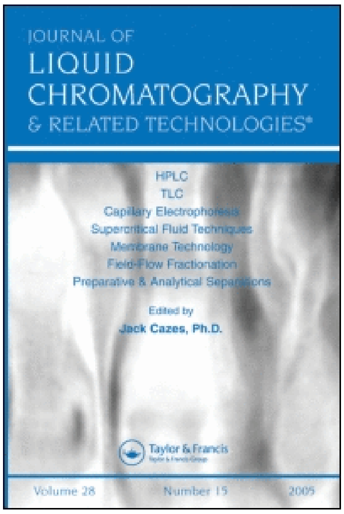

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Zakrzewski, Robert(2009) 'Determination of Methimazole in Pharmaceutical Preparations using an HPLC Method Coupled with an Iodine-Azide Post-Column Reaction', Journal of Liquid Chromatography & Related Technologies, 32: 3, 383 — 398

To link to this Article: DOI: 10.1080/10826070802631451

URL: http://dx.doi.org/10.1080/10826070802631451

Journal of Liquid Chromatography & Related Technologies®, 32: 383–398, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802631451

Determination of Methimazole in Pharmaceutical Preparations using an HPLC Method Coupled with an Iodine-Azide Post-Column Reaction

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Abstract: A simple and reliable high performance liquid chromatographic (HPLC) method with iodine-azide post-column reaction as a detection system has been developed and validated for determination of methimazole in tablets. A chromatographic separation was achieved on a C_{18} column with a mobile phase consisting of acetonitrile, water, and 2.5% sodium azide solution (pH 5.5) with isocratic elution (20:30:50, v/v/v). The detection is based on spectrophotometric measurement of the residual iodine ($\lambda = 350\,\mathrm{nm}$) from the post-column iodine-azide reaction induced by methimazole, after mixing an iodine solution containing iodide ions with the column effluent containing azide ions and methimazole. Obtained chromatograms for methimazole showed negative peaks as a result of the decrease in background absorbance. The method developed for methimazole was linear over the concentration range of 0.2–2 μmol L^{-1}. The method is accurate and precise (relative standard deviation, R.S.D. $< 4\%$). The detection limit (defined as $S/N = 3$) was 0.18 μmol L^{-1} (3.6 pmol injected amount) for the described compound. The elaborated method was applied to determine methimazole in tablets.

Keywords: HPLC, Iodine-azide reaction, Methimazole, Post-column reaction, Tablets analysis

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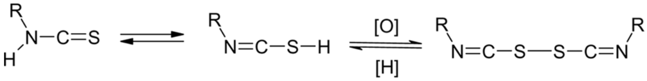
INTRODUCTION

Thioamides have some potential application in hyperthyroidism (Grave's disease) where an individual's own antibodies are attached to thyroid stimulating hormone receptors within cells of the thyroid gland and then trigger overproduction of thyroid hormone or hormone decreasing binding to transport proteins. Variation in their levels leads to great changes in many physiological functions. Thioamides belong to intrahyroidal inhibitors (goitrogens), which block thyroid hormone synthesis or reduce the release of hormones.[1] Such substances are referred to as thyreostats or antihormones. A search for a powerful, active, and less toxic substance led to employment of 2-mercapto-1-methylimidazole (methimazole). Its chemical structure is shown in Figure 1. The compound inhibits the iodination of thyroid hormones by reacting with liberated iodine according to the equation:[2]



That pharmaceutical has been also employed to promote water retention and increase weight and growth in animal for human consumption. Thyreostatic drugs are banned in the UE (directive 86/469 EU).

Different procedures have been developed to determine methimazole by potentiometric (with sodium tetraphenylborate, mercury(II) acetate and silver nitrate,[3] as well as iodine in an alkaline medium[4] as titrants) and coulometric titration (with iodine in an alkaline medium[5] and bromine and chloride in acid conditions[6]), conductometric,[7] and voltammetric[4] methods, high performance liquid chromatography with UV[8] and amperometric detection,[9] thin layer chromatography,[10] as well as capillary zone electrophoresis with amperometric detection[11] and supercritical carbon dioxide extraction.[12] Flow injection analysis with chemiluminescence[13] and spectrophotometric[14] detection have also been described. Methods based on iodine-azide reaction with

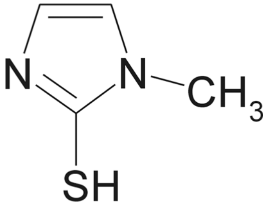


Figure 1. Structure of 2-mercapto-1-methylimidazole (methimazole).

volumetric titration,[15] as well as spectrophotometric measurement[16] have been introduced.

In this study, the reaction between iodine and sodium azide induced by methimazole was employed as a post-column reaction for its determination in tablets with high performance liquid chromatography.[17,18] The method is based on chromatographic separation of the thiol on a column with organic solvent sodium azide mixture. Subsequently, the residual iodine from the post-column iodine-azide reaction is measured with UV-Vis detector set at $\lambda = 350\,\text{nm}$ after mixing iodine solution with the column effluent containing sodium azide and an inductor. When constant iodine solution concentration in a post-column reagent and azide ions in the mobile phase are provided to the measurement system, a constant absorbance is maintained and recorded as a background from iodine absorption. However, when the chromatographic band with separated methimazole appears, the signal decreases due to consumption of iodine in the iodine–azide reaction. The studied thiol is detected as a negative peak and its area is proportional to the amount of the determined compound.

EXPERIMENTAL

HPLC System

The chromatographic separation was performed on Waters liquid chromatographic system equipped with Multisolvent Delivery System Model 600E, a 717plus autosampler, a variable wavelength LC spectrophotometer (2487 Dual λ). An analytical column, Symmetry C_{18} (150 mm × 3.9 mm i.d., 5 μm, Waters) was used for chromatographic separation at ambient temperature. The mobile phase consisted of a mixture of acetonitrile-2.5% sodium azide; pH 5.5-water (20:50:30, v/v/v) was delivered at a flow rate of 0.5 mL min^{-1} at ambient temperature. The iodine-azide post-column reaction was carried out on a Waters system provided with Reagent Manager as a single piston, pulse dampened pumping system for post-column reagent (mixture of 0.2 mmol L^{-1} iodine solution in 20 mmol L^{-1} potassium iodide) delivery at flow rate of 0.3 mL min^{-1} to the Post-column Reaction Module (the reaction tube, 6 m × 0.46 mm i.d.) with Temperature Control System. The chromatograms were integrated with Empower^{32} software (Waters). The injection volume was 20 μL and the detection was performed at a wavelength of $\lambda = 350\,\text{nm}$. A schematic diagram and optimal conditions for the separation of methimazole by HPLC and for its quantitation by the post-column reaction are depicted in Figure 2 and Table 1, respectively.

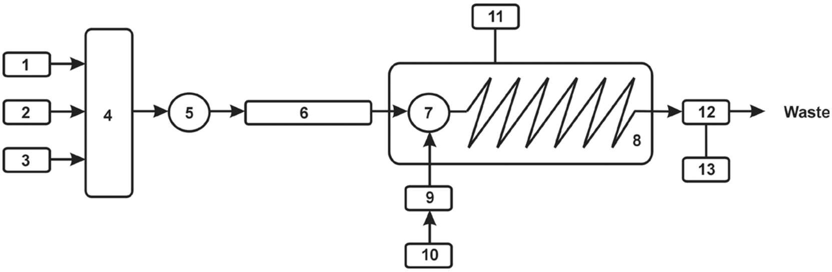


Figure 2. Flow diagram system with iodine–azide procedure detection. (1) sodium azide solution, (2) acetonitrile, (3) water, (4) pump, (5) injector valve, (6) analytical column, (7) mixing tee, (8) post-column reaction module, (9) pump, (10) iodine solution in potassium iodide solution, (11) temperature control system, (12) LC spectrophotometer, and (13) computer.

Chemicals, Reagents, and Standard Solution Preparation

All chemicals were of analytical or HPLC grade. Methimazole, sodium azide, hydrochloric acid, sodium hydroxide, phosphoric and acetic acid, sodium acetate, sodium dihydrogen phosphate dihydrate, sodium

Table 1. Chromatographic and post-column reaction conditions applied in the determination of methimazole

Parameter	Estimated value
Column	C_{18}
Composition of the mobile phase	Sodium azide:water: acetonitrile (50:30:20; v/v/v)
Flow-rate of the mobile phase [mL min^{-1}]	0.5
Sodium azide solution concentration [%]	2.5
Sodium azide solution pH	5.5
Iodine solution concentration c(I) [mmol L^{-1}]	0.2
Potassium iodide solution concentration c(KI) [mmol L^{-1}]	20
Post-column reaction solution flow rate [mL min^{-1}]	0.3
Post-column reaction module temperature [°C]	25
Injection volume [µL]	20

hydrogen phosphate heptahydrate, iodine, potassium iodide, methanol, and acetonitrile, as well as tetrahydrofuran were obtained from Aldrich (Steinheim, Germany), LAB-SCAN Analytical Sciences (Dublin, Ireland) or POCH (Gliwice, Poland). Methimazole tablets *Methizol* (ICN Polfa Rzeszów S.A., Rzeszów, Poland) and *Thyrozol* (Merck KGaA, Darmstadt, Germany) labeled to contain 5 mg of methimazole were obtained commercially.

All the solutions were freshly prepared daily. The water used in preparation of solutions was deionized water and then sparged for 15 min with helium.

A stock methimazole solution: 100 μmol methimazole was dissolved in 1 mL 1 mol L^{-1} sodium hydroxide solution and diluted to 100 mL with water. Working standard methimazole solutions (0.2–2 μmol L^{-1}) were prepared by appropriate serial dilution of stock solution with mobile phase. A 20 μL volume was injected into the HPLC system.

A mobile phase solution: 12.5 g sodium azide was dissolved in water and hydrochloric acid was added to obtain pH 5.5 and then was adjusted to 0.5 L with water. The mobile phase consisting of a mixture of acetonitrile, sodium azide solution (pH 5.5; 2.5% w/v), and water was mixed (20:50:30, v/v/v) with the HPLC pump according to Figure 2.

A post-column reagent solution: 6.3 g iodine and 20 g potassium iodide were dissolved and were adjusted with water to 0.5 L. To 500 μL of the solution mentioned above, 0.833 g of potassium iodide was added and diluted with water to 0.25 L.

The buffers pH was adjusted by potentiometric titration. The titration system was calibrated with standard pH solutions. All reagents were tested and found to be stable for unattended analysis.

Drug Purity Analysis

Ten tablets were weighed and crushed to 'fine' uniform particle size powder. After calculating the average tablet weight, a composite of the powder equivalent to the average of one tenth tablet was accurately weighed and quantitatively transferred into a 100 mL volumetric flask, and 1 mL 1 mol L^{-1} sodium hydroxide solution was added. The mixture was sonicated for 5 min. The flask was filled to volume with water and mixed well. Of the stock solution, 10 mL was centrifuged (10000 rpm) for 4 min.

Calibration Curve Method

The supernatant phase was separated and 1 mL of the solution was transferred into a 100 mL volumetric flask and diluted with mobile phase.

Additions Standard Method

The supernatant phase was separated and 1 mL of the solution was transferred into a 100 mL volumetric flask and 0.5, 1.0, and 2 μmol of methimazole was added. The solutions were diluted with the mobile phase.

RESULTS AND DISCUSSION

Validation

Validation of this method included assessment of solution stability, linearity, precision, detection, and quantitation limit, as well as accuracy.

The stock standard and sample solutions were prepared in alkaline conditions in order to dissolve methimazole in aqueous solution. It is well known that thiols solutions are not stable in the basic conditions because of their oxidation by oxygen. All solutions were prepared fresh daily. Repeated injection of the same sample demonstrated the stability of the studied thiol preserved in the mobile phase containing azide buffer solution pH 5.5 at ambient temperature within 5 h. The decay of the peak was not observed within the studied period, which is the time necessary to perform the entire HPLC analysis of the tablets.

Detector response linearity was determined by preparing seven calibration samples covering the concentration range of 0.2–2.0 μmol L^{-1}. Each sample was injected in quadruplicate. The chromatogram of methimazole in the tablets sample (Figure 3) clearly demonstrates the absence of any additional peak that could interfere with the analyte. Inter- and intra-assay precision and accuracy of the method are illustrated in Tables 2 and 3, respectively.

Linearity was obtained with $r^2 > 0.9975$, over a calibration range of the tested analyte. The regression equation was $A = 2.343 \pm 0.038c$, where A is the peak area [V × s] and c is the concentration of methimazole (μmol L^{-1}).

The limit of detection (LLOD) was estimated at a S/N ratio of 3. The LOD was determined by injecting standard solutions of various concentrations. The estimated limit of detection was 0.18 μmol L^{-1}. The lower limit of quantitation (LLOQ) taken as analyte concentration that generates a signal that is six times standard deviation of the background signal, was 0.2 μmol L^{-1} at R.S.D. of 1.2% ($n = 4$). The values (LLOD and LLOQ) were estimated by analyzing solutions of decreasing concentration of methimazole. Before analyzing each sample, water was processed in such a way. It was confirmed that there were no contaminants.

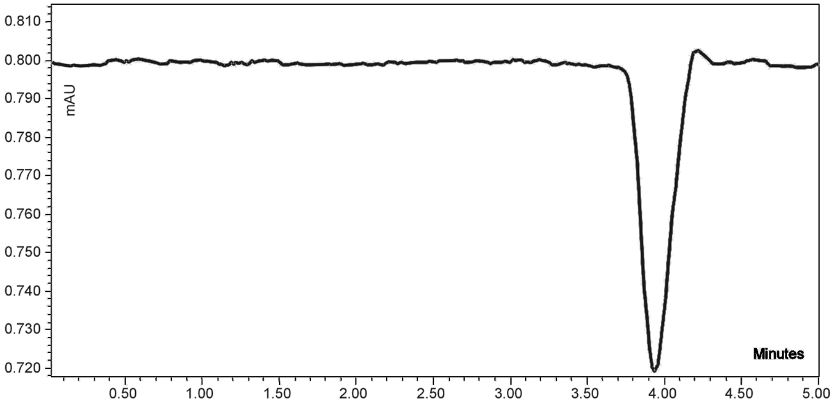


Figure 3. Chromatogram obtained for methimazole in tablets with iodine-azide reaction procedure detection (for chromatographic and post-column reaction conditions see Table 1).

As it is shown in the Tables 2 and 3, the mean recoveries of methimazole were 91–108% and 97–103% for inter- and intra-day analysis, respectively. These results show no clear relationship between concentration and recovery.

The overall precision of the method was evaluated by analyzing four consecutive injections of methimazole standard solutions at 0.2, 1, and 2 μmol L^{-1} representing the low, middle, and high concentration of the linearity concentration range. The intra-day precision (R.S.D.; $n = 4$), and inter-day precision (4 days, R.S.D.; $n = 16$) show that R.S.D. values are lower than 4.7%.

Table 2. Results of methimazole determination with iodine-azide post-column detection system (inter day); $n = 4$

Taken (μmol L^{-1})	Found $x \pm t_{0.95} \cdot \bar{s}$ (μmol L^{-1})	R.S.D. (%)	Recovery (%)
0.200	0.192 ± 0.003	1.2	96
0.300	0.273 ± 0.003	0.7	91
0.400	0.389 ± 0.004	0.8	97
0.600	0.610 ± 0.014	1.7	102
0.800	0.866 ± 0.008	0.7	108
1.00	1.04 ± 0.01	0.2	104
2.00	1.98 ± 0.01	0.4	99

Table 3. Validation of intra day assay; n = 16

Taken (μmol L^{-1})	Found $x \pm t_{0.95} \cdot \bar{s}$ (μmol L^{-1})	R.S.D. (%)	Recovery (%)
0.200	0.195 ± 0.005	4.7	97
1.00	1.03 ± 0.008	1.4	103
2.00	1.97 ± 0.012	1.1	99

Specificity and Separation

Different variables influence determination of methimazole in HPLC analysis using the iodine-azide detection system. Stationary phase is a variable which influences only the separation process. Concentration of iodine and iodide ions, flow rate of iodine in potassium iodine solution, temperature of the post-column reaction module, have impact only on iodine-azide detection procedure. Composition and flow rate of the mobile phase as well as concentration and pH of sodium azide solution affect both the separation process and the detection procedure. The aim of optimizing post-column reaction is to achieve the highest and fastest iodine consumption in the induced reaction. The analytical viewpoint defines this consumption as a way to measure the sulphur compound sensitivity in volumetric,[15] coulometric, spectrometric,[16] and flow injection analysis determination. Therefore, the conclusion is that the higher iodine consumption the lower the detection limit is. The adjustment of maximum iodine consumption in the post-column reaction was studied with the flow injection system in which the separating column was removed from the HPLC system.

Chromatographic System

Two different columns (C_{18}, CN) were investigated to obtain optimal separation conditions. The C_{18} column was chosen, as it gave a higher and more accurate peak with less width than the one achieved with the CN colulmn. The peaks obtained with the column were not sharp and integration was difficult. However, the retention times obtained with these columns were comparable.

The effect of the mobile phase composition on the separation process was studied. Acetonitrile and tetrahydrofuran, as organic modifiers of the mobile phase, were checked due to their high popularity in RP-HPLC. It should be noted, that sodium azide has limited solubility in aqueous/acetonitrile or aqueous/tetrahydrofuran mixtures with a high concentration of organic solvent. We did not observe any sodium azide precipitation within acetonitrile or tetrahydrofuran working concentration range (5–35%). The peaks obtained with tetrahydrofuran were not sharp and their retention time was comparable with the ones obtained with acetonitrile. The impact of the acetonitrile amount within the range of 5–35% in the mobile phase on the separation and the course of iodine-azide reaction was examined. The increase in the peak area was observed with an increase of the amount of acetonitrile into the mobile phase within the range of 5–25%. The amount of acetonitrile above 25% made no changes to the peak area. Analyses considering the relationship between acetonitrile concentration and the post-column reaction were performed with constant concentration of other components of the mobile phase. The results indicated 20% of acetonitrile as the most optimal percentage. Surprisingly, it was observed that organic solvent addition made the iodine consumption higher in the iodine–azide reaction. However, such a course of action was noted previously in the methanol–water mixture condition for the iodine–azide reaction induced by Vitamin B1[19] and in HPLC determination of thiopental.[18]

Neutral pH of buffer sodium azide is frequently applied to inhibit bacterial growth. It does not interact with proteins or change their chromatographic behavior. It also exhibits no activity towards proteins and does not affect their chromatographic behavior. This chemical is not recommended to be used in ion exchange chromatography due to its reactivity towards anion exchangers and blocks binding sites. Thus, sodium azide is excluded from addition into a mobile phase for sulfur anions determination.[20,21] To simplify the methimazole determination procedure, sodium azide was incorporated into the mobile phase as one of the reagents of iodine-azide reaction. To complete the post-column reaction, a lower concentration of sodium azide solution was employed in ion chromatography mode. The poisonous properties of sodium azide make it necessary to use it in very low amounts. In the study, sodium azide was used as a buffer that passed through the separating column. There were two main reasons for this: 1) suppress sodium azide dilution that decreases iodine consumption in the induced reaction, 2) to avoid the necessity of changing the buffer for separation process and for post-column detection system.

The effect of the azide ions solution concentration on the course of the iodine-azide reaction, was established in the concentration range of 0.1–5% with flow injection analysis as a tool (Figure 4). The peak area increased with an increase of sodium azide solution concentration. The optimal concentration of sodium azide was chosen to be 2.5%, as the further increase of sodium azide solution concentration brings smaller changes to peak area. The peak width dropped at the chosen sodium azide solution concentration.

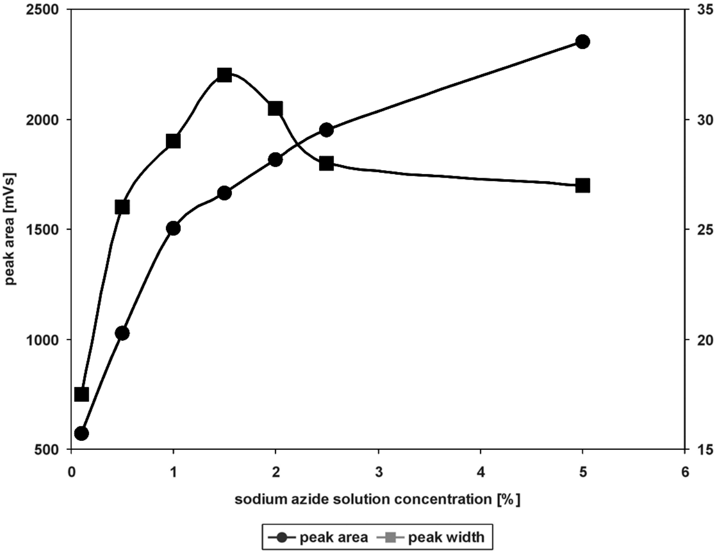


Figure 4. The influence of concentration of sodium azide solution on the peak area and peak width; $c_{met} = 0.5\,\mu mol\ L^{-1}$ and for flow injection analysis conditions see Table 1.

Sodium azide solution pH is found to have an impact on separation and the course of the iodine–azide reaction. The pH reagent solution has an effect upon iodine consumption. A solution at pH lower than 5.5 should not be applied because of the emission of the poisonous, volatile hydrazoic acid. Under pH above 8.0, iodine forms iodate (I) which does not take part in the iodine–azide reaction. Methimazole analysis was performed within pH range 5.5–7.0. As a result, the decrease in the peak in the flow injection analysis with increasing pH was observed. For chromatographic determination of methimazole, the value of pH 5.5 was found to be the most optimal. Similar observations were made in the course of the iodine-azide reaction when measured in aqueous medium with volumetric titration[15] and spectrophotometric measurements.[16]

Additionally, the influence of phosphate and acetate buffer (0.05 mol L^{-1}, pH 5.5) incorporated into the mobile phase with the C_{18} column on the separation process were examined. Applying these solutions as a component of mobile phase is useless and unnecessarily complicates the procedure since it did not improve the shape of the peak. Methimazole peak retention time did not change. Phosphate and acetate buffer do not have any impact on the separation step and on the course of the iodine-azide reaction induced by methimazole within the studied range.

Hence, acetonitrile, 2.5% sodium azide solution and water (30:50:20; v/v/v) mixture was applied as a mobile phase for determination of methimazole in tablets.

Flow Rate of Mobile Phase and Post-column Reagent Solution as the Key to Optimize the Post-column Reaction Time and Efficiency of HPLC Separation.

Sufficient conduct of the reaction requires adequate contact time between the eluate (containing methimazole and azide ion) and the post-column reaction solution (containing iodine solution). According to results from flow injection analysis the peak area decreased with an increase in mobile phase flow rate within the range of 0.5–1.1 mL min^{-1}. These data support the statement that induction time of iodine–azide reactions induced by methimazole is longer than the contact time. The maximum iodine consumption (the highest peak) was obtained when the mobile phase was pumped relatively slowly. It is well known that the decreasing flow rate brings a longer run time and band spreading. The flow rate of 0.5 mL min^{-1} was chosen as a settlement between the separation and detection procedure. The retention time obtained in these conditions was 3.9 min with reasonable bandwidth (less than 0.5 min.)

The flow rate of iodine solution was studied as an indicator of LLOQ (maximum consumption of iodine in the post-column reaction; maximum peak area) and LOD (maximum ratio of methimazole peak area and noise peak area). The increase in iodine solution flow rate above 0.4 mL min^{-1} resulted in a decrease in the peak area. Thus, it can be concluded that the reaction was incomplete due to not enough time for methimazole to induce the iodine–azide reaction. There was some background noise derived from the pump applied to the iodine solution. It was observed that iodine solution high flow rate was responsible for noise peak area. Consequently, an essential step was to establish the relation between detection limit and methimazole peak area and noise peak area (Figure 5). The value of 0.1 mL min^{-1} of iodine solution flow rate was detected to give the highest signal-to-noise ratio. The optimal flow rate of iodine solution (0.3 mL min^{-1}) is a result of the influence between two factors: an increase of peak area with the increase of post-column solution flow rate up to the value of 0.4 mL min^{-1} (lowering the quantitation limit), and the highest signal-to-noise ratio (detection limit).

Post-Column Reaction Conditions

The post-column reaction is influenced by the concentration of iodine and iodide ions. The delivery of iodine solution in potassium iodide is performed after the separation step (Figure 2). The studies of dependence

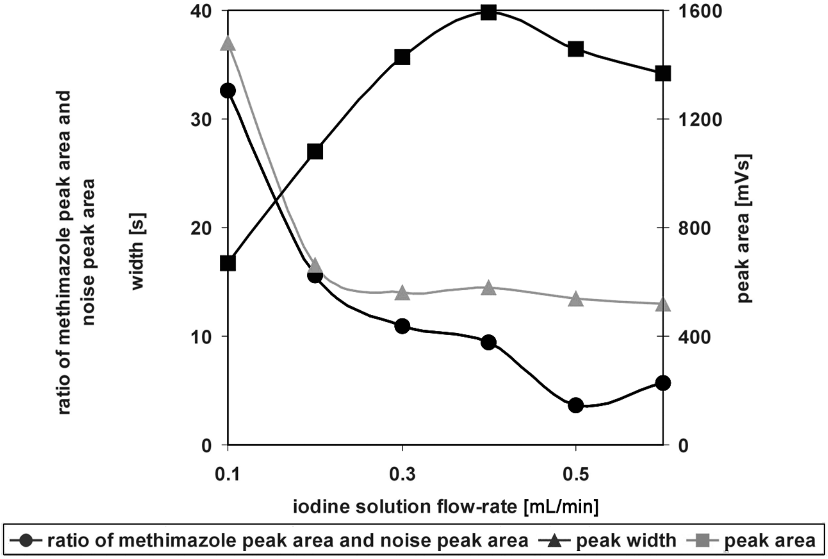


Figure 5. The influence of flow rate of iodine solution on the ratio of methimazole peak area and noise peak area, peak area and peak width; $c_{met} = 0.5\,\mu mol\ L^{-1}$ and for conditions see Table 1.

of iodine and iodide ions on the course of iodine-azide reactions induced by methimazole were conducted without separation column. The results showed no impact of iodine solution concentration on methimazole peak area in the range of 0.05–0.5 mmol L^{-1}. The highest consumption of iodine in the induced iodine-azide reaction was when the iodine amount left was at the level of 20–30% besides the initial amount of iodine.[22] Applying a lower concentration of iodine led to the higher rate of reaction[23] and lower detection limit. Applying too low a concentration of iodine caused a complete consumption of iodine in the induced reaction. This means that the absorbance level decreases to *ca.* 0 AU and the peak area is not proportional to the methimazole amount. The optimal conditions of iodine solution were found to be $c(I) = 0.2\,mmol\ L^{-1}$ and $c(KI) = 20\,mmol\ L^{-1}$ with flow rate $0.3\,mL\ min^{-1}$. The obtained absorbance under such conditions was *ca.* 0.8 AU.

In the presence of potassium iodide, solubility of iodine in water increases and evaporation of iodine decreases due to the formation triiodide ions. In the proposed method, the iodide ions influence only the course of the iodine–azide reaction. To establish the relation between iodide ions concentration and methimazole peak area, the concentration range of 1–50 mmol L^{-1} of potassium iodide solutions was applied. The increase in the peak area was observed at the concentration range of 1–20 mmol L^{-1}. The explanation is connected with a further shift in the equilibrium of iodine-iodide ions to the right that makes triiodide ions concentration increase. As a consequence, a higher absorbance is detected with a constant concentration of iodine solution. Within the range of 20–50 mmol L^{-1} the peak area does not change, this indicates no iodide ions impact on the course of iodine–azide reaction induced by methimazole. A similar relationship was observed in spectrophotometric measurements of iodine consumed in induced reactions.[16] The potassium iodide content of 20 mmol L^{-1} was found to be optimal.

An investigation was made into the temperature of the post-column reaction module and its influence on the peak are within the range of 25–40°C under optimal conditions. A peak area decrease was reported with increasing temperature, which is, in turn, the effect of a non-inducing compound creation from methimazole in an iodimetric reaction as a consequence of the increase in the oxidation rate. According to the research, the increase in the temperature results in the iodine consumption decrease. During the analysis the temperature in a post-column reaction module was kept at a steady level of 25°C. The decrease in the temperature below the ambient was ensured in all experiments by the commercial post-column reaction module.

The optimal conditions of methimazole determination with post-column reaction are summarized in Table 1.

Drug Purity Analysis

Elaborated HPLC method with iodine-azide reaction as a detection system was then applied to the determination of methimazole from two commercial products (Table 4) using two techniques: calibration curve and standard addition. In all the investigated drugs the amount of methimazole determined by the HPLC method with post-column iodine-azide reaction was in good agreement with the declared content. The recoveries of methimazole from *Methizol* and *Thyrosol* tablets were calculated within the range 99–103% with R.S.D. values within the range 2.9–4.0% (n = 6).

Table 4. Results of methimazole determination in tablets

Tablets name	Declared value (mg)	Determination technique	Found $x \pm t_{0.95} \cdot \bar{s}$ (mg)	R.S.D. (%)	Recovery (%)
Metizol	5	Calibration curve	5.08 ± 0.20	2.9	102
		Standard addition	4.97 ± 0.38	3.4	99
Thyrozol	5	Calibration curve	4.95 ± 0.35	4.0	99
		Standard addition	5.13 ± 0.46	3.8	103

Interferences

As they are listed below, there can be three sources of possible interferences when the iodine-azide post-column reaction system is applied:

1. Negative peak from iodine-azide reaction inductors (e.g. cysteine or cystine).[18]
2. Negative peaks from compounds that react with iodine under experimental conditions (e.g. ascorbic acid).[18]
3. Positive peaks from compounds that react with iodide ion (e.g. bromate(V), iodate(V), nitrate(III)).[21]

The peaks from these mentioned above compounds may interfere with the analyte when the retention times are similar. It was observed that tablet excipients do not interfere in these specific circumstances of the proposed application.

CONCLUSIONS

This paper describes an HPLC method with iodine-azide post-column reaction for the determination of methimazole in tablets. The method was found to be specific, accurate, precise, and suitable for the analysis of methimazole in these pharmaceutical formulations. The results of this study show the methimazole peak response to be precise and linear over the range of 0.2–2 μmol L^{-1}. The recovery of the studied compound was determined to be in the range of 91–108% (R.S.D. < 4.7%) as a standard and 99–103% (R.S.D. < 4.0%) for tablets. This method appears to be reliable and convenient for the direct analysis of methimazole in pharmaceutical preparations.

Previous studies on tablet samples in HPLC analysis reached the levels of 1 μmolL^{-1}[9] respectively. The detection limits were obtained with a modified electrode as an electrochemical detection system. The proposed method allows decreasing the detection limit (0.2 μmol L^{-1}). Since the iodine-azide reaction takes place only in the presence of sulfur(II) compounds, the method is sensitive only towards sulfur(II) compounds. The sulfur(II) impurities (included compounds which do not absorb in UV region) of methimazole tablets can be only seen on the chromatogram.

ACKNOWLEDGMENTS

This work was supported by grant No. N204 14732/3728 from the Ministry of Science and High Education, Poland. The author wishes to thank Prof. W. Ciesielski and Dr D. Kaźmierczak for reviewing the manuscript.

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Received March 8, 2008
Accepted April 25, 2008
Manuscript 6318