

## Determination of Thiamazole in Tablet Formulation by Using Reversed Phase Liquid Chromatographic Method

Kader Poturcu<sup>1+</sup>, Ebru Çubuk Demiralay<sup>1\*</sup>

<sup>1</sup>Arts & Science Faculty, Suleyman Demirel University, Isparta, Turkey

\*Corresponding author: [ebrucubuk@sdu.edu.tr](mailto:ebrucubuk@sdu.edu.tr)

+Speaker: [kaderpoturcu@sdu.edu.tr](mailto:kaderpoturcu@sdu.edu.tr)

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**Abstract** – In this study, the amount of thiamazole (methimazole) in pharmaceutical tablet formulation was determined by using reversed phase liquid chromatography method (RPLC). Chromatographic separation was carried out by using YMC Triart C18 (150 mm × 4.6 mm, 3µm, YMC, USA) column. 5% (v/v) acetonitrile-water binary mixture at pH 9.5 was used as a mobile phase. Metronidazole was chosen as an internal standard. Flow rate was 0.8 ml/min and column temperature was 25 °C in chromatographic separation. The studied wavelengths for thiamazole and metronidazole are 260 and 340 nm, respectively. This proposed method was suitably validated with respect to accuracy, precision, linearity, the limit of detection (LOD) and limit of quantification (LOQ). The calibration graph of thiamazole was linear from 4 ppm to 14 ppm. The recovery of the 5 mg thiamazole containing commercial tablet (Thyromazol) was 100.059%. The proposed RPLC method was successfully applied to the determination of thiamazole in commercial tablet formulation.

**Keywords** –Thiamazole, tablet formulation, RPLC method, method validation, recovery.

### I. INTRODUCTION

During the last decades, there has been increasing evidence that many of the chemicals are not only toxic at high concentrations, but can cause disruption of the endocrine system at levels which do not cause obvious ill-health. The endocrine system is the chemical communicational system of the body which regulates such activities as body fluid homeostasis, management of stress, and reproduction and fertility which are necessary for propagation of the species [1,2].

Anti-thyroid drugs are used to treat hyperthyroidism as they normalise thyroid function through binding to the thyroid peroxidase enzyme. These drugs are known as thioamides that containing a thiocarbonyl group and a thiourea moiety within a heterocyclic structure. The common agents used are methimazole, carbimazole and propylthiouracil [3,4].

Thiamazole, 1-methyl-2-mercaptoimidazole, is a widely used in medicine for the treatment of hyperthyroidism, and as adjuncts in the treatment of thyrotoxicosis or thyroid storm [5-10]. The structures of thiamazole is shown in Fig 1. It can be seen that this drugs share a common thiocarbamide group which is essential for antithyroid activity of drug candidate [3]. Thiamazole (methimazole) is also the active metabolite of carbimazole [11].

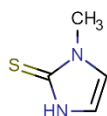


Fig. 1. Chemical structure of thiamazole and metronidazole (IS) [12]

In the last years thiamazole became also a fashioned model substance for endocrine disruption (thyroid axis) studies thus inhibits the production of the thyroid hormones.

Predicted pK<sub>a</sub> value of the methimazole by Pallas software related to (N-3) nitrogen atom is given 4.41, whereas SPARC software is determined this value as 5.91. Calculated pK<sub>a</sub> value of methimazole predicts a basic characteristic on the nitrogen and a protonated form of it under pH 2.4 and an unprotonated form below pH 6.5. It can be read out from the predicted value, the methimazole is very polar. Thiamazole contains ionisable group, which affects the chromatographic behavior of this compound [13].

When separating very polar, water-soluble compounds, highly aqueous (>95%) eluents are often required to achieve sufficient retention. However, operating a conventional C18 column under such conditions can lead to dewetting or 'phase collapse' which can result in poor chromatographic reproducibility [14]. Therefore, chromatographically correct quantitative and qualitative determination of small molecular weight polar substances, is still one of the most problematic tasks in the RPLC, what was verified by our experiments also. Moreover, the hydrophobic stationary phase gives unsatisfactory retention of the polar analytes although silanol groups can retain polar compounds due to the silanophil interaction. In this case, peak broadening or tailing can decrease the effectivity of the separation [15]. To overcome these limitations, new generation RPLC columns consisting hybrid silica based stationary phases that is stable under the use of 100% aqueous mobile phase is preferred. YMC-Triart C18's innovative surface modification technology results in excellent peak shapes even for the basic compounds that often

exhibit tailing shapes on conventional silica and hybrid silica based ODS columns [14].

The aim of this study was to develop and validate a simple, sensitive and reliable method for quantification of thiamazole in tablet formulations. To achieve this purpose, a reversed-phase liquid chromatographic method was developed and a validation study of thiamazole was carried out according to International Conference on Harmonisation (ICH) [16]. The proposed RPLC method has been applied for commercial Thyromazol tablet containing a 5 mg active substance.

## II. MATERIALS AND METHOD

### A. Chemicals and Reagents

Thiamazole was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN), ammonia (NH<sub>3</sub>) and ammonium chloride (NH<sub>4</sub>Cl), potassium hydrogen phthalate were analytical grade and supplied by Merck (Darmstadt, Germany). Potassium hydrogen phthalate was used as a primary standard reference substance for pH electrode calibration of ACN-water mixtures used as a mobile phase in chromatographic analyses [17].

### B. Instrumentation and Apparatus

The HPLC (Shimadzu, Japan) consisted of a pump (LC-20AD), degasser (DGU-20A3), UV-Vis detector (SPD-20A), and a column oven (CTO-20A). RPLC analyses were carried out on an YMC Triart C18 (150 mm X 4.6 mm, 3µm) column.

HPLC grade water used for solution preparation was obtained from Millipore (Direct Q3 UV, France). The pH measurements were done with a combination glass electrode (In Lab 412) connected to a pH meter (Mettler Toledo MA 235, Switzerland). The pH values of the mobile phases were measured versus a 0.05 m potassium hydrogen phthalate solution as primary standard substance, dissolved in the appropriate ACN-water binary mixtures according to IUPAC rules [17].

### C. Chromatographic System and Conditions

The validation studies of the pharmaceutical tablet formulation of thiamazole were carried out in 5% (v/v) ACN-water binary mixture at pH 9.5. Chromatographic analyses were performed at 25°C, with a flow rate at 0.8 mL/min. The injected volume of analyte into the column was 20 µL for each run; thiamazole and metronidazole (IS) were detected at 240 and 360 nm, respectively.

### D. Preparation of Solutions

#### Thiamazole Tablet Formulation

The commercial tablet formulation Thyromazol® (Abdi İbrahim, İstanbul) containing 5 mg thiamazole were analyzed for method development and validation studies.

#### Thiamazole Pharmaceutical Dosage Form

Ten tablets from Thyromazol® were accurately weighed, finely powdered and an accurately weighed amount of the powder equivalent to a tablet was transferred into a 100 mL volumetric flask; 50 mL ACN-water binary mixture was added; to dissolve obtained mixture, sonication was carried out approximately 30 min and then filtered. An appropriate amount of clear filtrate was taken and the internal standard (IS,

metronidazole) was added to this solution. For final concentration, the solution was diluted with the mobile phase (pH 9.5) and 20 µL of this solution was injected into the HPLC column.

### E. Method Validation

#### Specificity

The ability to separate thiamazole and IS was demonstrated by assessing the resolution between the peaks according to Purnell equation [18]. The tailing factor (Tf) for thiamazole and IS was also assessed. The identification of retention time of thiamazole in the tablet formulation and IS was carried out comparing retention time values of thiamazole and IS pure stock solutions.

#### Linearity

The calibration graph was constructed by plotting the ratio of peak area (analyte/IS) (y) as a function of the analyte concentration (x) in µg/mL. The calibration function of the graph was calculated with the standard deviation (SD) values of slope and intercept. LOD and LOQ values of the proposed method were calculated on the basis of response and slope of the calibration function.

#### Accuracy, Precision, and Repeatability

The two most important parameters of chromatographic analyses are accuracy and precision. Accuracy is a measure of the closeness of the experimental value to the accepted either as a conventional true value or an accepted reference value. Precision measures of how close individual measurements are to each other. [19]. Accuracy of the proposed RPLC method was tested by recovery studies of 5 mg thiamazole active substance containing Thyromazol® tablet. The precision of the method was determined by in terms of within-day precision (repeatability) and between-day (intermediate precision) precision. Within-day and between-day precision values were estimated containing two different concentrations of thiamazole standard solution (8 and 12 µg/mL). RSD% values were obtained by performing the experiment 5 times on a day and repeating for three separate days.

#### System Suitability Tests

Retention time, theoretical plate number and tailing factor of the chromatographic analyses were assessed under the title of system suitability parameters. These parameters were evaluated by six replicate analyses of thiamazole and compared with standard values. The acceptance criteria are RSD% of peak areas not more than 2%, theoretical plates numbers (N) at least 2000 per each peak and tailing factors not more than 2.0 for system suitability parameters of thiamazole validation studies [20].

#### Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ values of the developed RPLC method were calculated by using the signal-to-noise ratio. LOD and LOQ values were calculated using  $\bar{x}+3s$  and  $\bar{x}+10s$  formulas, respectively [21].

## III. RESULTS AND DISCUSSIONS

### A. Optimization of Chromatographic Conditions

Optimum chromatographic separation for validation studies of thiamazole was achieved by using YMC Triart C18 (150 mm × 4.6 mm, 3µm) at 25 °C. Selected conditions, sharp peak shapes and precise retention time were obtained. The system suitability tests are summarized in Table 1.

Table 1. System suitability tests parameter

Parameters	Compounds	
	Thiamazole	Metronidazole (IS)
Retention time	6.884	16.148
Capacity factor (k)	1.512	4.893
Selectivity (α)	-	3.236
Resolution (Rs)	-	15.541
Tf	0.966	1.018
Theoretical plate numbers	6584	11741

The retention time values for thiamazole and IS (metronidazole) were found to be 6.884 and 16.148 minutes, respectively. Total run time was approximately 18 minutes. The calculated resolution factor (Rs) > 1.5 value corresponds to the requirements. It can be concluded from the parameters that studied RPLC system was found suitable for the validation studies of thiamazole.

Calibration plots for proposed method were evaluated and checked by analyzing standard solutions at six points: 4, 6, 8, 10, 12, 14 µg/mL, respectively. Calibration standards were prepared by diluting stock solutions with mobile phase and injected in 4 parallel measurements. Linearity range was obtained from 4-14 µg/mL. LOD and LOQ values as 1.046 and 3.486 µg/mL were calculated. The measured slope, intercept, correlation coefficient of each calibration curves and the calculated LOD and LOQ as well as the precision are shown in Table 2. The results are summarized in Table 2.

Table 2. Statistical evaluation of the calibration data by RPLC

Calibration parameters	Thiamazole
Retention time (min)	6.884
Linearity range (µg/mL)	4-14
Slope	1.306
Intercept	0.810
Correlation coefficient	0.993
Limit of detection (LOD) (µg/mL)	1.046
Limit of quantification (LOQ) (µg/mL)	3.486

The linearity of standard calibration curve was confirmed by the high values of the correlation coefficient. Correlation coefficient shows high fitting of the linear on the calibration point and so the linearity of the curve is verified.

The proposed method was applied to the determination of thiamazole in Thyromazol tablet formulation. Meanwhile, the recovery of standard addition were performed. Within-day precision and between-day precision values of the method were determined using five replicate injections of two concentrations (8 and 12 µg/mL) analyzed on the same day and repeating for three separate days. The results are given in Table 3.

Table 3. Within-day precision and between-day precision of thiamazole

Precision results	Thiamazole
Theoretical concentration (µg/mL)	8.00
	12.00
Within-day measured concentration mean (µg/mL)	8.059
	11.900
Between-day precision	7.914
	11.721
RSD (%)	1.264
	0.813

Accuracy of the method showed satisfactory results, all values were less than 5% measured on the same day and less than 10% measured on another day [16]. Fig. 2 showed us, The representative chromatogram of the thiamazole containing Thyromazol tablet.

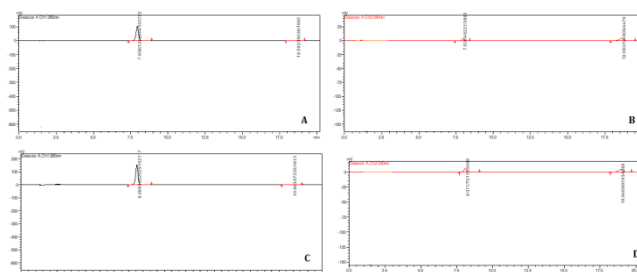


Figure 2. The representative chromatogram of the thiamazole containing Thyromazol tablet 8 ppm thiamazole direct tablet fomulation A) 260 nm B) 340 nm; 12 ppm standard added tablet fomulation C) 260 nm D) 340 nm

Method precision and accuracy were tested using tablet sample of thiamazole including 5 mg of active substance. Three samples of tablets were prepared for each experiment. Development method was applied to the direct determination of this compound in its tablet dosage form, using the related calibration straight line without any sample extraction or evaporation other than filtration and adequate dilution steps.

Table 4. Analysis result of thiamazole in tablet formulation

Amount of Thiamazole in tablets (mg)	Recovery%	Bias%
5.074 ± 0.012	100.059	1.480

The results obtained are satisfactorily accurate and precise as indicated by the excellent recovery% (Table 4).

#### IV. CONCLUSION

A liquid chromatographic method was developed and validated for thiamazole in pharmaceutical tablet formulations. Since thiamazole is a widely used test substance in endocrine disruption and in other biochemical and physiological studies (N-methylthiourea also), this method will have a wide application potential in view on these fields also. In addition, the methods (due to its popularity especially the HPLC method) can serve as strong basis for further developments of determination of other low molecular weight and high polar substances. Especially other imidazole

substances, derivatives, structural similar forms are concerned due their important and versatile biological function. The developed new separation method is simple, effective and enough sensitive. It can be shortly summarized that a novel separation method was successfully developed. The separation conditions were optimized and an optimal separation was achieved.

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