

## STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF DIETHYLCARBAMAZINE CITRATE, GUAIPHENESIN AND CHLORPHENIRAMINE MALEATE

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### ABSTRACT

**Objective:** The present work describes the development and subsequent validation of a simple, precise and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate in tablet dosage forms.

**Methods:** A simple, accurate, precise and robust RP-HPLC method was developed and validated for the estimation of diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate. The chromatographic separation of all the three active components was achieved by using luna phenyl-hexyl column (250 mmx4.6 mm, dp=5 μm) with a mobile phase consisting of isocratic method with 0.1% triethylamine as buffer along with orthophosphoric acid adjusted to P<sup>H</sup> 2.5: acetonitrile (50:50v/v) at a flow rate 1.0 ml/min and ultraviolet detection at 210 nm.

**Results:** The retention time of chlorpheniramine maleate, guaiphenesin and diethylcarbamazine citrate were 2.86, 4.89 and 7.76 min respectively. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. The established method was linear in the range of 1-15, 0.6-9, 0.02-0.3 μg/ml and correlation coefficient was 0.999, 0.9991, and 0.993 for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate respectively.

**Conclusion:** The proposed method can be used for the quantitative analysis of diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate.

**Keywords:** Diethylcarbamazine citrate, Guaiphenesin and Chlorpheniramine maleate

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### INTRODUCTION

Diethylcarbamazine citrate, chemically *N, N*-diethyl-4-methyl piperazine-1-carboxamide dihydrogen citrate [1] is one of the essential medicines needed in a basic health system, suggested by world health organisation (WHO) [2]. It is used in the treatment of filariasis including lymphatic filariasis, tropical pulmonary eosinophilia and loiasis.

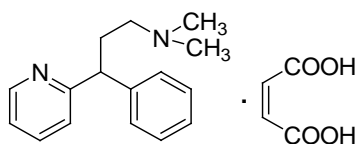


Fig. 1: Chemical structure of diethylcarbamazine citrate

Guaiphenesin, chemically (*S, S*)-2-methylamino-1-phenylpropan-1-ol hydrochloride [3, 4], mainly used as a cough remedy. It has been given to patients which have altered nasal mucociliary clearance associated with HIV. It is used to remove phlegm from the airways in acute respiratory tract infections.

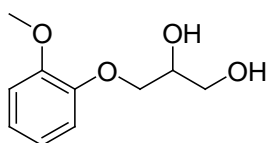


Fig. 2: Chemical structure of guaiphenesin

As chlorpheniramine maleate, chemically (RS)-3-(4-chlorophenyl)-3-(pyridin-2-yl) propyl dimethylamine hydrogen maleate [5], has the

relatively less sedative effect it is most commonly used as an antihistamine in small animal veterinary practices.

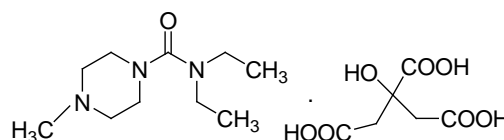


Fig. 3: Chemical structure of chlorpheniramine maleate

The literature survey revealed that several analytical methods have been reported for the estimation of diethylcarbamazine citrate [6], guaiphenesin [7] and chlorpheniramine maleate [8, 9] individually or in combination with other drugs by UV-visible spectrophotometry, nuclear magnetic resonance spectroscopy, high-performance liquid chromatography methods [10-13]. No method has been developed for the simultaneous determination of diethylcarbamazine citrate, guaiphenesin, chlorpheniramine maleate both in bulk and pharmaceutical dosage forms.

On the meticulous observance of the potential applications of these three active drugs, we aimed to develop and validate a new, rapid and sensitive RP-HPLC method for simultaneous estimation of diethylcarbamazine citrate, guaiphenesin, and chlorpheniramine maleate. Degradation studies (stress studies) were carried out to establish the stability characteristics of the three ingredients under heat, acid, base, peroxide, light and reductive stress conditions as recommended in the ICH guidelines Q1A (R2).

### MATERIALS AND METHODS

#### Instrumentation

The analysis was performed on waters alliance-2695 chromatographic system, equipped with a quaternary pump and PDA

detector-2996. Chromatographic software empower-2.0 was used for data collection and processing.

#### Chemicals and reagents

Acetonitrile (HPLC grade), triethylamine (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) were purchased from Merk (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate as reference standards were procured from Supriya life Sciences, Goregaon (E), Mumbai, India (99.7-99.9% purity).

#### Chromatographic conditions

Chromatographic analysis was done using isocratic elution and acetonitrile: 0.1% triethylamine  $P^H$  adjusted to 2.5 with OPA (50:50 by volume) as a mobile phase and was filtered through 0.45 $\mu$

membrane filter paper. The flow rate of the mobile phase was monitored at 1 ml/min and eluents were detected at 210 nm. Operating pressure 3000 psi was maintained at room temperature by injecting the volume 10  $\mu$ l with a run time 10 min.

#### Selection of wavelength

By using photodiode spectrophotometer the absorption spectra of the solution of the three drugs in acetonitrile were scanned in the UV region 200-400 nm against acetonitrile as blank and spectra are shown in fig. From the fig. the spectra of the diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate shows different  $\lambda_{max}$  viz. 254.5, 255.6 and 369.4 nm respectively. By considering the chromatographic parameter, sensitivity and selectivity of a method for three drugs 210 nm was selected as the detection wavelength for HPLC chromatographic method.

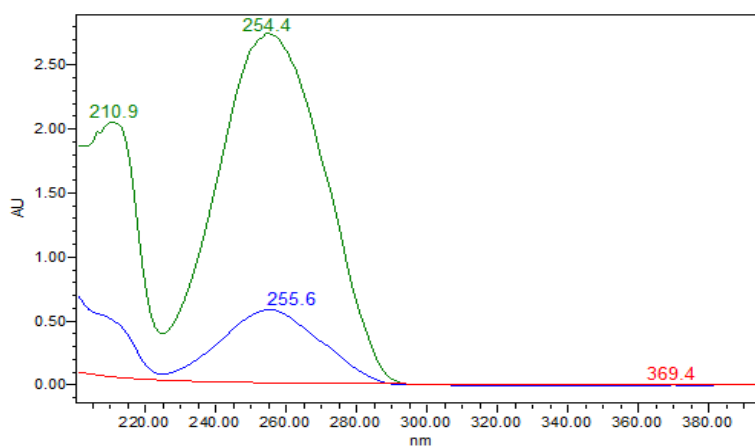


Fig. 4: PDA spectrum for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate

#### Preparation of standard solution

100 mg of diethylcarbamazine citrate, 60 mg of guaiphenesin, 2 mg of Chlorpheniramine maleate (working standard) were weighed accurately and transferred into a 100 ml volumetric flask. 70 ml of mobile phase was added to the above flask and then sonicated about 20 min for uniform mixing and then diluted 1 ml of the above solution to 10 ml with the mobile phase and again 1 ml of the solution was diluted to 10 ml with same mobile phase.

#### Preparation of sample solution

10 tablets were weighed and pulverised to powder form, from which one equivalent weight (437.5 mg) was taken into 100 ml volumetric flask. 70 ml of mobile phase was added to the above flask and then sonicated about 20 min for uniform mixing. 1 ml of the above

solution was diluted to 100 ml with the mobile phase and filtered through 0.45 $\mu$  nylon syringe filter.

#### Validation

The optimized chromatographic separation was aimed to obtain a resolution above 1.5 between all components, tailing factor is less than 2.0 and plate count will be more than 2000 with respect to the stationary, mobile phase compositions, flow rate, sample volume, detection wavelength and temperature.

#### Validation procedure

In the present method validation was done with the aspect of system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), forced degradation and stability according to the ICH guidelines [14-18].

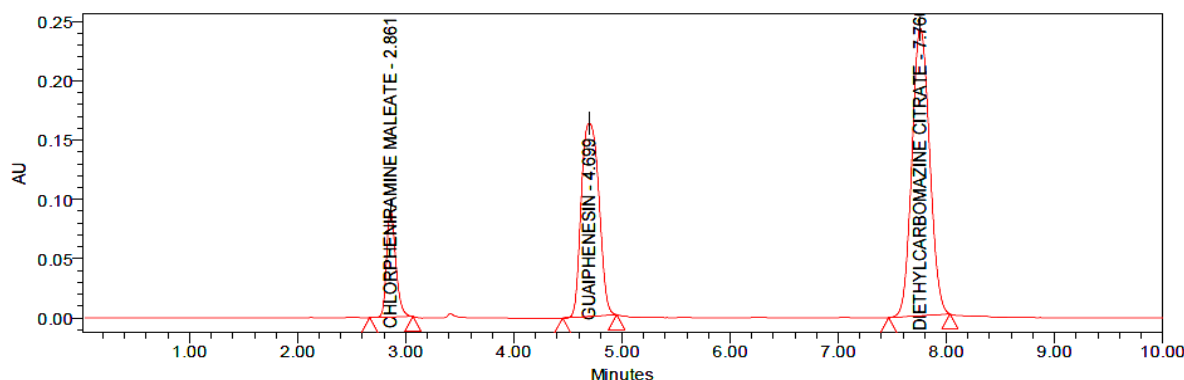


Fig. 5: Typical chromatogram for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate

### System suitability

As per the test method the standard and check standard solutions were prepared and injected into HPLC system [10, 11], from which the evaluated system suitability parameters are found to be within the limits.

### Specificity

The analyte was assessed unequivocally to know the components impurity which may be expected to be present with the help of specificity. As per test method blank was prepared and injected. No blank peak was eluted in the retention time of analyte peak. Placebo solutions were prepared in duplicate and injected as per test method. It was found that no placebo peaks were interfered at the retention time of the main peak.

### Accuracy

Three different concentrations such as lower quantitation limit, medium quantitation limit, and higher quantitation limit were used to evaluate the accuracy of the RP-HPLC method. The amount of the drugs present, percentage recovery and RSD were calculated by giving a minimum of three injections from each concentration.

### Precision

The precision of the test method was evaluated by considering six different concentrations. The amount of the drugs present, percentage recovery and RSD were calculated by giving a minimum of six preparations.

### Linearity and range

Six series of standard solutions were selected for assessing linearity range. By using peak area versus concentration of the standard solution calibration curve was plotted and the regression equations were also calculated. The slope, intercept and the correlation coefficient was calculated by least squares method.

### LOD and LOQ

By using optimized chromatographic conditions in accordance with 3.3 s/n and 10 s/n criteria, where s/n indicates signal-to-noise ratio,

the LOD and LOQ were determined by injecting progressively lower concentrations of the standard solutions into the HPLC column.

### Forced degradation

In chromatogram of forced degradation there should be no interference between peaks and were well separated from each other with the resolution at least 1.0 and the peak purity of the principal peaks should pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

### Robustness

Small changes such as  $\pm 5\%$  in the ratio of acetonitrile in the mobile phase,  $\pm 0.2$  ml/min in the flow rate and  $\pm 5$  nm in the wavelength were made to demonstrate the robustness method. The separation factor, retention time and peak asymmetry were calculated.

### Stability

Standard and the sample solutions were subjected to 24 h stability studies. The stability of these solutions was studied and observed for changes in the area and retention time of the peaks which were then compared with the pattern of the chromatogram of the freshly prepared solution.

## RESULTS AND DISCUSSION

### Method validation

In this method system suitability, linearity, precision, accuracy, robustness, LOD (Limit detection), LOQ (Limit of quantification), forced degradation and the stability are validated for the selected diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate drugs.

### System suitability

10  $\mu$ l of working standard solution (10  $\mu$ g/ml of diethylcarbamazine citrate, 6  $\mu$ g/ml of guaiphenesin and 0.2  $\mu$ g/ml of chlorpheniramine maleate) was prepared and injected into the system. It was determined by making six replicate injections and all the parameters were found to be within the limits. The results are given table 1.

**Table 1: System suitability parameters for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate**

| System suitability parameter | Diethylcarbamazine citrate | Guaiphenesin | Chlorpheniramine maleate |
|------------------------------|----------------------------|--------------|--------------------------|
| Retention time (min)         | 7.850                      | 4.715        | 2.900                    |
| Theoretical plate number (N) | 10909                      | 4416         | 5810                     |
| Tailing factor (T)           | 1.062                      | 1.145        | 1.271                    |
| Resolution (R)               | 10.693                     | 8.386        | -                        |

### Linearity

The linearity of the proposed method was constructed by considering concentration on the x-axis and peak area on the y-axis. It was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear in the range of 1-

15, 0.6-9, 0.02-0.3  $\mu$ g/ml for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate respectively. The regression equation for calibration curve was  $Y=561967x+13655$  ( $r^2=0.999$ ) for diethylcarbamazine citrate,  $Y=474141x+21692$  ( $r^2=0.999$ ) for guaiphenesin and  $Y=951864x+4648$  ( $r^2=0.999$ ) for chlorpheniramine maleate. The results are given in table 2.

**Table 2: Linearity data for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate**

| Diethylcarbamazine citrate |             | Guaiphenesin    |             | Chlorpheniramine maleate |             |
|----------------------------|-------------|-----------------|-------------|--------------------------|-------------|
| Conc $\mu$ g/ml            | Area counts | Conc $\mu$ g/ml | Area counts | Conc $\mu$ g/ml          | Area counts |
| 1.00                       | 560581      | 0.60            | 278415      | 0.02                     | 190320      |
| 2.50                       | 1419062     | 1.50            | 687456      | 0.05                     | 487956      |
| 5.00                       | 2878887     | 3.00            | 1358742     | 0.10                     | 954786      |
| 10.00                      | 5602579     | 6.00            | 2786284     | 0.20                     | 1923654     |
| 12.50                      | 7041847     | 7.50            | 3568745     | 0.25                     | 2378462     |
| 15.00                      | 8443101     | 9.00            | 4254810     | 0.30                     | 2854810     |
| Corr Coef                  | 0.999       | Corr Coef       | 0.999       | Corr Coef                | 0.999       |
| Slope                      | 561966.74   | Slope           | 474141.22   | Slope                    | 9518964.73  |
| Intercept                  | 13655.29    | Intercept       | 21692.26    | Intercept                | 4648.64     |

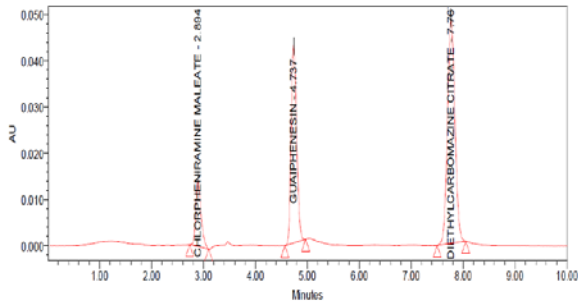


Fig. 6: Chromatogram for linearity-1

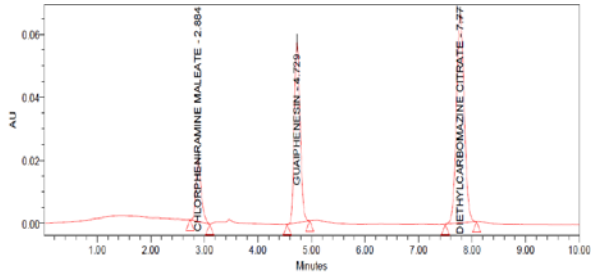


Fig. 7: Chromatogram for linearity-2

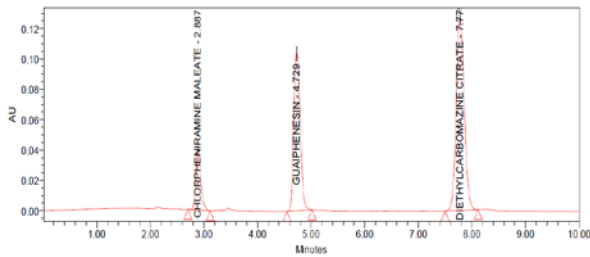


Fig. 8: Chromatogram for linearity-3

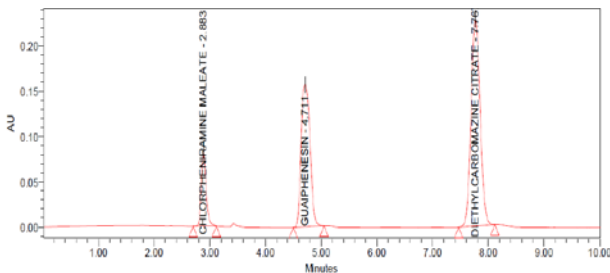


Fig. 9: Chromatogram for linearity-4

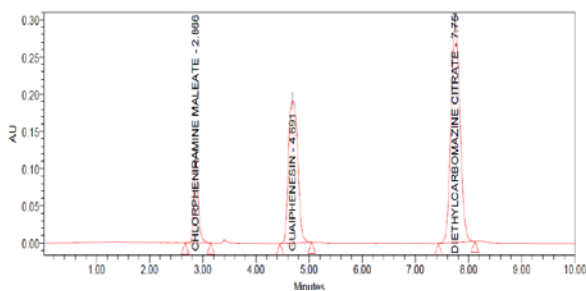


Fig. 10: Chromatogram for linearity-5

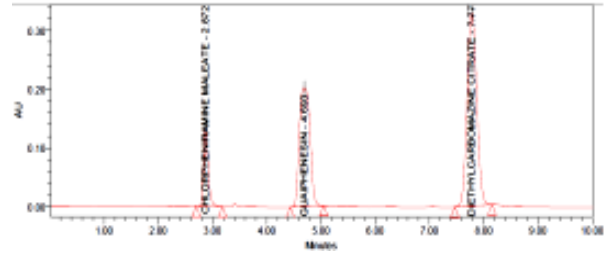


Fig. 11: Chromatogram for linearity-6

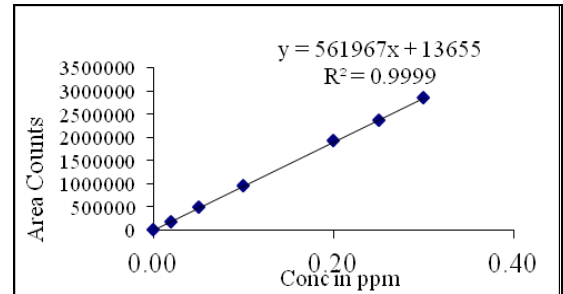


Fig. 12: Linearity plot for diethylcarbamazine citrate

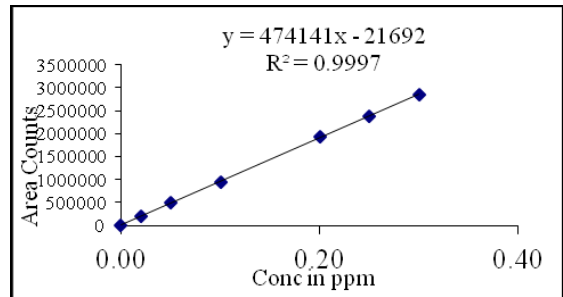


Fig. 13: Linearity plot for guaiphenesin

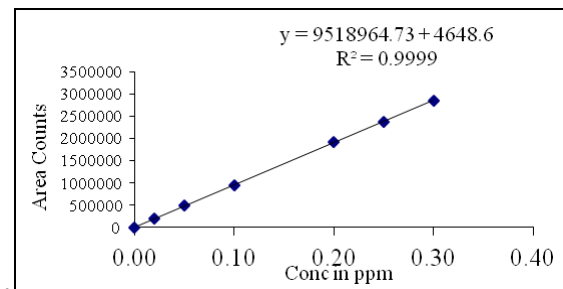


Fig. 14: Linearity plot for chlorpheniramine maleate

**Accuracy**

In this method, accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 5, 10 and 15 µg/ml of diethylcarbamazine citrate; 3, 6 and 9 µg/ml of guaiphenesin; and 0.1, 0.2 and 0.3 µg/ml of chlorpheniramine maleate were prepared. As per the test method, the test solution was injected three times for each spike level and the assay was performed. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of 100.34-100.81% for diethylcarbamazine citrate and 100.51-100.18% for guaiphenesin and 100.64-100.34% for chlorpheniramine maleate. RSD values were found to be less than 2%. The results are given in table 3, 4 and 5.

Table 3: Accuracy data for diethylcarbamazine citrate

| Accuracy | Amount of drug conc $\mu\text{g/ml}$ | Amount added $\mu\text{g/ml}$ | Amount obtained $\mu\text{g/ml}$ | Area counts | % Recovery | Mean recovery, $\pm\text{RSD}$ |
|----------|--------------------------------------|-------------------------------|----------------------------------|-------------|------------|--------------------------------|
| 50%      | 10.01                                | 5.08                          | 5.031                            | 1383585     | 100.34     | 100.26,                        |
|          | 10.01                                | 5.04                          | 5.012                            | 1379603     | 100.28     | 0.10                           |
|          | 10.01                                | 5.11                          | 5.143                            | 1313241     | 100.15     |                                |
| 100%     | 10.01                                | 10.18                         | 10.541                           | 2789315     | 100.27     | 100.26,                        |
|          | 10.01                                | 10.12                         | 10.324                           | 2784571     | 100.18     | 0.07                           |
|          | 10.01                                | 10.16                         | 10.148                           | 2799874     | 100.32     |                                |
| 150%     | 10.01                                | 15.47                         | 15.478                           | 4189106     | 100.24     | 100.5                          |
|          | 10.17                                | 15.28                         | 15.369                           | 4196329     | 100.45     | 0.28                           |
|          | 10.17                                | 15.19                         | 15.214                           | 4145316     | 100.81     |                                |

Table 4: Accuracy data for guaiphenesin

| Accuracy | Amount of drug conc $\mu\text{g/ml}$ | Amount added $\mu\text{g/ml}$ | Amount obtained $\mu\text{g/ml}$ | Area counts | % Recovery | Mean recovery, $\pm\text{RSD}$ |
|----------|--------------------------------------|-------------------------------|----------------------------------|-------------|------------|--------------------------------|
| 50%      | 6.12                                 | 3.01                          | 3.124                            | 904105      | 100.51     | 100.37,                        |
|          | 6.12                                 | 3.05                          | 3.131                            | 904979      | 100.35     | 0.12                           |
|          | 6.12                                 | 3.14                          | 3.214                            | 894400      | 100.27     |                                |
| 100%     | 6.12                                 | 6.15                          | 6.528                            | 1871153     | 100.38     | 100.45,                        |
|          | 6.12                                 | 6.21                          | 6.374                            | 1880820     | 100.47     | 0.07                           |
|          | 6.12                                 | 6.28                          | 6.484                            | 1892189     | 100.52     |                                |
| 150%     | 6.12                                 | 9.57                          | 9.428                            | 2847220     | 100.61     | 100.42,                        |
|          | 6.12                                 | 9.26                          | 9.569                            | 2867896     | 100.48     | 0.21                           |
|          | 6.12                                 | 9.15                          | 9.214                            | 2816024     | 100.18     |                                |

Table 5: Accuracy data for chlorpheniramine maleate

| Accuracy | Amount of drug conc $\mu\text{g/ml}$ | Amount added $\mu\text{g/ml}$ | Amount obtained $\mu\text{g/ml}$ | Area counts | % Recovery | Mean recovery, $\pm\text{RSD}$ |
|----------|--------------------------------------|-------------------------------|----------------------------------|-------------|------------|--------------------------------|
| 50%      | 0.21                                 | 0.15                          | 0.148                            | 254967      | 100.64     | 100.49,                        |
|          | 0.21                                 | 0.14                          | 0.142                            | 257431      | 100.58     | 0.19                           |
|          | 0.21                                 | 0.11                          | 0.147                            | 252908      | 100.27     |                                |
| 100%     | 0.21                                 | 0.21                          | 0.215                            | 547450      | 100.56     | 100.41,                        |
|          | 0.21                                 | 0.22                          | 0.218                            | 546146      | 100.38     | 0.13                           |
|          | 0.21                                 | 0.23                          | 0.223                            | 541044      | 100.29     |                                |
| 150%     | 0.21                                 | 0.30                          | 0.347                            | 793703      | 100.17     | 100.24,                        |
|          | 0.21                                 | 0.31                          | 0.311                            | 792084      | 100.21     | 0.08                           |
|          | 0.21                                 | 0.32                          | 0.318                            | 793544      | 100.34     |                                |

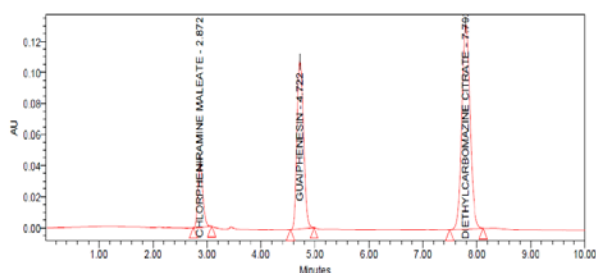


Fig. 15: Chromatogram for accuracy 50%-1

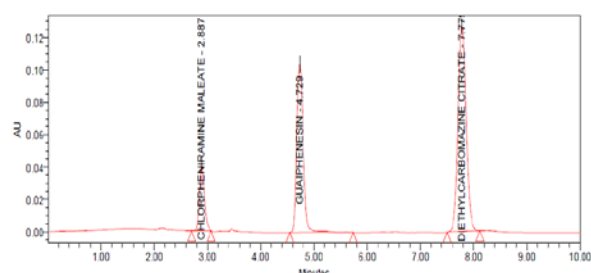


Fig. 17: Chromatogram for accuracy 50%-3

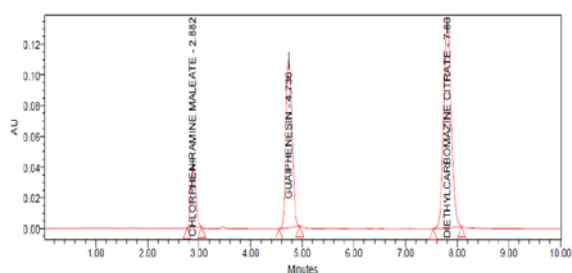


Fig. 16: Chromatogram for accuracy 50%-2

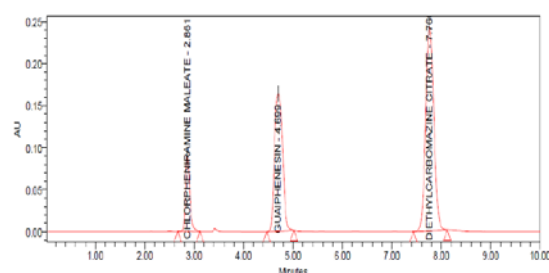


Fig. 18: Chromatogram for accuracy 100%-1

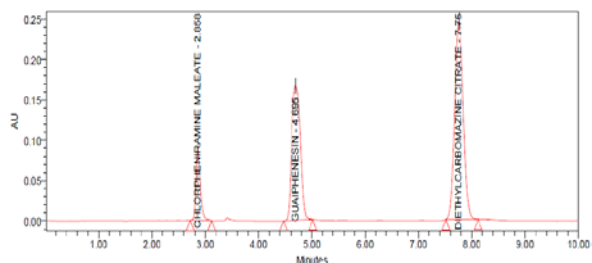


Fig. 19: Chromatogram for accuracy 100%-2

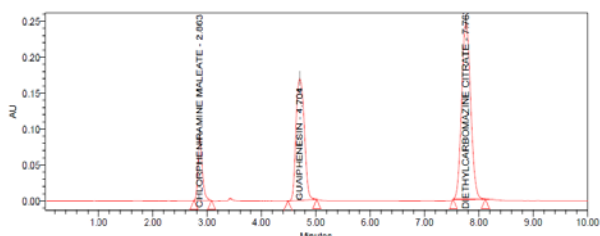


Fig. 20: Chromatogram for accuracy 100%-3

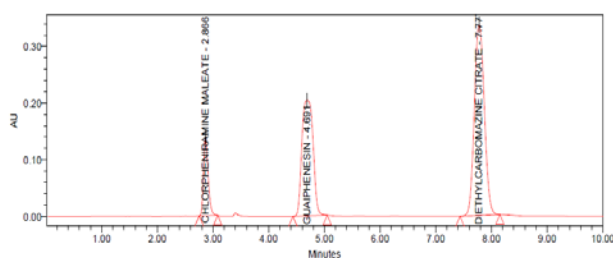


Fig. 21: Chromatogram for accuracy 150%-1

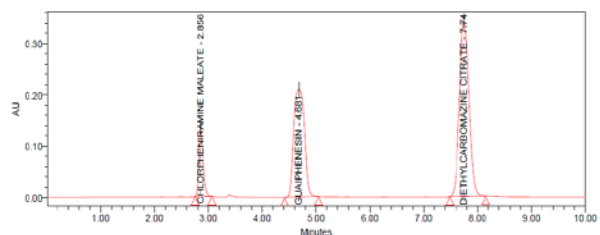


Fig. 22: Chromatogram for accuracy 150%-2

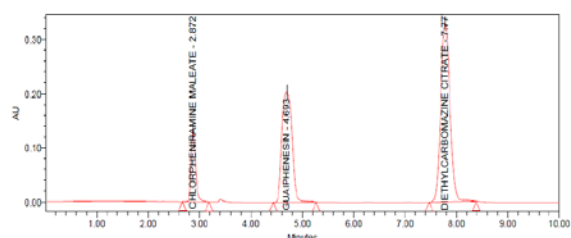


Fig. 23: Chromatogram for accuracy 150%-3

**Precision**

**Repeatability**

Repeatability was calculated by injecting standard solution six times containing diethylcarbamazine citrate (10µg/ml), guaiphenesin (6µg/ml) and chlorpheniramine maleate (0.2µg/ml). Peak areas and % RSD were calculated.

**Intraday precision**

Six replicates of a sample solution containing diethylcarbamazine citrate (10µg/ml), guaiphenesin (6µg/ml) and chlorpheniramine maleate (0.2µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

**Interday precision**

Six replicates of a sample solution containing diethylcarbamazine citrate (10µg/ml), guaiphenesin (6µg/ml), and chlorpheniramine maleate (0.2µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 6 and 7.

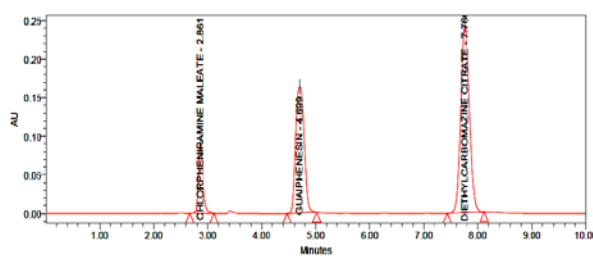


Fig. 24: Chromatogram for method precision-1

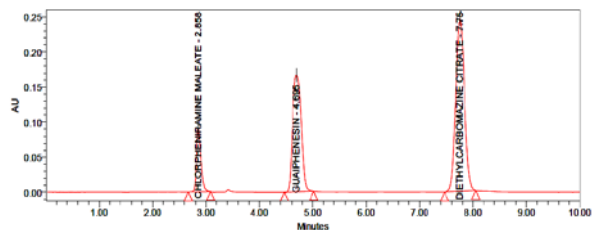


Fig. 25: Chromatogram for method precision-2

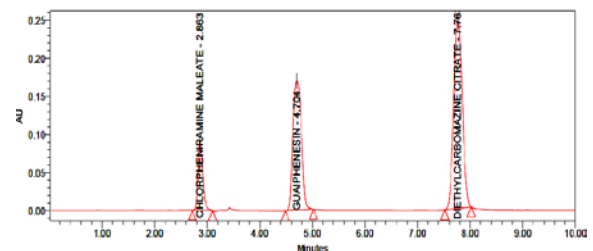


Fig. 26: Chromatogram for method precision-3

Table 6: Intraday data for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate

| Diethylcarbamazine citrate |             |               | Guaiphenesin |             |               | Chlorpheniramine maleate |             |               |
|----------------------------|-------------|---------------|--------------|-------------|---------------|--------------------------|-------------|---------------|
| Conc (µg/ml)               | Area counts | % assay as is | Conc (µg/ml) | Area counts | % assay as is | Conc (µg/ml)             | Area counts | % assay as is |
| 10.0                       | 2789315     | 100.42        | 6.0          | 1871153     | 100.68        | 0.2                      | 547450      | 100.25        |
|                            | 2788941     | 100.68        |              | 1880820     | 100.54        |                          | 541665      | 100.37        |
|                            | 2764650     | 100.54        |              | 1892189     | 100.42        |                          | 549553      | 100.48        |
|                            | 2790843     | 100.28        |              | 1892461     | 100.37        |                          | 546337      | 100.51        |
|                            | 2789315     | 100.64        |              | 1871153     | 100.26        |                          | 547450      | 100.68        |
|                            | 2789421     | 100.37        |              | 1892189     | 100.15        |                          | 545149      | 100.75        |
| % RSD                      | 1.66        |               | 0.67         |             | 0.49          |                          |             |               |

Table 7: Interday data for diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate

| Diethylcarbamazine citrate |             |               | Guaiphenesin |             |               | Chlorpheniramine maleate |             |               |
|----------------------------|-------------|---------------|--------------|-------------|---------------|--------------------------|-------------|---------------|
| Conc (µg/ml)               | Area counts | % assay as is | Conc (µg/ml) | Area counts | % assay as is | Conc (µg/ml)             | Area counts | % assay as is |
| 10.0                       | 2764851     | 100.56        | 6.0          | 1841523     | 100.43        | 0.2                      | 541282      | 100.38        |
|                            | 2758945     | 100.37        |              | 1847456     | 100.68        |                          | 541365      | 100.56        |
|                            | 2768748     | 100.41        |              | 1848752     | 100.36        |                          | 541878      | 100.37        |
|                            | 2775358     | 100.52        |              | 1845896     | 100.38        |                          | 541758      | 100.58        |
|                            | 2787486     | 100.78        |              | 1847893     | 100.55        |                          | 541785      | 100.41        |
|                            | 2778952     | 100.54        |              | 1847895     | 100.47        |                          | 541478      | 100.36        |
| % RSD                      | 0.84        |               | 0.73         |             |               | 0.68                     |             |               |

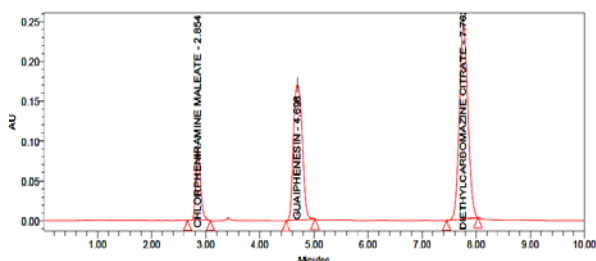


Fig. 27: Chromatogram for method precision-4

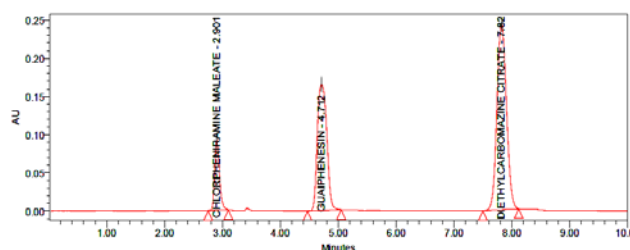


Fig. 31: Chromatogram for intermediate precision-2

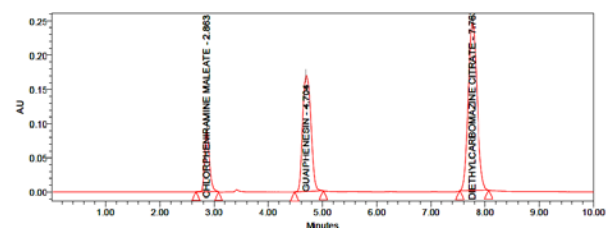


Fig. 28: Chromatogram for method precision-5

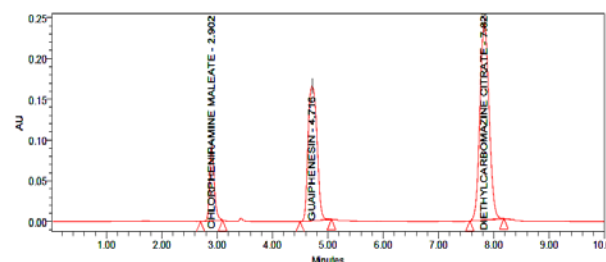


Fig. 32: Chromatogram for intermediate precision-3

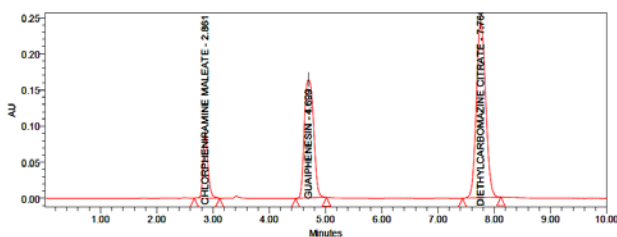


Fig. 29: Chromatogram for method precision-6

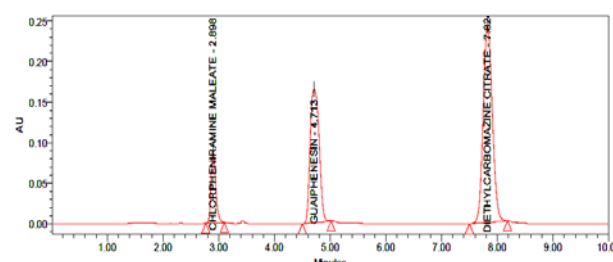


Fig. 33: Chromatogram for intermediate precision-4

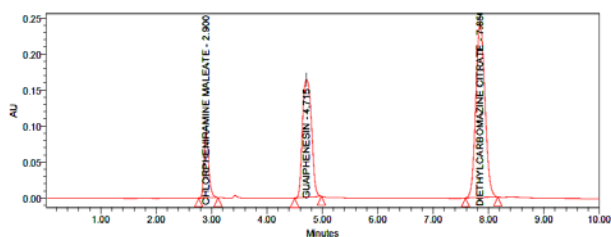


Fig. 30: Chromatogram for intermediate precision-1

LOD and LOQ

LOD and LOQ minimum concentration level at which the analyte can be reliably detected, quantified by using the standard formulas (3.3times  $\sigma/s$  and 10times  $\sigma/s$  for LOD and LOQ respectively) were found to be 0.1 and 0.2 µg/ml for diethylcarbamazine citrate, 0.06 and 0.12 µg/ml for guaiphenesin and 0.002 and 0.004 µg/ml for chlorpheniramine maleate. The low values of LOD and LOQ indicate the high sensitivity of method. The results are given in table 8 and 9.

Table 8: Results of LOQ

| Diethylcarbamazinicitrate |     | Guaiphenesin |     | Chlorpheniramine maleate |     |
|---------------------------|-----|--------------|-----|--------------------------|-----|
| Conc (µg/ml)              | s/n | Conc (µg/ml) | s/n | Conc (µg/ml)             | s/n |
| 0.2                       | 17  | 0.12         | 15  | 0.004                    | 18  |

Table 9: Results of LOD

| Diethylcarbamazine citrate |     | Guaiphenesin |     | Chlorpheniramine maleate |     |
|----------------------------|-----|--------------|-----|--------------------------|-----|
| Conc (µg/ml)               | s/n | Conc (µg/ml) | s/n | Conc (µg/ml)             | s/n |
| 0.1                        | 5   | 0.06         | 4   | 0.002                    | 6   |

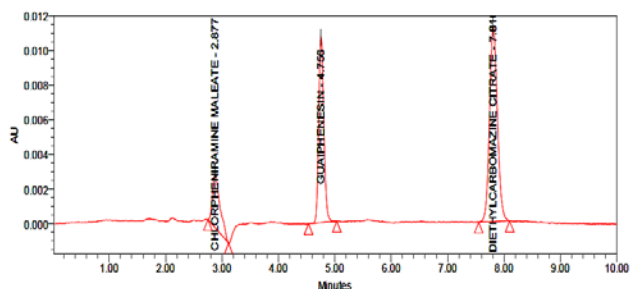


Fig. 36: Chromatogram for LOD

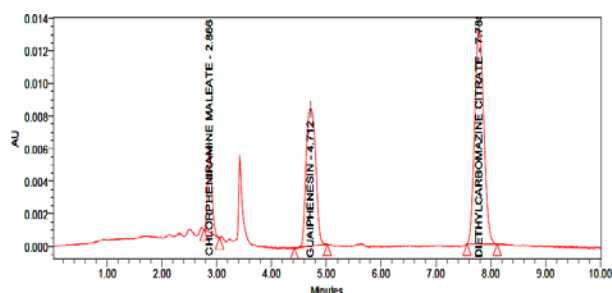


Fig. 37: Chromatogram for LOQ

#### Forced degradation

Stress degradation conditions such as acidic, basic, oxidative, reduction, thermal, hydrolysis and photolytic stresses were attempted as per ICH guidelines Q1A (R2).

#### Acid degradation

Acid degradation studies were carried out by weighing 27 mg of sample and transferred to a 10 ml volumetric flask, to this add 5 ml of diluent dissolve it and add 0.1 ml of 5N HCl. The mixture was refluxed at 70 °C for 1 hour. Then the solution was neutralized with 0.1 ml of 5N NaOH and diluted with the mobile phase up to the mark and mixed well. 0.1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the HPLC system and chromatograms were recorded.

#### Alkali degradation

Alkali degradation studies were carried out by weighing 27 mg of sample and transferred to a 10 ml volumetric flask, to this add 5 ml

of diluent dissolve it and add 0.1 ml of 5N NaOH. The mixture was refluxed at 70 °C for 1 hour. Then the solution was neutralized with 0.1 ml of 5N HCl and diluted with the mobile phase up to the mark and mixed well. 0.1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the system and chromatograms were recorded.

#### Peroxide degradation

Peroxide degradation studies were carried out by weighing 27 mg of sample and transferred to a 10 ml volumetric flask, to this add 5 ml of diluent dissolve it and add 0.1 ml of 15% H<sub>2</sub>O<sub>2</sub>. The mixture was refluxed at 70 °C for 30 min. 1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the system and chromatograms were recorded.

#### Reduction degradation

Reduction degradation studies were carried out by weighing 27 mg of sample and transferred to a 10 ml volumetric flask, to this add 5 ml of diluent dissolve it and add 0.1 ml of 10% sodium bisulphate. The mixture was refluxed at 70 °C for 1 hour. 0.1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the system and chromatograms were recorded.

#### Hydrolysis degradation:

Hydrolysis degradation studies were carried out by weighing 27 mg of sample and transferred to a 10 ml volumetric flask, to this add 5 ml of diluent and add 0.1 ml of water and sonicated to disperse, dissolve and refluxed at 70 °C for 30 min. 0.1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the system and chromatograms were recorded.

#### Thermal degradation

Thermal degradation studies were carried out by weighing 27 mg of sample and exposed to a temperature of 80 °C for 72 h in hot air oven. Then the sample was transferred to a 10 ml volumetric flask, dissolves in 5 ml of diluent and diluted with mobile phase up to the mark. 1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the system and chromatograms were recorded.

#### Photolytic degradation

Photolytic degradation studies were carried out by weighing 27 mg of sample and exposed to 1.2 Million lux hours of light. Then the sample was transferred to a 10 ml volumetric flask, dissolved in 5 ml of diluent and diluted with the mobile phase up to the mark. 1 ml of the same solution was diluted to 10 ml with the diluent. 10 µl of the above solution was injected into the system and chromatograms were recorded.

Table 10: Results of force degradation studies of diethylcarbamazine citrate

| Stress condition       | Time   | % assay | % degradation | Purity angle | Purity threshold |
|------------------------|--------|---------|---------------|--------------|------------------|
| Acid degradation       | 1h     | 86.8    | 13.2          | 0.12         | 0.25             |
| Alkaline degradation   | 1h     | 94.8    | 5.2           | 0.14         | 0.28             |
| Oxidative degradation  | 30 min | 96.5    | 3.5           | 0.18         | 0.24             |
| Reduction degradation  | 1h     | 93.6    | 6.4           | 0.16         | 0.30             |
| Thermal degradation    | 3h     | 87.2    | 12.8          | 0.14         | 0.28             |
| Photolytic degradation | 72h    | 84.9    | 15.1          | 0.11         | 0.25             |
| Hydrolysis degradation | 30 min | 91.1    | 8.9           | 0.15         | 0.24             |



Table 11: Results of force degradation studies of guaiphenesin

| Stress condition       | Time   | % assay | % degradation | Purity angle | Purity threshold |
|------------------------|--------|---------|---------------|--------------|------------------|
| Acid degradation       | 1h     | 85.4    | 14.6          | 0.10         | 0.27             |
| Alkaline degradation   | 1h     | 93.6    | 6.4           | 0.17         | 0.32             |
| Oxidative degradation  | 30 min | 95.3    | 4.7           | 0.23         | 0.37             |
| Reduction degradation  | 1h     | 92.7    | 7.3           | 0.24         | 0.36             |
| Thermal degradation    | 3h     | 86.5    | 13.5          | 0.25         | 0.45             |
| Photolytic degradation | 72h    | 84.2    | 15.8          | 0.16         | 0.28             |
| Hydrolysis degradation | 30 min | 91.8    | 8.2           | 0.18         | 0.39             |

Table 12: Results of force degradation studies of chlorpheniramine maleate

| Stress condition       | Time   | % assay | % degradation | Purity angle | Purity threshold |
|------------------------|--------|---------|---------------|--------------|------------------|
| Acid degradation       | 1h     | 85.4    | 13.7          | 0.14         | 0.34             |
| Alkaline degradation   | 1h     | 93.6    | 5.8           | 0.17         | 0.36             |
| Oxidative degradation  | 30 min | 95.3    | 4.2           | 0.25         | 0.39             |
| Reduction Degradation  | 1h     | 92.7    | 7.5           | 0.19         | 0.42             |
| Thermal degradation    | 3h     | 86.5    | 12.8          | 0.23         | 0.41             |
| Photolytic Degradation | 72h    | 84.2    | 16.4          | 0.18         | 0.51             |
| Hydrolysis Degradation | 30 min | 91.8    | 9.4           | 0.15         | 0.43             |

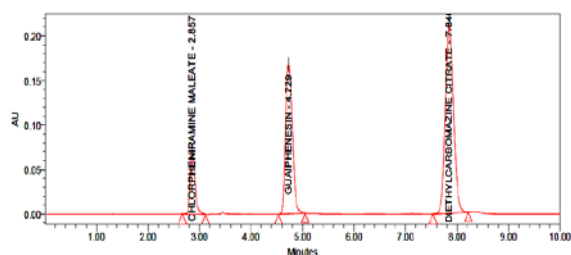


Fig. 38: Chrom for acid degradation

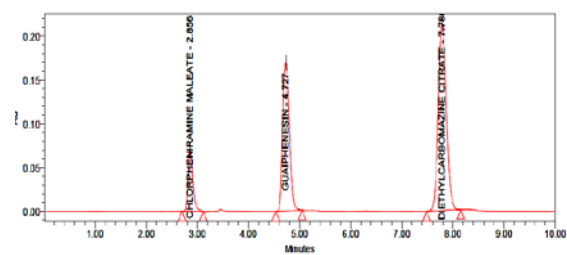


Fig. 42: Chrom for thermal degradation

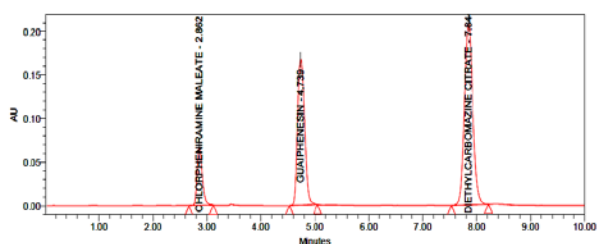


Fig. 39: Chrom for alkali degradation

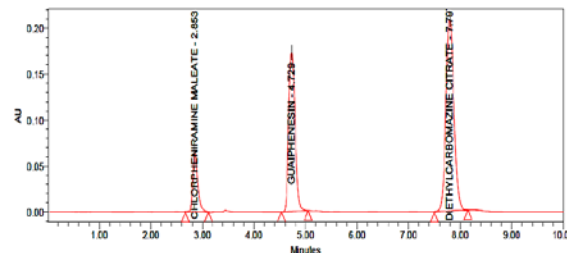


Fig. 43: Chrom for photolytic degradation

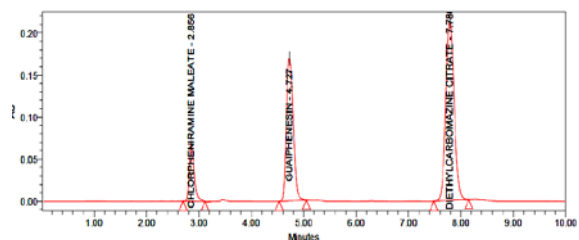


Fig. 40: Chrom for peroxide degradation

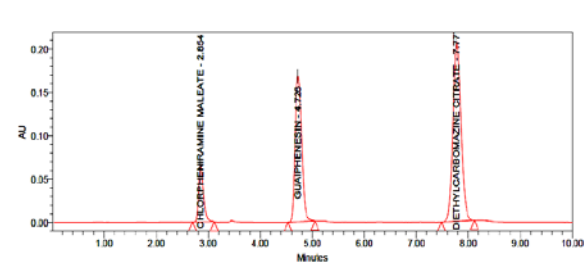


Fig. 44: Chrom for hydrolysis degradation

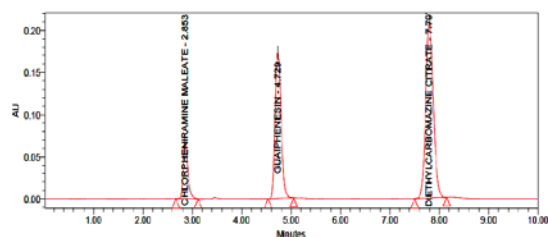


Fig. 41: Chrom for reduction degradation

### Robustness

The proposed method was found to be Robust as the % RSD was found to be less than 2%. Slight variations were done in the optimised method parameters like flow rate ( $\pm 0.2\%$ ), organic content in mobile phase ( $\pm 5\%$ ), pH ( $\pm 0.2$ ) and wavelength of detection ( $\pm 5\%$ ).

### Flow rate variation

This study was conducted to find the effect of variation in flow rate. Standard and check standard solutions were prepared as per test

method and injected into HPLC system with a flow rate of 1.0 ml/min. System suitability parameters were evaluated and found to be within the specified limits as per test method and RT of the main peak was monitored.

#### Organic phase variation

This study was conducted to find the effect of variation in organic phase. Standard and check standard solutions were prepared as per the test method and injected into HPLC system with mobile phases of 0.1% triethylamine as a buffer along with orthophosphoric acid adjusted to P<sup>H</sup> 2.5: acetonitrile(50:50v/v) and wavelength of 210 nm. System suitability parameters are found to be within the specified limits and RT of the main peak was monitored for 50:50 v/v (mixed 0.1% triethylamine buffer).

#### PH variation

This study was conducted to find the variation in P<sup>H</sup>. Standard and check standard solutions were prepared as per test method and injected into HPLC system with different buffer P<sup>H</sup>. System suitability parameters were evaluated and found to be within the specified limits as per test method and RT of the main peak was monitored.

#### Wavelength variation

This study was conducted to find the effect of variation in wavelength. Standard and check standard solutions were prepared as per test method and injected into HPLC system with different buffer wavelengths. System suitability parameters were evaluated and found to be within the specified limits as per test method and RT of the main peak was monitored.

Table 13: Results for robustness

| Parameter                            | Diethylcarbamazine citrate |             | Guaiphenesin    |             | Chlorpheniramine maleate |             |
|--------------------------------------|----------------------------|-------------|-----------------|-------------|--------------------------|-------------|
|                                      | USP plate count            | USP tailing | USP plate count | USP tailing | USP plate count          | USP tailing |
| Less flow rate (0.8 ml/min)          | 3200                       | 0.86        | 4320            | 0.08        | 5896                     | 0.14        |
| High flow rate (1.2 ml/min)          | 3450                       | 0.78        | 3548            | 0.12        | 4100                     | 0.11        |
| Less wavelength (205 nm)             | 3525                       | 0.45        | 3896            | 0.45        | 4752                     | 0.86        |
| High Wavelength (215 nm)             | 4272                       | 0.52        | 3868            | 0.53        | 5962                     | 0.86        |
| Less organic phase composition (-5%) | 3984                       | 0.68        | 3796            | 0.63        | 4635                     | 0.86        |
| High organic phase composition (+5%) | 3582                       | 0.67        | 3863            | 0.45        | 3785                     | 0.86        |
| Less pH variation (-0.2)             | 3985                       | 0.73        | 3981            | 0.58        | 3868                     | 0.86        |
| High pH variation (+0.2)             | 4584                       | 0.59        | 3789            | 0.67        | 4589                     | 0.86        |

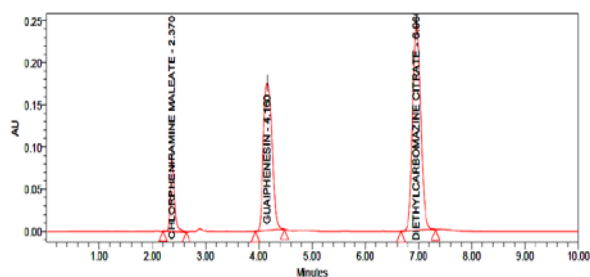


Fig. 45: Chromatogram for flow plus

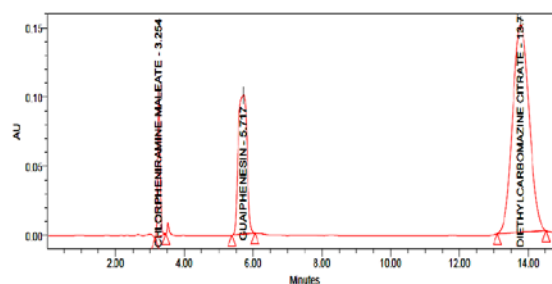


Fig. 48: Chromatogram for org minus

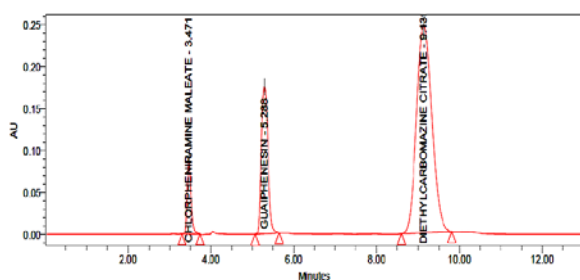


Fig. 46: Chromatogram for flow minus

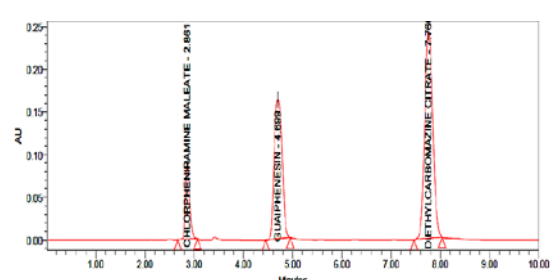


Fig. 49: Chromatogram for wave plus

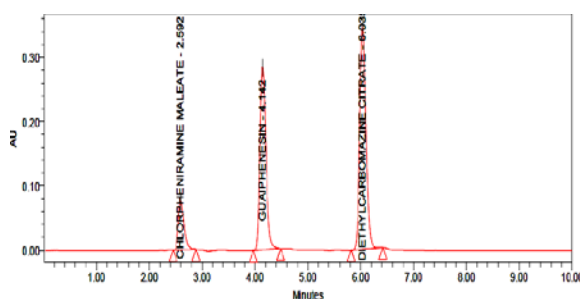


Fig. 47: Chromatogram for org plus

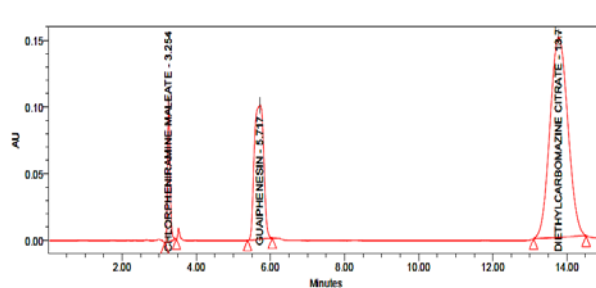


Fig. 50: Chromatogram for wave minus

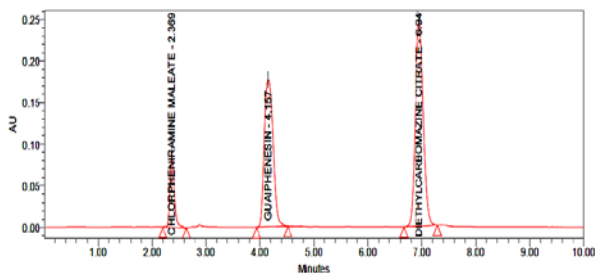


Fig. 51: Chromatogram for pH plus

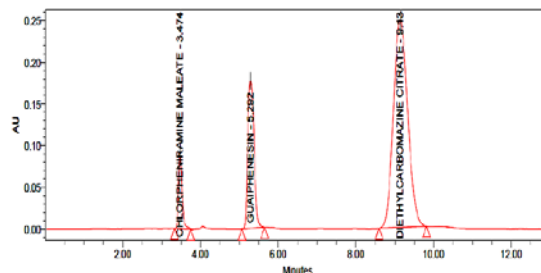


Fig. 52: Chromatogram for pH minus

Table 14: Results of stability studies

| Stability | % assay | % deviation |
|-----------|---------|-------------|
| Initial   | 100.2   | 0.00        |
| 6 h       | 100.8   | 0.06        |
| 12 h      | 100.4   | 0.02        |
| 18 h      | 100.3   | 0.01        |
| 24 h      | 100.5   | 0.03        |

**Solution stability**

Sample solutions were analysed initially to 24 h at different intervals of time at room temperature and the results were recorded. The % deviation should not be more than 5.0%. The results are given in table 14.

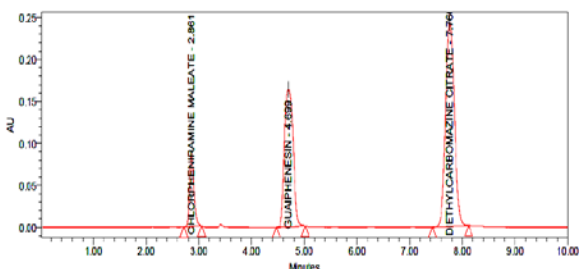


Fig. 53: Chromatogram for stability initial

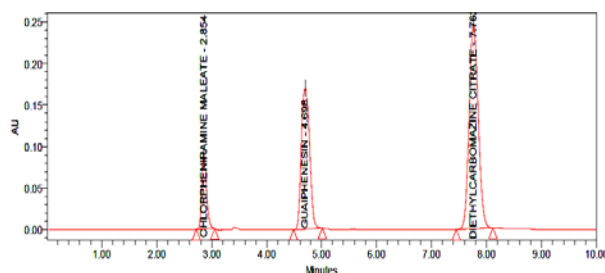


Fig. 56: Chromatogram for stability 18 h

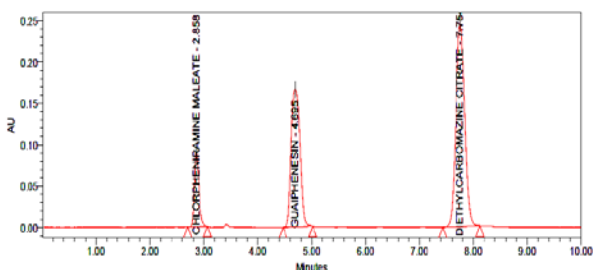


Fig. 54: Chromatogram for stability 6 h

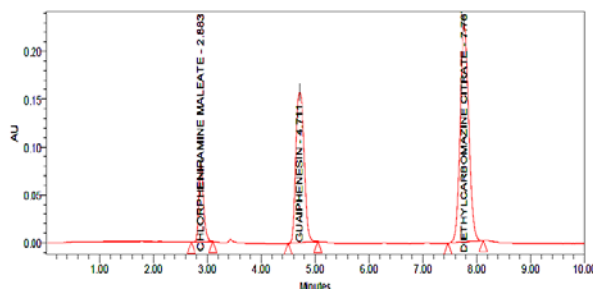


Fig. 57: Chromatogram for stability 24 h

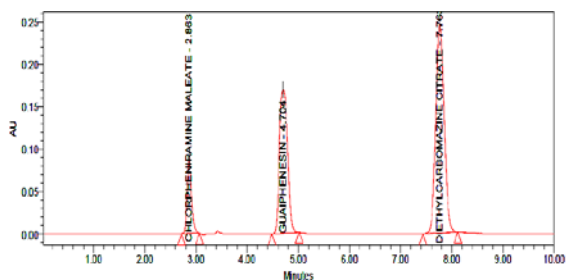


Fig. 55: Chromatogram for stability 12h

**CONCLUSION**

Stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate in pharmaceutical formulations as per ICH guidelines. The developed method was found to be accurate, precise and reliable with %RSD less than 2%. Therefore, the developed method is simple, accurate, precise and robust. The present method was found to be stability indicating as the degradation of drug substance was between 5-20%. Finally, this method can be used for better analysis of pharmaceutical formulations of diethylcarbamazine citrate, guaiphenesin and chlorpheniramine maleate drug.

**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICT OF INTERESTS**

Declared none

## REFERENCES

1. <http://en.wikipedia.org/wiki/Diethylcarbamazine>; 2009. [Last accessed on 20 Jul 2017].
2. WHO. Model List of Essential Medicines. World Health Organization; 2013.
3. Indian pharmacopoeia, Ghaziabad, Indian Pharmacopoeia; 2007. p. 558-59, 1005-6.
4. British Pharmacopoeia. London; British Pharmacopoeia Commission; 2009. p. 2861, 5094.
5. Indian Pharmacopoeia. The Indian pharmacopoeia commission, Ghaziabad, Govt. of India. Ministry of health and Family Welfare. Vol. II; 2007. p. 919-20, 1029-30.
6. Michael UA, Kenneth CO, Anthony AA. Spectrophotometric and thermodynamic studies of the charge-transfer interaction between diethylcarbamazine citrate and chloranilic acid. *Chem Pharm Bull* 1999;47:463-6.
7. Lee AR, Hu TM. Determination of guaiphenesin in antitussive pharmaceutical preparations containing dextromethorphan by first- and second-derivative UV spectroscopy. *J Pharm Biomed Anal* 1994;12:747-52.
8. Khalode KD, Waikar SB, Pasmane SP. A validated UV spectrophotometric method for the simultaneous estimation of Dextromethorphan hydrobromide and chlorpheniramine maleate. *Int J Pharm Technol* 2012;4:4690-9.
9. Joshi RS, Pawar NS, Sawant RL. Validated spectrophotometric methods for simultaneous estimation of acetaminophen, chlorpheniramine maleate and caffeine in pue and tablete dosage form. *Latt Am J Pharm* 2010;29:1226-30.
10. Mahesh RJ, Jeyaprakash MR, Madhuri K, Meyyanathan SN, Elango K. A sensitive RP-HPLC method for simultaneous estimation of diethylcarbamazine and levocetirizine in tablet formulation. *Indian J Pharm Sci* 2011;73:320-3.
11. Hamide C, Tuncel O. Simultaneous high-performance liquid chromatographic determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in pharmaceutical dosage forms. *J Chromatogr Sci* 2002;40:97-100.
12. Suzen S, Akay C, Cevheroglu S. *Formaco* 1999;54:705-9.
13. Kulikov AU, Aleksey GV. LC determination of lercanidipine and its impurities using DryLab software and experimental design procedures. *J Chromatogr* 2008;67:5-6.
14. Anand P, Varun R, Anagha D. Simultaneous analysis of intestinal permeability markers, caffeine, paracetamol and sulfasalazine by Reverse phase liquid chromatography: a tool for standardization of rat everted gut sac model. *Asian J Pharm Clin Res* 2010;3:204-7.
15. Suraj S, Prasanna KP, Sagar KM, Sabuj S. HPLC method development for simultaneous estimation of hydrochlorothiazide and perindopril in tablet dosage form. *Asian J Pharm Clin Res* 2012;5:136-8.
16. Code Q2A-text on validation of analytical procedure step-3 Consensus Guideline; 1994.
17. ICH Harmonised Tripartite Guideline; 2015.
18. Code Q2B-Validation of analytical procedure methodology step-4 consensus guideline, ICH Harmonised Tripartite Guideline; 1994.
19. Prakash K, Sireesha KR. Liquid chromatographic method for simultaneous determination of lomefloxacin hydrochloride and dexamethasone sodium phosphate in eye drops. *Asian J Pharm Clin Res* 2012;5:79-82.