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Effect of Solvent on the absorbance maxima (λ_{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 (λ_{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 λ_{max} value. All the stock solutions of Thiocolchicoside were scanned in UV region i.e. from 200 nm-400 nm.

***Keywords*---**Thiocolchicoside, THC, peak, λ_{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 (λ_{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 ($E_{1\%, 1cm}$).

Materials

Thiocolchicoside, distilled water, NaCl, KCl, Na_2HPO_4, KH_2PO_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 100µg/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. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml were prepared. Highest dilution i.e. 50µg/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 KH_2PO_4, 710 mg Na_2HPO_4, 373 mg KCl and 293 mg NaCl in 500 ml distilled water. Stock solution was prepared of concentration of 100µg/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. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml were prepared. Highest dilution i.e. 50µg/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 100µg/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 50µg/ml scanned in UV region i.e. from 200 nm-400 nm and no peak was observed and then stock solution of concentration of 100µg/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. 10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/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 100µg/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. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml were prepared. Highest dilution i.e. 50µg/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 100µg/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 50µg/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. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/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 µg/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 50µg/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. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/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 µg/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 50µg/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. 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/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. (µg/ml)	Absorbance	λ_{max} (nm)
10	0.212	292
20	0.415	
30	0.623	
40	0.829	
50	1.045	

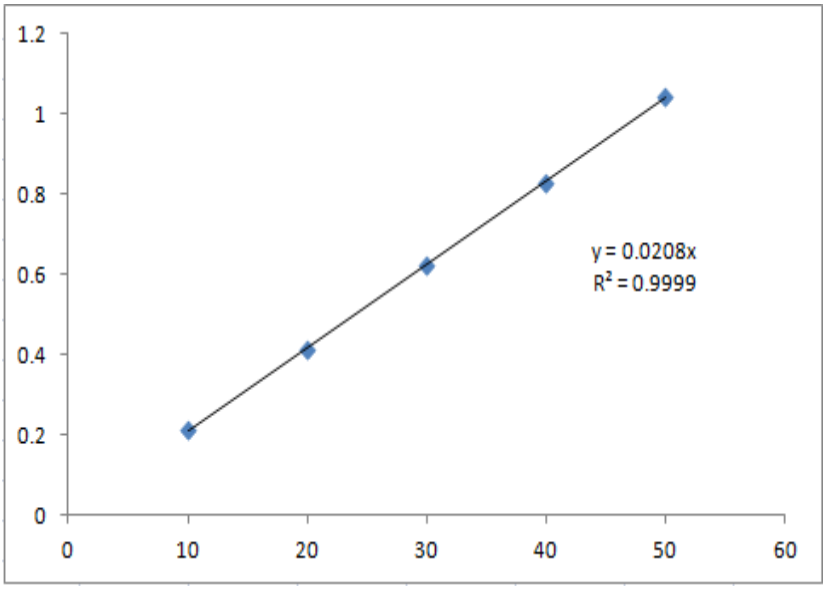

Fig 1: Calibration plot of Thiocolchicoside in distilled water

Table 2
Summary of Thiocolchicoside in DW

Sr. No.	Conc. of drug in dilution (µg/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. (µg/ml)	Absorbance	λ_{max} (nm)
10	0.095	284
20	0.169	
40	0.431	
60	0.697	
80	0.863	
100	1.035	

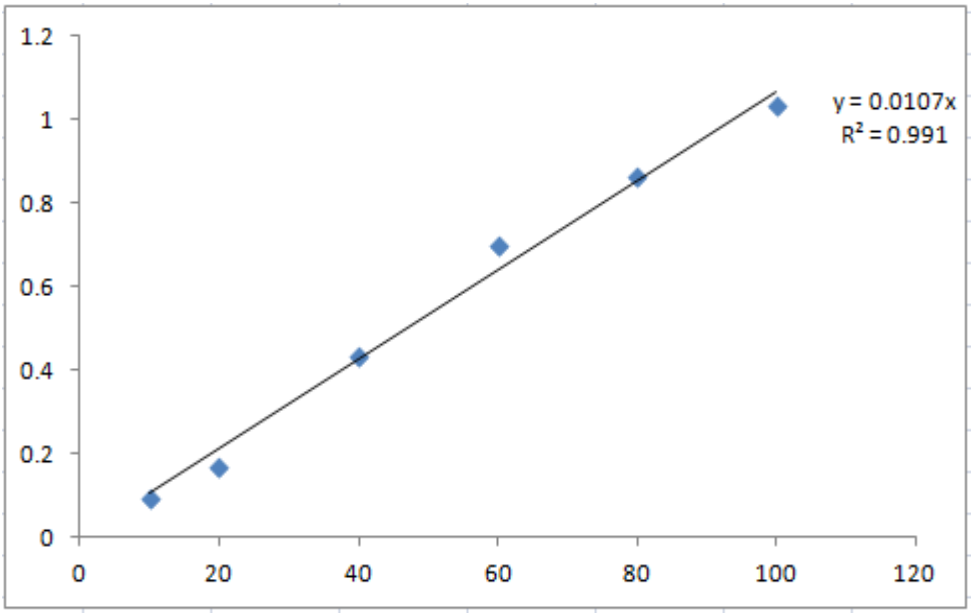

Fig 2: Calibration plot of Thiocolchicoside in ethanol

Table 4
Summary of Thiocolchicoside in Ethanol

Sr. No.	Conc. of drug in dilution (µg/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. (µg/ml)	Absorbance	λ_{max} (nm)
10	0.182	248
20	0.369	
30	0.541	
40	0.719	
50	0.963	

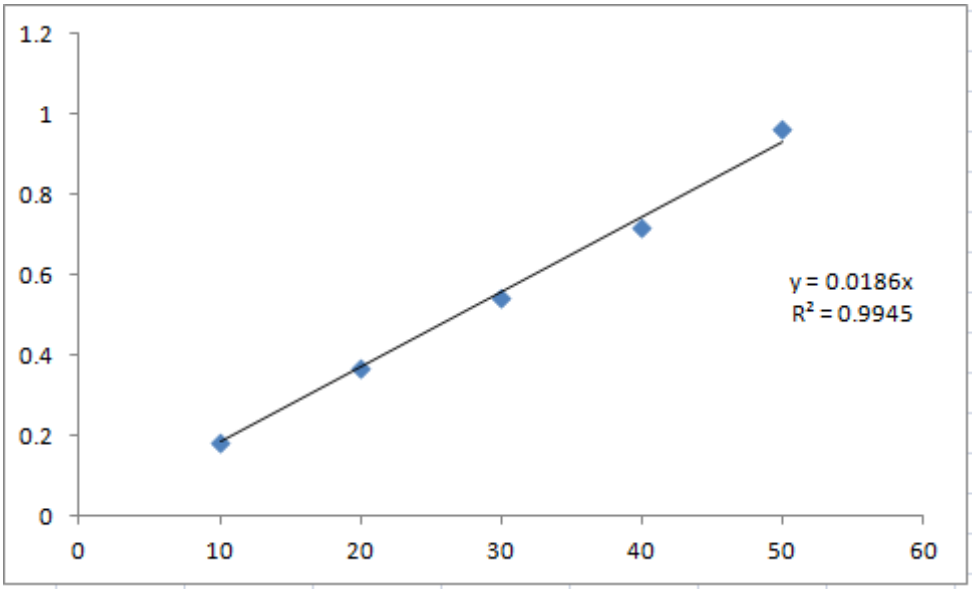

Fig 3: Calibration plot of Thiocolchicoside in methanol

Table 6
Summary of Thiocolchicoside in Methanol

Sr. No.	Conc. of drug in dilution (µg/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. (µg/ml)	Absorbance	λ_{max}
10	0.212	302
20	0.416	
30	0.665	
40	0.837	
50	1.058	

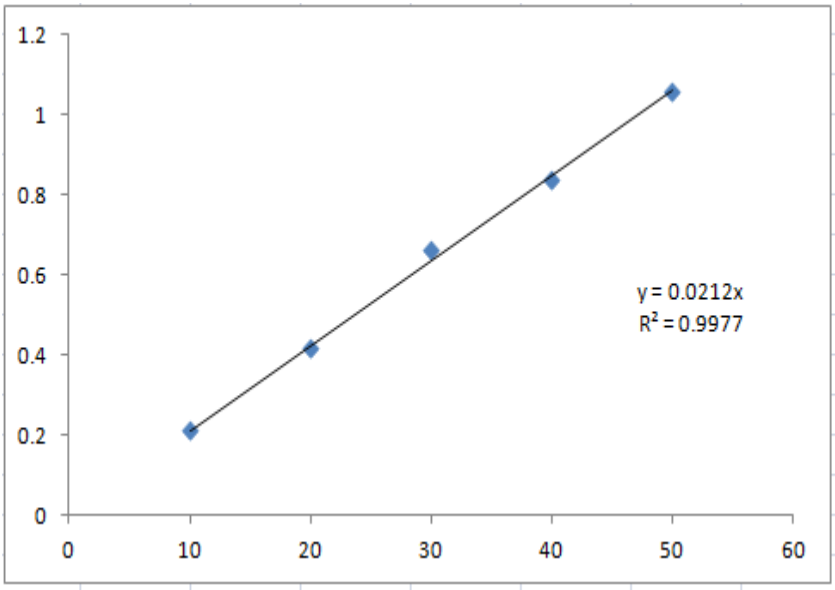

Fig 4: Calibration plot of Thiocolchicoside in 0.01M PBS 7.4

Table 8
Summary of Thiocolchicoside in PBS 7.4

Sr. No.	Conc. of drug in dilution (µg/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. (µg/ml)	Absorbance	λ_{max}
10	0.200	280
20	0.458	
30	0.593	
40	0.942	
50	1.044	

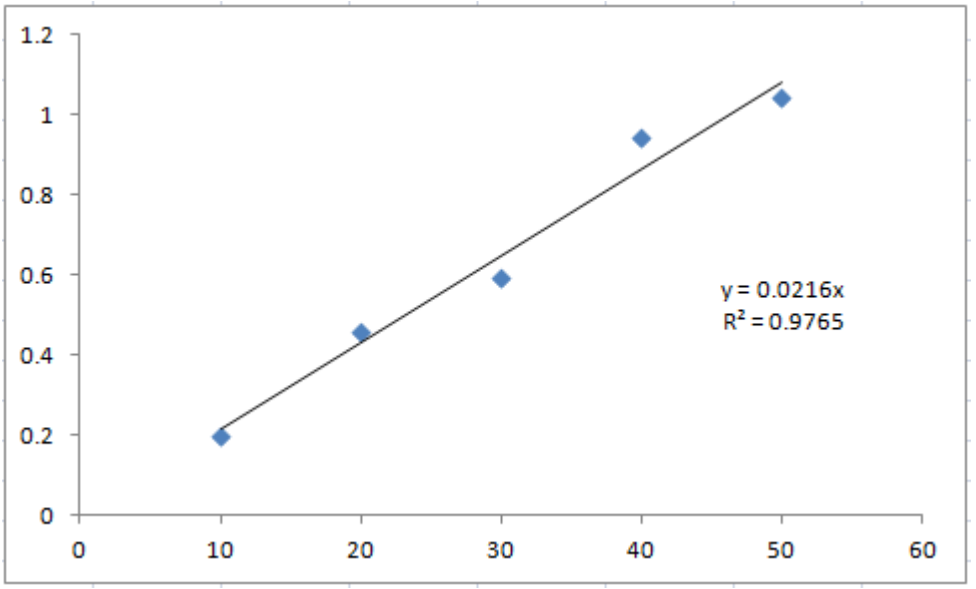

Fig 5: Calibration plot of Thiocolchicoside in n-octanol

Table 10
Summary of Thiocolchicoside in n-Octanol

Sr. No.	Conc. of drug in dilution (µg/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. (µg/ml)	Absorbance	λ_{max}
10	0.235	290
20	0.455	
30	0.661	
40	0.868	
50	1.095	

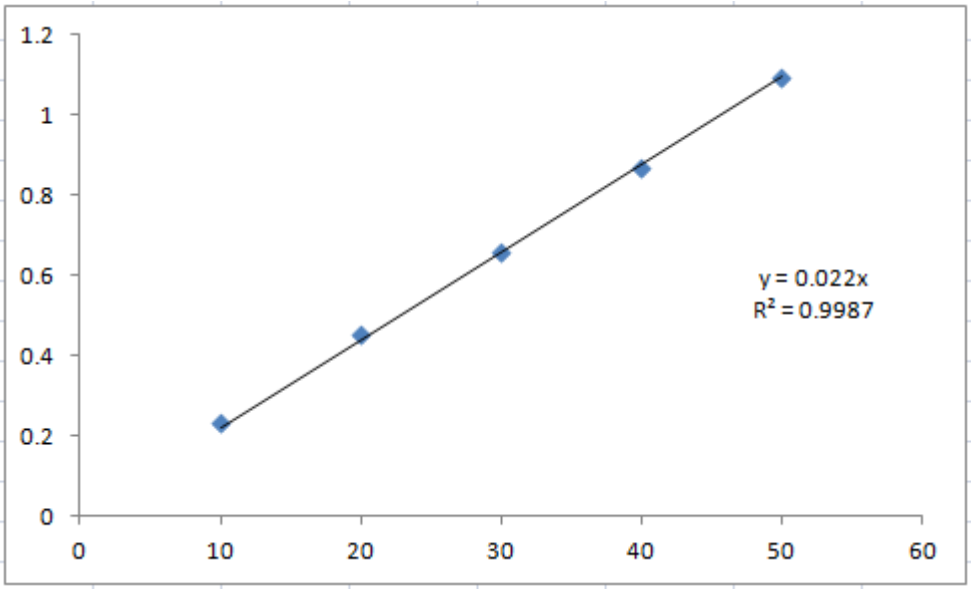

Fig 6: Calibration plot of Thiocolchicoside in 0.9% w/v NaCl

Table 12
Summary of Thiocolchicoside in NaCl

Sr. No.	Conc. of drug in dilution (µg/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. (µg/ml)	Absorbance	λ_{max} (nm)
10	0.217	294
20	0.468	
30	0.655	
40	0.864	
50	1.047	

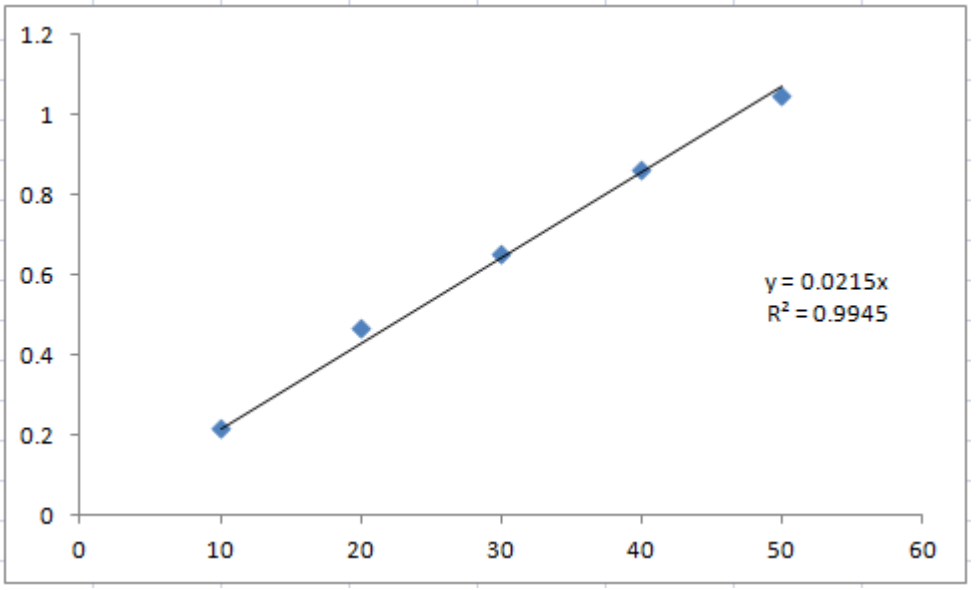

Fig-7 Calibration plot of Thiocolchicoside in Acetonitrile: water (70:30)

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

Sr. No.	Conc. of drug in dilution (µg/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 λ_{max} with solvent for calibration plots of THC:

Table 15: λ_{max} summary in various solvents

Sr. No	Solvent	Peak of drug (λ_{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

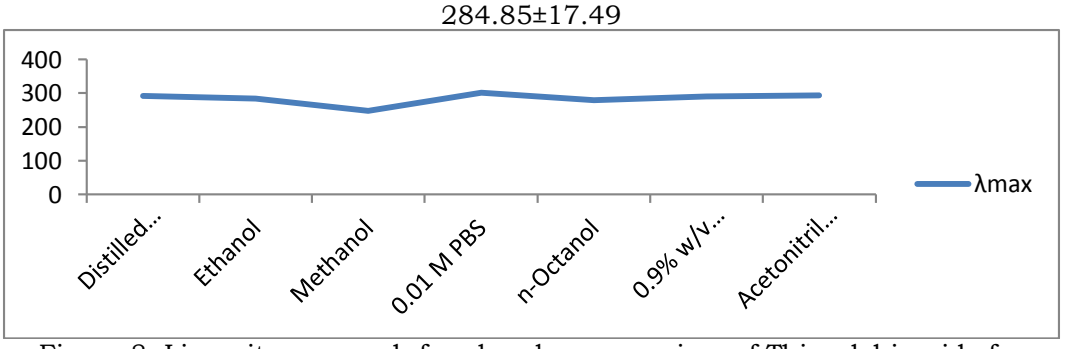

Figure 8: Linearity approach for absorbance maxima of Thiocolchicoside for various solvents

Summary:

Table 16
Regression coefficient, slope and MEC data for Thiocolchicoside

Sr. No.	Solvent	Regression coefficient (r^2)	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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