

# Development and Validation of Spectrophotometric Methods Manipulating Ratio Spectra for Determination of Tetramisole Hydrochloride in the Presence of its Alkali-Induced Degradation Product

## Abstract

Development and validation of four simple, accurate, selective and sensitive UV spectrophotometric methods manipulating ratio spectra for the determination of tetramisole hydrochloride (TZH) in the presence of its alkali-induced degradation (DTZH) product without preliminary separation. These methods are (A) Ratio difference (RD), where the difference in peak amplitudes were measured at 235 and 215nm. (B) Derivative ratio (<sup>1</sup>DD), where the peak amplitudes of the first derivative of the ratio spectra were measured at 220nm. (C) Mean centering (MC), where the amplitudes of mean centered values were measured at 235nm. (D) Continuous wavelet transform (CWT). Where The amplitudes of the transformed signals were measured at 239 nm. The developed methods were validated according to ICH guidelines and accuracy, precision, repeatability and robustness were found to be within the acceptable limit.

**Keywords:** Tetramisole hydrochloride; Ratio difference (RD); Derivative ratio (<sup>1</sup>DD); Mean centering (MC); Continuous wavelet transform (CWT)

## Research Article

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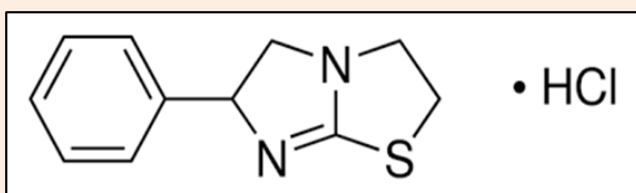
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## Introduction

Tetramisole hydrochloride is ( $\pm$ )-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-b]thiazole hydrochloride (Figure 1). It is an anthelmintic used in veterinary medicine for the control of nematode infections [1]. Tetramisole hydrochloride was determined by several techniques including spectrophotometry [2-6], potentiometric [7,8] and HPLC [9,10].



**Figure 1:** Structural formula of tetramisole hydrochloride.

The aim of this work is to develop and validate simple, sensitive, selective and cost effective spectrophotometric methods for the determination of tetramisole hydrochloride in the presence of its alkali-induced degradation product without preliminary separation. These methods namely ratio difference [11,12], derivative ratio [13,14], mean centering [15,16] and continuous wavelet transform [17,18].

## Experimental

### Instruments

Shimadzu dual beam UV-Visible 1800 Spectrophotometer,

(Tokyo, Japan), equipped with 10 mm matched quartz cells. The bundled software, UV-Probe personal spectroscopy software version 2.21 (SHIMADZU). Mean centering and continuous wavelet transform were implemented in MATLAB 8.2.0.701 (R2013b) using PLS toolbox version 2.1. The *t*-test and *F*-test were performed using Microsoft Excel.

### Samples

Both pure tetramisole hydrochloride (99.8%) (B. NO.20160920) and Anthimizole® 10% veterinary powder (B. NO. 150632) were kindly supplied by Pharma-Swede, Egypt. 10<sup>th</sup> of Ramadan city, Egypt.

### Chemicals and solvents

Hydrochloric acid, sodium hydroxide and methanol (El-Nasr Co., Egypt). Solvent used throughout the work was distilled water.

### Standard solution

A stock solution of tetramisole hydrochloride (100 $\mu$ g/ml) was prepared by dissolving 10mg of tetramisole hydrochloride in 50ml of distilled water and complete to 100ml with the same solvent.

### Degraded sample

Accelerated alkali-induced degradation was performed by refluxing 100mg of pure tetramisole hydrochloride with 50ml of 1N sodium hydroxide solution for 2 hours. The solution was cooled to room temperature then neutralized to pH 7 by addition of 1N hydrochloric acid solution, and then evaporated to dryness under vacuum. The obtained residue was extracted with methanol

(3 x 25ml), filtered into a 100ml volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradate derived from 1mg/ml of tetramisole hydrochloride. Working solution of degradate (100µg/ml) was obtained by further dilution of the stock solution with the distilled water.

## Procedure

### Construction of calibration curves

Different aliquots equivalent to (20-120µg) of both tetramisole hydrochloride and its alkali-induced degradation product were accurately transferred from their standard solutions (100µg/ml) into two separate series of 10-ml volumetric flasks and completed to volume with distilled water. The absorption spectra (from 200 to 400nm) of these solutions were recorded using distilled water as a blank and stored in computer. The stored spectra of tetramisole hydrochloride are divided by the spectrum of 8µg/ml of tetramisole degradate to get the ratio spectra.

### Ratio difference method (RD)

The calibration curve was constructed by plotting the amplitudes difference of the ratio spectra at 235 and 215nm ( $\Delta P_{235-215}$ ) versus the corresponding concentrations in µg/ml and the regression equation was derived.

### Derivative ratio method (DD)

To the obtained ratio spectra, the first derivative of ratio spectra was employed using  $\Delta\lambda = 4\text{nm}$  and scaling factor 1. The calibration curve was constructed by plotting the amplitudes of the first derivative values at 220nm versus the corresponding concentrations in µg/ml and the regression equation was derived.

### Mean centering method (MC)

The ratio spectra were mean centered using MATLAB. The calibration curve was constructed by plotting the amplitudes of the mean centered values at 235nm versus the corresponding concentrations in µg/ml and the regression equation was derived.

### Continuous wavelet transform (CWT)

The ratio spectra were transferred to the wavelet domain and the wavelet coefficients were calculated using bior 2.4 family and [scale value (a) =25]. The amplitudes of the transformed signals at 239nm were measured. The calibration curve was constructed by plotting the amplitudes values at 239nm versus the corresponding concentrations in µg/ml and the regression equation was derived.

### Application to laboratory prepared mixtures

Different aliquots equivalent to (100-20µg) of tetramisole hydrochloride and (20-100µg) of tetramisole degradate were accurately transferred from their standard solutions (100µg/ml) into a series of 10-ml volumetric flasks to prepare mixtures containing different ratios of both. The volumes were completed with distilled water and the absorption spectra (from 200 to 400nm) of these prepared mixtures were recorded using distilled water as a blank. The recorded spectra of mixtures are divided by the spectrum of 8µg/ml of tetramisole degradate to get the ratio spectra. The concentrations of tetramisole hydrochloride were calculated as described under linearity from the corresponding regression equation for each proposed method.

### Application to pharmaceutical preparation

Appropriate weight of Anthimazole® 10 % veterinary powder equivalent to 10mg of tetramisole hydrochloride was transferred into 100-mL volumetric flask and the volume was made up to 75ml with distilled water. The solution was shaken vigorously then sonicated for 10 min and filtered through Whatman filter paper no 41. The volume was completed to 100-ml with the same solvent to obtain solution claimed to contain 100µg/mL. The procedures stated under linearity were repeated using aliquots covering the working concentration range. The concentrations of tetramisole hydrochloride in veterinary powder were calculated from the corresponding regression equations.

## Results and Discussion

### Identification and interpretation of degradation product

Complete degradation was achieved, as investigated by thin layer chromatography using methanol: toluene: chloroform (14:36:50, by volume) as a developing solvent, where one spot of the degradation product obtained with significant separation from that of intact one. The structure of the isolated degradation product was elucidated using IR, <sup>1</sup>HNMR and MS spectrometry. Infrared (IR) spectrum of the degradation product showed appearance of abroad peak at 3419cm<sup>-1</sup> which may be assigned to NH group, also appearance of peak at 2550cm<sup>-1</sup> for SH group and at 1681 cm<sup>-1</sup> for the carbonyl group, (Figure 2 & 3). <sup>1</sup>HNMR of the degradate showed appearance of proton of NH group at 6.00 ppm also, appearance of proton of SH proton at 1.5ppm as shown in Figure 4 & 5. Mass interpretation for degradate reveal that molecular weight of tetramisole degradate is 222 as shown in Figure 6. The suggested degradation pathway is shown in Figure 7.

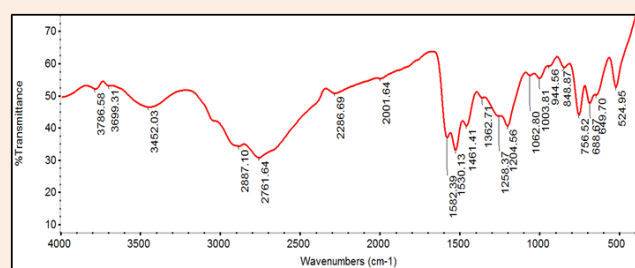


Figure 2: IR spectrum of intact tetramisole on KBr disc.

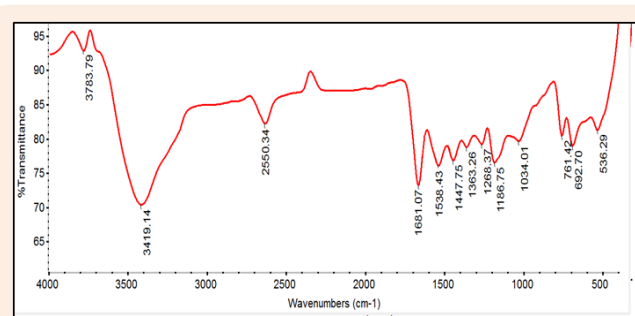


Figure 3: IR spectrum of tetramisole degradation product on KBr disc.

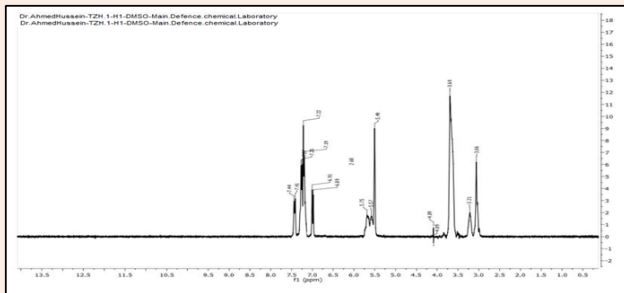


Figure 4:  $^1\text{H}$ NMR spectrum of tetramisole in DMSO.

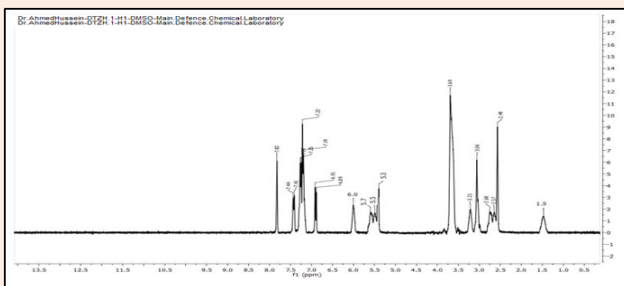


Figure 5:  $^1\text{H}$ NMR spectrum of tetramisole degradation product in DMSO.

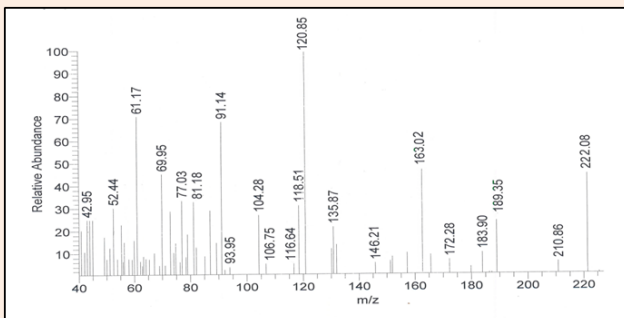


Figure 6: Mass spectrum of tetramisole degradation product.

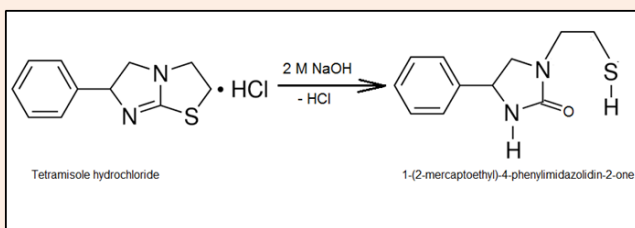


Figure 7: Suggested degradation pathway of tetramisole.

### Spectral characteristics and optimization of the methods

The zero-order absorption spectra of tetramisole hydrochloride and its degradation product shows severe overlapping, as shown in Figure 8. To overcome the interference from the degradation product, we developed four spectrophotometric methods that manipulate ratio spectra namely ratio difference, derivative ratio, mean centring

and continuous wavelet transform. These methods were found to be very easy to apply, rapid, simple, sensitive, accurate and precise.

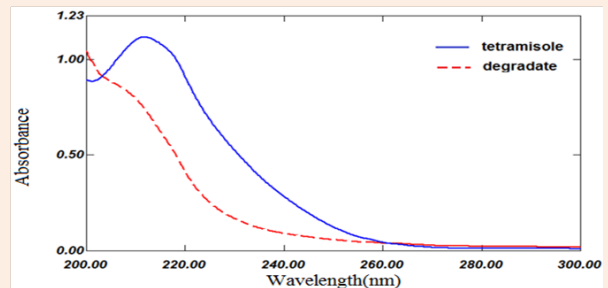


Figure 8: Zero-order absorption spectra of tetramisole hydrochloride ( $12\mu\text{g/ml}$ ) and its alkaline degradation product ( $12\mu\text{g/ml}$ ) (.....) in distilled water.

### Ratio difference method (RD)

In this method, the absorption spectra of the drug were divided by the absorption spectrum of the degradation product ( $8\mu\text{g/ml}$ ), as a divisor, to get the ratio spectra, as shown in Figure 9. The interference from degradation product can be removed by measuring the difference in peak amplitudes at 235 and 215 nm. This difference is zero for degradation product, while it is directly proportional to the concentration of the drug.

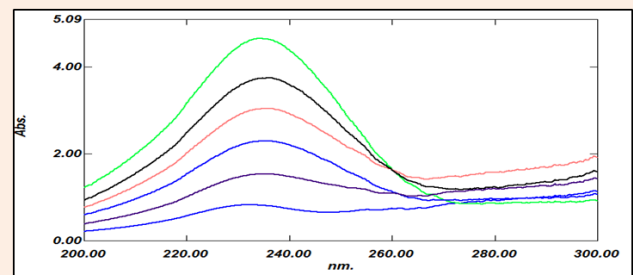


Figure 9: Ratio spectra of tetramisole hydrochloride ( $2\text{-}12\mu\text{g/ml}$ ) using  $8\mu\text{g/ml}$  its degradation product as a divisor.

### Derivative ratio method (1DD)

In this method, first derivative corresponding to each ratio spectrum was recorded. The amplitudes of the first derivative of the ratio spectra at 220 nm were proportional to the concentrations of the drug without interference from its degradation product, as shown in Figure 10.

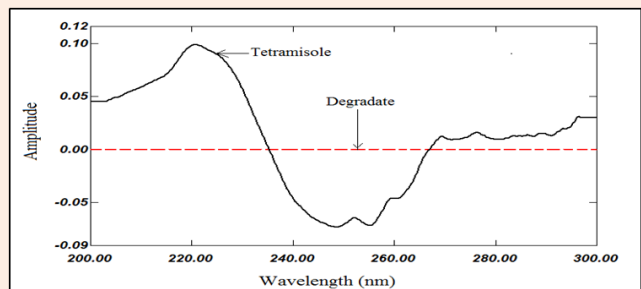


Figure 10: First derivative of the ratio spectra of tetramisole hydrochloride ( $8\mu\text{g/ml}$ ) using  $8\mu\text{g/ml}$  of its degradation product as a divisor.

### Mean centering method (MC)

In this method, the obtained ratio spectra were mean centered. The mean centered values at 235nm were proportional to the concentrations of the drug without interference from its degradate, as shown in Figure 11.

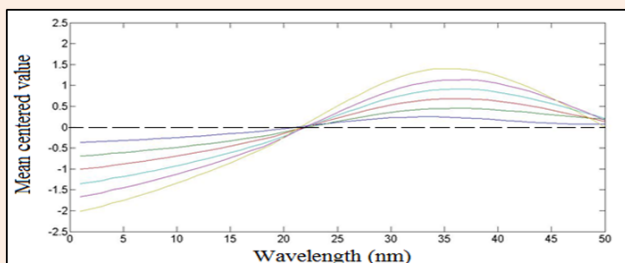


Figure 11: Mean centering of the ratio spectra of tetramisole hydrochloride (2-12 µg/ml) using 8 µg/ml of its degradate as a divisor.

### Continuous wavelet transform (CWT)

In this method, The ratio spectra were transferred to the wavelet domain and the wavelet coefficients were calculated. The amplitudes of the transformed signals at 239nm were measured which are proportional to the concentrations of tetramisole hydrochloride without interference from its degradate, as shown in Figure 12.

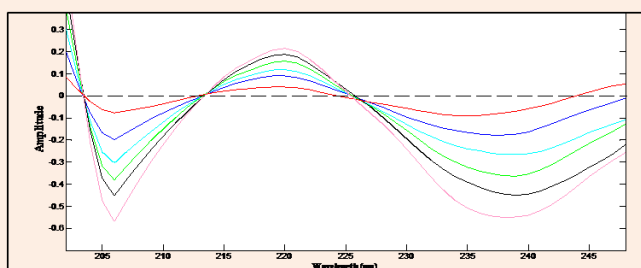


Figure 12: Continuous wavelet transform of tetramisole hydrochloride (2-12 µg/ml) using 8 µg/ml of its degradate as a divisor.

### Methods Validation

Methods validation was performed according to ICH guidelines [19] for all the proposed methods. Linearity, range, LOD, LOQ, accuracy and precision of the proposed methods were shown in

Table 2: Determination of tetramisole hydrochloride in laboratory prepared mixtures with tetramisole degradate by the proposed methods.

TZH (µg/ml)	DTZH (µg/ml)	Degradate %	RD	<sup>1</sup> DD	MC	CWT
10	2	16.67	99.65	99.65	100.68	101.59
8	4	33.33	101.06	99.78	99.77	99.04
6	6	50.00	100.13	100.15	98.79	99.79
4	8	66.67	101.49	98.23	98.34	100.96
2	10	83.33	98.47	98.67257	99.48	98.18
Mean ±RSD			100.16±1.190	99.29±0.813	99.41±0.913	99.91±1.385

Table 1. The selectivity of the methods was checked by the analysis of laboratory prepared mixtures of the drug with its alkali-induced degradation product as shown in Table 2. The validity of the proposed procedures is further assessed by applying the standard addition technique and the results obtained in Table 3 showing no excipients interference. The developed methods have been also applied for determination of tetramisole hydrochloride in Anthimazole® veterinary powder and the results obtained were acceptable with small RSD % values. Results obtained by the proposed methods were statistically compared to those obtained by the reported method [6] and no significant difference was observed Table 4. One-way ANOVA was applied for the purpose of comparison of developed methods, Table 5. showed that there was no significant difference between the proposed methods for the determination of tetramisole and the reported method.

Table 1: Regression and analytical parameters of the proposed spectrophotometric methods for determination of tetramisole hydrochloride.

Parameters	RD	<sup>1</sup> DD	MC	CWT
Wavelength (nm)	235&215	220	235	239
Range (µg/mL)	12-Feb			
Slope (b)	0.1766	0.0113	0.1115	0.0471
Intercept (a)	0.0222	0.0072	0.0088	-0.0168
Correlation coefficient(r)	0.9998	0.9998	0.9998	0.9997
LOD	0.181	0.182	0.157	0.199
LOQ	0.548	0.551	0.476	0.603
Accuracy <sup>d</sup>	100.34	99.94	99.69	99.77
<b>Precision</b>				
Repeatability (RSD) <sup>b</sup>	0.837	1.006	1.024	0.896
Intermediate precision (RSD) <sup>c</sup>	0.925	0.95	0.936	0.706

<sup>a</sup>Average of three determinations for three concentrations (4, 8 and 12 µg/mL) for tetramisole hydrochloride repeated three times.

<sup>b</sup>The intraday (n = 3), average of three concentrations (4, 8 and 12 µg/mL) for tetramisole hydrochloride repeated three times within the day.

<sup>c</sup>The interday (n = 3), average of three concentrations (4, 8 and 12 µg/mL) for tetramisole hydrochloride repeated three times in three days.



**Table 3:** Application of standard addition technique to the analysis of Anthimazole® veterinary powder by applying the proposed methods.

Dosage conc. (µg/mL)	Standard Added (µg/mL)	RD	<sup>1</sup> DD	MC	CWT
		Recovery % of Pure Found			
4	4	100.99	100.88	99.51	98.46
	6	98.28	98.23	98.90	99.22
	8	101.67	99.12	100.26	98.30
Mean ± RSD		100.31±1.787	99.41±1.360	99.56±0.683	98.66±.498

**Table 4:** Statistical comparison between the results obtained by applying the proposed methods and the reported method for determination of tetramisole hydrochloride in Anthimazole® veterinary powder.

Parameter	RD	<sup>1</sup> DD	MC	CWT	Reported Method [6]
Mean	100.03	100.04	100.30	100.29	99.93
SD	1.019	0.972	0.969	1.212	0.882
RSD%	1.018	0.971	0.966	1.208	0.883
N	5	5	5	5	5
Variance	1.038	0.944	0.939	1.469	0.778
<i>t</i> -test*	0.157 (2.31)	0.178 (2.31)	0.621 (2.31)	0.536 (2.31)	--
<i>F</i> -value*	1.334 (6.39)	1.213 (6.39)	1.208 (6.39)	1.888 (6.39)	---

\*The values in the parenthesis are the corresponding theoretical values of *t* and *F* at (*P* = 0.05).

**Table 5:** One-way ANOVA testing for the different proposed methods used for the determination of tetramisole hydrochloride in Anthimazole® veterinary powder.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	<i>F</i> Value
Between exp.	4	0.556	0.139	0.135 ( 2.866)
Within exp.	20	20.670	1.033	

The values between parentheses are the theoretical *F* values. The population means are not significantly different.

## Conclusion

The presented work concerns with the development and validation of simple, accurate and precise spectrophotometric methods for determination of tetramisole hydrochloride in bulk, pharmaceutical formulation and in the presence of its degradation product without sample pretreatment and without interference from excipients or degradate. The developed methods do not require sophisticated techniques or instruments and can be easily applied for quality control and routine analysis of the studied drug.

## Acknowledgement

None.

## Conflict of Interest

None.

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