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Extractive Spectrophotometric Methods for the determination of Levamisole Hydrochloride

Johnson Misquith*[1], Padmavathi P.Prabhu[1], E.V.S.Subrahmanyam[1], A.R.Shabaraya[2]

[1]Department of Quality Assurance, Srinivas College of Pharmacy, Valachil-574143, India.
[2]Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil-574143, India.

***Corres. Author: jmisquith@gmail.com**
Tel: +91-824-2274722, Fax: +91-824-2274725,Cell:+919964023728

Abstract: Simple and sensitive extractive spectrophotometric methods have been developed for the estimation of LevamisoleHydrochloride[LH] in pure and pharmaceutical dosage forms. The developed methods are based on the formation of colored chloroform extractable ion-association complex of the drug with bromocresol green (BCG). The extracted complexes showed absorbance maxima at 420nm. Beer's law is obeyed in the concentration ranges between 1.5-7.5 µg/ml respectively. The effectiveness of volume of coloring agent, buffer solutionhave been studied and optimized. This methodis validated as per the guidelines of ICH. The methods are applied for the determination of drugs in commercial tablets and results of analysis were validated statistically through recovery studies.

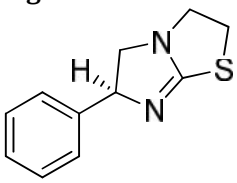
Keywords: LevamisoleHydrochloride[LH], Extractive spectrophotometric methods, Bromocresol Green indicator.

INTRODUCTION:

LevamisoleHydrochloride [LH](Fig 1) is the *S*-enantiomer of tetramisole- a synthetic imidazo-thiazole derivativeacting as an anthelmintic [1]. The single enantiomer was introduced in 1969 since the other enantiomer (dexamisole) showed more adverse effects[1,2]. Levamisole proved to be also effective in combination with 5-fluorouracil as adjuvant therapy in patients with colon carcinoma and current investigations of Levamisole HCl are focused on its immunomodulatory effects. The literature survey reveals that several methods are reported for the determination of LH, by HPLC[7,8,12,15,16],LC-MS[9,13,14], GC[17], GLC[18]and few Extractive spectrophotometric methods for Tetramisole[2,3,4,5] have been reported. In this paper, we reportedasimple and sensitive extractive spectro-photometric method for the assay of drug. The methods are based on ion-pair complexation of drug with dye as bromocresol green and measuring the

absorbance of the colored complexes by extracting into chloroform.

Fig 1: Levamisole Hydrochloride



. HCl

MATERIALS AND METHODS:

AShimadzu, UV Spec-1700, digital spectrophotometer with 1 cm matched quartzcells was used for all spectral and absorbance measurements. ASystronics µ pH system-361digital pH meter was used for all pH measurements. Pure drugwas procured from β-Pharmaceuticals and Research Ltd., Mangalore as a gift sample. The colouring reagentnamely BCG (AR Grade) supplied by SD Fine Chemicals Ltd,Mumbai are used without any further purification. The dyes were used as0.04% solutions, Potassium Hydrogen phthalate was used as a buffer and 0.1N HCl was used for maintainingthe pH in the medium. Chloroform (AR Grade) supplied by SD Fine ChemicalsLtd, Mumbai is used throughout the work.

EXPERIMENTAL:

a) Standard drug preparation

Stock solution of Levamisole Hydrochloride [LH] was prepared by accurately weighing 100mg of pure drug into a 100 ml volumetric flask and dissolved it in a 25 ml distilled water and the volume was made up to the mark with distilled water to get a concentration of 1 mg/ml.(Stock Solution).The working standard solution-1 of LH was prepared by pipetting out 5 ml of the standard stock solution into a 100 ml volumetric flask and the volume was made up to the mark with distilled water to get a concentration of 50µg/ml.

b) For Pharmaceutical formulations

A tablet marketed formulation, **Dicaris (*Johnson & Johnson*)** was obtained for all analytical study. Twenty tablets each containing 100 mg of LH were weighed and powdered. The powder equivalent to 100 mg of LH was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the distilled water and sonicated for 10 min. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000 µg/ml (Stock Solution-1).

The above solution was carefully filtered through Whatmann filter paper (No. 41). From this solution 10ml was pipetted out and diluted to 100 ml with distilled water in a 100 ml volumetric flask to give a concentration of 100 µg/ml (Stock Solution-2) from this 6ml of the solution was pipetted into a 100ml volumetric flaskand a 6 µg/ml was prepared as a working solution.

From the working standard drug solutions 5ml of (6µg/ml) drug solution were placed in a 250 ml capacity separating funnel. Into this 5 ml of 0.04% BCG was added followed by 5 ml buffer solution, 10 ml of chloroform was then added to extract the drug from the mixture. Shake the funnel vigorously for 2 min. and then the reaction mixture was kept aside for 10 min for the clear separation of the phases and completion of reaction. The chloroform layer was separated and the absorbance was measured against a reagent blank at a 420 nm, the results are recorded in Table 2.

c) Preparation of calibration curve

Calibration curves were constructed according to the optimum conditions. From the working standard drug solution (50µg/ml) pipette out 6, 12, 18, 24, and 30 ml (which gives 3-15µg/ml) drug solution were placed in 5 different 250 ml capacity separating funnels. Into this 5 ml of 0.04% BCG was added followed by 5 ml buffer solution, 10 ml of chloroform was then added to extract the drug from the mixture. Shake the funnels vigorously for 2 min. and then the reaction mixture was kept aside for 10 min for the clear separation of the phases and completion of reaction. The chloroform layer was separated and the absorbance was measured against a reagent blank at a 420 nm, the results are recorded in Figure 3.

d) Optimum conditions

The optimum conditions for the developed methods are fixed based on the study of the effects of various parameters such as volume of the acid buffer, volume of the dye. Control experiments are carried out by measuring absorbance at 420nm of series of the solutions varying one and fixing the other parameters.

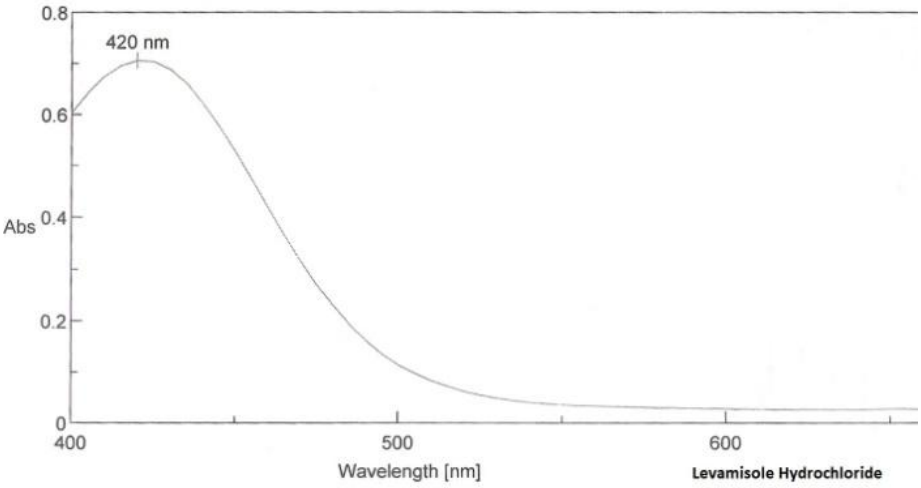


Figure 2: Absorption spectra of LH with BCG

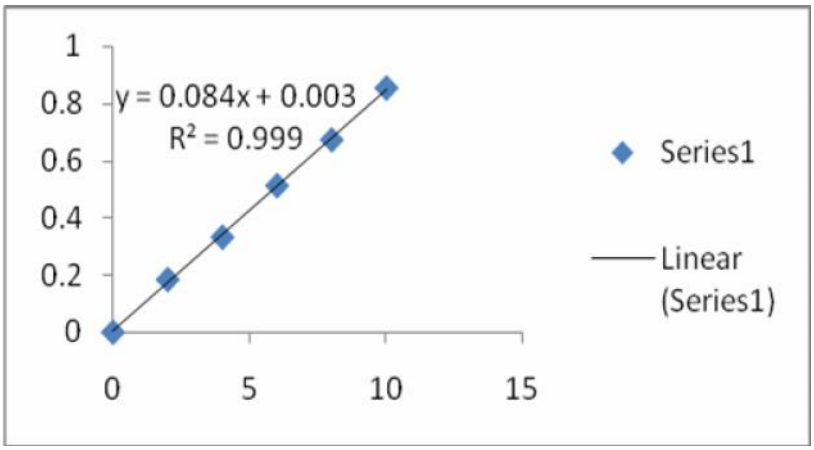


Figure 3: Beer's Law plot of LH with BCG

Table 1: Optical characteristics of the proposed methods for LH in BCG

Parameter	**LH (at 420 nm)**
Linear Range (µg/ml)	1.5-7.5
Molar absorptivity (1/mol.cm)	1.04×10^4
Regression Equation* (y)	y=bx+a
Slope (b)	0.1036
Intercept (a)	0.0012
Correlation coefficient (R^2)	0.9996
Standard deviation of slope	0.00011
Standard deviation of intercept	0.00085
Limit of Detection (µg/ml)	0.0035
Limit of Quantitation (µg/ml)	0.0106

**Y = bx+a, where 'Y' is the absorbance and x is the concentration of LevamisoleHydrochlorideµg/ml. For six replicates*

Table 2: Determination of LevamisoleHCl in Pharmaceutical formulations

Formulation	Actual concentration of LH(µg/ml)	Amount obtained of LH (µg/ml)	%LH
Tablet	08	7.95	99.37

Table 3: Determination of Accuracy, Recovery studies

Amt. of sample LH µg/ml	Amt. of Pure drug LH%	Amt. of Pure drug LHµg/ml	Amt. of drug recovered LH µg/ml	Mean % Recovery ± SD
5	80%	4	3.92	98.08+0.31
5	100%	5	5.01	100.2+0.48
5	120%	6	5.98	99.72+0.61

RESULTS AND DISCUSSION:

Levamisole Hydrochloride[LH] forms ion-association complex in acidic medium with Bromocresol Green indicator and these complexes are quantitatively extracted into chloroform. The absorbance spectra of the ion-association complexes are by plotting absorbance against wavelength (Fig.2 and 3). From the respective absorbance spectra the maximum absorbance are found to be at 420nm.

OPTICAL CHARACTERISTICS:

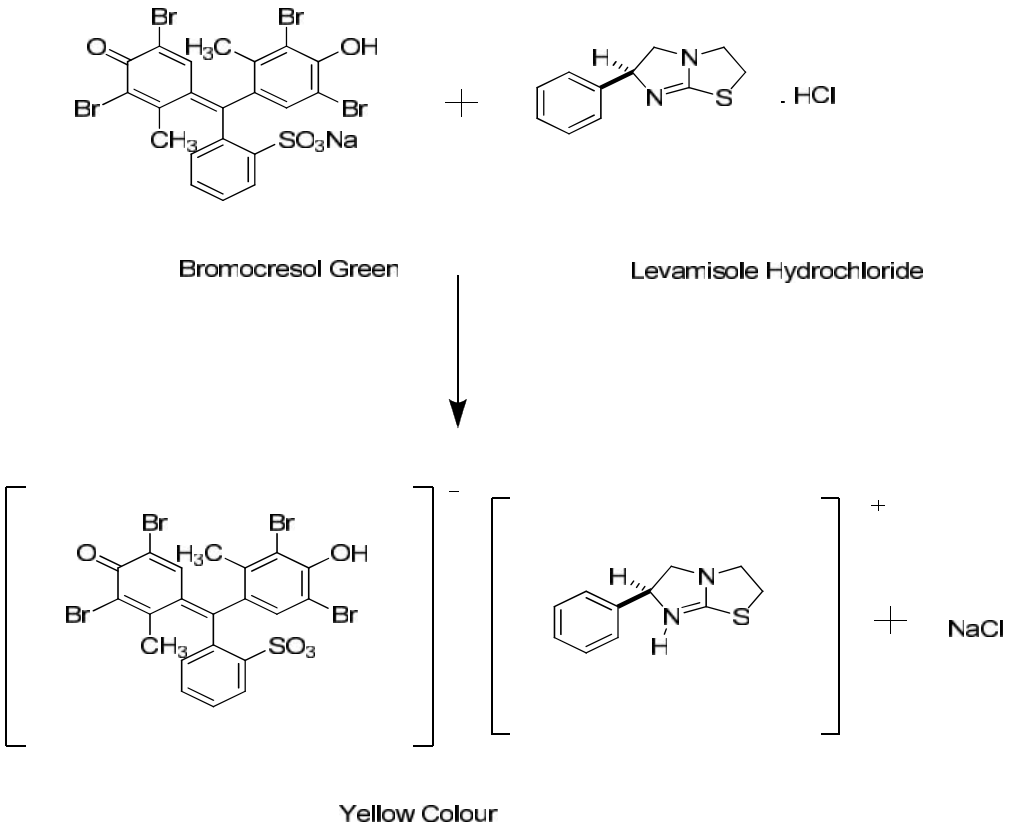
The optical characteristics such as Beer's law limits,Molar Extinction coefficient, standard deviation of slope, standard deviation of intercept, LOD, LOQ and correlation coefficient were calculated for this method and results are summarized in Table1. The values obtained for the determination of Levamisole Hydrochloride in Pharmaceutical formulations (Tablets) by the proposed methods are presented in Table 2. Studies reveal that the common excipients and other additives usually present in the tablets did not interfere in the proposed methods.

RECOVERY STUDIES:

Recovery studies by the standard addition method were performed to study the accuracy of the proposed method. Preanalysed samples of LH (5 µg/ml) were spiked with 80, 100 and 120 % extra LH standard and the mixture were analysed with the proposed method. Accuracy was assessed as the % Recovery at each concentration level. Data obtained from accuracy study are given inTable 3.

Scheme of the colored products:

Levamisole Hydrochloride involves in ion association complex formation with Colouring agent BCG which is extractable into chloroform from aqueous phase. Levamisole Hydrochloride is expected to attract the oppositely charged part of the BCG and behaves as single unit being held together by electrostatic attraction. Based on analogy the structure of ion association complexes are shown in the following scheme.



CONCLUSION:

A simple extractive spectrophotometric methods for the determination of Levamisole Hydrochloride in pure as well as in its dosage form were developed.Proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. The method is found to be simple, precise, economic and less time consuming. The method has been statistically evaluated and results obtained are accurate, precise and insensitive and free from the interferences of other additives present in the formulation. The proposed Extractive Colorimetric method can be used for determination of Levamisole Hydrochloride in tablets.

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