

Research Article

Stability-Indicating High-Performance Liquid Chromatographic Determination of Levamisole Hydrochloride in Bulk and Dosage Forms

Amal Abdalraheem,^{1,2} Shaza Shantier ,¹ Imad Abureid,¹ and Elrasheed A. Gadkariem¹

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, P. O. Box 1996, Khartoum, Sudan

²National Medicines Quality Control Laboratory, Khartoum, Sudan

Correspondence should be addressed to Shaza Shantier; sshantier@yahoo.com

Received 18 August 2020; Revised 21 September 2020; Accepted 24 September 2020; Published 9 October 2020

Academic Editor: Victor David

Copyright © 2020 Amal Abdalraheem et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A selective, accurate, and precise stability-indicating HPLC method for analysis of levamisole hydrochloride in bulk and in their injection and oral solution formulations has been developed and validated in accordance with ICH guidelines. The chromatographic separation was carried out on a C₈ column (250 × 4.6 mm with a particle size of 5 micrometer) using a mixture of phosphate buffer pH 8.0 and acetonitrile (70 : 30 v/v) as the mobile phase pumped at a flow rate of 1.5 ml/min with UV detection at 215 nm. The calibration curve was linear over the 10–50 µg/ml concentration range with a correlation coefficient of 1.0000. The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.29 µg/ml and 0.89 µg/ml, respectively. The accuracy and the precision of the developed method were significantly good (RSD < 2%). The validity of the proposed method was further confirmed through the statistical comparison of the obtained data with those of the official method.

1. Introduction

Levamisole hydrochloride (Figure 1) has anthelmintic and immunomodulating activities. Due to the risk of serious side effects and the availability of more effective replacement medications, now it is used only in veterinary medicine [1, 2].

The official method for the determination of levamisole in both injection and oral solution dosage forms, according to the *British Pharmacopeia* (BP), is based on nonaqueous titration after liquid/liquid extraction of the drug as a base [3], while in the *United States Pharmacopeia* (USP), the determination of levamisole in the tablet dosage form is carried out by reversed-phase high-performance liquid chromatography (RPHPLC) using the gradient elution mode [4]. Literature review revealed that other analytical methods were also used for the determination of levamisole in different dosage forms, including direct spectrophotometry at 215 nm [5], spectrophotometry after ion-pair extraction [6, 7], reversed-phase liquid chromatography

[8, 9], thin-layer chromatography [10], and high-performance thin-layer chromatography [11]. Various chromatographic methods for its determination in animal and human biological fluids were also reported [12–17].

In this study, we are reporting a simple, cost-effective, accurate, and precise stability-indicating high-performance liquid chromatographic method for the determination of levamisole in the presence of its degradation products.

2. Materials and Methods

2.1. Instruments and Apparatus. The experiments were performed on a Shimadzu Prominence HPLC system consisted of the following: degasser (Model DGU-20A5), pump (Model LC-20AD), Rheodyne manual injector fitted with 20 µl loop, and variable wavelength UV-VIS detector (Model SPD-20A). The chromatographic separations were carried out on Intersustain® (C₈, 5 µm particle size, 250 mm × 4.6 mm ID) from GL Sciences Inc., Japan. UV/VIS

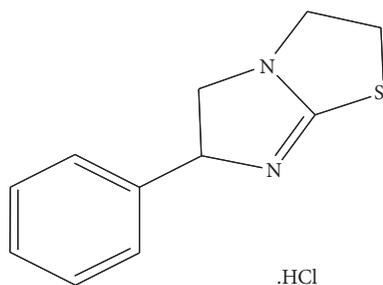


FIGURE 1: Chemical structure of levamisole hydrochloride.

scanning of wavelength was carried using a Shimadzu UV-1800 240V spectrophotometer.

2.2. Chemicals and Reagents. Levamisole hydrochloride working standard (100.73%) was kindly provided by National Medicines Quality Control Laboratory (NMQCL), Sudan. The following commercial product injections and oral solutions were purchased from the local market: leozide oral solution labelled to contain 2.5% w/v levamisole hydrochloride (manufactured by PDH, Pakistan), levamisole hydrochloride 10% w/v injection (manufactured by Bash Pharma, Sudan), and Levapan injection labelled to contain 10% w/v levamisole hydrochloride (manufactured by Pharma Swede, Egypt).

Potassium dihydrogen phosphate, potassium hydroxide pellets, phosphoric acid (analytical grade), methanol (HPLC grade), and acetonitrile (HPLC grade) were purchased from Scharlau, Spain. Laboratory-produced distilled water was used as a diluent throughout this work.

2.3. Potassium Phosphate Buffer Solution 0.05 M, pH 8.0. The buffer was prepared by dissolving 6.8 g of potassium dihydrogen phosphate anhydrous in about 500 ml of distilled water, the volume was then completed to 1000 ml with distilled water, and the pH was adjusted using 85% phosphoric acid and/or 5 M potassium hydroxide. The solution was then filtered through a 0.45 μm filter.

2.4. Preparation of Standards and Sample Solutions

2.4.1. Levamisole Hydrochloride Standard Stock Solution. The solution was prepared by initially dissolving accurately weighed 100 mg of levamisole hydrochloride in 30 ml of distilled water in a 100 ml volumetric flask. The content of the flask was then sonicated for 10 minutes, cooled, and adjusted to the mark using the same diluent (solution A, 1000 $\mu\text{g}/\text{ml}$).

Solution A was further diluted by transferring 15 ml into a 100 ml volumetric flask, and the volume was completed to the mark with the diluent (solution B, 150 $\mu\text{g}/\text{ml}$).

2.4.2. Linearity Standard Solution. Five-point calibration solution was prepared by transferring aliquot volumes (1–5 ml) from solution A into five separate 100 ml

volumetric flasks; the volumes of the flasks were then adjusted to the mark with the diluent.

2.5. Sample Preparation

2.5.1. Injection Dosage Form. An accurately pipetted volume from the injection containing 190 mg levamisole hydrochloride was transferred into a 250 ml volumetric flask, and the volume was completed to the mark with the diluent. 2 ml of the resultant solution was further diluted to 50 ml with the diluent (solution C, 30 $\mu\text{g}/\text{ml}$).

2.5.2. Oral Dosage Form. An accurately pipetted volume from the oral solution containing 50 mg levamisole hydrochloride was transferred into a 50 ml volumetric flask, and the volume was then completed to the mark with the diluent. 3 ml of the resultant solution was further diluted to 100 ml with the diluent (solution D, 30 $\mu\text{g}/\text{ml}$).

2.6. Procedure

2.6.1. Method Development and Optimization. Initially, levamisole hydrochloride standard solution (30 $\mu\text{g}/\text{ml}$) was spectrophotometrically scanned over the range of 190–400 nm against the diluents to determine the drug's wavelength of maximum absorption.

Various mobile phase ratios such as 30 : 70 and 40 : 60% v/v (acetonitrile : 0.05 M phosphate buffer adjusted to pH 8.0) pumped at 1.5 ml/minute flow rate using C_{18} were initially experimented. A second experiment was performed using C_8 instead while maintaining the other experimental conditions the same. A third experiment was performed using the C_8 column while changing the mobile phase composition to 50 : 50% v/v.

The standard containing 30 $\mu\text{g}/\text{ml}$ levamisole hydrochloride was injected, and detection was carried out at 215 nm.

2.6.2. Method Validation

(1) Linearity. Triplicate injections 20 μl each were made from each linearity standard solution. The calibration curve was obtained by plotting its average peak area against its corresponding concentration. The regression analysis data (slope, intercept, and correlation coefficient) were calculated; further, the limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the regression analysis data according to the following formula [18]:

$$\text{LOD} = 3.3 \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \frac{\sigma}{S44}$$
(1)

where σ is the average standard deviation of the responses obtained from the linearity determination and S is the slope of the calibration curve.

Solutions C and D were treated as in the calibration graph procedure. The content percentage was then calculated using the proportional relation between the sample peak area and that of the standard and their corresponding concentrations.

(2) *Accuracy.* To assess the accuracy of the method, recovery studies were carried out by spiking a sample of known concentration with the standard to 60%, 100%, and 150% of the nominal sample concentration ($n = 3$). The recovery % was then calculated using the following equation [18]:

$$\% \text{ recovery} = (C_s - C_u)/C_A \times 100, \quad (2)$$

where C_s is the concentration of spiked samples, C_u is the concentration of unspiked samples, and C_A is the concentration of the analyte added to the test sample.

(3) *Precision.* The method's intraday precision (repeatability) was determined by analyzing six sample solutions (100% of the target concentration). Each sample was injected three times; the mean, standard deviation (SD), and relative standard deviation (RSD) were calculated. To determine the interday precision, the procedure was repeated by a different analyst on a different day using a different instrument.

(4) *Robustness.* Robustness was investigated by varying the following method critical parameters: pH (± 0.1), organic solvent ratio ($\pm 1\%$), flow rate (± 0.1 ml/min), column supplier (different brands), and detection wavelength (± 5 nm).

(5) *Specificity.* The specificity of the method was studied by testing its ability to separate levamisole hydrochloride from its degradation products, produced under the influence of heat, acid, alkali, and oxidative conditions.

2.6.3. Forced Degradation Study

(1) *Heating Condition.* An aliquot of solution B (5 ml) was transferred into a 50 ml volumetric flask, and the volume was made up to the mark with the diluent. The solution was then heated in reflux at 100°C for 1 hour. After cooling, the solution was injected into the HPLC, and the peak area was recorded and compared to the control one.

(2) *Alkaline, Acidic, and Oxidative Conditions.* An aliquot of solution B (5 ml) was transferred into a 50 ml volumetric flask, 5 ml of 0.5 M sodium hydroxide was added, and the volume was made up to the mark with the diluent. Then, the solution was heated under reflux at 100°C for 5 hours. After cooling, the solution was injected into the HPLC.

The above procedure was repeated using 0.5 M HCL instead of NaOH and heated for 2 hours.

The effect of oxidative conditions on levamisole hydrochloride was investigated by repeating the above procedure using 5 ml of 30% hydrogen peroxide solution instead of NaOH or/and HCl and heating under reflux at 100°C for 30 min.

3. Results and Discussion

Although various methods were reported for determination of levamisole hydrochloride in human and animal biological fluids, they still have some disadvantages. The *British Pharmacopeia* method for the analysis of the levamisole oral solution and injection was based on the nonaqueous titration after extracting the active ingredient using perchloric acid as the titrant and the toxic extracting solvent chloroform. It is well known that the nonaqueous titrimetric method has many drawbacks including using of large amount of sample and reagents, requiring a greater level of operator skill than routine instrumental methods, and nonselective and inaccuracy due to possible interference from the related substances, impurities, and degradation products leading to false results. In addition, using the toxic solvent chloroform can lead to emulsion formation during the extraction step resulting in more tedious process and error.

In regard to the USP official method, levamisole was assayed using gradient elution in which the retention time was 15 minutes. The nonjustifiable gradient elution that was applied for its determination is mainly used for complex mixtures, samples with high interfering matrixes, or failure to use the stability-indicating isocratic HPLC method to resolve degradation products. In addition, the long elution time (15 minutes) and repeated use of the alkalisising competing base (diisopropyl amine) can lead to harmful effect on the column.

Based on the aforementioned points, in this study, the chromatographic conditions were optimized to get a peak with good system suitability parameters and reasonable retention time, using water as the sample diluent and the detection wavelength at 215 nm.

3.1. Method Development and Optimization. Mobile phases with different compositions and polarities were investigated to obtain good resolution with suitable retention time. A mobile phase consisting of acetonitrile and ammonium phosphate (30:70% v/v, pH 7.2) using methanol 60% as a diluent with a C_{18} (250 mm * 4.6 mm, $5\ \mu\text{m}$) column resulted in an asymmetric peak with a bad baseline at 3.2 minutes (S1). Changing the diluent to water gave a better baseline but resulted in a shouldered peak.

Buffer was then prepared using potassium dihydrogen phosphate powder instead of ammonium phosphate, and this eluent gave a tailed peak at 8.9 minutes (tailing factor 2.4) (S2). Increasing the buffer pH to 8 resulted in a symmetric peak (tailing factor = 1.3) with a long retention time of 13.3 minutes (S3). Altering the mobile phase polarity by increasing the acetonitrile ratio up to 40% resulted in a short retention time at 7.5 minutes with good symmetry (S4). The column polarity was also altered using C_8 instead of C_{18} which resulted in better resolution with shorter elution time (≈ 6 min). In addition, the method was successfully applied for stability indication as the C_8 column will resolve the degradation products more effectively than the more hydrophobic column C_{18} which resulted in drug and degradation product peaks overlapping.

TABLE 1: System suitability parameters.

Column	Eluent	Retention time	Peak area	Theoretical plates no.	Tailing factor
C ₈	A	6.210	1140457	11162	1.4
	B	6.210	1578835	8090	1.3
C ₁₈	A	13.350	17516036	15812	1.3

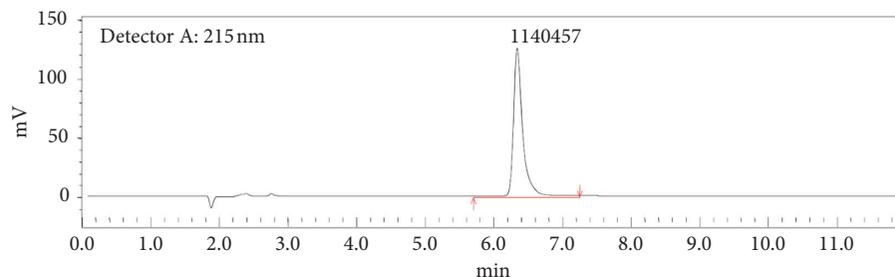


FIGURE 2: Typical chromatogram for levamisole using 30 : 70% v/v acetonitrile : 0.05 M phosphate buffer pH 8.0.

TABLE 2: Regression analysis data for levamisole.

Parameter	Eluent A
Retention time	6.210 minutes
Range	10–50 µg/ml
LOD	0.29 µg/ml
LOQ	0.89 µg/ml
Slope ± SE (* 10 ³)	53.84 ± 0.145
Intercept ± SE (* 10 ³)	10.3 ± 4.95
Correlation coefficient (r)	1.00

TABLE 3: Percentage recovery at three levels (60, 100, and 150%).

Dosage form	Percentage recovery (mean ± RSD); n = 3		
	60% (18 µg/ml)	100% (30 µg/ml)	150% (45 µg/ml)
Injection	(100.3 ± 0.63)	(100.9 ± 0.54)	(99.3 ± 0.30)
Oral solution	(98.4 ± 1.54)	(100.1 ± 1.27)	(100.7 ± 0.26)

An effective elution of the analyte satisfying all the system suitability parameters with a relatively short runtime of ≈6 minutes and a tailing factor of 1.4 was thus obtained with a flow rate of 1.5 mL/min on a C₈ column using a ratio of 30 : 70% v/v (acetonitrile : 0.05 M phosphate buffer pH 8.0) or 50 : 50% v/v (methanol : 0.05 M phosphate buffer pH 8.0) (Table 1 and Figure 2). Although 50% methanol gave the same result as 30% acetonitrile (S5), the acetonitrile containing mobile phase was preferred because of its low viscosity and consequent low system pressure. The developed method is thus superior to the BP and USP in terms of its simplicity and cost-effectiveness considering the analysis time and chemical consumption.

3.2. Method Validation. The developed method was then validated in accordance with ICH guidelines [18]. Linearity,

precision, accuracy, specificity, and robustness were studied as method validation parameters.

3.2.1. Linearity. The constructed curves were found to be linear over a concentration range 10–50 µg/ml with a correlation coefficient of 1.000 (S6). Table 2 summarizes the obtained regression analysis data. These results indicate that the developed method has excellent linearity. Low values of LOD and LOQ reflect the sensitivity of the method.

3.2.2. Accuracy. Accuracy of the developed method was determined by sample spiking at three levels (60, 100, and 150%) (Table 3). The obtained recovery percentages were in the range of 99.3–100.9% for injection and 98.4–100.7% for oral dosage forms. These highly recovered percentages for

TABLE 4: Robustness of the developed method ($n = 6$) (assay and statistical evaluation).

Parameter	RSD for area for six injections	Theoretical plates no.	Tailing factor	Retention time
Developed method (default)	0.143	11162	1.3	6.210
New column	0.784	14903	1.3	9.532
29% acetonitrile (-1%)	0.409	6556	1.3	7.232
31% acetonitrile (+1%)	0.182	11747	1.2	6.437
Buffer pH 7.8 (-0.2)	0.387	11186	1.4	6.123
Buffer pH 8.2 (+0.2)	0.949	12392	1.2	6.809
Wavelength 210 (-5)	0.3190	12331	1.2	6.183
Wavelength 220 (+5)	0.297	11475	1.2	6.110
Flow rate 1.35 (-0.15)	0.156	13075	1.3	6.884
Flow rate 1.65 (+0.15)	0.126	11450	1.3	5.685

TABLE 5: Content percentage results.

Dosage form	Sample name	Content % \pm RSD
Oral solution	Brand 1	103.2 \pm 0.2
	Brand 2	102.9 \pm 0.9
	Brand 3	122.1 \pm 0.9
Injection	Brand 1	102.1 \pm 0.5
	Brand 2	96.1 \pm 0.4
	Brand 3	102.1 \pm 1.0
Powder for oral solution	Brand 1	102.1 \pm 1.5

TABLE 6: Statistical comparison between the developed method and the official method.

	Developed method	Official method
Content % 1	103.40	99.20
Content % 2	105.90	97.10
Content % 3	100.40	100.00
Content % 4	100.00	94.90
Content % 5	99.80	100.50
Average	101.90	98.34
STDEV	2.67	2.32
t -value*	2.25 (2.31)	
F -value*	5.38 (6.38)	

*Calculated at 95% confidence limit for 8 degrees of freedom.

injection and oral dosage forms reflected the accuracy of the developed method and freedom from interference.

3.2.3. Precision. Method's precision was determined as repeatability by analyzing six sample solutions (100% of the target concentration) and intermediate precision on a different day by a different analyst using a different instrument. The method was proved to be precise with the repeatability RSD% values of 1.5 and 0.92, $n = 6$, for injection and oral dosage forms, respectively (S7). Intermediate precision performed with a different analyst on different days using

different instruments resulted in RSD% values of 0.54 and 0.86 for injection and oral dosage forms, respectively (S8).

3.2.4. Robustness. The investigated critical parameters of the developed HPLC method reflected the robustness of the developed method. Results showed that the method can withstand small but deliberate changes with all system suitability parameters being within the acceptable limits (Table 4).

The developed method was also applied for the determination of the content of different brands for different

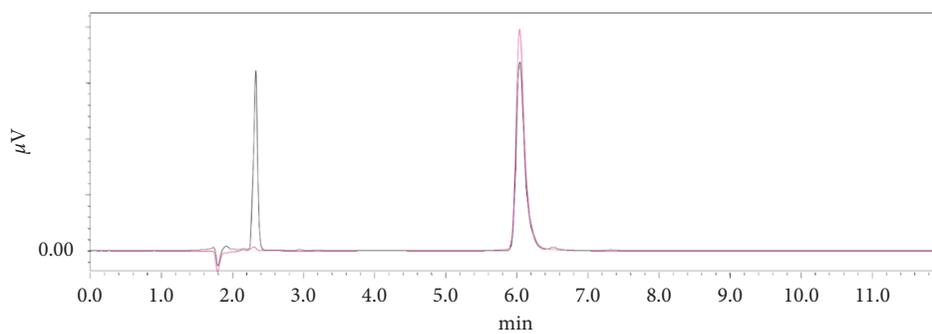


FIGURE 3: Typical chromatogram for the degraded levamisole standard with heating (—: intact drug; —: degraded drug).

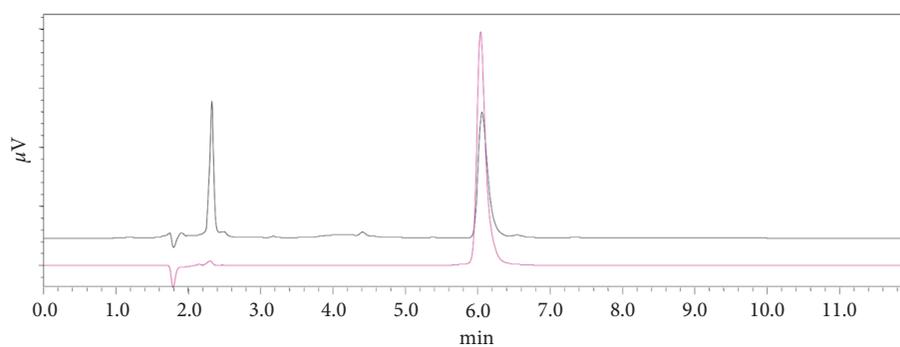


FIGURE 4: Typical chromatogram for the degraded levamisole standard with 0.5 M NaOH (—: intact drug; —: degraded drug).

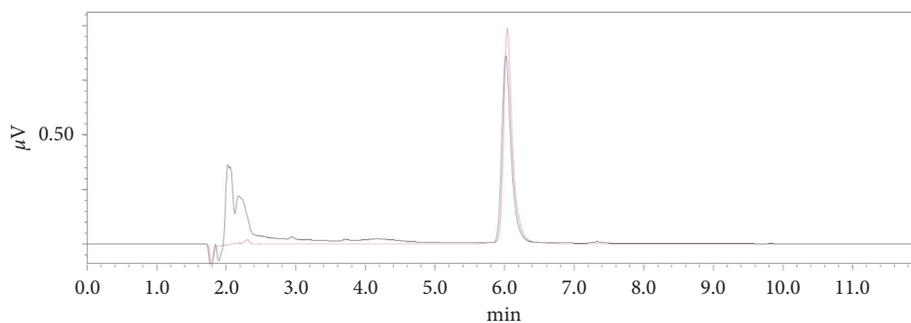


FIGURE 5: Typical chromatogram for the degraded levamisole standard with 0.5 M HCL (—: intact drug; —: degraded drug).

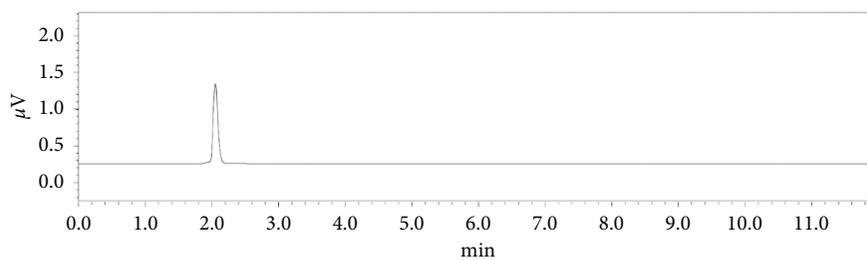


FIGURE 6: Typical chromatogram for the degraded levamisole standard with 30% H₂O₂.

dosage forms. Obtained assay results were found satisfactory for injection, oral, and powder dosage forms (Table 5).

The accuracy and precision of the developed method were further confirmed by the statistical comparison with the BP official method. The t -value and the F -value for eight degrees of freedom were 2.25 and 5.38, respectively (Table 6). As the calculated values are less than the tabulated ones (t -value = 2.31 and F -value = 6.38), this indicates no significant difference between the developed method and the official ones.

3.3. Forced Degradation Study. The developed method was applied as stability indicating to investigate the effect of different stress conditions on the drug solution. The obtained results revealed that the degradation product is more polar than the intact drug appearing at a low k value ($RT \approx 2$ minutes) in the C_8 column.

Heating the solution of levamisole for one hour resulted in about 15.5% reduction of its peak area and appearance of a large peak with an RT of 2.5 minutes (Figure 3).

While heating the drug solution in the presence of sodium hydroxide for half an hour, 27% reduction of its peak area was observed with the appearance of large and small peaks with an RT at 2.3 and 4.4 minutes, respectively (Figure 4). Furthermore, heating the drug solution with an acid resulted in 5% reduction of its peak area and appearance of broad unresolved peaks of RT 2.1 minutes (Figure 5).

Oxidation of the drug by adding hydrogen peroxide to the solution resulted in appearance of a large peak at 2 minutes and disappearance of the normal drug peak (Figure 6).

4. Conclusion

A selective, accurate, and precise stability-indicating HPLC method for analysis of levamisole hydrochloride in bulk and in their injection and oral solution formulations has been developed and validated in accordance with ICH guidelines. The developed method was found to be suitable for the routine analysis as well as for stability studies of this drug.

Stability studies showed that the drug solution was unstable under heat, acidic, alkaline, and oxidative conditions. It showed major degradation in the basic condition (41%) and minor degradation in acidic condition (5%) which indicates that pH of drug solutions is better to be slightly acidic. Further studies are recommended for isolation and identification of these degradation products using hyphenated techniques such as LC/MS.

Data Availability

All data underlying the results are available as part of the article and in the supplementary materials.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are thankful to the Faculty of Pharmacy, University of Khartoum, and National Medicines Quality Laboratory, Sudan.

Supplementary Materials

S1: typical chromatogram for the drug using ACN: ammonium phosphate buffer pH 7.2 (30::70% v/v) using C_{18} and methanol 60% v/v as the diluent. S2: typical chromatogram for the drug using ACN: KH_2PO_4 buffer pH 7.2 (30:70 v/v), C_{18} column. S3: typical chromatogram for the drug using ACN: KH_2PO_4 pH 8.0 (30:70 v/v), C_{18} column. S4: typical chromatogram for the drug using ACN: KH_2PO_4 pH 8 (40:60 v/v), C_{18} column. S5: typical chromatogram for the drug using methanol: KH_2PO_4 pH 8 (50:50 v/v), C_8 column. S6: calibration curves of the developed method. S7: repeatability of the developed method. S8: intermediate precision results for levamisole injection and oral solution. (*Supplementary Materials*)

References

- [1] Kluwer Academic, *Levamisole Hydrochloride*, U. S Pharmacopeia National Formulary *Usp38*, NF33, Kluwer Academic, South Holland, Netherlands, 2015.
- [2] Levamisole (Ergamisol®), Drug Enforcement Administration Office of Diversion Control Drug & Chemical Evaluation Section, 2013.
- [3] Levamisole Oral Solution, Levamisole Injection Monograph, British Pharmacopeia (Veterinary) 201.
- [4] Levamisole Hydrochloride Tablet, USP Monographs, U.S Pharmacopeia National Formulary *USP3*, NF33, 2015.
- [5] J. Misquith and A. Dias, "Validated ultra violet spectroscopic methods for the determination of levamisole hydrochloride," *International Journal of PharmTech Research*, vol. 4, pp. 1631–1637, 2012.
- [6] A. M. El-Didamony, "Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 69, no. 3, pp. 770–775, 2008.
- [7] J. Misquith, P. P. Prabhu, E. V. Subrahmanyam, and A. R. Shabaraya, "Extractive spectrophotometric methods for the determination of levamisole hydrochloride," *International Journal of PharmTech Research*, vol. 4, pp. 1215–1220, 2012.
- [8] P. Ravisankar and G. D. Rao, "Development and validation of RP-HPLC method for determination of levamisole in bulk and dosage form," *Asian Journal of Pharmaceutical Clinical Research*, vol. 6, pp. 169–173, 2013.
- [9] F. Chiadmi, A. Lyer, S. Cisternino et al., "Stability of levamisole oral solutions prepared from tablets and powder," *Journal of Pharmacy and Pharmaceutical Sciences*, vol. 8, pp. 322–5, 2005.
- [10] B. Asghari, S. Ebrahimi, F. Mirzajani, and H. Aboul-Enein, "Development and validation of a simple stability-indicating TLC method for the determination of levamisole in pharmaceutical tablet formulation," *Journal of Planar Chromatography-Modern TLC*, vol. 24, no. 5, pp. 419–422, 2011.
- [11] H. Panchal and M. Patel, "HPTLC method for the simultaneous estimation of oxyclozanide and levamisole

- hydrochloride in tablet dosage form,” *International Journal of Pharmaceutical Sciences and Research*, vol. 8, pp. 4000–4005, 2017.
- [12] H. El-Kholy and B. W. Kemppainen, “Liquid chromatographic method with ultraviolet absorbance detection for measurement of levamisole in chicken tissues, eggs and plasma,” *Journal of Chromatography B*, vol. 796, no. 2, pp. 371–377, 2003.
- [13] E. Dreassi, G. Corbini, C. La Rosa, N. Politi, and P. Corti, “Determination of levamisole in animal tissues using liquid chromatography with ultraviolet detection,” *Journal of Agricultural and Food Chemistry*, vol. 49, no. 12, pp. 5702–5705, 2001.
- [14] J. L. Du Preez and A. P. Lotter, “Solid-phase extraction and HPLC determination of levamisole hydrochloride in sheep plasma,” *Journal of Veterinary Research (South Africa)*, vol. 63, pp. 209–211, 1996.
- [15] L. Tong, L. Ding, Y. Li et al., “A sensitive LC-MS/MS method for determination of levamisole in human plasma: application to pharmacokinetic study,” *Journal of Chromatography B*, vol. 879, no. 5-6, pp. 299–303, 2011.
- [16] R. Woestenborghs, L. Michielsen, and J. Heykants, “Determination of levamisole in plasma and animal tissues by gas chromatography with thermionic specific detection,” *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 224, no. 1, pp. 25–32, 1981.
- [17] T. F. Vandamme, M. Demoustier, and B. Rollmann, “Quantitation of levamisole in plasma using high performance liquid chromatography,” *European Journal of Drug Metabolism and Pharmacokinetics*, vol. 20, no. 2, pp. 145–149, 1995.
- [18] I. H. Guideline, “Validation of analytical procedures: text and methodology Q2 (R1),” in *Proceedings of the International Conference on Harmonization*, pp. 11-12, Geneva, Switzerland, November 2005.