Journal of Cardiovascular Disease Research
ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

A Validated UV Visible Spectroscopic Methods For Determination Of Levamisole Hcl

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ABSTRACT

The simple and sensitive extractive spectrophotometric method has been developing for estimation of levamisole coordination compound [LH] that is in pure and pharmaceutical indefinite quality forms. In the present study suitable UV spectroscopic method was developed using 0.1M H_2SO_4 as a solvent at wavelength of 220.8nm and then accurate and precise with a regression correlation of 0.998 and can be used for routine analysis. Then related to visible the developed methods are base on formations of colored chloroform extractable ion-association complex of the drug with bromothymol blue.

The effectiveness of volume of coloring agent, buffer solution have been studied and optimised. Beer's law is obeyed in the concentration ranges between 1-5µg/ml respectively. The area unit applied for determination of medication in business tablets and result of study were valid statistically through recovery study. The developed drug used in analytical chemistry for quantitative determination of different analytes.

KEYWORDS:
Spectrophotometry, Levamisole HCL, Bromothymol blue, Absorptive reaction.

INTRODUCTION

Levamisole hydrochloride chemically known as 2, 3,5,6-tetra hydro-6- phenylimidazo [2,1b],thiazole hydrochloride[1,3].Levamisole is an antihelmintic drug that commonly used to treat parasitic, viral and bacterial infections[1]. It can stimulates the formation of antibodies to various antigens, to enhance the T-cells which responses and leads to activation and proliferation and also macrophage functions which are phagocytosis and chemotaxis, also increase neutrophil mobility[2].

The mode of action of this drug as antiparasitic agent, which can treat ascariasis, related to its agonist activity to L-subtype nicotinic acetylcholine receptors in nematode muscles[3].Because of immunomodulatory effect, this drug may used in study to treat in various immunemediated diseases results in positive initiated.[4]

This drug with combinations with others leads to treat the various cancers. Potentiate monocyte then increase neutrophil mobility adherence and chemotaxis and inhibit alkaline phosphatase (6). It also has cholinergic activity. It works as a nicotinic acetylcholine receptor agonist that causes continued stimulation of parasitic worm muscles, which leads to paralysis (5). Present preparations are intended for veterinary use such as dewormer in cattle, pigs and sheep (7).

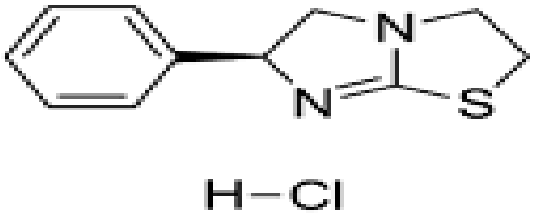

Figure 01: Structure of levamisole HCL

Depend upon the amount of light and its wavelength which absorbs by sample, UV and Visible spectroscopy is bases on absorption of light by sample solutions. Absorbance was noticed also the purity of sample then concentration of drug that presents in sample should identified(8). The amount of absorbed light is related to the amount of sample and quantitative analysis is possible by optical spectroscopy. The new spectrophotometers will additionally support the customer work flow with fast, easy to use and reliable analytical instrumentations (9).

The parameters of validation includes accuracy, precision, LOD, LOQ, recovery study and range were evaluated. The methods that applied for determination of drugs in tablets and results of analysis will be validated statistically through recovery studies.[10]

MATERIALS&METHODS:

METHODS

METHOD-A:

INSTRUMENTATION:

A UV-Visible double beam spectrophotometer (UV-1600 SHIMADZU) was used for spectrophotometric method. All weighing are done on analytical weighing balance (Model SHIMADZU).

REAGENTS &CHEMICALS:

Levamisole HCL, tablet of 50mg, 0.1M H_2SO_4, Distilled water.

PREPARATION OF STANDARD STOCK:

Standard drug solution of levamisole HCL was prepared accurately weighing 10mg of the drug and dissolved in 0.1m H_2SO_4 and the volume was made up to 100ml to obtain stock solution (100µg/ml).Take a series of 1 ml of stock solution and make upto 10ml with 0.1M dose type or bulk type is identified. The static development ensures the quantity of specific drug is simply determined. The validation parameters ensure that the development technique is precise, correct and reproducible and might be used for routine analysis of levamisole in bulk and combined dose type.

METHOD B:

MATERIALS&METHODS:

A SHIMADZU, Double beam UV Visible spectrophotometer with 1cm matched quartz cells was used for all of spectral and absorbance measurements. A digital hydrogen ion concentration measurement. The coloring reagent namely BTB (AR Grade) are used without any further purification. The dyes were used 50mg in solutions, Potassium Hydrogen phthalate was used as a buffer and 0.1N HCL was used for maintaining the pH in the medium. Chloroform used to separate and done throughout the work.

EXPERIMENTAL:

REAGENTS PREPARATIONS:

1) 50mg of drug in 50ml of distilled water.
2) 0.1N HCL preparation (8.5ml in 100ml) from that solution 0.1n HCL take 1ml and dilute with distilled water makeup to 100ml.
3) Dye- 50mg of bromothymol blue in 100ml volumetric flask then add distilled water upto mark and sonicate it for 2mins.
4) Potassium hydrogen phthalate preparation (0.2m) 4gms with 100ml distilled water.
5) 0.2M HCL preparation (17ml in 1000ml).
6) Acid phthalate buffer-Take 25ml of 0.2m potassium hydrogen phthalate add 24.75ml of 0.2m HCL and add water to makeup to100ml.

[A] STANDARD DRUG PREPARATION:

Stock solution of Levamisole Hydrochloride [LH] was prepared by accurately weighing 50mg of pure drug into 50ml volumetric flask and dissolved it in a 25ml distilled water and the volume was made upto the mark with distilled water to get a concentration of 1mg/ml.(stock solution).The working standard solution-1 of LH was prepared by pipetting out 1ml of standard stock solution into 50ml volumetric flask and the volume was made upto the mark with distilled water to get a concentration of 50µg/ml.

[B] FOR PHARMACEUTICAL FORMULATIONS

A tablet marketed formulation, tablets each containing 350mg of accurately weighed and powdered. The powder equivalents to 50mg of LH was accurately weighed and transferred to volumetric flask of 50ml capacity containing 25ml of distilled water and sonicate it for 10min. The flask was shaken and volume was make upto the mark with distilled water to give a solution of 1000µg/ml.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

The higher than answer was fastidiously filtered through Whattman paper. From this solution 1ml was pipette out and diluted to 50ml with distilled water in a 50ml volumetric flask to give a concentration of 100 µg/ml from this 1ml of solution was pipette into 50ml volumetric flask and a 1µg/ml was prepared as a working solution.

From the operating customary drug answers 5ml of drug solution were placed in an exceedingly 250 cc unit capacity separating funnel. Into this 5ml of 0.1n HCL and5ml acid phthalate buffer followed by 5ml of BTB. Shake it vigorously for 5mins and add 10ml chloroform and shake vigorously and kept a side for 15mins for clear separation of phases and completion of reaction. The chloroform layer was separated and the absorbance was measured against a reagent blank at 450nm, the results are recorded.

CONSTRUCTION OF CALIBRATION CURVE:

Calibration curves were construct according to the optimum conditions. From the working standard drug solution (50 µg/ml) pipette out 2,4,6,8 and 10ml (which gives 2-10 µg/ml) drug solution were placed in 5 different 250ml capacity separating funnels Into this 5ml of 0.1N HCL (diluted) then 5ml of BTB was added followed by 5ml acidic phthalate buffer solution shake it for 5mins, 10ml of chloroform was then superimposed to extract the drug from the mixture. Shake the funnels vigorously for 2mins and then the reaction mixture was kept aside for 15mins for clear separation of phases for completion of reaction. The chloroform layer was separated and the absorbance was measured against a reagent blank at 450nm, the results were recorded.

RESULTS:

METHOD A:

ANALYTICAL WAVELENGTH:

The maximum absorption was found to be at the wavelength of 220.8nm.Hence, wavelength for levamisole HCL as shown in figure 2.

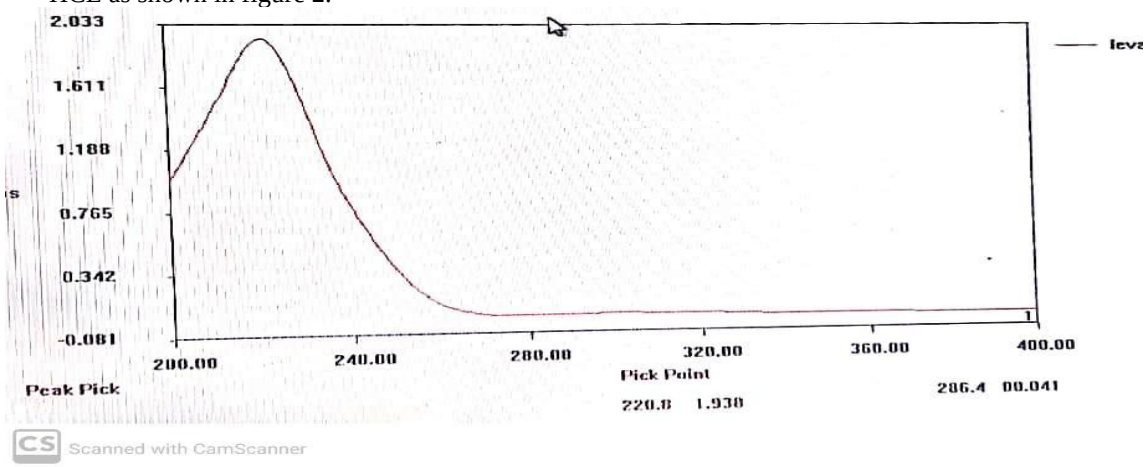

Figure 02: A typical UV spectrum of Levamisole HCL at 220.8nm.

CALIBRATION CURVE:

The results of absorbance for the prepared concentration were plotted i.e. Concentration Vs Absorbance the method was found to be linear over the prepared concentration range with standard equation y=0.1728x+0.0422 and regression value was found to be 0.998 as shown in figure 03. From the calibration data obtained it was found that the regression coefficient was <1 which is within the limits of Beer Lamberts law.

SR.NO	Concentration(µg/ml)	Absorbance
1	1	0.199
2	2	0.379
3	3	0.595
4	4	0.755
5	5	0.875

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Table 01: Calibration Curve Data of Levamisole HCL

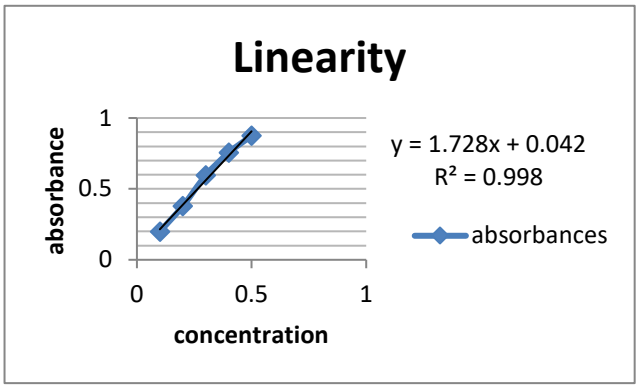

FIGURE 03: Calibration graph of Levamisole HCL at 220.8nm

PRECISION:

Precision of the method was evaluated for Levamisole HCL .The reproducibility of this method was evaluated in same laboratory.

The values obtained to be précised in respect to reproducibility and as well as repeatability.

	ABSORBANCE		
ANALYTE	0 HR	3 HR	6HR
MEAN	0.589	0.581	0.568
SD	0.0007	0.00122	0.0013
%RSD	0.1277	0.2099	0.2288

Table 02: Determination Intra-day precision by UV

ANALYTE	ABSORBANCE		
	0 HR	24 HR	48HR
MEAN	0.589	0.568	0.538
SD	0.0007	0.0013	0.0018
%RSD	0.1277	0.2288	0.3438

Table 03: Inter-day precision by UV

ACCURACY (RECOVERY STUDY):

Accuracy of method bases on recovery experiments. The recovery was performed at three levels 80,100,120 % of Levamisole HCL standard concentration. Three samples were prepared for each and all recovery level. The solutions were then analyzed and the % recoveries were calculated from the calibration curve values.The recovery value for Levamisole HCL was 99.30±0.616 and RSD was 0.6409 which is less than 2, which shows that the method has good reproducibility.

STATISTICS	LEVEL OF RECOVERY		
	80%	100%	120%
AMOUNT PRESENT	1	2	4
AMOUNT OF STANDARD ADDED	1	1.9	3.3
TOTAL AMOUNT RECOVER	2	3.9	7.3
% RECOVERY	100	96	100
MEAN	99.30		
STANDARD DEVIATION	0.001		

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

%RSD	0.4577

Table 04: Recovery Studies

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

Limit of detection is the lowest amount of analyte which can be detected but not necessarily quantified, and limit of quantification is the lowest possible concentration that can be quantified LOD and LOQ were found to be 0.3437 µg/ml &1.0416 µg/ml respectively.

SPECIFICITY:

Specificity is the ability of the method which accurately measures the analyte response in the presence of all potential sample components. The results that compares with analysis of standard Levamisole and tablet formulations. Excipient of the solid dosage form did not interrupt with analyte that shows method has good specificity.

VALIDATION PARAMETERS:

All the validation parameters as reported in table 05 were found to be with in desired range which depicts that the method was found to be reproducible with respect to all the validation parameters and can be a useful tool for routine evaluation of drug in bulk and combined dosage forms.

PARAMETERS	RESULTS
LINEARITY RANGE	1-5 µg/ml
REGRESSION EQ	Y=x+0.0422
CORRELATION COEFFICIENT	0.998
SLOPE(m)	0.1728
Y INTERCEPT	0.2708
Λ MAX	220.8nm
LOD	0.34375
LOQ	1.416 µg/ml
INTER DAY PRECISION	0.01802
INTRADAY PRECISION	0.0981
ACCURACY(%MEAN RECOVERY)	99.30

Table 05: Validation Parameters

METHOD B:

PARAMETER	LH(AT 450NM)
LINEAR RANGE (µG/ML)	2-10
MOLAR ABSORPTIVE	329.77
REGRESSION EQUATION* (Y)	1.0047
SLOPE (M)	0.0959
INTERCEPT (C)	0.0305
CORRELATION COEFFICIENT (R2)	0.999
SANDAL'S SENSITIVITY	0.00964
LIMIT OF DETECTION	0.0485
LIMIT OF QUANTIZATION	0.1470
REGRESSION COEFFICIENT	1.0047

Table 06: Optical Characters of the proposed methods for LH in BTB

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

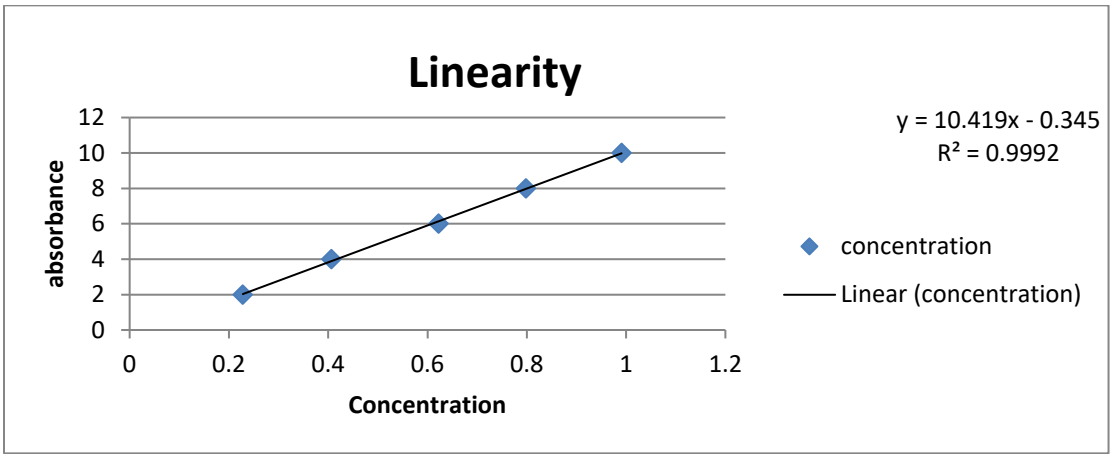

Figure 04: Beer's Law plot of LH with BTB

ACCURACY (RECOVERY STUDIES):

Accuracy of the method bases on recovery experiments. The recovery was performed at three levels 80,100 and 120 percents of Levamisole HCL standard concentration. Three samples were prepared for each and all recovery level. The solutions were then analyzed and the percentage recoveries were calculating from the calibration curve values.The recovery value for Levamisole HCL was 98.86±0.616 and RSD was 0.6598 which is less than 2, which shows that the method has good reproducibility.

STATISTICS	LEVEL OF RECOVERY		
	80%	100%	120%
AMOUNT PRESENT	1	3	4
AMOUNT OF STANDARD ADDED	1	2.9	4
TOTAL AMOUNT RECOVER	2	5.9	8
%RECOVERY	100	96	100
MEAN	98.66		
SD	0.001		
%RSD	0.15873		

PRECISION:

Precision of the method was evaluated for Levamisole HCL. The reproducibility of method and repeatability was evaluated in same laboratory.

The values obtained were as per Table 07 and table 08.From the data obtained in the method was found to be précised in respect to reproducibility and as well as repeatability.

ANALYTE	ABSORANCE		
	0 HR	3 HR	6 HR
MEAN	0.698	0.671	0.633
SD	0.00147	0.0089	0.0021

%RSD	0.2111	0.13329	0.36112

Table 08: Determination Intra- day Precision by UV

ANALYTE	ABSORBANCE		
	0 HR	3 HR	6 HR
MEAN	0.698	0.631	0.621
SD	0.00147	0.0116	0.0013
%RSD	0.2111	0.1852	0.2197

Table 09: Inter-day Precision by UV

DISCUSSION:

METHOD A:

A simple, rapid and properly validated UV spectroscopic method was developed for estimation of Levamisole hydrochloride.

OPTIMUM CONDITIONS:

The optimum conditions for the developed ways are mounted supported the study of the consequences of assorted parameters like volume of the acid buffer, volume of the dye, concentration of drug. Control experiments are carried out by measuring absorbances at 450 nm of series of the solutions varying one and fixing the other parameters.

METHOD B:

Levamisole HCL forms ion-association complex in acidic medium with Bromothymol blue indicator and these complexes are quantitatively extracted through chloroform. The absorbance spectra of the ion-association complexes are by plotting absorbance against wavelength and from the respective absorbance spectra the maximum absorbance are found to be 450nm.

OPTICAL CONDITIONS:

The optical characteristics such as limits of Beer's law, Molar extinction coefficient, and standard deviation of intercept, LOD, LOQ and correlation coefficient were calculated for this method and results are summarized. The values obtained for determination Levamisole HCL in pharmaceutical formulations by the planned strategies. Studies revels that the common excipients at all.

RECOVERY STUDIES:

Recovery studies by the standard addition methods were performed to study the accuracy of the method.Pre analyzed the of Levamisole hydrochloride spiked with 80,100 and 120% extra LH standard and the mixture were analyzed with the proposed method. Accuracy was assessed as the percentage recovery at each concentration level. Data obtain from the accuracy study are shown in table 4.

CONCLUSIONS:

Levamisole Hydrochloride involves in ion association complex formation with coloring agent bromo thymol blue which is extractable into chloroform from aqueous phase. Levamisole Hydrochloride is expected to attract the opositively charged part of the BTB and behaves as single unit being held together by electrostatic attractions. Bases on analogy the structure of an ion association complex are shown in following scheme.

In the present study a suitable UV Spectroscopic method was developed for Levamisole hydrochloride in 0.1n H_2SO_4 as dissolution medium for drug and method was validated for different parameters as accuracy, precision, specificity, standard deviation, remittance, beer's law, LOD, LOQ and recovery. It can be concluded that the developed method has good reproducibility, repeatability that can be routinely used for estimation of Levamisole hydrochloride in bulk and combined formulation.

ABBREVIATTIONS

LH	LEVAMISOLE HYDROCHLORIDE
HCL	HYDROCHLORIDE
BTB	BROMO THYMOL BLUE
LOD	LIMIT OF DETECTION
LOQ	LIMIT OF QUANTIFICATION
RSD	RELATIVE STANDARED DEVIATION
SD	STANDARED DEVIATION
µg/ml	MICROGRAM PER MILLILITER
UV	ULTRAVIOLET
M	MOLAR

TABLES

Table 01: Calibration Curve Data of Levamisole HCL

SR.NO	Concentration(µg/ml)	Absorbance
1	1	0.199
2	2	0.379
3	3	0.595
4	4	0.755
5	5	0.875

Table 02: Determination Intra-day precision by UV

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Table 04: Recovery Studies

STATISTICS	LEVEL OF RECOVERY		
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AMOUNT PRESENT	1	2	4
AMOUNT OF STANDARD ADDED	1	1.9	3.3
TOTAL AMOUNT RECOVER	2	3.9	7.3
% RECOVERY	100	96	100
MEAN	99.30		
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%RSD	0.4577		

Table 05: Validation Parameters.

PARAMETERS	RESULTS
LINEARITY RANGE	1-5 µg/ml
REGRESSION EQ	Y=x+0.0422
CORRELATION COEFFICIENT	0.998
SLOPE(m)	0.1728
Y INTERCEPT	0.2708
Λ MAX	220.8nm
LOD	0.34375
LOQ	1.416 µg/ml
INTER DAY PRECISION	0.01802
INTRADAY PRECISION	0.0981
ACCURACY(%MEAN RECOVERY)	99.30

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PARAMETER	LH(AT 450NM)
LINEAR RANGE (µG/ML)	2-10
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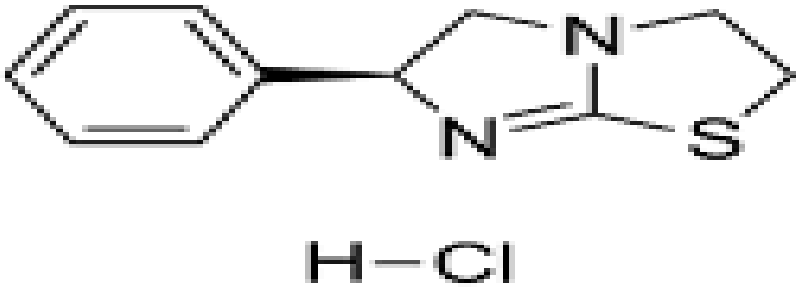
REGRESSION EQUATION* (Y)	1.0047
SLOPE (M)	0.0959
INTERCEPT (C)	0.0305
CORRELATION COEFFICIENT (R2)	0.999
SANDAL'S SENSITIVITY	0.00964
LIMIT OF DETECTION	0.0485
LIMIT OF QUANTIZATION	0.1470
REGRESSION COEFFICIENT	1.0047

FIGURES

Figure 01: Structure of Levamisole HCL

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Figure 02: A Typical UV Spectrum of Levamisole HCL At 220.8nm.



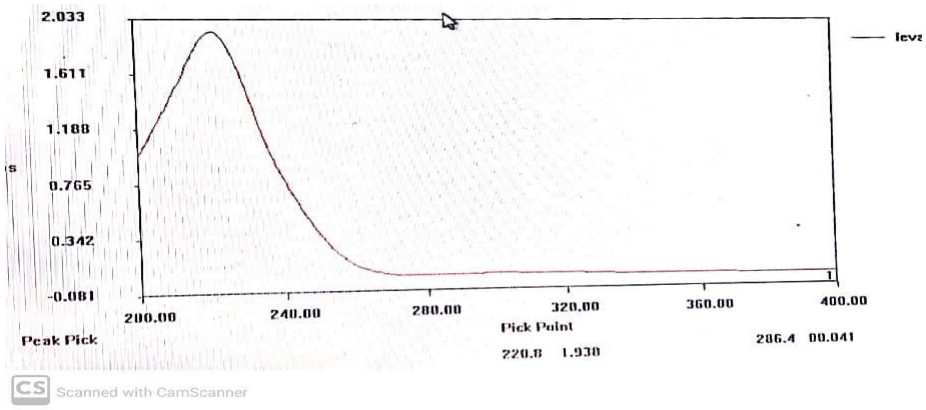


Figure 03: Calibration graph of Levamisole HCL at 220.8nm

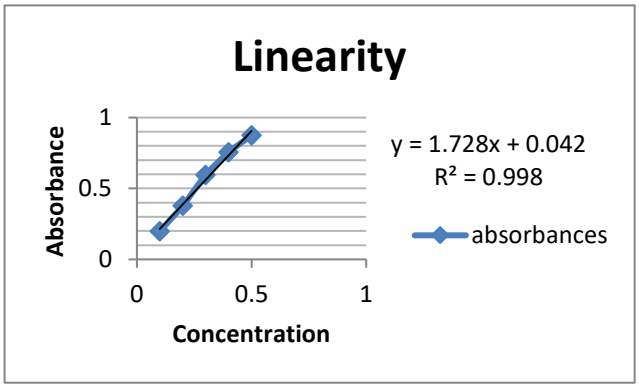
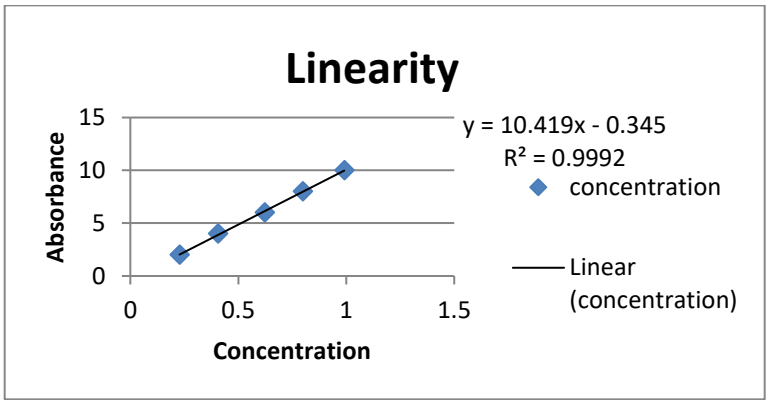


Figure 04: Beer's Law plot of LH with BTB

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021



ACKNOWLEDGEMENT:

We are thankful to management and staff, SIR C R REDDY COLLEGE OF PHARMACEUTICAL SCIENCES, ELURU, WEST GODAVARI.

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