INTRODUCTION

The genus Colchicum belongs to the colchicaceae family. Molecular formula of COLC C₂₅H₃₀NO₆ (Fig. 1) with IUPAC name N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl] acetamide and molecular weight is 399.44 g/mol.

It is a highly poisonous alkaloid containing various species of colchicum. COLC is the main alkaloid obtained from the bulb and seeds of colchicum. It is used in human and veterinary medicine. The medicinal value of colchicum is due, to the presence of (-)-colchicine, the main alkaloid, which was isolated from all species of colchicum. It is widely used in breeding studies and as a drug to treat gout but is also valuable for other diseases such as familial Mediterranean fever, primary biliary cirrhosis and breast cancer [1-7]. Generic COLC is available in tablets. (-)-Colchicine, is a phenylethyl isoquinoline derived alkaloid, and it is a poisonous, lipid-soluble alkaloid with a unique 7-memberd aromatic tropolone ring [8-10].

MATERIALS AND METHODS

Apparatus and chromatographic conditions

All HPLC measurements were made on a Shimadzu Corporation system (Analytical instruments Division, Kyoto, Japan). The system consists of an LC 10AT solvent pump, an SPD10DVP detector and a data station with Win-chrome software, version 3.1. A stock solution of COLC was prepared by dissolving 100 mg of COLC in mobile phase at a flow rate of 1.2 ml/min with an operating pressure of 131-133 kg/cm². A Hamilton 702 µlR injector with a 25 L loop was used for the injection of the samples. The mobile phase was filtered through 0.45 µm membrane filter and degassed by using sonicator for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. Detection was performed at 254 nm with a sensitivity of 0.2 AUFS. All determinations were performed at ambient temperature. The injection volume was 20 µl and the total run time was 10 minutes.

Materials and Reagents

All chemicals used were of analytical or pharmaceutical grade. Acetonitrile (HPLC grade) and dihydrogen orthophosphate (AR grade) supplied by S.D. Fine-Chem, Ltd., India and Merck (India) Ltd., respectively were used. COLC standard was purchased from Sigma Aldrich. Dosage forms of COLC, manufactured by different firms, were obtained commercially. The body fluids which were received in forensic science laboratory for toxicological analysis were used. These body fluids were said to be collected from a body of a person who suspected to be died due to the consumption of plant material of Gloriosa superba.

Standard COLC solution

A stock solution of COLC was prepared by dissolving 100 mg of COLC in mobile phase in a 100 ml calibrated flask to get 1000 µg/ml. The standard solutions were prepared by further dilution of the stock.
standard solution with the specified mobile phase to reach the concentration range of 7-130 μg/ml.

Recommended general procedure
Suitable amounts of aliquots of standard COLC were transferred in to a series of 5 ml calibrated flasks and diluted to the volume with acetonitrile and mixed well. Then, 20 μl of the solution was injected and a chromatogram was noted. A calibration graph was plotted.

Procedure for pharmaceutical formulations (tablets)
Twenty tablets of COLC were weighed and finely powdered and an amount equivalent to 100 mg of COLC was dissolved in mobile phase and filtered. After keeping for 5 min in an ultrasonic, the solution was diluted to volume, filtered through 0.45 μm Millipore membrane filter and degassed. A 20 μl solution was then injected into chromatographic system.

Procedure for biological fluids
Biological fluids (1 ml each) were extracted with 1 ml saturated NH₄Cl solution and adjusted to pH 9.6 with ammonia and 5 ml of dichloromethane with 5 % 2-propanol. After 10 min agitation, the tubes were centrifuged for 5 min at 3500 rpm. The organic layer was transferred into glass tubes and then evaporated to dryness at 45ºC. The residues were reconstituted in 75 μl mobile phase. An aliquot was analysed using the procedure described earlier.

RESULTS
Method development
The mobile phase was chosen after several trials with methanol, acetonitrile, water and buffer in various proportions and at different pH values. A mobile phase consisting of acetonitrile : methanol: water (32:48:20 v/v; pH adjusted to 5.2 with phosphoric acid) was selected to achieve the maximum separation and sensitivity. Flow rates between 0.5 and 2.0 ml/min were tried. It was observed that a flow rate of 1.2 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time.

Using a reverse-phase C₁₈ column, the retention time for COLC was observed to be 8.767 min. A total time of 10 min was necessary for the analysis. COLC exhibited absorption maxima at 254 nm. Under optimum conditions, the chromatograms of a series of COLC standard solutions were recorded. A computer controlled data station with Win-chrome software was used to plot the peak area versus the concentration in μg/ml.

Analytical features
To set up a linearity range, a series of COLC solutions of different concentrations were prepared in mobile phase in the range of 7-130 μg/ml. The reproducibility of the detector response at each concentration level was examined by carrying out the experiment in triplicate. A typical chromatogram is shown in Fig. 2.

![HPLC Chromatogram of Colchicin standard (5μg/ml)](Image)

A calibration curve was obtained by plotting the peak area of the standard to the COLC concentration. Further, COLC showed linearity in the concentration range of 5-130 μg/ml. A good correlation between COLC peak areas and drug concentration was observed with r² ≥ 0.9998. The minimum detectable and quantifiable concentration of COLC under the optimised conditions was found to be 2 μg/ml and 5 μg/ml respectively. Precision of the method was studied with 5 replicates of the standard solution. The results are shown in Table 1 and 2. The low values of the relative standard deviation indicate the high precision of the method.

Table 1: Rt, linear regression, LOD and LOQ for COLC

<table>
<thead>
<tr>
<th>Standard</th>
<th>Rt Min</th>
<th>Regression equation</th>
<th>r²</th>
<th>% SD</th>
<th>LOD (μg/ml)</th>
<th>LOQ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>8.76</td>
<td>Y = 1938.887 + 13.948*X</td>
<td>0.9998</td>
<td>2.65</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>a Correlation coefficient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Analysis of pharmaceutical preparations of COLC

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Label claim mg/tablet</th>
<th>Reported method [15]</th>
<th>Recovery** ± SD %</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goutnil (Tablet)</td>
<td>0.5</td>
<td>100.18 ± 0.15</td>
<td>99.2 ± 0.88, F=1.73; t=0.85</td>
<td></td>
</tr>
<tr>
<td>Colchicindon (Tablet)</td>
<td>0.5</td>
<td>100.04 ± 0.13</td>
<td>10.1 ± 0.92, F=1.50; t=0.87</td>
<td></td>
</tr>
</tbody>
</table>

**Recovery value by the proposed method is the mean of five determinations. The calculated F- and t- values refer to 95 % confidence limits.

Recovery studies
To study the accuracy and reproducibility of the proposed method, recovery experiments were carried out. The recovery of the added standard was studied at five different concentration levels. Each level was repeated five times. To an aliquot of the analysed preparations, a known concentration of the standard solution was added. The COLC contents were once again determined by the proposed method. From the amount of the drug present, the percent recovery was calculated using the following formula:

\[
\text{% Recovery} = \frac{N (\Sigma xy) - (\Sigma y)(\Sigma x)}{N (\Sigma x^2) - (\Sigma x)^2}
\]

Where, x is the amount of standard drug added, y is the amount of drug found by proposed method, and N is the number of observations. The results obtained are shown in Table 3.
Table 3: COLC recovery investigations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount of standard added (μg/ml)</th>
<th>Amount of standard detected (μg/ml)</th>
<th>Recovery (%)</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>100</td>
<td>103.35 ± 2.92</td>
<td>103.35</td>
<td>2.82</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>252.61 ± 6.08</td>
<td>101.04</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>497.20 ± 4.28</td>
<td>99.44</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* Recovery value by the proposed method is the mean of five determinations.

Table 4: Analysis of biological fluids

<table>
<thead>
<tr>
<th>Biological fluids</th>
<th>Concentrations ng/ml</th>
<th>Reported method [16]</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Blood</td>
<td>21.9</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>Cardiac Blood</td>
<td>22.8</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>148.5</td>
<td>152.5</td>
<td></td>
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</table>

CONCLUSION

The proposed method gives a good accuracy and reproducibility. The total time of analysis was only 10 min. The method is simple, rapid and does not involve complicated sample preparation. High percent recovery values show that the method was free from interference by the excipients used in the preparations. Hence, the present method could be used for routine quality control as well as to analyse biological fluids.

ACKNOWLEDGEMENTS

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REFERENCES
