DETERMINATION OF THE THIAMAZOLE RELATED SUBSTANCES IN PHARMACEUTICAL FORMULATIONS BY HPLC-UV METHOD

Lela Munjas Jurkić[1], Danijela Štanfel[1],
Iva Valentić Žunić[2], Maša Safundžić Kučuk[3]

[1] JGL d.d., R&D Analytical Development, Svilno 20, Rijeka, Croatia
[2] JGL d.d., R&D Pharmaceutical Development, Svilno 20, Rijeka, Croatia
[3] JGL d.d., R&D Stability Development, Svilno 20, Rijeka, Croatia
e-mail: lela.munjas@jgl.hr

INTRODUCTION

Thiamazole is an active substance used in drug products for the treatment of hyperthyroidism. It is a thyreostatic that inhibits the synthesis of natural thyroid hormones T3 and T4 by inhibiting the incorporation of iodine. Thin-layer chromatography has been recommended for determination of the thiamazole related substances in official pharmacopoeias. On the other hand, HPLC method has been rarely used for this purpose though the spectrum of thiamazole molecule shows intensive band at ~250 nm due to the internal transitions within the coordinated ligand [1]. Therefore, we developed and validated a new HPLC method with UV detection for determination of the thiamazole related substances.

MATERIALS AND METHODS

The HPLC method was developed after the optimization of several chromatographic parameters. All peaks of impurities were well separated from each other and from the main thiamazole peak (figure 1).
External standard calibration was used. The method was validated according to the ICH guidelines for validation of analytical procedures [2] in terms of specificity, linearity, quantitation limit (LOQ), detection limit (LOD), accuracy, precision and robustness (Table 2). Validation was performed on the Athyrazol tablets, a drug manufactured by JGL.

Table 1. HPLC chromatographic conditions

Column:	Zorbax SB-C18, Rapid Resolution 150 mm x 4.6 mm, I.D. 3.5 µm
Column temperature:	25°C
Mobile phase:	A gradient mixture of the mobile phase consisting of 0.03 M ammonium acetate buffer and acetonitrile
Flow rate:	0.7 ml/min
Injection volume:	10 µl
Detection:	UV detector set at 252 nm
Run time:	30 min.

RESULTS AND DISCLUSION

We have found eight unknown impurities and thiamazole related substance C in the control sample. The peaks of thiamazole and impurity C are well separated from the peaks of the other unknown impurities and of the placebo, providing the specificity of the method. Precision was validated by testing the repeatability of the sample preparation and intermediate precision obtained by the two analysts (Table 2).
The method was accurate in the range 0.5 µg/ml - 10.1 µg/ml for impurity C and 0.2 µg/ml - 4.0 µg/ml for thiamazole (figure 2). Linearity was achieved for concentration from 0.5 µg/ml to 12.6 µg/ml for impurity C and 0.2 µg/ml - 5.0 µg/ml for thiamazole with high R^2 and low bias (figure 3). Detection and quantitation limits for impurity C were measured to be 0.17 µg/ml (0.017%) and 0.5 µg/ml (0.05%), respectively. Detection and quantitation limits of 0.06 µg/ml (0.006%) and 0.20 µg/ml (0.020%) for any other unknown impurities derived from the measurements of thiamazole were found. The proposed method proved to be precise, robust and specific, with no interference from excipients.

Table 2. Summary of the validation results (*c – concentration* of the thiamazole related compounds relative to the thiamazole content)

Parameter	Results
Specificity with forced degradation study	The method adequately separates all impurities and placebo peaks from each other and from the main peak of thiamazole with the resolution corresponding to the peak to valley ratio of at least 1.5
Precision a) Repeatability of the method b) Intermediate precision	RSD≤2.17% (*c* > 0.5%); RSD≤5.78% (0.05% < *c* ≤ 0.5%) RSD≤8.92% (*c* ≤ 0.05%) RSD≤8.51% Difference between 2 analysts ≤7.31%
Linearity	thiamazole: R^2= 0.99994 (Bias = 0.382%) impurity C: R^2= 0.99998 (Bias = -1.017%)
Range	*thiamazole:* **0.2 µg/ml -4.0 µg/ml;** 0.02%-0.40% in relation to the concentration of thiamazole in sample solution *impurity C:* **0.5 µg/ml -10.1 µg/ml;** 0.05% -1.0% in relation to the concentration of thiamazole in sample solution
Accuracy	*thiamazole:* from 95.65 % to 100.09% *impurity C:* from 96.20% to 101.98%
Detection limit (LOD)	thiamazole (for unknown impurities): 0.06 µg/ml (0.006%) impurity C: 0.17 µg/ml (0.017%)
Quantitation limit (LOQ)	thiamazole (for unknown impurities): 0.20 µg/ml (0.020%) impurity C: 0.50 µg/ml (0.050%)
Robustness	Impurities with content higher than LOQ are stable at least 59 hours at 10°C SST solution is stable at least 68 hours Diff. ≤ 1.61%

REFERENCES

[1] D. Cvijanović, V. Damjanović, I. Picek, and B. Foretić, "Spectroscopic Studies of Methimazole Reactivity toward the Aquapentacyanoferrate (II) Ion in Aqueous Solutions," *Spectroscopy: An International Journal*, vol. 27, no. 5-6, Article 136058, 6 p., 2012

[2] European Medicines Agency, Note for Guidance on Validation of Analytical Procedures: Text and Methodology, ICH Topic Q 2 (R1) (CPMP/ICH/381/95), available at http://www.ema.europa.eu.

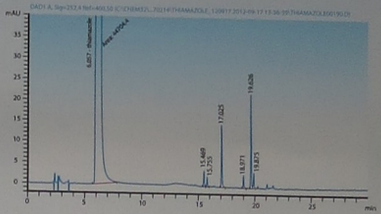

Figure 1a. Chromatogram of the sample solution of Athyrazol tablets.

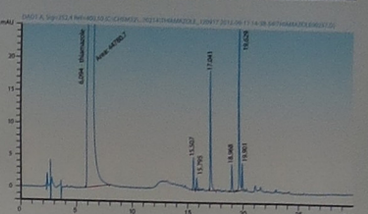

Figure 1b. Chromatogram of the sample solution of Athyrazol tablets after the exposure to UV radiation.

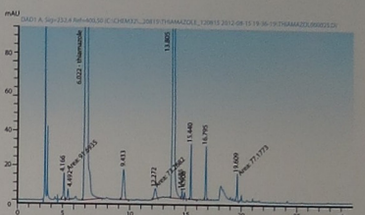

Figure 1c. Chromatogram of the sample solution of Athyrazol tablets after the exposure to oxidation treatment.

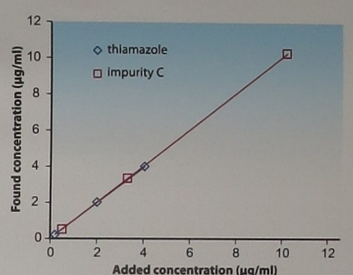

Figure 2. Accuracy of the method for thiamazole and impurity C.

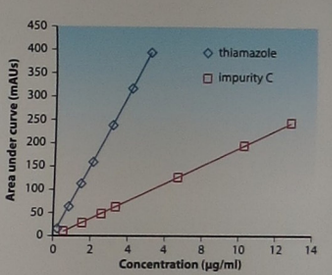

Figure 3. Linearity of the method for thiamazole and impurity C.

Forced degradation study showed that the thiamazole was susceptible to the degradation when exposed to oxidative environment and prolonged UV radiation (Figures 1a, b and c), while it was more resilient to increased temperature and degradation in acid and alkaline environments (Table 3). Impurity C is the main product of the acid hydrolysis and can be considered a degradation product. Oxidation also lead to the formation of eight unknown impurities previously not detected in control samples.

Table 3. Relative retention times of detected impurities with their content in different degradation environments (n.d. – not detected).

	Oxidation	UV	Acid hydrolisis	Alkaline hydrolisis	Heating
Impurity C	n.d.	n.d.	0.3 %	n.d.	n.d.
All unknown impurities	7.9 %	5.9 %	0.2 %	0.2 %	0.6 %
Total impurities	7.9 %	5.9 %	0.5 %	0.2 %	0.6 %

The HPLC method was compared to the previously validated TLC method, a less expensive LC method commonly used in the past by pharmaceutical laboratories. For TLC method we used a mixture of toluene, 2-propanol and concentrated ammonia as the mobile phase and a visual estimation of the spot intensity under the UV light at 254 nm. The new HPLC method has been proved to be far superior to the TLC method. TLC showed much lower detection and quantitation limits of 1 µg/ml and 2 µg/ml for impurity C respectively, when compared to HPLC method (Table 4). Similarly, the TLC method was linear only in the range between 5 and 25 µg/ml for impurity C, while HPLC method covers range from 0.5 µg/ml.

Table 4. Comparison between HPLC and TLC methods.

PARAMETAR:	*HPLC METHOD:*	*TLC METHOD:*
LOD	0.5 µg/ml	1 µg/ml
LOQ	0.17 µg/ml	2 µg/ml
RANGE	0.5 µg/ml-10.1 µg/ml for impurity C	5 µg/ml-25 µg/ml for impurity C

CONCLUSION

A novel high-performance liquid chromatography method with UV detection at 252 nm was proposed for determination of the thiamazole related substances in pharmaceutical formulation. We have shown that the HPLC method is highly reliable, sensitive, accurate, precise, robust, effective and moderately fast to be used for determination of the thiamazole related substances in pharmaceutical products. It is far superior to the previously used TLC method.