MODESTUM
OPEN ACCESS

Eurasian Journal of Analytical Chemistry, 2018, 13(4), em36
ISSN:1306-3057
Research Paper https://doi.org/10.29333/ejac/91925

Development and Validation of Stability-Indicating High Performance Liquid Chromatographic Method for Determination of Tetramisole Hydrochloride in Pharmaceutical Formulation

Mohammed Nassar [1], Khalid Abdel-Salam [1], Ahmed Abdel-Halim [1], Ragab Said [1], Ahmed Abdel-Monem [1*]

[1] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University,11751, Nasr City, Cairo, EGYPT

Received 3 April 2018 ▪ Revised 20 April 2018 ▪ Accepted 25 April 2018

ABSTRACT

Objective: Development of simple and selective stability-indicating HPLC method for determination of tetramisole hydrochloride in presence of its degradation products. **Method**: Analysis was performed on SUPELCO C18 column (250mm length, 4.6mm width, 5µm particle size) using acetonitrile : methanol : water (50:33:17, by volume) as a mobile phase. The analysis process was carried out at ambient temperature with flow rate of 1 mL/min and the separated peaks were detected by UV at 212 nm. Drug was exposed to forced degradation condition like hydrolysis, oxidation, thermal and photolysis. **Results**: Method can well resolve all degraded product as compare to tetramisole hydrochloride. The method has been linear for the range of 1 – 6 µg/mL with r^2 0.9997. **Conclusion**: This work focused on study of the stability of tetramisole hydrochloride and its behavior when exposed to different stress conditions. The proposed method was successfully applied for determination of tetramisole hydrochloride in pharmaceutical preparation without interference from excipients or degradation products and the method was found to be valid according to International Conference on Harmonization (ICH) guidelines.

Keywords: tetramisole hydrochloride, HPLC, stability indicating method

INTRODUCTION

Tetramisole hydrochloride (**Figure 1**) is (±)-2,3,5,6-Tetrahydro-6-phenyl imidazo[2,1-b] thiazole hydrochloride. It is an anthelmintic drug used in veterinary medicine for treatment of nematode infections [1].

The Literary Survey reveals that many techniques have been used to determine tetramisole hydrochloride which include spectrophotometry [2-7], Potentiometry [8,9], Polarography [10] and HPLC [11-13].

This work aims to develop simple, selective and cost effective stability-indicating HPLC method for determination of tetramisole hydrochloride.

EXPERIMENTAL

Apparatus

HPLC, Consta Metric® 4100 LDC equipped with Diode-array UV-Visible detector and auto sampler (Spectra SYSTEM AS3000). Analytical pump (Milton Roy, USA). ChromQuest 4.2.34, version 3.1.6 data analysis program was used for chromatographic analysis.

✉ mw-nassar@yahoo.com ✉ dr.khalid64@yahoo.com ✉ elhashash3a@yahoo.com ✉ Ragabmsaid@yahoo.com ✉ ahmednaggar111@yahoo.com ahmednaggar111@azhar.edu.eg **(*Correspondence)**

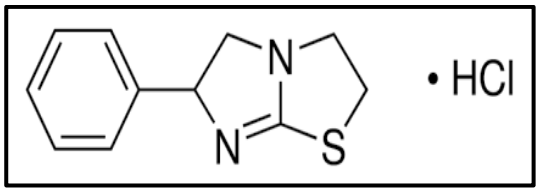

Figure 1. Chemical structure of tetramisole hydrochloride

Materials

Both pure standard tetramisole hydrochloride (certified to contain 99.8%) (Batch number 20160920) and Anthimizole® 10 % powder (Batch number 150632) were kindly supplied by Pharma-Swede company, Tenth of Ramadan City, Egypt.

Chemicals and Solvents

All solvents and reagents used were of analytical grade. HPLC grade acetonitrile and methanol (Sigma-Aldrich, Germany). Distilled water (freshly distillated). Hydrochloric acid, sodium hydroxide, and hydrogen peroxide (El-Nasr Company, Egypt).

Chromatographic Conditions

Isocratic separation was carried out on SUPELCO C18 column (250mm length, 4.6mm width, 5µm particle size) at ambient temperature using acetonitrile : methanol : water (50:33:17, by volume) as a mobile phase which degassed by a degasser before pumped through the column at flow rate 1 mL/min. The injection volume of the sample solutions was 20 µl and the separted peaks were detected by UV at 212 nm.

Preparation of Standard Solutions and Construction of Calibration Graph

Stok solution of tetramisole hydrochloride (100 µg/mL) was prepared by dissolving 10 mg of tetramisole hydrochloride powder in 50 mL of the mobile phase and complete the volume to 100 mL with the mobile phase. Working standard solutions of tetramisole hydrochloride (1 - 6 µg/mL) were prepared by dilution of aliquots of the stock solutions with the mobile phase. Into HPLC column, 20 µl from each solution was injected and eluted by the action of the mobile phase under the previously described chromatographic conditions. Calibration graph was constructed by plotting the peak area against the corresponding concentrations and the linear regression equations was derived.

Application on Pharmaceutical Dosage Form

Accurate weight of Anthimizole® 10 % powder equivalent to 10 mg of tetramisole hydrochloride was obtained and transferred into 100-mL volumetric flask. Add 75 mL of the mobile phase and shake vigorously for 10 min then filter the solution through membrane filter. Completed the volume to 100-mL with mobile phase. From the obtained solution prepare serial dilution covering concentration range and inject them into HPLC. Calculate the concentrations of tetramisole hydrochloride in pharmaceutical samples by using regression equation.

Forced Degradation Studies of Tetramisole Hydrochlorid

Acidic hydrolysis was carried out using 2 mL of 0.1 N hydrochloric acid for 24 hr. at ambient temperature. Basic hydrolysis were carried out using 2 mL of 0.1 N sodium hydroxide for 4 hr. at ambient temperature, neutralization of the samples with alkali or acid was performed before dilution. Study of neutral hydrolysis was carried out by using 2 mL of deionized water for 6 hours at 90°C. Oxidative degradation was carried out using 1 mL of 3 % H_2O_2 for 6 hours at room temperature. Photosensitivity of the drug was studied by exposing the drug solution to sunlight for 48 hours, for thermal study 0.2 g of the powder sample was placed in a vial in a controlled-temperature oven at 100 °C for 6 hours. Tetramisole hydrochloride at a concentration of 5 µg/mL was used in all the degradation studies. From the above resultant stressed solutions 20 µl was injected onto the column, and the chromatograms were obtained under the previously described conditions.

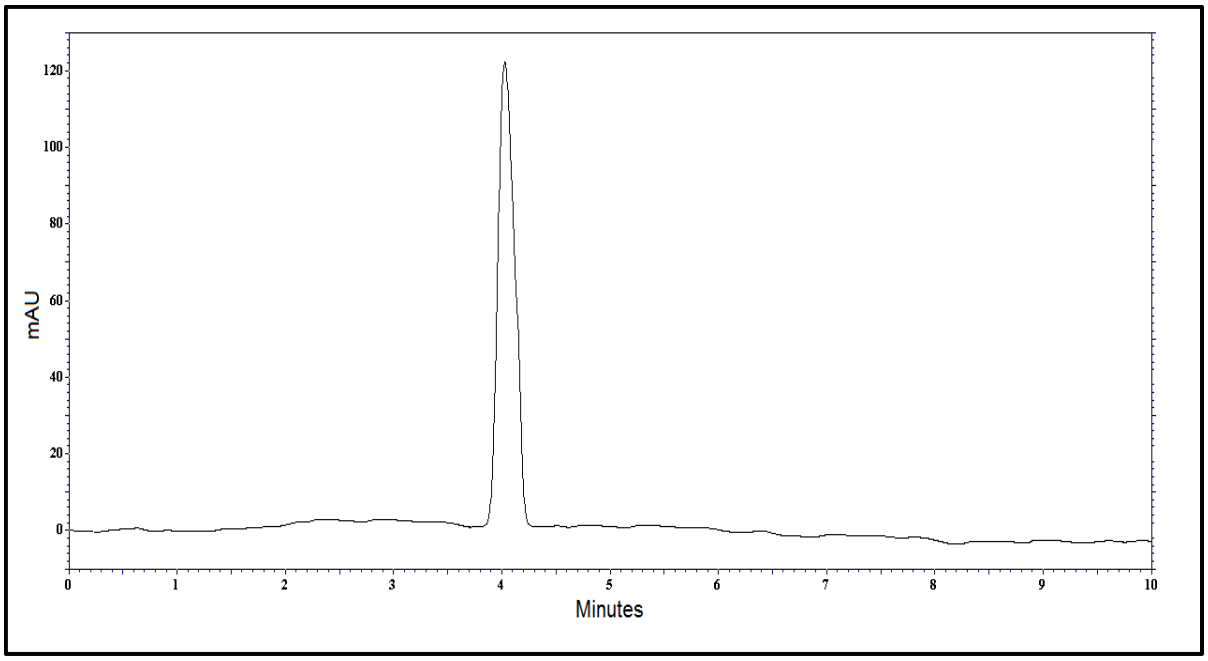

Figure 2. HPLC chromatogram of 5 µg/mL tetramisole hydrochloride at 212 nm

RESULTS AND DISCUSSION

Optimization of HPLC Method

Three parameters should be studied and optimized carefully in order to enhance the resolution and sensitivity of the HPLC method. The first parameter is the mobile phase selection. Several mobile phase compositions were tried including acetonitrile : methanol : water in different ratios. A good separation and satisfactory peak symmetry for tetramisole hydrochloride was achieved by using acetonitrile : methanol : water (50:33:17, v/v/v) as a mobile phase at flow rate 1 mL/min. The second parameter is the choice of the sutible stationary phase. Different columns were also tried such as SUPELCO® C18 column and Discovery® HS C18 column. Good separation was carried out on SUPELCO C18 column. The third parameter is the choice of the optimum wavelength at which the determination of tetramisole htdrochloride was done. Several wavelengths were tried but 212 nm which is the λmax of tetramisole htdrochloride was chosen as this wavelength gave the best results regarding accuracy and sensitivity. **Figure 2** shows a typical chromatogram for tetramisole hydrochloride with retention time of 4.05 min.

Degradation Behaviour

The instability of the drug may lead to loss of its activity and increase the risk of excepted adverse effects through formation of degradation products, so different stress conditions were applied to evaluate the stability of tetramisole hydrochloride.

Hydrolytic conditions: Upon exposing the drug to stress hydrolytic conditions the drug showed 22.63% degradation towards acidic hydrolysis with appearing of one degradation peak at retention time 2 min. (**Figure 3**), and showed 20.35 % degradation towards basic hydrolysis with appearing of one degradation peak at retention time 2.91 min. (**Figure 4**), but no degradation products were found when the drug exposed to neutral hydrolysis.

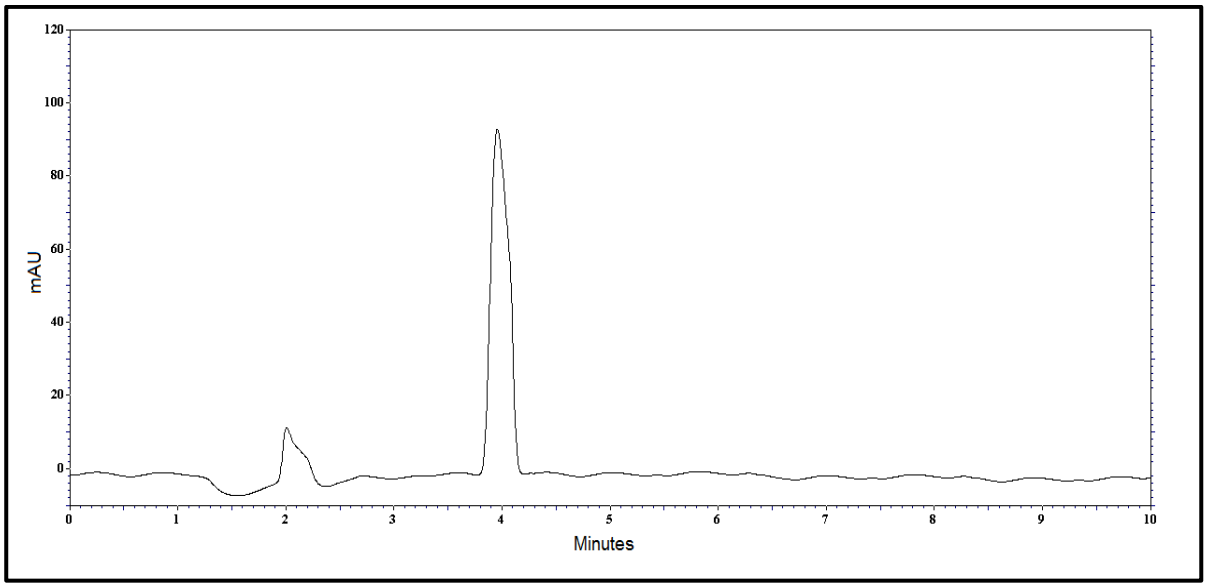

Figure 3. HPLC chromatogram for acidic hydrolysis of tetramisole hydrochloride after exposure to 0.1 N HCl for 24 hr. at ambient temperature

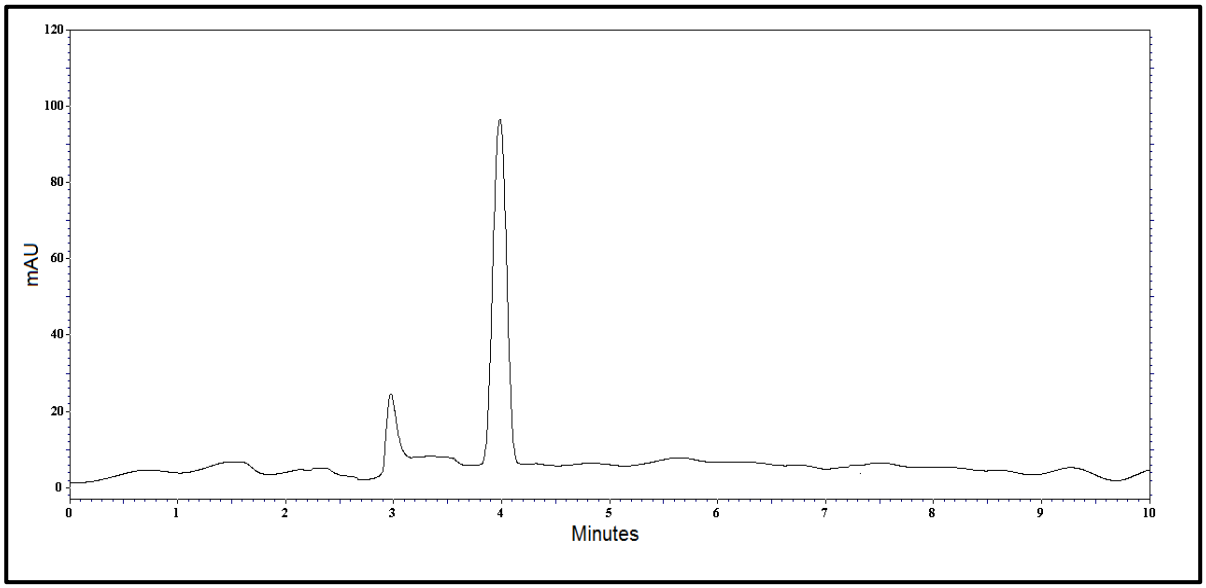

Figure 4. HPLC chromatogram for basic hydrolysis of tetramisole hydrochloride after exposure to 0.1 N NaOH for 4 hr. at ambient temperature

Oxidative studies: The drug showed 18.58 % degradation towards oxidative stress with appearing of one degradation peak at retention time 3.35 min. (**Figure 5**).

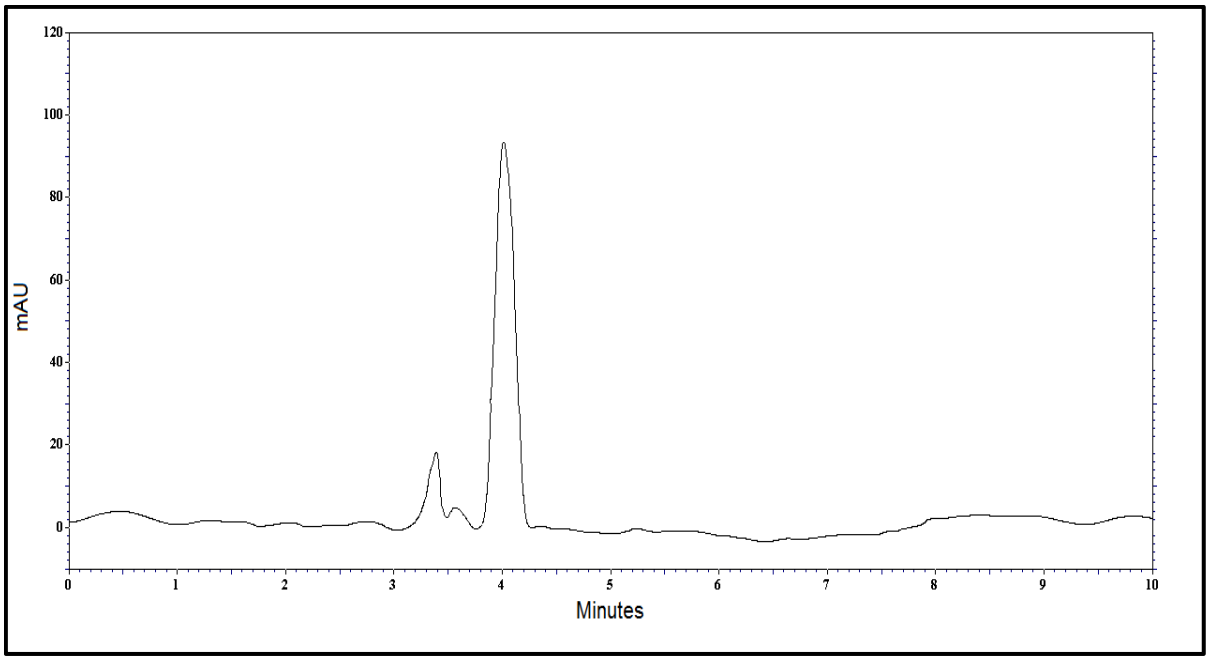

Figure 5. HPLC chromatogram of oxidative degradation behavior of tetramisole hydrochloride after exposing to 3 % H_2O_2 for 6 hour at ambient temperature

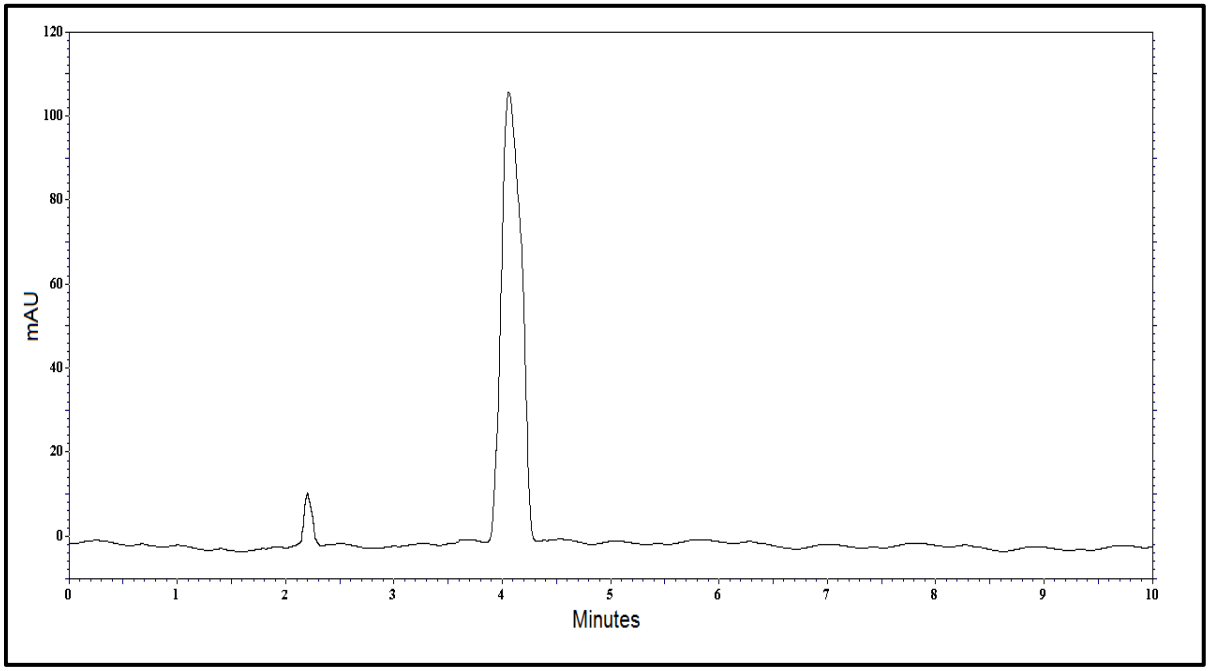

Figure 6. HPLC chromatogram of photolytic degradation behavior of tetramisole hydrochloride after exposing to sunlight for 48 hours

Photolytic studies: When drug solution was exposed to sun light for 48 hours the drug showed 12.25 % degradation with appearing of one degradation peak at retention time 2.15 min. (**Figure 6**).

Thermal studies: There was no degradation of solid tetramisole hydrochloride on exposure to heat at 100°C for 24 hrs, which indicated that the drug was thermally stable. Results of stability studies of tetramisole hydrochloride are shown in **Table 1**.

Table 1. Summary of Forced Degradation Studies

Stress conditions	Number of degradates (t_R)	Percent of degradation
0.1 N HCL at ambient temperature for 24 hours	1 (2.00)	22.63
0.1 N Sodium hydroxide at ambient temperature for 4 hours	1 (2.91)	20.35
Neutral hydrolysis at 90°C for 6 hours	0	0
3 % H_2O_2 at room temperature for 6 hours	1 (3.35)	18.58
Sunlight for 48 hours	1 (2.15)	12.25
Thermal at 100°C for 24 hours	0	0

Table 2. Results of System Suitability for the Determination of Tetramisole hydrochloride by the Proposed HPLC Method

Parameters	Tetramisole	Reference value [14]
Retention time (t_R)	4.05±0.152	-
Capacity factor (K´)	4.71	1-10 acceptable
Theoretical plates (N)	2966	> 2000
Tailing factor (T)	1.2	< 2
Injection precision (%RSD)	0.615	< 1
Resolution (Rs)		
0.1 N HCL (24 hour)	4.71	> 2
0.1 N NaOH (4 hours)	2.73	> 2
3 % H2O2 (6 hours)	2.00	> 2
photolysis (48 hours)	4.60	> 2

System Suitability Test

System suitability of HPLC method was achieved by calculating some parameters including number of theoretical plates (N), capacity factor (k), resolution factor (R) and tailing factor (T). Results of all parameters were good and indicative of the specificity of the method for evaluating of the stability of tetramisole hydrochloride when compared to USP reference values [14], as shown in **Table 2**.

Method Validation

The proposed HPLC method was validated in terms of linearity, specificity, precision, accuracy and robustness according to ICH guidelines [15].

Linearity and range. A linear correlation was obtained by plotting the peak area versus drug concentrations in µg/ml in the range of 1 – 6 µg/mL with coefficient of determination [r^2] = 0.9997

Accuracy. In order to ensure the accuracy of the proposed HPLC we calculate the mean percent recovery of three determination for three different concentrations of pure tetramisole hydrochloride and it was found to be 100.20.

Precision. Method precision was checked by measuring % RSD of three different concentrations of tetramisole hydrochloride repeated three times within the same day (repeatability) and in three different days (intermediate precision). Small values of % RSD indicating high precision of the method.

Specificity. The specificity of the developed method was assessed by resolving tetramisole hydrochloride from its possible degradation products.

Robustness. Analytical procedure robustness was studied by measuring the capacity of the method to remain unaffected by small changes in method parameter such as small changes in flow rate (±0.1 mL/min), mobile phase ratio and wavelength of detection (± 1 nm). The peak area or retention time of tetramisole hydrochloride not affected by these small changes in parameter which confirms the robustness of the method. The summary of these parameters and other validation and regression parameters are shown in **Table 3**.

Table 3. Validation Sheet and Regression Parameters of Tetramisole hydrochloride by the Proposed HPLC Method

Parameters	Proposed HPLC method
Wavelength (nm)	212
Range (µg/mL)	1 - 6
Regression Equation	$y^a = b\,x^b + a$
Slope (*b*)	360607
Intercept (*a*)	2283
Coefficient of determination (r^2)	0.9997
Accuracy	100.20
Precision (RSD)	
Repeatability	1.117
Intermediate precision	0.956
Robustness (%RSD)	
Change flow rate (±0.1 mL/min)	0.872
Change wavelength of detection (±1 nm)	1.057
LOD	0.114
LOQ	0.346

[a] Peak area of tetramisole

[b] Concentration of tetramisole

Table 4. Standard Addition Technique of the Proposed HPLC Method

Pharmaceutical taken (µg/mL)	Pharmaceutical found (µg/mL)	Pure add (µg/mL)	Pure found (µg/mL)	% Recovery
2	2.03	2	2.01	100.72
		3	2.95	98.45
		4	3.98	99.47
	Mean			99.54
	RSD			1.144

Table 5. Statistical comparison between the results obtained by applying the proposed methods and the reported method for determination of tetramisole hydrochloride in Anthimizole® veterinary powder

Parameter	Proposed method	Reported method
Mean	99.44	99.93
SD	1.019	0.882
RSD%	1.018	0.883
N	5	5
Variance	0.782	0.778
t-test[a]	0.892 (2.31)	——
F-value[a]	1.006 (6.39)	——

[a] The values in the parenthesis are the tabulated values of *t* and *F* at (P = 0.05)

Application of the Proposed Method to Marketed Formulation

The proposed method was successfully applied for the determination of the drug in its marketed formulation Anthimizole® powder. There was no interference from the excipients commonly present in the assayed powder. The results obtained from the application of standard addition technique confirming that the proposed HPLC method could be determine tetramisole hydrochloride in marketed formulation without any interference from excipient or additives as shown in **Table 4**.

Statistical Analysis

By comparing the results obtained from the developed HPLC method with that of the reported method [6], we found that the *t* and *F* values obtained are less than the tabulated ones which indicate that there is no significant differences between the developed HPLC method and the reported method regarding both accuracy and precision as shown in **Table 5**.

CONCLUSION

This work focuses on the study of the stability of tetramisole hydrochloride and its degradation behavior under different stress conditions. The developed HPLC methods gave accurate, precise and selective results for determination of tetramisole hydrochloride in the presence of its degradation products without prior separation and can be applied for routine analysis and for checking quality of pharmaceutical preparations during storage of the drug. Moreover, the proposed HPLC method uses simple reagents, with minimal preparation procedures and short run time encouraging application of the method in routine analysis.

REFERENCES

1. Sweetman SC. Martindale, the complete drug reference 36th Ed., Pharmaceutical Press, London, UK, 2009.
2. Sane RT, Sapre DS, Nayak VG. An extractive spectrophotometric method for the determination of tetramisole hydrochloride in pharmaceutical preparations; Talanta. 1985;32:148–149. https://doi.org/10.1016/0039-9140(85)80045-X
3. Amin AS, Dessouki HA. Facile colorimetric methods for the quantitative determination of tetramisole hydrochloride; Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2002;58:2541–2546. https://doi.org/10.1016/S1386-1425(02)00005-7
4. Sastry CSP, Aruna M, Reddy MN, Sankar DG. Extractive spectrophotometric determination of some anthelmintics using fast green FCF or orange-II; Indian Journal of Pharmaceutical Science. 1988;50:140–142.
5. Amin AS. Quantitative determination of some pharmaceutical veterinary formulations using bromocresol purple and bromocresol green; Analytical Letters. 2006;30:2503–2513. https://doi.org/10.1080/00032719708001761
6. Patil H, Wani M, Kuchekar BS. Development and validation of UV-spectrophotometric method for the estimation of tetramisole hydrochloride in bulk and pharmaceutical dosage form; Indo American Journal of Pharmaceutical Research. 2014;4:1903–1909.
7. Nassar MWI, Attia KAM, Mohamad AA, Said RA, Abdel-monem AH. Development and validation of spectrophotometric methods manipulating ratio spectra for determination of tetramisole hydrochloride in the presence of its alkali-induced degradation product Journal of analytical & pharmaceutical research. 2018;7(1):67-72.
8. Gupta VK, Singh AK, Gupta B. Potentiometric sensor for the high throughput determination of tetramisole hydrochloride; Combinatorial Chemistry & High Throughput Screening. 2007;10:583–594. https://doi.org/10.2174/138620707782152371
9. Issa YM, Rizk MS, Shoukry AF, Abdel-Aziz R, Abdel-Fattah HM, Atia EM. Construction and performance characteristics of new tetramisole selective plastic membrane electrodes; Talanta. 1994;41:135–141. https://doi.org/10.1016/0039-9140(94)80179-7
10. Holbrook A, Scales B. polarographic determination of tetramisole hydrochloride in extracts of animal tissue. Analytical Biochemistry. 1967;18:46-53. https://doi.org/10.1016/0003-2697(67)90054-1
11. Wagh JS, Mokashi AA, Datta A. High-performance liquid chromatographic method for the monitoring of the synthesis of the precursor for tetramisole; Journal of Chromatography. 1993;644:428–433. https://doi.org/10.1016/0021-9673(93)83357-X
12. Mourot D, Delepine B, Boisseau J, Gayot G. High-pressure liquid chromatographic analysis of veterinary anthelmintics I: quantitative determination of tetramisole; Journal of Pharmaceutical Sciences. 1979;68:796–797. https://doi.org/10.1002/jps.2600680636
13. Bertuccia C, Tedescoa D, Fabinia E, Di Pietraa AM, Rossib F, Garagnanib M, Borrellob ED, Andrisano V. Determination of levamisole and tetramisole in seized cocaine samples by enantioselective high-performance liquid chromatography and circular dichroism detection. Journal of Chromatography. 2014;1363:150–154. https://doi.org/10.1016/j.chroma.2014.07.069
14. The United States Pharmacopeia, National Formulary 35, United States Pharmacopeia Convention Inc., 30th edn, 2012.
15. International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2 (R1), Current step 4 version. 2005.