

## Research Article

# Development and Validation of HPTLC Method for Simultaneous Estimation of Levamisole Hydrochloride and Oxyclozanide in its Bulk and Pharmaceutical Dosage Form

Patel MB<sup>1</sup>, Patel RK<sup>1</sup>, Patel SG<sup>2</sup> and Patel AJ<sup>1\*</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Kadi Sarva Vishwavidyalaya, India

<sup>2</sup>Department of Pharmaceutical Chemistry and Analysis, CHARUSAT University, India

\*Corresponding author: Patel AJ, Department of Pharmaceutical Chemistry, Kadi Sarva Vishwavidyalaya, India

Received: December 28, 2016; Accepted: January 30, 2017; Published: February 01, 2017

## Abstract

Levamisole Hydrochloride and Oxyclozanide are anthelmintics drugs. A High Performance Thin Layer Chromatographic Method (HPTLC) was developed and validated for the simultaneous estimation of Oxyclozanide and Levamisole hydrochloride in their bulk form and tablets. Chromatography was performed using pre-coated silica gel aluminium plate 60F254, (10×10 cm) as stationary phase and Acetone: Toluene: Ammonia (5:5:0.04, v/v) as mobile phase. Detection was carried out at 225 nm. The R<sub>f</sub> values for Oxyclozanide and Levamisole hydrochloride were found to be 0.28±0.007 and 0.49±0.009 respectively. The method was validated according to ICH guidelines and can be used to estimate aforementioned compounds in their combined tablets.

**Keywords:** Levamisole hydrochloride; Oxyclozanide; HPTLC; ACM; Anthelmintic

## Introduction

Levamisole Hydrochloride is chemically, (6S)-2, 3, 5, 6-Tetrahydro-6-phenylimidazo [2,1-b] thiazole [1]. It is official in IP, BP and USP. It acts as autonomic ganglionic stimulant and has nicotinic action. Oxyclozanide is chemically 2, 3, 5-trichloro-N-(3,5-dichloro-2-hydroxyphenyl)-6 hydroxy benzamide. It is official in IP. It acts as an uncoupler of the oxidative phosphorylation in the liver fluke and in combination they are used as anthelmintic in animals and birds [2].

Based on the literature review, it was found that a number of studies involving method development for individual estimation of Oxyclozanide and Levamisole hydrochloride and in combination with other drugs have been carried out in formulations/biological fluids. Thus, a number of analytical methods including RP-HPLC [3-8], LC-MS [9,10], LC-MS/MS [11,12] and UV-spectrometry [13-15] have been developed for estimation of Oxyclozanide and Levamisole hydrochloride separately. So far, for analyzing combination of aforementioned drugs as per our knowledge few methods such as HPLC, UV (first derivative spectrophotometry) and HPTLC have been reported [16]. But the reported methods were performed using binary mixture instead of formulation (tablets). It was felt that there is a need to develop new and simpler analytical methods such as HPTLC for the simultaneous estimation of Levamisole hydrochloride and Oxyclozanide in its bulk & pharmaceutical dosage form.

Nowadays, HPTLC is becoming a routine analytical technique with numerous advantages of low operating cost; quick conductance of several trials and need for minimum sample clean up. The major advantage of HPTLC is that several samples can be run in chorus using small amount of mobile phase; in contrast to HPLC, thus time saving

and cost effective. UV spectroscopy methods are easy to execute and possible to perform even at smaller units for quality control [17,18].

The present work was undertaken with an aim to develop and validate of analytical methods as per ICH guidelines [19,20] for simultaneous estimation of Oxyclozanide and Levamisole hydrochloride in the bulk drug and tablets.

## Materials and Methods

### Chemicals

Levamisole hydrochloride and Oxyclozanide were kindly supplied as a gift samples from Nucare Laboratories, Mehsana, Gujarat, India. AR grade Methanol, Acetone, Toluene, Chloroform, Ethyl acetate, Glacial acetic acid and Ammonia (Finar laboratories, Ahmedabad, India).

### Formulation

The tablets containing 2000 mg Levamisole hydrochloride and 4000 mg Oxyclozanide were procured from local pharmacy.

### High performance thin layer chromatography

**Preparation of standard solutions:** Accurately weighed portions of Levamisole hydrochloride (10 mg) and Oxyclozanide (20 mg) were transferred separately to 10 ml volumetric flasks, dissolved and diluted to the mark with methanol to obtain standard solutions having concentrations of Levamisole hydrochloride (1000 µg/mL) and Oxyclozanide (2000 µg/mL). The aliquots of these solutions were taken and diluted with methanol to get working standard solution mixture having concentration of 200 µg/mL Levamisole hydrochloride and 400 µg/mL Oxyclozanide.

**Preparation of test solution:** Twenty tablets were taken and

weighed accurately. The tablets were finely powdered using mortar and pestle. A quantity of 42.7 mg of tablet powder was taken out containing 10 mg of Levamisole hydrochloride and 20 mg of Oxyclozanide in 10 ml volumetric flask. The contents were mixed with methanol. Methanol was added up to the mark to get the test solution containing 1000 µg/mL Levamisole hydrochloride and 2000 µg/mL Oxyclozanide. The solution was further diluted 5 times and used for spotting on pre coated plates.

### Conditions for HPTLC

A Camag HPTLC system (Switzerland) with Linomat V automatic sample applicator and Camag TLC Scanner III Camag (Muttentz, Switzerland) were used for development of method. Pre-coated silica gel aluminum plate 60F254, (10×10 cm; E. Merck, Darmstadt, Germany) were used for separation of components. Analytical balance (CP224S, Sartorius, Gottingen, Germany) was used for weighing of compounds. UV cabinet with dual wavelength UV lamp (254 nm & 366 nm) was used for visualization of spots. Plates were scanned using Camag winCATS software. Ultrasonic bath (Frontline ultrasonic bath) was used to prepare solutions from standards and tablets. The working standard of Levamisole hydrochloride and Oxyclozanide were spotted in band width 6 mm using Hamilton 100 µl syringe on precoated plates using automatic application device. Linear ascending development was carried out in 10×10 cm twin trough glass chamber saturated with the mobile phase for 20 min. The plate was removed from the chamber, subsequently dried in a current of air and densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorption mode at 222 nm and operated by win CATS software. Peak areas were recorded for all the peaks.

### Validation of HPTLC

The method was validated as per ICH guidelines.

**Linearity and range:** To set up the linearity range (200-600 ng/band for Levamisole hydrochloride and 400-1200 ng/band Oxyclozanide) aliquots of 1, 1.5, 2, 2.5, 3 µl from the combined working standard solution (400 µg/ml of Oxyclozanide and 200 µg/ml of Levamisole hydrochloride) were spotted on the TLC plate and developed and analyzed. The calibration curve for Levamisole hydrochloride and Oxyclozanide were constructed by plotting peak area *versus* concentration (ng/band) corresponding to each band.

**Specificity:** The specificity of the method was ascertained by analyzing standard drug and sample. The bands for Levamisole hydrochloride and Oxyclozanide in individual samples were confirmed by comparing the R<sub>f</sub> of the band with those obtained from standard.

### Precision

**System precision:** System precision experiment was performed by application of 2 µl of combined working standard solution (800 µg/ml of Oxyclozanide and 400 µg/ml of Levamisole hydrochloride) for six times on same TLC plate. Plate was developed and analyzed as described in section 3.2.1. The areas of six replicate bands were measured and % CV was calculated.

**Method precision:** Method precision was performed by preparing the combined working standard solution for six times and

2 µl of each solution was applied on same TLC plate (800 ng/band of Oxyclozanide and 400 ng/band of Levamisole hydrochloride). Plate was developed and analyzed as described in section 3.2.1. The areas of six replicate bands were measured and % CV was calculated.

**Intermediate precision (Reproducibility):** The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of Oxyclozanide and Levamisole hydrochloride at 100% test concentration (800 ng/band of Oxyclozanide and 400 ng/band of Levamisole hydrochloride) on the same day and on different days. The results were reported in terms of relative standard deviation (% RSD).

### Accuracy (% Recovery study)

The accuracy of the methods was determined by calculating recoveries of Levamisole hydrochloride and Oxyclozanide by the standard addition method. Known amounts of standard solution of Levamisole hydrochloride and Oxyclozanide were added at 80%, 100% and 120% levels to pre-quantified sample solutions of Levamisole hydrochloride and Oxyclozanide.

### Limit of Detection (LOD) & Limit of Quantitation (LOQ)

The limits of detection and quantification of the developed method were calculated from the standard deviation of the intercepts and mean slope of the calibration curves of Oxyclozanide and Levamisole hydrochloride using the formulae as given below.

$$\text{LOD} = 3.3X\sigma/S$$

$$\text{LOQ} = 10X\sigma/S$$

Where,  $\sigma$ =the standard deviation of the response

S=slope of the calibration curve.

### Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage it was.

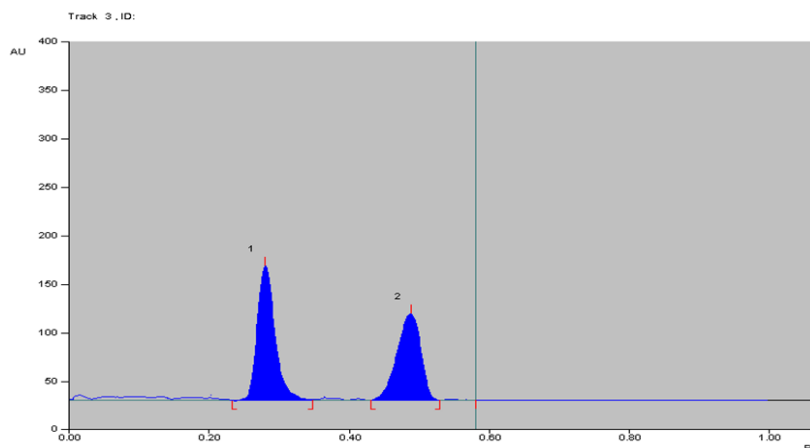
## Results and Discussion

Method A: High performance thin layer chromatography

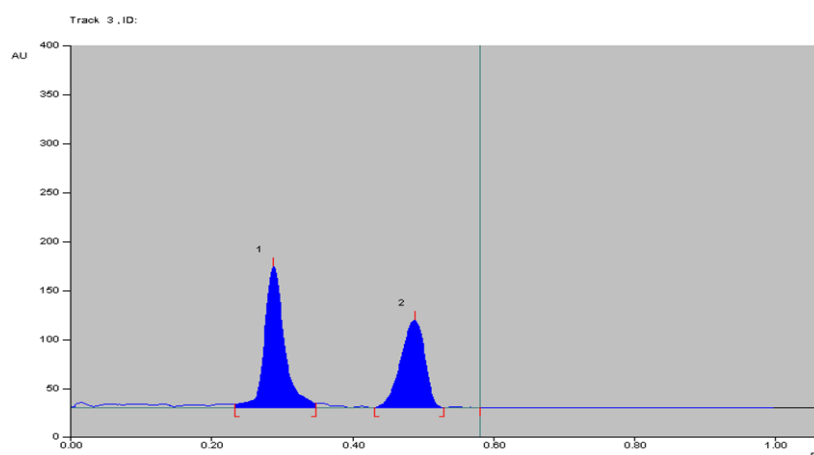
### Optimization of mobile phase for separation of drugs

The HPTLC procedure was optimized with a view to develop an assay method. Both, Levamisole hydrochloride and Oxyclozanide were applied on TLC plates and run in different mobile phase systems. The mixture of Toluene: acetone: ammonia (5:5:0.04, v/v) was proven to be better than the other mixtures in terms of resolution and peak shape. The chromatograms obtained using optimized mobile phase for standard solution is shown in (Figure 1).

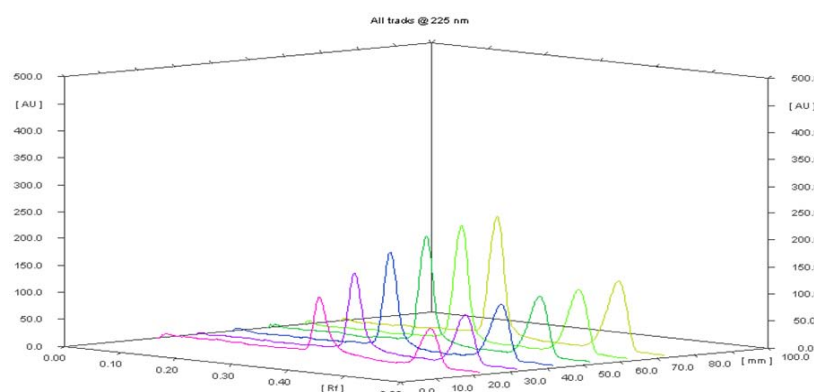
**Validation of HPTLC method:** The method was found to be linear in the range of 400-1200 ng/band for Oxyclozanide and 200-600 ng/band for Levamisole hydrochloride. Peak area was directly proportional to the concentrations of drugs in this region. Correlation co-efficient for calibration curve of Levamisole hydrochloride and Oxyclozanide were found to be 0.996 and 0.997 respectively. Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition.



**Figure 1:** Chromatogram of Oxyclozanide and Levamisole hydrochloride (800 ng/band and 400 ng/band respectively) in Toluene: acetone: ammonia (5:5:0.04, v/v).



**Figure 2:** Chromatogram of test sample of Oxyclozanide and Levamisole hydrochloride in Toluene: acetone: ammonia (5:5:0.04, v/v).



**Figure 3:** 3D chromatogram of Levamisole hydrochloride (200-600 ng/band) and Oxyclozanide (400-1200 ng/band).

The % recovery of Oxyclozanide and Levamisole hydrochloride was found to be 100.42 and 99.67 respectively. The % RSD of system precision of Oxyclozanide and Levamisole hydrochloride were found to be 0.84 and 0.75 respectively. The % RSD of method precision of Oxyclozanide and Levamisole hydrochloride were found to be 1.01 and 0.87 respectively. Mean RSD for intra-day

and inter day precision of Levamisole hydrochloride was found to be 1.22 and 1.52 respectively. The Mean RSD for intra-day and inter day precision of Oxyclozanide was found to be 1.20 and 1.51 respectively. The LOD and LOQ values were found to be 46.77 ng/ml and 141.74 ng/ml for Oxyclozanide and 28.9 ng/ml and 87.5 ng/ml for Levamisole hydrochloride, respectively. The robustness

of the method was studied by changing the composition of mobile phase (toluene: acetone (ammonia 0.04 ml); 5.5:4.5, 5:5, 4.5:5.5, v/v) where % RSD values were 0.855 and 1.13 of Oxytoclozanide and Levamisole hydrochloride respectively. Change in volume of mobile phase (7.5, 10, 12.5 ml), gave % RSD values of 0.793 and 0.528 for Oxytoclozanide and Levamisole hydrochloride respectively. Change in saturation time (20, 25, 35 min) gave % RSD values of 1.169 and 0.74 for Oxytoclozanide and Levamisole hydrochloride respectively. The % RSD was <2% in each study indicating the variability of the method is within control and the developed method is reproducible, accurate and sensitive for estimation of both drugs.

### Analysis of marketed formulations (tablets) using HPTLC

The assay results for the analysis of marketed tablets containing Oxytoclozanide (4000 mg) and Levamisole hydrochloride (2000 mg) were in good agreement with the labeled contents. The % drug obtained was 98.81% of Oxytoclozanide and 98.13% of Levamisole hydrochloride. No interference of the excipients with the peaks of interest appeared; so the method is applicable for the routine estimation of Oxytoclozanide and Levamisole hydrochloride in pharmaceutical dosage forms (Figures 2 & 3).

### Conclusion

All these factors leads to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully for the estimation of Oxytoclozanide and Levamisole hydrochloride in bulk drug and in commercially available combined dosage formulation without inference and with good selectivity.

### References

- Maryadele J, Neil O. The Merck Index an Encyclopedia of Chemicals, Drugs and Biological. USA: Merck Research Laboratories. 2013; 5511: 7057.
- Sweetman SC. Martindale the Complete Drug Reference. London: Pharmaceutical Press. 2005.
- Ravisankar P, Rao GD. Development and Validation of RP-HPLC Method for Determination of Levamisole in Bulk and Dosage Form. Asian J Pharma and Clin Res. 2013; 6: 169-173.
- Dreassi E, Corbini G, La Rosa C, Politi N, Corti P. Determination of Levamisole in Animal Tissues using Liquid Chromatography with Ultraviolet Detection. J Agri Food Chem. 2012; 49: 5702-5705.
- Ulavapalli KR, Sriramulu J, Bobarala V, Mallu VR. RP-HPLC Method for Simultaneous Estimation of Levamisole, Mebendazole and Albendazole in Pharmaceutical Products. Ind J Novel Drug Delivery. 2011; 3: 134-142.
- Kumar AR, Shetty AS. Development and Validation of RP-HPLC Method for the Estimation of Oxytoclozanide in Bulk Drug and Pharmaceutical Formulation. Int J PharmTech Res. 2012; 4: 700-704.
- Jo K, Cho HJ, Yi H, Cho SM, Park JA, Kwon CH, et al. Determination of Oxytoclozanide in Beef and Milk using High-Performance Liquid Chromatography System with UV Detector. Lab Animal Res. 2011; 27: 37-40.
- Tonder EC, Villiers MM, Malan C, Preez JL. Simple, Robust and Accurate High Performance Liquid Chromatography Method for the Analysis of Several Anthelmintics in Veterinary Formulation. J Chroma A. 1996; 729: 267-272.
- Jedziniak P, Juszkiwicz TS, Olejnik M. Determination of Benzimidazoles and Levamisole Residues in Milk by Liquid Chromatography-Mass Spectrometry: Screening Method and Validation. J. Chroma A. 2009; 1216: 8165-8172.
- Sakamoto M, Takeba M, Sasamoto T, Kusano T, Kanai S, Nagayama T. Determination of Bithionol, Bromophen, Nitroxylin, Oxytoclozanide and Tribromsalan in Milk with Liquid Chromatography Coupled with Tandem Mass Spectrometry. J AOAC Int. 2010; 93: 1340-1346.
- Tang L, Ding L, Wang Z, Wang J, Yang L. A Sensitive LC-MS/MS Method for Determination of Levamisole in Human Plasma: Application to Pharmacokinetic Study. J. Chromatogr B Analyt Biomed Life science. 2011; 879: 299-303.
- Baere SD, Cherlet M, Croubels S, Baert K, Backer PD. Liquid Chromatography Determination of Levamisole in Animal Plasma: Ultraviolet versus Tandem Mass Spectrometric Detection. Analytica Chimica Acta. 2003; 483: 215-224.
- Ei-Didamony AM. Spectrophotometric Determination of Benzylamine HCl, Levamisole HCl and Mebeverine HCl through Ion-Pair Complex Formation with Methyl Orange. Spectrochimica Acta Part A. 2008; 69: 770-775.
- Misquith J, Prabhu PP, Subrahmanyam E, Shabaraya AR. Extractive Spectrophotometric Methods for the Determination of Levamisole Hydrochloride. Int J PharmTech Res. 2012; 4: 1215-1220.
- Dinc E, Onur F. Comparative Study of the Ratio Spectra Derivative Spectrophotometry, Derivative Spectrophotometry and Vierordts Method Applied to the Analysis of Oxfendazole and Oxytoclozanide in A Veterinary Formulation. Analysis. 1997; 25: 55-59.
- Jaenchen DE. Thin Layer (Planer) Chromatography in Handbook of Instrumental techniques for Analytical Chemistry. 1<sup>st</sup> edn. 1997; 221-235.
- Ranger B. Quantitative Planer Chromatography as a Tool in Pharmaceutical Analysis. J AOAC Int. 1993; 76: 7-13.
- ICH. Validation of Analytical Procedures: Definitions and Terminology, Q2A (R1), Geneva. 1996.
- ICH. Validation of Analytical Procedures: Methodology, Q2 B (R1), Geneva. 1996.