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## Effect of Solvent on the absorbance maxima ( $\lambda_{\max}$ ) of Standard Plot of Thiocolchicoside

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**Abstract**---Standard plot of Thiocolchicoside was constructed in distilled water, 0.01M PBS, n-octanol, ethanol, methanol, 0.9% w/v NaCl and mixture of acetonitrile: water (70:30). Peak of stock solution was observed at a particular wavelength ( $\lambda_{\max}$ ) and various dilutions of that stock solution were prepared and absorbance value of every dilution was observed at that particular wavelength and corresponding to this regression coefficient ( $r^2$ ), slope value (m) of plot and molar extinction coefficient of drug was observed. As stock solution of Thiocolchicoside was prepared in various solvents with different concentration of stock solution at particular  $\lambda_{\max}$  value. All the stock solutions of Thiocolchicoside were scanned in UV region i.e. from 200 nm-400 nm.

**Keywords**---Thiocolchicoside, THC, peak,  $\lambda_{\max}$ , regression coefficient, slope, molar extinction coefficient, linearity etc.

### Introduction

The effect of solvent and pH on the wavelength for absorption and wavelength for emission on various compounds can be determined [1]. Linearity, slope value (m), molar extinction coefficient (MEC) and regression coefficient ( $r^2$ ) of drug in particular solvents depends on absorbance value. Drug is to be treated with various solvents to evaluate the linearity or distribution of drug in solvent/s uniformly, for this purpose a stock solution of drug to be prepared. Stock solution to be scanned for its wavelength ( $\lambda_{\max}$ ) at which drug solution will show a peak. A multiple linear regression technique confirms that some complexes are not compatible with some solvent parameters at bulk and molecular levels [2]. In the field of Pharmaceutical development, it is generally required to increase the

aqueous solubility of water soluble drugs. The efforts to increase aqueous solubility can be successful to study the compatibility profile of drugs with various solvents. Various organic solvents can be employed to increase the aqueous solubility of drugs [3]. A validated analytical method generally required for the quantification of particular drug in different dosage forms [4]. The confirmation of an assay of pure compound in various organic solvents through spectrophotometric method is employed to comply the ICH guidelines [5]. Various bichromatic and spectrophotometric methods are used for the estimation of an absorption maximum for the drugs. These methods may be more or less complex while the outcomes obtained through the derivative spectroscopy provides more complex spectrum [6]. Consequently dilutions to be prepared in accordance to the stock concentration of drug solution. Slope value of drug in that particular solvent is to be observed, which would be helpful for further calculations in research. Stock solution of drug is to be subjected to observe regression coefficient ( $r^2$ ), slope value (m) and molar extinction coefficient ( $E_{1\%, 1\text{cm}}$ ).

## Materials

Thiocolchicoside, distilled water, NaCl, KCl,  $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ , n-octanol, ethanol, methanol, NaCl, HPLC grade acetonitrile and HPLC water. All chemicals were used of analytical grade.

## Experimental

**Calibration plot of THC in distilled water:** For the preparation of stock solution of Thiocolchicoside in distilled water, stock solution of  $100\mu\text{g/ml}$  concentration was prepared i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml of distilled water was poured gradually and take care that stock solution should be without any visible particle or should be absolutely clear or homogenous. Corresponding to this five dilutions i.e.  $10\mu\text{g/ml}$ ,  $20\mu\text{g/ml}$ ,  $30\mu\text{g/ml}$ ,  $40\mu\text{g/ml}$  and  $50\mu\text{g/ml}$  were prepared. Highest dilution i.e.  $50\mu\text{g/ml}$  is scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 292 nm and corresponding to this absorbance value of all dilutions is taken and on the behalf of observed values of absorbance standard plot was plotted and regression coefficient and slope value was measured.

**Calibration plot of THC in 0.01M PBS:** For the preparation of stock solution of Thiocolchicoside in 0.01M phosphate buffer saline (PBS) solution, firstly 0.01M PBS solution was prepared by dissolving 680 mg  $\text{KH}_2\text{PO}_4$ , 710 mg  $\text{Na}_2\text{HPO}_4$ , 373 mg KCl and 293 mg NaCl in 500 ml distilled water. Stock solution was prepared of concentration of  $100\mu\text{g/ml}$  i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml of pre-prepared 0.01M PBS poured gradually and take care that stock solution should be without any visible particle or should be absolutely clear or homogenous. Corresponding to this five dilutions i.e.  $10\mu\text{g/ml}$ ,  $20\mu\text{g/ml}$ ,  $30\mu\text{g/ml}$ ,  $40\mu\text{g/ml}$  and  $50\mu\text{g/ml}$  were prepared. Highest dilution i.e.  $50\mu\text{g/ml}$  scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 302 nm and corresponding to this absorbance value of all dilutions were taken and on the behalf of observed values of absorbance the standard plot was plotted and regression coefficient and slope value was measured.

**Calibration plot of THC in ethanol:** For the preparation of stock solution of Thiocolchicoside in ethanol, stock solution of 100 $\mu$ g/ml concentration was prepared i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml of ethanol was poured gradually and absolutely clear stock solution was prepared. Stock solution with dilution of concentration of 50 $\mu$ g/ml scanned in UV region i.e. from 200 nm-400 nm and no peak was observed and then stock solution of concentration of 100 $\mu$ g/ml scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 284 nm. Corresponding to this six dilutions i.e. 10 $\mu$ g/ml, 20 $\mu$ g/ml, 40 $\mu$ g/ml, 60 $\mu$ g/ml, 80 $\mu$ g/ml and 100 $\mu$ g/ml were prepared and corresponding to this absorbance value of all dilutions were taken and on the behalf of observed values of absorbance standard plot was plotted and regression coefficient and slope value was measured.

**Calibration plot of THC in methanol:** For the preparation of stock solution of Thiocolchicoside in methanol, stock solution of 100 $\mu$ g/ml concentration was prepared i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml of methanol was poured gradually and clear stock solution was prepared. Corresponding to this five dilutions i.e. 10 $\mu$ g/ml, 20 $\mu$ g/ml, 30 $\mu$ g/ml, 40 $\mu$ g/ml and 50 $\mu$ g/ml were prepared. Highest dilution i.e. 50 $\mu$ g/ml scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 248 nm and corresponding to this absorbance value of all dilutions were taken and on the behalf of observed values of absorbance standard plot was plotted and regression coefficient and slope value was measured.

**Calibration plot of THC in n-Octanol:** For the preparation of stock solution of Thiocolchicoside in n-octanol, stock solution of 100 $\mu$ g/ml concentration was prepared i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml of n-octanol was poured gradually and clear stock solution was prepared. Stock solution with dilution of concentration of 50 $\mu$ g/ml scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 280 nm. Corresponding to this five dilutions i.e. 10 $\mu$ g/ml, 20 $\mu$ g/ml, 30 $\mu$ g/ml, 40 $\mu$ g/ml and 50 $\mu$ g/ml were prepared and absorbance value of all dilutions were taken and on the behalf of observed values of absorbance standard plot is plotted and regression coefficient and slope value was measured.

**Calibration plot of THC in 0.9% w/v NaCl:** Stock solution of Thiocolchicoside in isotonic solution of sodium chloride i.e. 0.9% w/v NaCl was prepared, for this 900 mg NaCl was dissolved in 1000 ml distilled water and stock solution of 100  $\mu$ g/ml concentration was prepared i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml of 0.9% w/v solution of NaCl was poured gradually and clear stock solution was prepared. Stock solution with dilution of concentration of 50 $\mu$ g/ml scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 290 nm. Corresponding to this five dilutions i.e. 10 $\mu$ g/ml, 20 $\mu$ g/ml, 30 $\mu$ g/ml, 40 $\mu$ g/ml and 50 $\mu$ g/ml were prepared and absorbance value of all dilutions were taken and on the behalf of observed values of absorbance standard plot was plotted and regression coefficient and slope value was measured.

**Calibration plot of THC in Acetonitrile:water (70:30):** For the preparation of stock solution of Thiocolchicoside in mixture of acetonitrile (ACN) and water of HPLC grade in ratio of 70:30 i.e. 70 ml ACN and 30 ml HPLC grade water were

taken and stock solution of 100  $\mu\text{g}/\text{ml}$  concentration was prepared i.e. 10 mg drug directly taken in 100 ml volumetric flask and 100 ml mixture of acetonitrile (ACN) and water of HPLC grade in ratio of 70:30 was poured gradually and clear stock solution was prepared. Stock solution with dilution of concentration of 50  $\mu\text{g}/\text{ml}$  scanned in UV region i.e. from 200 nm-400 nm and peak was observed at 294 nm. Corresponding to this five dilutions i.e. 10  $\mu\text{g}/\text{ml}$ , 20  $\mu\text{g}/\text{ml}$ , 30  $\mu\text{g}/\text{ml}$ , 40  $\mu\text{g}/\text{ml}$  and 50  $\mu\text{g}/\text{ml}$  were prepared and absorbance value of all dilutions were taken and on the behalf of observed values of absorbance standard plot was plotted and regression coefficient and slope value was measured.

## Results

Table 1  
Concentration and absorbance data of Thiocolchicoside in DW

| Conc. ( $\mu\text{g}/\text{ml}$ ) | Absorbance | $\lambda_{\text{max}}$ (nm) |
|-----------------------------------|------------|-----------------------------|
| 10                                | 0.212      | 292                         |
| 20                                | 0.415      |                             |
| 30                                | 0.623      |                             |
| 40                                | 0.829      |                             |
| 50                                | 1.045      |                             |

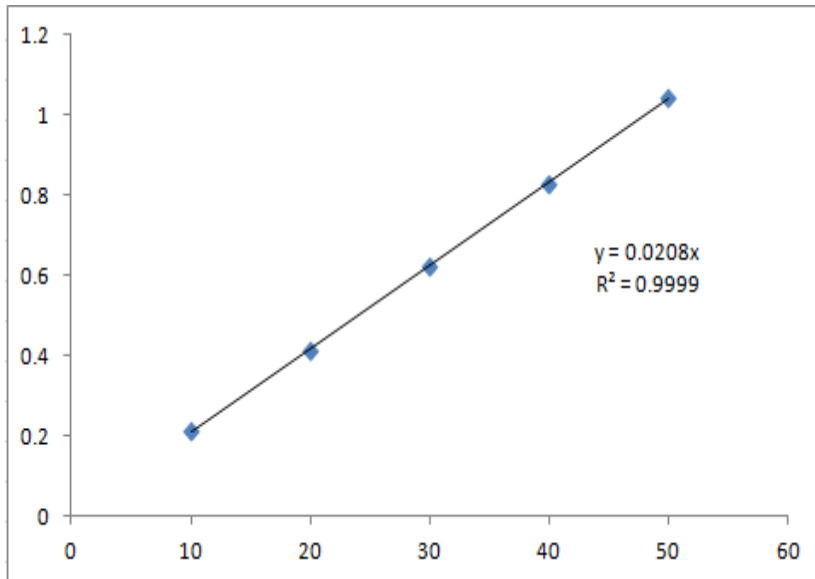


Fig 1: Calibration plot of Thiocolchicoside in distilled water

Table 2  
Summary of Thiocolchicoside in DW

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in distilled water |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|--------------------------------------------|
| 1       | 10.19                                          | 101.9                          | 0.9999                                   | 0.0208                                     |
| 2       | 19.95                                          | 99.8                           |                                          |                                            |
| 3       | 29.95                                          | 99.8                           |                                          |                                            |
| 4       | 39.86                                          | 99.65                          |                                          |                                            |
| 5       | 50.24                                          | 100.5                          |                                          |                                            |

Table 3  
Concentration and absorbance data of Thiocolchicoside in Ethanol

| Conc. ( $\mu\text{g/ml}$ ) | Absorbance | $\lambda_{\text{max}}$ (nm) |
|----------------------------|------------|-----------------------------|
| 10                         | 0.095      | 284                         |
| 20                         | 0.169      |                             |
| 40                         | 0.431      |                             |
| 60                         | 0.697      |                             |
| 80                         | 0.863      |                             |
| 100                        | 1.035      |                             |

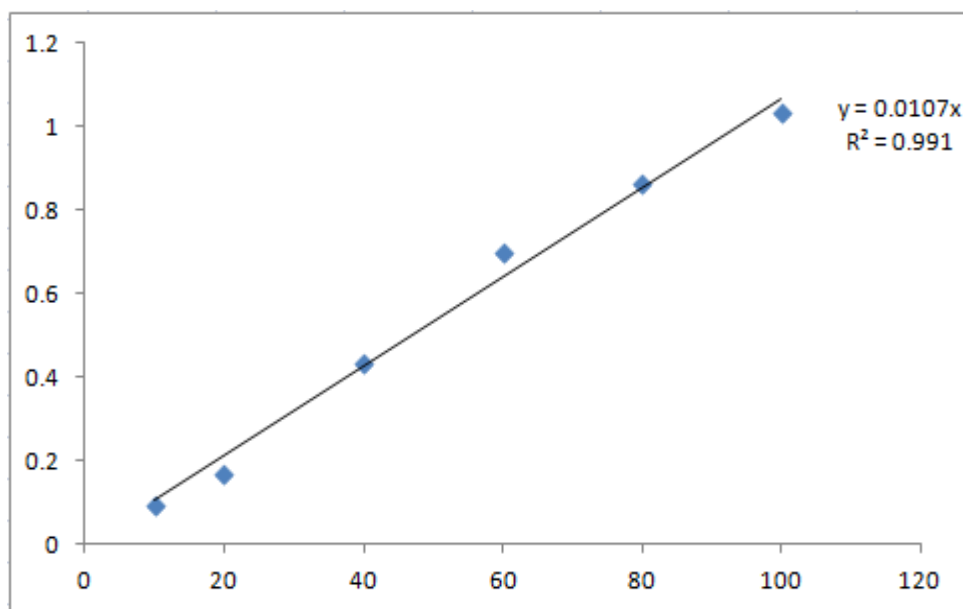


Fig 2: Calibration plot of Thiocolchicoside in ethanol

Table 4  
Summary of Thiocolchicoside in Ethanol

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in ethanol |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|------------------------------------|
| 1       | 8.88                                           | 88.8                           | 0.991                                    | 0.0107                             |
| 2       | 15.79                                          | 79                             |                                          |                                    |
| 3       | 40.28                                          | 100.7                          |                                          |                                    |
| 4       | 65.14                                          | 108.6                          |                                          |                                    |
| 5       | 80.65                                          | 100.8                          |                                          |                                    |
| 6       | 96.73                                          | 96.73                          |                                          |                                    |

Table 5  
Concentration and absorbance data of Thiocolchicoside in Methanol

| Conc. ( $\mu\text{g/ml}$ ) | Absorbance | $\lambda_{\text{max}}$ (nm) |
|----------------------------|------------|-----------------------------|
| 10                         | 0.182      | 248                         |
| 20                         | 0.369      |                             |
| 30                         | 0.541      |                             |
| 40                         | 0.719      |                             |
| 50                         | 0.963      |                             |

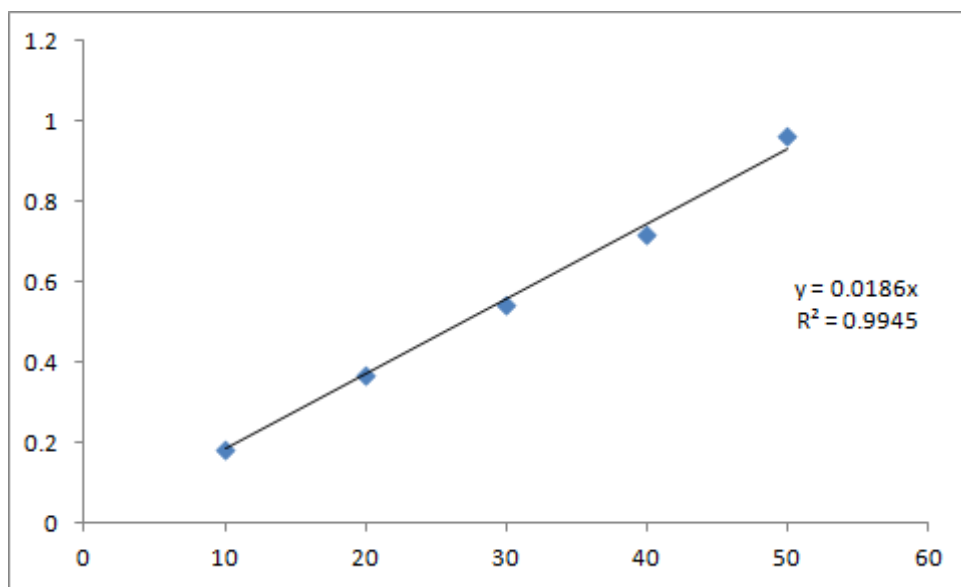


Fig 3: Calibration plot of Thiocolchicoside in methanol

Table 6  
Summary of Thiocolchicoside in Methanol

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in methanol |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|-------------------------------------|
| 1       | 9.78                                           | 97.8                           | 0.9945                                   | 0.0186                              |
| 2       | 19.84                                          | 99.2                           |                                          |                                     |
| 3       | 29.09                                          | 97                             |                                          |                                     |
| 4       | 38.66                                          | 96.6                           |                                          |                                     |
| 5       | 51.77                                          | 103.5                          |                                          |                                     |

Table 7  
Concentration and absorbance data of Thiocolchicoside in PBS 7.4

| Conc. ( $\mu\text{g/ml}$ ) | Absorbance | $\lambda_{\text{max}}$ |
|----------------------------|------------|------------------------|
| 10                         | 0.212      | 302                    |
| 20                         | 0.416      |                        |
| 30                         | 0.665      |                        |
| 40                         | 0.837      |                        |
| 50                         | 1.058      |                        |

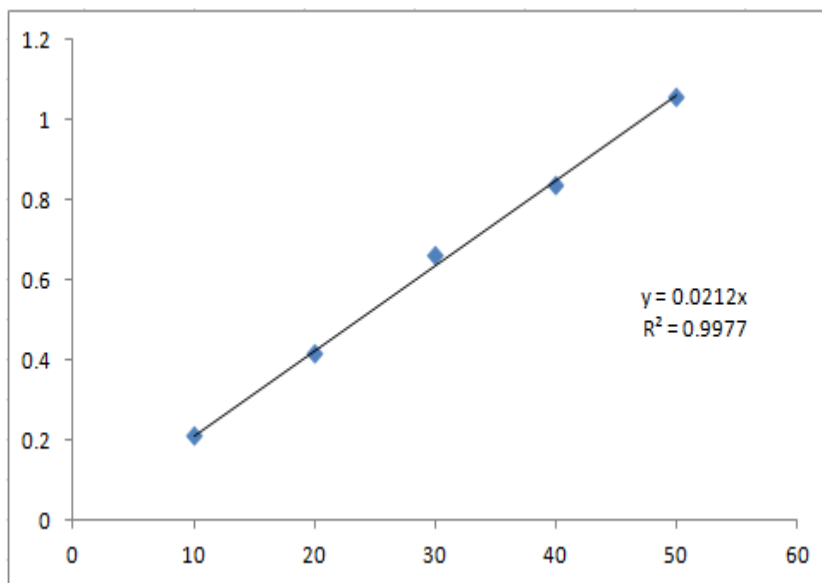


Fig 4: Calibration plot of Thiocolchicoside in 0.01M PBS 7.4

Table 8  
Summary of Thiocolchicoside in PBS 7.4

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in 0.01M PBS 7.4 |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|------------------------------------------|
| 1       | 10                                             | 100                            | 0.9977                                   | 0.0212                                   |
| 2       | 19.62                                          | 98.1                           |                                          |                                          |
| 3       | 31.37                                          | 104.6                          |                                          |                                          |
| 4       | 39.48                                          | 98.7                           |                                          |                                          |
| 5       | 49.90                                          | 99.8                           |                                          |                                          |

Table 9  
Concentration and absorbance data of Thiocolchicoside n-Octanol

| Conc. ( $\mu\text{g/ml}$ ) | Absorbance | $\lambda_{\text{max}}$ |
|----------------------------|------------|------------------------|
| 10                         | 0.200      | 280                    |
| 20                         | 0.458      |                        |
| 30                         | 0.593      |                        |
| 40                         | 0.942      |                        |
| 50                         | 1.044      |                        |

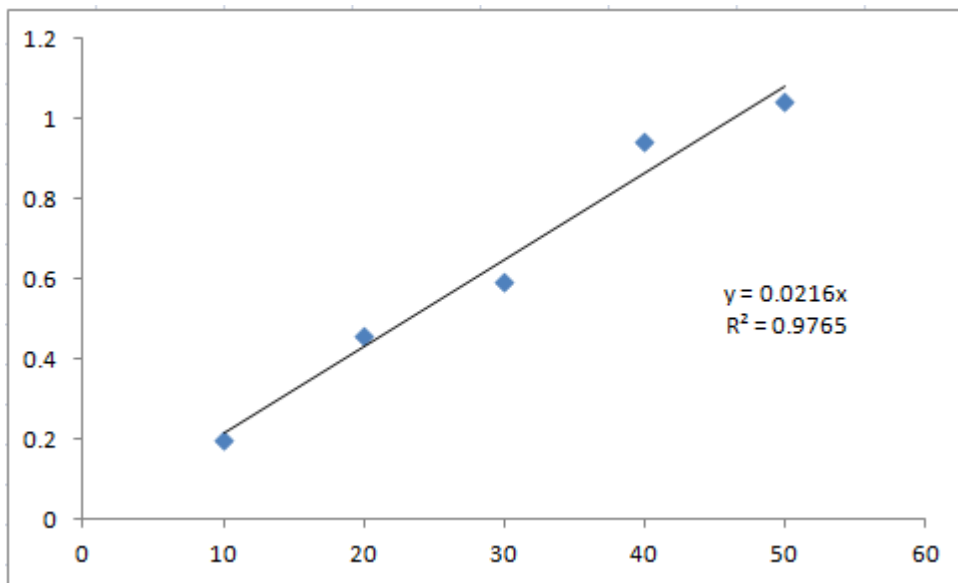


Fig 5: Calibration plot of Thiocolchicoside in n-octanol



Table 10  
Summary of Thiocolchicoside in n-Octanol

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in n-octanol |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|--------------------------------------|
| 1       | 9.26                                           | 92.6                           | 0.9765                                   | 0.0216                               |
| 2       | 21.20                                          | 106                            |                                          |                                      |
| 3       | 27.45                                          | 91.5                           |                                          |                                      |
| 4       | 43.61                                          | 109                            |                                          |                                      |
| 5       | 48.33                                          | 96.7                           |                                          |                                      |

Table 11  
Concentration and absorbance data of Thiocolchicoside in NaCl

| Conc. ( $\mu\text{g/ml}$ ) | Absorbance | $\lambda_{\text{max}}$ |
|----------------------------|------------|------------------------|
| 10                         | 0.235      | 290                    |
| 20                         | 0.455      |                        |
| 30                         | 0.661      |                        |
| 40                         | 0.868      |                        |
| 50                         | 1.095      |                        |

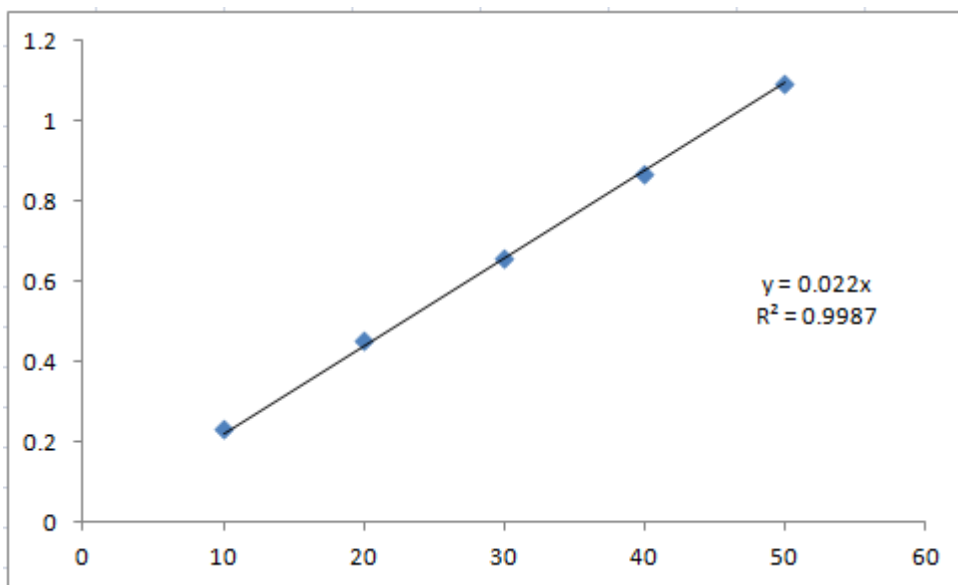


Fig 6: Calibration plot of Thiocolchicoside in 0.9% w/v NaCl

Table 12  
Summary of Thiocolchicoside in NaCl

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in 0.9% w/v NaCl |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|------------------------------------------|
| 1       | 10.68                                          | 106.8                          | 0.9987                                   | 0.022                                    |
| 2       | 20.68                                          | 103.4                          |                                          |                                          |
| 3       | 30.04                                          | 100.1                          |                                          |                                          |
| 4       | 39.45                                          | 98.6                           |                                          |                                          |
| 5       | 49.77                                          | 99.5                           |                                          |                                          |

Table 13  
Concentration and absorbance data of Thiocolchicoside in Acetonitrile:water (70:30)

| Conc. ( $\mu\text{g/ml}$ ) | Absorbance | $\lambda_{\text{max}}$ (nm) |
|----------------------------|------------|-----------------------------|
| 10                         | 0.217      | 294                         |
| 20                         | 0.468      |                             |
| 30                         | 0.655      |                             |
| 40                         | 0.864      |                             |
| 50                         | 1.047      |                             |

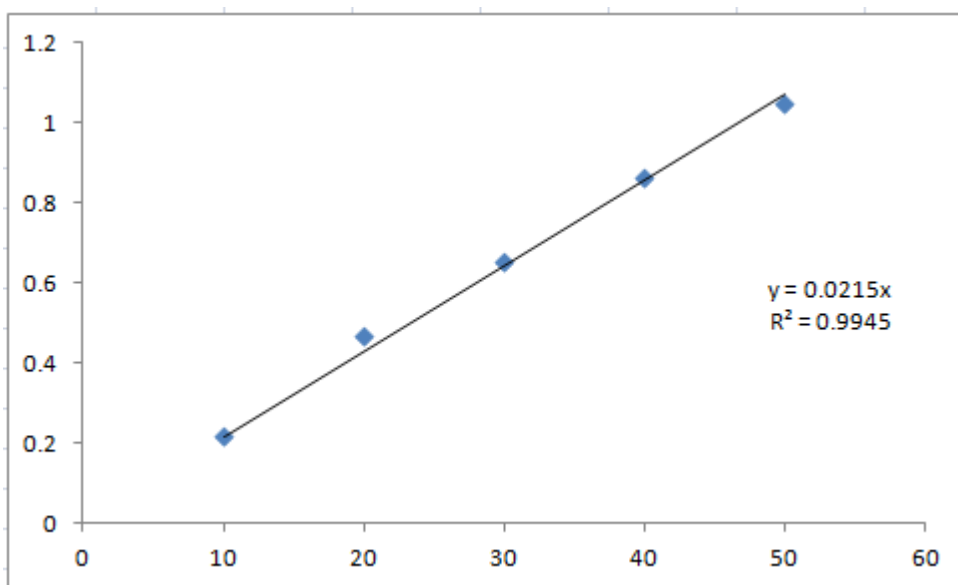


Fig-7 Calibration plot of Thiocolchicoside in Acetonitrile: water (70:30)

Table 14  
Summary of Thiocolchicoside in Acetonitrile: water (70:30)

| Sr. No. | Conc. of drug in dilution ( $\mu\text{g/ml}$ ) | % Recovery of drug in dilution | Regression coefficient ( $r^2$ ) of plot | Slope value (m) of drug in Acetonitrile:water (70:30) |
|---------|------------------------------------------------|--------------------------------|------------------------------------------|-------------------------------------------------------|
| 1       | 10.09                                          | 100.9                          | 0.9945                                   | 0.0215                                                |
| 2       | 21.77                                          | 108.8                          |                                          |                                                       |
| 3       | 30.46                                          | 101.5                          |                                          |                                                       |
| 4       | 40.19                                          | 100.5                          |                                          |                                                       |
| 5       | 48.70                                          | 97.4                           |                                          |                                                       |

### Discussion/Conclusion

Spectrophotometric analysis of Thiocolchicoside was conducted in various solvents and the observed data is presented in tabular form. Pharmaceutical analysis and quality control of drug substances require procedures and methods with good performance characteristics [7].

I. relationship of  $\lambda_{\text{max}}$  with solvent for calibration plots of THC:

Table 15:  $\lambda_{\text{max}}$  summary in various solvents

| Sr. No | Solvent                    | Peak of drug ( $\lambda_{\text{max}}$ ) in nm |
|--------|----------------------------|-----------------------------------------------|
| 1      | Distilled water            | 292                                           |
| 2      | Ethanol                    | 284                                           |
| 3      | Methanol                   | 248                                           |
| 4      | 0.01 M PBS                 | 302                                           |
| 5      | n-Octanol                  | 280                                           |
| 6      | 0.9% w/v NaCl              | 290                                           |
| 7      | Acetonitrile:water (70:30) | 294                                           |

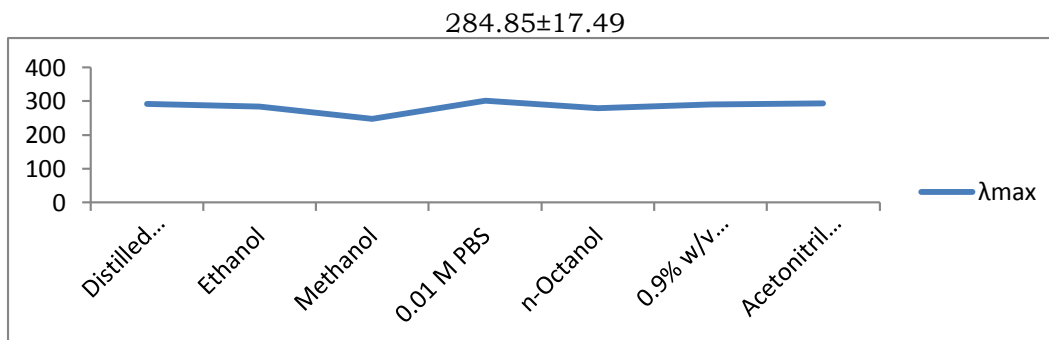


Figure 8: Linearity approach for absorbance maxima of Thiocolchicoside for various solvents

## Summary:

Table 16  
Regression coefficient, slope and MEC data for Thiocolchicoside

| Sr. No. | Solvent                    | Regression coefficient (r <sup>2</sup> ) | Slope  | MEC |
|---------|----------------------------|------------------------------------------|--------|-----|
| 1       | Distilled water            | 0.9999                                   | 0.0208 | 208 |
| 2       | Ethanol                    | 0.9910                                   | 0.0107 | 107 |
| 3       | Methanol                   | 0.9945                                   | 0.0186 | 186 |
| 4       | 0.01 M PBS                 | 0.9977                                   | 0.0212 | 212 |
| 5       | n-Octanol                  | 0.9765                                   | 0.0216 | 216 |
| 6       | 0.9% w/v NaCl              | 0.9987                                   | 0.0220 | 220 |
| 7       | Acetonitrile:water (70:30) | 0.9945                                   | 0.0215 | 215 |

## II. Statistical Determination Of Observed Parameters:

Table 17  
Statistical data of Thiocolchicoside for regression coefficient, slope and absorptivity coefficient

| Sr. No. | Mean ± SD of regression coefficient | Mean ± SD of slope | Mean ± SD of MEC  |
|---------|-------------------------------------|--------------------|-------------------|
| 1       | 0.993257±0.007984                   | 0.019486±0.004031  | 194.8571±40.30893 |

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