



Received on 04 February, 2017; received in revised form, 07 April, 2017; accepted, 25 June, 2017; published 01 September, 2017

HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF OXYCLOZANIDE AND LEVAMISOLE HYDROCHLORIDE IN TABLET DOSAGE FORM

H. Panchal ^{*1} and M. Patel ²

Department of Pharmacognosy ¹, Anand Pharmacy College, Anand - 388001, Gujarat, India.

Department of Pharmacognosy ², Indukaka Ipcowala College of Pharmacy, New V.V. Nagar - 388121, Gujarat, India.

Keywords:

HPTLC analysis, Levamisole hydrochloride, Oxyclozanide

Correspondence to Author:

Hiteksha Panchal

Assistant Professor,
Department of Pharmacognosy,
Anand Pharmacy College, Anand -
388001, Gujarat, India.

E-mail: hitekshapanchal22@yahoo.com


ABSTRACT: This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of Oxyclozanide and Levamisole hydrochloride as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminium plates pre-coated with silica gel 60 F254 as the stationary phase and the solvent system consisted of Toluene: acetone: ammonia (5:5:0.04 v/v/v). Densitometric evaluation of the separated zones was performed at 225 nm. The two drugs were satisfactorily resolved with R F values 0.28 ± 0.007 and 0.49 ± 0.009 for Oxyclozanide and Levamisole hydrochloride, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (400-1200 ng/band for Oxyclozanide) and (200-600 ng/band for Levamisole hydrochloride), precision (intra-day % RSD was 1.20 and inter-day % RSD was 1.51 for Oxyclozanide, and intra-day % RSD was 1.22 and inter-day % RSD was 1.52 for Levamisole hydrochloride), accuracy (0.556 for Oxyclozanide and 1.31 for Levamisole hydrochloride), and specificity in accordance with ICH guidelines.

INTRODUCTION: The combination of Oxyclozanide (OCL) and Levamisole hydrochloride (LMH) has recently been introduced in the market. Chemically Oxyclozanide is 2, 3, 5-trichloro-N-(3, 5 - dichloro 2- hydroxyphenyl) - 6 hydroxy benzamide. Levamisole hydrochloride is (6S)-2, 3, 5, 6- Tetrahydro-6-phenylimidazo [2, 1-b] thiazole both are used for the treatment of Anthelmintic ¹⁻². Literature survey reveals that, so far no HPTLC method has been reported for the simultaneous estimation of OCL and LMH in formulation.

Several methods were reported for the individual estimation of OCL and LMH but not for the combined form ³⁻⁹. In the present investigation, an attempt has been made to develop a rapid, accurate, precise and cost effective HPTLC method for simultaneous estimation of OCL and LMH in combined dosage form.

Experimental:

MATERIALS: Levamisole hydrochloride and Oxyclozanide was kindly supplied as a gift samples from Nucare Laboratories, Mehsana, Gujarat, India. Silica gel 60 F₂₅₄ TLC plates (20×10 cm, layer thickness 0.2 mm, E. Merck) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Finar laboratories, Ahmedabad. Marketed formulation OCL (4000 mg) and LMH (2000 mg) was used for the analysis.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.8(9).4000-05</p>
<p>Article can be accessed online on: www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8(9).4000-05</p>	

Instrumentation: The instrument used in the present study was Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100 μ l), Camag TLC Scanner 3, Camag Win CATS software, Camag Twin-trough chamber (20 \times 10 cm). Ultrasonicator was used for extraction of the drugs from the tablets.

Preparation of Standard Stock Solutions: Accurately weighed portions of Levamisole hydrochloride (10mg) and Oxyclozanide (20mg) were transferred to separate 10mL volumetric flasks and dissolved and diluted to the mark with methanol to obtain standard solutions having concentrations of Levamisole hydrochloride (1000 μ g/mL) and Oxyclozanide (2000 μ g/mL). The aliquots of these solutions were taken and diluted with methanol to get working standard solution mixture having concentration of 200 μ g/mL levamisole hydrochloride and 400 μ g/mL oxyclozanide.

Sample Preparation: A quantity of 42.7mg of tablets was taken out containing 10mg of Levamisole hydrochloride and 20mg of Oxyclozanide in 10mL volumetric flask. The contents were mixed with methanol. Methanol was added up to the mark to get the test solution containing 1000 μ g/mL Levamisole hydrochloride and 2000 μ g/mL oxyclozanide. The solution was further diluted 5 times to have a concentration of 200 μ g/mL Levamisole hydrochloride and 400 μ g/mL Oxyclozanide.

Optimization of HPTLC Method: To carry out HPTLC analysis, the TLC plates were pre-washed with methanol. Activation of plates was done in an oven at 60° for 5 min. The chromatographic conditions maintained were pre-coated silica gel 60F₂₅₄ aluminum sheets (20 \times 100 mm) as stationary phase, toluene: acetone: ammonia (5:5:0.04 v/v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance allowed was 80 mm, wavelength scanning was done at 265 nm keeping the slit dimension at 6 \times 0.45 mm. Five microlitres of standard solutions of OCL and LMH were spotted and developed at constant temperature. Wavelength was selected by scanning standard solutions of both drugs over 200 nm to 400 nm wavelengths. OCL showed maximum absorbance

at 325nm and LMH at 222nm. Both components showed reasonably good response at 225nm, therefore photometric measurements were performed at 225nm in absorption mode with Camag TLC scanner 3 using Win CATS software. Aliquots of standard solutions OCL and LMH were applied on the TLC plate. TLC plate was dried, developed and analyzed as described earlier.

Method Validation:

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_f of the band with those obtained from standard.

Linearity: 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 μ L from the combined working standard solution (400 μ g/mL of oxyclozanide and 200 μ g/ml of levamisole hydrochloride) were spotted on the TLC plate and developed and analysed. The above working standard of levamisole hydrochloride and oxyclozanide were spotted in band width 6mm using Hamilton 100 microlitre syringe on pre-coated silica gel aluminium plate 60 F254 using automatic application device. Linear ascending development was carried out in 10 \times 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 222nm and operated by winCATS software. Peak areas were recorded for all the peaks.

The calibration curve for Levamisole hydrochloride and Oxyclozanide were constructed by plotting peak area versus concentration (ng/band) corresponding to each band. Range is the interval between upper and lower concentration (amount) of analyte in sample in sample for which it has been demonstrated that the analytical method has suitable level of precision accuracy and linearity. The linear response was observed over a range of 200-600 ng/band for Levamisole hydrochloride and 400-1200 ng/band Oxyclozanide.

Precision:

System precision: System precision experiment was performed by application of 2 μ L of combined working standard solution (800 μ g/mL of Oxytocin and 400 μ g/mL of Levamisole hydrochloride) for six times on same TLC plate. Plate was developed and analyzed. 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 μ L of each solution was applied on same TLC plate (800 ng/band of oxytocin and 400 ng/band of levamisole hydrochloride). Plate was developed and analyzed. The areas of six replicate bands were measured and % CV was calculated.

Intermediate Precision (Reproducibility): The intraday and inter-day precision of the proposed method was determined by analyzing mixed standard solution of oxytocin and levamisole hydrochloride at 100% test concentration (800 ng/band of oxytocin and 400 ng/band of levamisole hydrochloride) on the same day and on different days. The results are 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 oxytocin by the standard addition method. Known amounts of standard solution of levamisole hydrochloride and oxytocin were added at 80%, 100% and 120% levels to pre-quantified sample solutions of levamisole hydrochloride and oxytocin.

Limit of Detection (LOD) and 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 Oxytocin and Levamisole hydrochloride using the formulae as given below.

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

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

Where, σ = 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. Typical parameter evaluated during method robustness: Mobile phase ratio, Saturation time.

Analysis of Drugs in Combined Dosage Form: Assay of marketed tablets formulation (Faskleen of Dips Vetcare Pvt. Ltd.) containing Levamisole hydrochloride (2000mg) and Oxytocin (4000mg) was performed by preparing the sample solutions as described in the previous section. Six of the prepared sample and standard solutions were applied on TLC plate. The assay was done densitometrically at 225nm for quantitation of levamisole hydrochloride and oxytocin. The contents of levamisole hydrochloride and oxytocin in the sample solutions were determined by fitting the response into the respective regression line equation for levamisole hydrochloride and oxytocin.

RESULT AND DISCUSSION:

Specificity: The specificity of the method was ascertained by analyzing standard drug and sample. The bands for Levamisole hydrochloride and Oxytocin in individual samples were confirmed by comparing the R_f of the band with those obtained from standard. The chromatograms of test and standard oxytocin and levamisole hydrochloride are shown in Fig. 1.

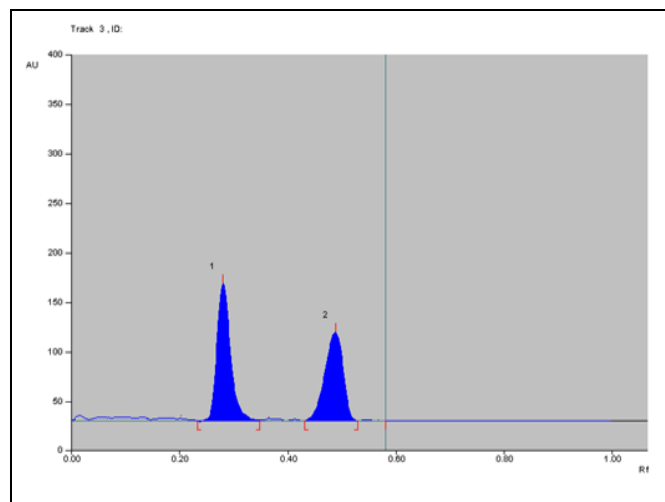


FIG. 1: PEAK DISPLAY OF OXYCLOZANIDE AND LEVAMISOLE HYDROCHLORIDE (800ng/BAND AND 400ng/BAND RESPECTIVLY) MOBILE PHASE: TOLUENE: ACETONE: AMMONIA (5:5:0.04, V/V)

Linearity: Linear responses were observed in the concentration range of 400-1200 ng/band for Oxyclozanide and 200-600 ng/band for Levamisole hydrochloride. Correlation co-efficient for calibration curve of levamisole hydrochloride and oxyclozanide were found to be 0.996 and 0.997 respectively. 3D chromatogram of standard levamisole hydrochloride and oxyclozanide in linearity range is depicted in **Fig. 2** and **Fig. 3**. Calibration curves of Levamisole hydrochloride and Oxyclozanide are shown in **Fig. 4** and **Fig. 5** respectively. Results of linearity are shown in **Table 1**. The regression line equations for levamisole hydrochloride and oxyclozanide are as following $y = 4.745x + 1134$ for levamisole hydrochloride $y = 3.615x + 1339$ for oxyclozanide.

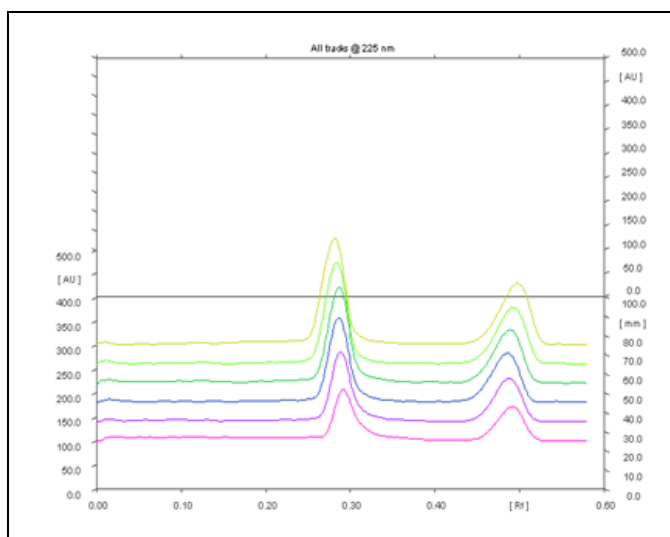


FIG. 2: 2D CHROMATOGRAM OF LEVAMISOLE HYDROCHLORIDE (200-600 ng/BAND) AND OXYCLOZANIDE (400-1200 ng/BAND)

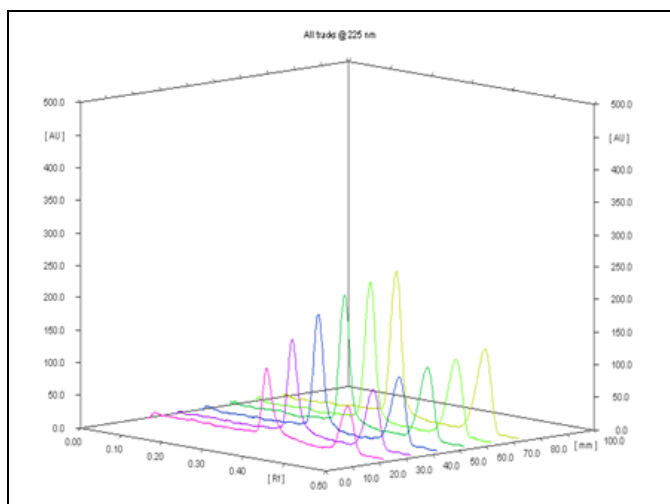


FIG. 3: 3D CHROMATOGRAM OF LEVAMISOLE HYDROCHLORIDE (200-600 ng/BAND) AND OXYCLOZANIDE (400-1200 ng/BAND)

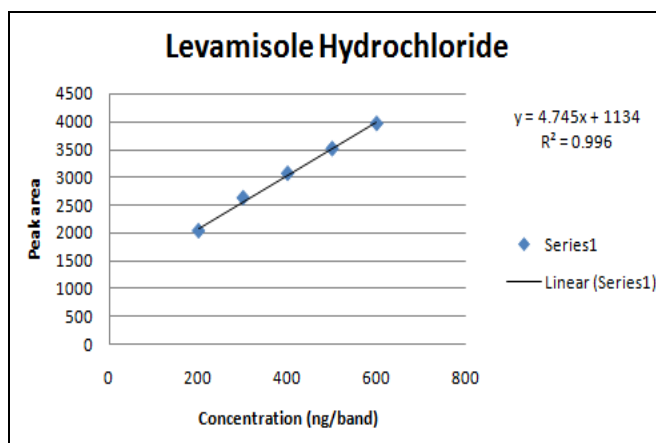


FIG. 4: CALIBRATION CURVE OF LEVAMISOLE HYDROCHLORIDE AT 225 nm

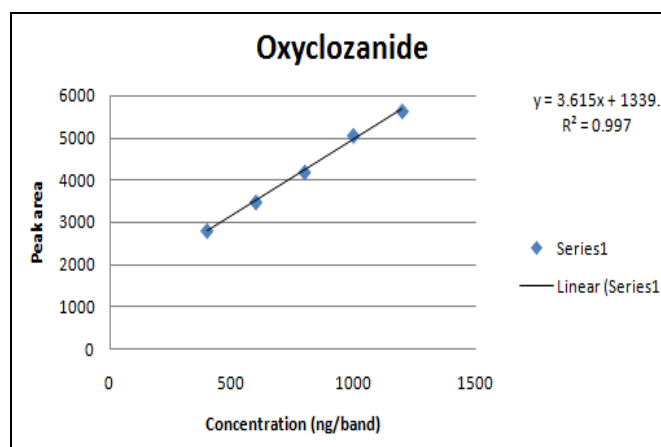


FIG. 5: CALIBRATION CURVE OF OXYCLOZANIDE AT 225 nm

TABLE 1: RESULTS OF LINEARITY FOR LEVAMISOLE HYDROCHLORIDE AND OXYCLOZANIDE

Parameters	Levamisole hydrochloride	Oxyclozanide
Linearity range	200- 600 ng/band	400- 1200 ng/band
Regression line equation	$y = 4.745x + 1134$	$y = 3.615x + 1339$
Slope \pm S.D. (n= 3)	4.745 ± 0.056	3.615 ± 0.074
Y- intercept \pm S.D. (n= 3)	1134 ± 41.52	1339 ± 51.24
Correlation coefficient (R2)	0.996	0.997

Precision:

System and Method Precision: 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.

Intra-day and Inter-day Precision: 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.

Accuracy: Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The % recovery of Oxyclozanide and Levamisole hydrochloride was found to be 99.47 and 100.1 respectively.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD for Oxyclozanide and Levamisole hydrochloride were found to be 20.89 ng/band and 6.43 ng/band respectively. The LOQ for Oxyclozanide and Levamisole hydrochloride were found to be 63.31 ng/band and 19.47 ng/band respectively.

Analysis of Marketed Formulations (Tablets) by Developed Method: The assay results for the analysis of marketed tablets containing Oxyclozanide (4000mg) and Levamisole hydrochloride (2000mg) were in good agreement with the labeled contents. The average contents of Oxyclozanide and Levamisole hydrochloride were 0.9813 mg/mL (0.09813% w/v) and 3.9524 mg/mL tablets (0.39524 %, w/v), respectively.

So the % drug obtained was 98.81% of Oxyclozanide 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 Oxyclozanide and Levamisole hydrochloride in pharmaceutical dosage forms. Results obtained are shown in following **Table 2**.

TABLE 2: ESTIMATION OF OXYCLOZANIDE AND LEVAMISOLE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION (TABLETS)

Drug	Label Claim	Mean Peak Area \pm S.D	Mean Amount Found	Mean % Content
Oxyclozanide	4000 mg	4165.2 \pm 46.3	3908.9 mg	97.72 %
Levamisole hydrochloride	2000 mg	2984.06 \pm 30.7	1949.48	97.47 %

TABLE 3: SUMMARY OF VALIDATION PARAMETERS

Sr. no	Parameters	Oxyclozanide	Levamisole Hydrochloride
1	Linearity Range	400-1200 ng/band	200-600 ng/band
2	Correlation Co-efficient (R ²)	0.997	0.996
3	Precision (% RSD)		
	System precision (n=6)	0.84	0.75
	Method precision (n=6)	1.01	0.877
	Intraday precision (n=6)	1.20	1.22
	Inter day precision (n=6)	1.51	1.52
4	% Recovery	100.42%	99.67%
5	Limit of Detection (LOD) (ng/band)	46.77	28.87
6	Limit of Quantification (LOQ) (ng/band)	141.74	87.50

CONCLUSION: Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC technique is precise, specific, and accurate. Statistical analysis proves that the method is suitable for the analysis of Oxyclozanide and Levamisole hydrochloride as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Oxyclozanide and Levamisole hydrochloride, also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

ACKNOWLEDGEMENT: The authors are thankful to Mr. Mitesh Patel, Ms. Archita Patel, Assistant Professor, K.B. Institute of Pharmaceutical education and research for their kind support.

REFERENCES:

1. Sweetman SC: Martindale The complete drug reference. London: Pharmaceutical Press. 2005; 34: 107-111.
2. Maryadele J, Neil O: The Merck Index An encyclopedia of chemicals, drugs and biological. USA: Merck Research Laboratories. 2013; 15: 5511.
3. Indian Pharmacopoeia 2010, Volume-II, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India, Ministry of Health and Family Welfare 1572-1573, 2676.

4. C.S.R. Lakshmi and M. N. Reddy: Determination of oxcyclozanide by using visible spectrophotometry. *Indian Drugs*, 1998; 35(9): 583-585.
5. E. C. Van-Tonder, M. M. De-Villiers, J. S. Handford, C. E. P. Malan and J. L. Du-Preez: Simple robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations. *J. Chromatogr.-A.*, 1996; 729(1): 267-272.
6. E. Dinc and F. Onur: Comparative study of the ratio spectra derivative spectrophotometry, derivative spectrophotometry and Vierordt's method applied to the analysis of oxfendazole and oxcyclozanide in a veterinary formulation. *Analisis*. 1997; 25(3): 55-59.
7. E. Dinc and M. Kanbur: Spectrophotometric multicomponent resolution of a veterinary formulation containing oxfendazole and oxcyclozanide by multivariate calibration -prediction techniques. *J. Pharm. Biomed. Anal.*, 2002; 28(3-4): 779-788.
8. E. Dinc, G. Pektas, and D. Baleanu: Continuous Wavelet Transform and Derivative Spectrophotometry for the Quantitative Spectral Resolution of a Mixture containing Levamisole and Triclabendazole in Veterinary Tablets. *Reviews in Analytical Chemistry*. 2009; 28(2): 79-92.
9. E. Dreassi, G. Corbini, C. la -Rosa, N. Politi and P. Corti; Determination of levamisole in animal tissues using liquid chromatography with ultraviolet detection. *J. Agric. Food. Chem.*, 2001; 49(12): 5702-5705.

How to cite this article:

Panchal H and Patel M: HPTLC method for the simultaneous estimation of Oxcyclozanide and Levamisole hydrochloride in tablet dosage form. *Int J Pharm Sci Res* 2017; 8(9): 4000-05. doi: 10.13040/IJPSR.0975-8232.8(9).4000-05.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)