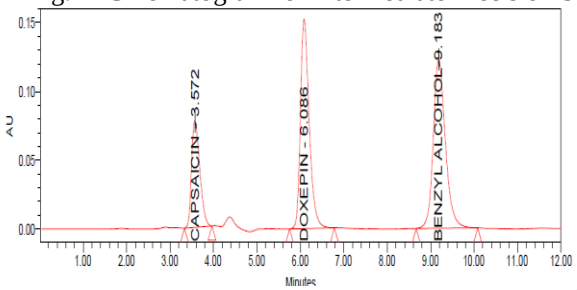


Fig.44-Chromatogram for Intermediate Precision-5

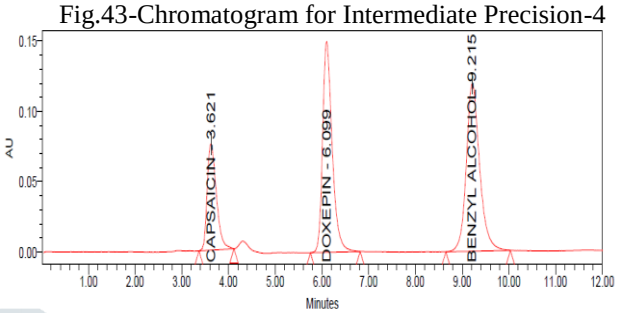


Fig.45-Chromatogram for Intermediate Precision-6

LOD & LOQ

LOD and LOQ were separately determined by calibration curve method [25]. LOD and LOQ of the compounds were determined by injecting progressively lower concentrations of standard solutions using the developed RP-HPLC method. The LOD concentrations for doxepin, benzyl alcohol and capsaicin were found 0.06µg/ml, 0.02µg/ml and 0.001µg/ml respectively and their s/n values are 3, 5 and 7. The LOQ concentrations were found to be 0.66µg/ml, 0.20µg/ml and 0.01µg/ml for doxepin, benzyl alcohol and capsaicin respectively and their s/n values are 23, 25 and 28.

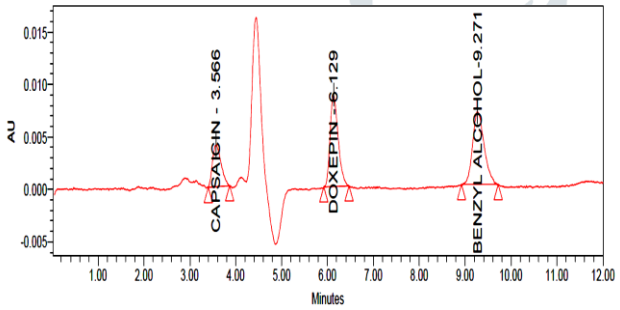


Fig.46-Chromatogram for LOD

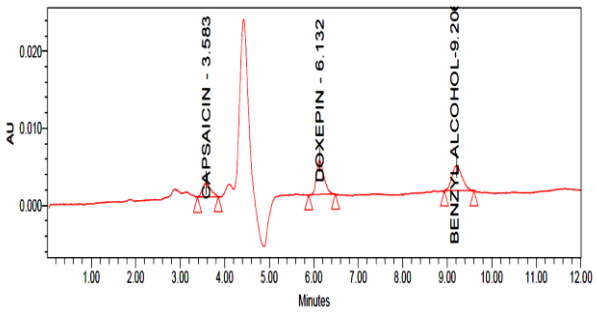


Fig47-Chromatogram for LOQ

Forced Degradation

The proposed analytical method can be used for release and stability studies for effective evaluations and can be considered as stability indicating method. The forced degradation study was carried out according to the ICH requirements include acid (0.2N, 0.1N and 0.05N HCl), base (0.2N, 0.1N and 0.05N NaOH), hydrogen peroxide, reduction, thermal and photolytic degradation. From the chromatograms, it is evident that the selected drugs were stable under the applied stress conditions though the degraded peaks were observed. Results were shown in table.

Stress condition/duration/solution	Degradation
Acid degradation (0.2N,0.1N and 0.05 N HCl, 1 hr)	22%
Alkaline degradation (0.2N,0.1N and 0.05 N NaOH, 1 hr)	24%
Oxidative degradation (5 % H_2O_2,80°C for 30 min)	26%
Reduction degradation (10 % $NaHSO_4$,80°C for 15 min)	23%
Thermal degradation (Solid sample, 80°C, 3 hr)	28%
Photolytic Degradation (sample expose sun light 6 hr)	25%

Table 11: Results of Forced degradation studies

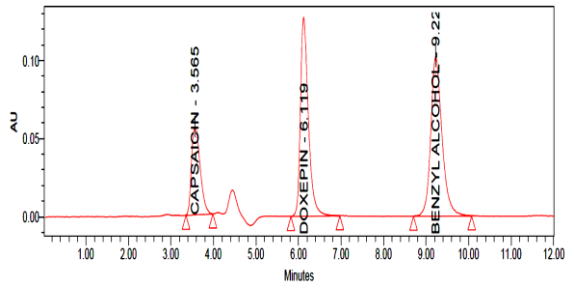


Fig48-Chromatogram for Acid Degradation

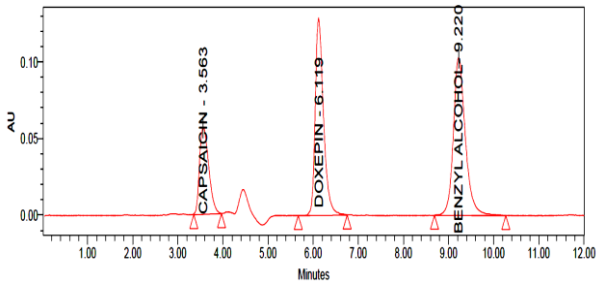


Fig.49-Chromatogram for Alkali Degradation

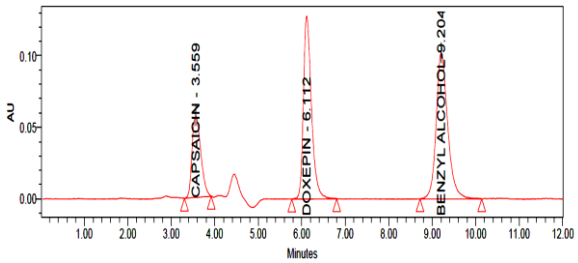


Fig.50-Chromatogram for Peroxide Degradation

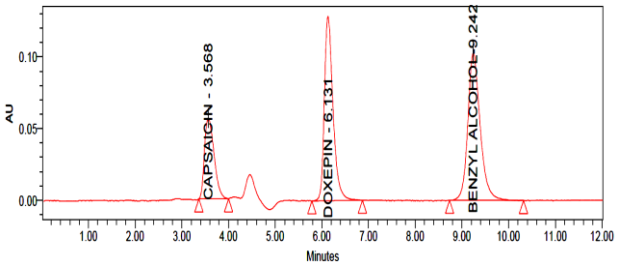


Fig.51-Chromatogram for Reduction Degradation

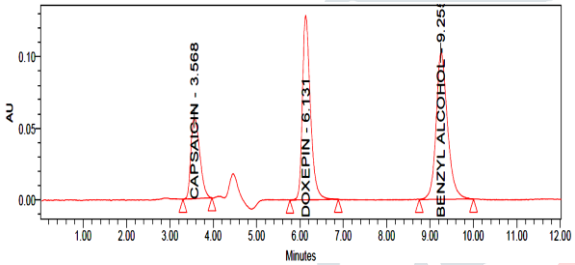


Fig.52-Chromatogram for Thermal Degradation

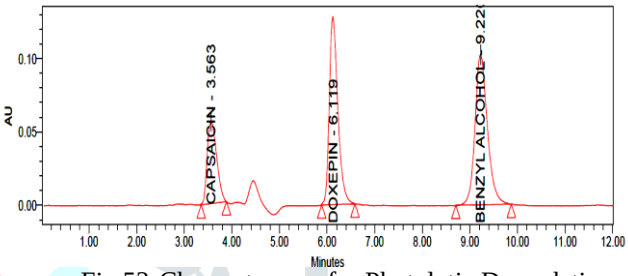


Fig.53-Chromatogram for Photolytic Degradation

Robustness

As per ICH norms, small but deliberate variations were made in the method parameters such as change in the flow rate (±0.2), organic content in the mobile phase (±2%), wavelength of detection (±5) and pH (±0.5) to check the method capacity to remain unaffected. The robustness of the method was evaluated by observing the effect of the modified parameters on retention time, tailing factor, area, percentage content. The degree of reproducibility of the results which were obtained by small deliberate variations has proven that the method is robust.

Change in parameter	**% RSD for** Doxepin	**% RSD for** Benzyl alcohol	**% RSD for** Capsaicin
Flow (0.8 ml/min)	0.42	0.91	0.31
Flow (1.2 ml/min)	0.10	0.26	0.45
Organic phase composition (+2%)	0.08	0.18	0.17
Organic phase composition (-2%)	0.15	0.26	0.21
Wavelength (278 nm)	0.15	0.31	0.29
Wavelength (268 nm)	0.46	0.29	0.36
pH of the Buffer (+0.5)	0.28	0.45	0.51
pH of the Buffer (-0.5)	0.32	0.48	0.38SS

Table 12: Results of Robustness studies

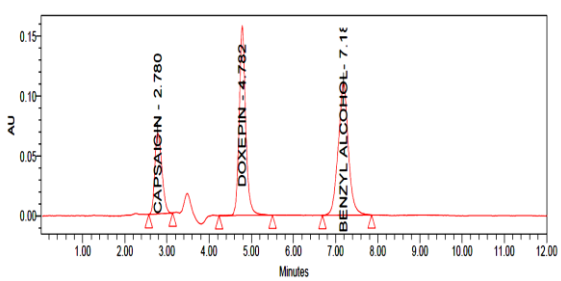


Fig. 54-Chromatogram for Flow Plus

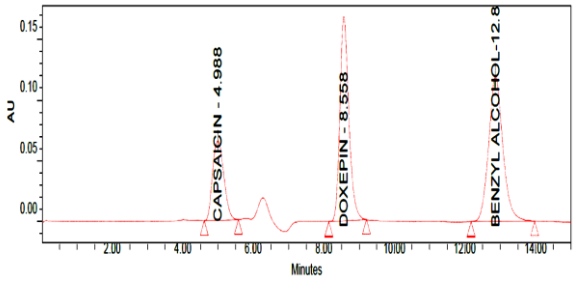


Fig.55-Chromatogram for Flow Minus

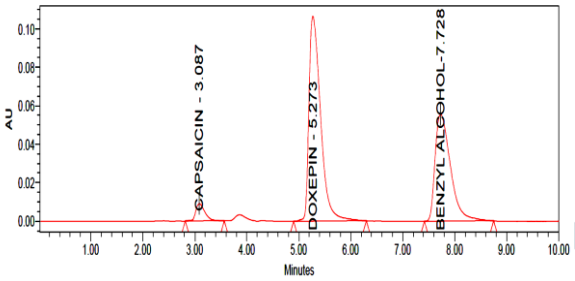


Fig.56-Chromatogram for Organic Plus

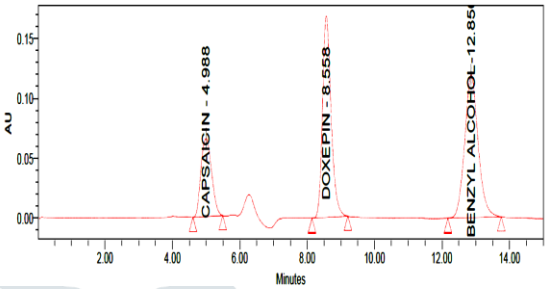


Fig.57-Chromatogram for Organic Minus

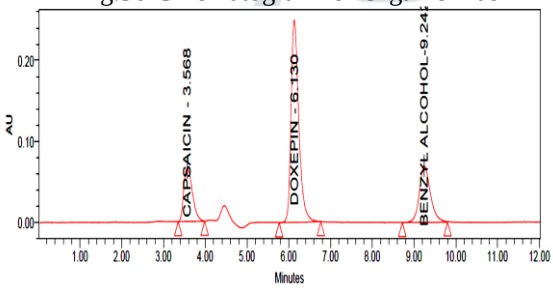


Fig.58-Chromatogram for Wavelength Plus

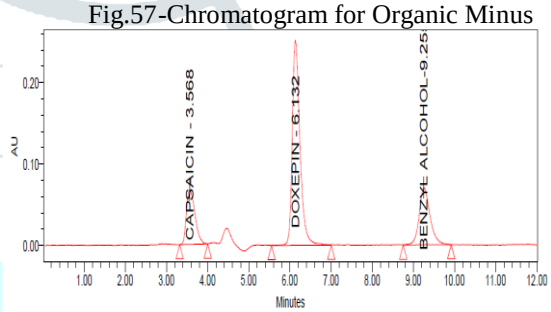


Fig.59-Chromatogram for Wavelength Minus

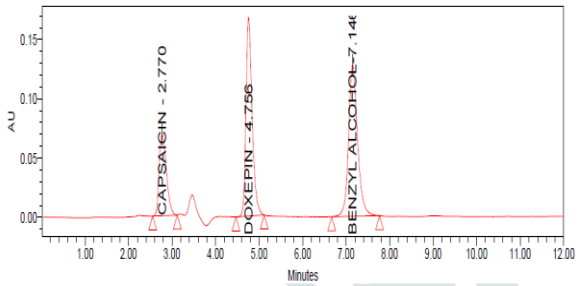


Fig.60-Chromatogram for pH Plus

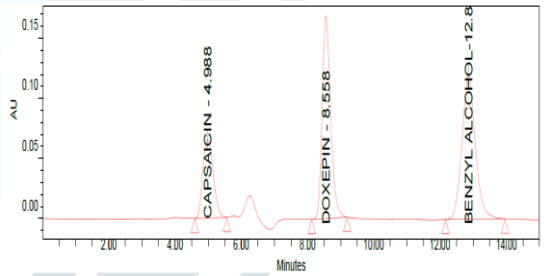


Fig.61-Chromatogram for pH Minus

Stability

To assess the stability of sample solutions, they were analysed initially to 24hr at different intervals of time at room temperature. No significant degradation was observed during this period and the % deviation was not more/less than 5.0%, suggesting that the solutions were stable for at least 24 hr, which was sufficient for the whole analytical procedure. Results are furnished below.

Stability	**% Label Claim** Doxepin	**% Deviation** Doxepin	**% Label Claim** Benzyl alcohol	**% Deviation** Benzyl alcohol	**% Label Claim** Capsaicin	**% Deviation** Capsaicin
Initial	100.8	0.0	100.5	0.0	100.1	0.0
6 Hr	100.3	0.5	100.1	0.4	100.5	-0.4
12 Hr	100.1	0.7	100.3	0.2	99.9	-0.2
18 Hr	100.4	0.4	100.6	-0.1	100.3	-0.2
24 Hr	100.5	1.3	100.8	-0.3	100.8	-0.7

Table 13:Results of Stability studies

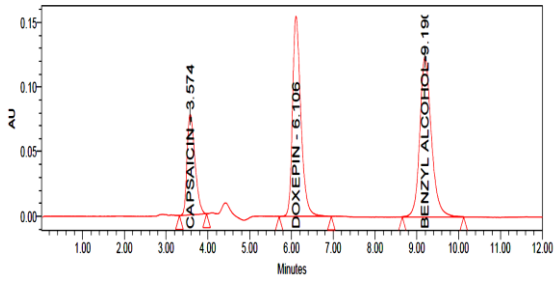


Fig.62-Chromatogram for Stability Initial

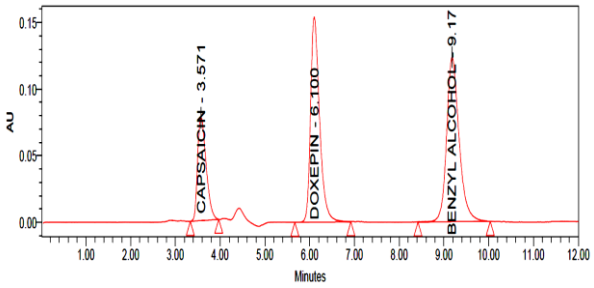


Fig.63-Chromatogram for Stability 6hr

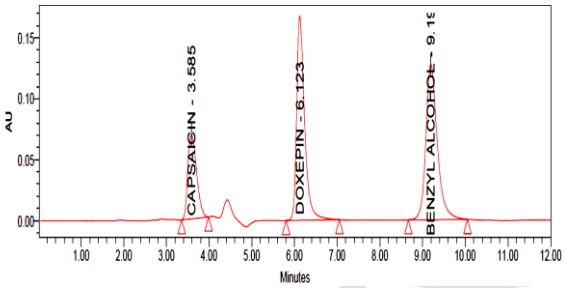


Fig.64-Chromatogram for 12hr

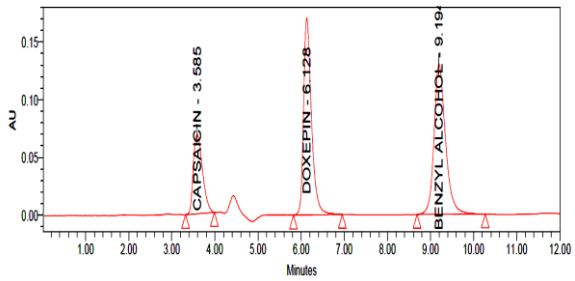


Fig.65-Chromatogram for Stability 18hr

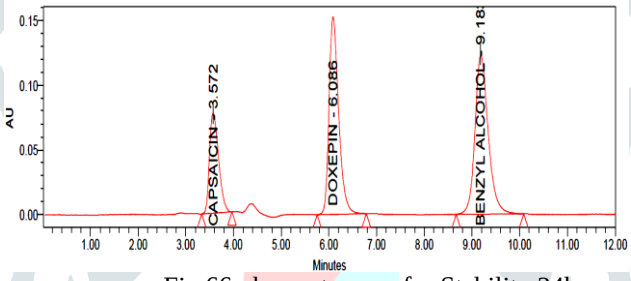


Fig.66-chromatogram for Stability 24hsr

Conclusion

In this study a novel, simple, rapid, economical, sensitive and easily available HPLC method was developed for the simultaneous determination of doxepin, benzyl alcohol and capsaicin in bulk and tablet dosage form. The main advantages of this method over the previously reported HPLC methods are its availability, shorter run times, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a large number of samples are to be analysed. The validation of all the parameters like linearity, accuracy, specificity, robustness, stability was done and found to be within the acceptance criteria. The RSD values for all parameters were found to be less than 2, which indicates the validity of method and results obtained by this method are in fair agreement. So the proposed method could be easily applied for the routine analysis and pharmaceutical formulations of doxepin, benzyl alcohol and capsaicin in quality control laboratories without any preliminary separation.

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