Journal of Chromatography B, 869 (2008) 67–74

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of methimazole in urine with the iodine-azide detection system following its separation by reversed-phase high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 7 January 2008
Accepted 8 May 2008
Available online 16 May 2008

Keywords:
Iodine-azide reaction
Methimazole assay
Urine
HPLC

ABSTRACT

The iodine-azide detection system to determine methimazole following its separation by RP-HPLC is described in this paper. The reaction between iodine and azide ions induced by methimazole was applied as a post-column reaction detection system. Neither extraction nor preconcentration of the sample was necessary. The methimazole standards added to normal urine show that the response of the detector, set at 350 nm (corresponding to unreacted iodine in the post-column iodine-azide reaction), was linear within the concentration range 2–10 nmol/mL of urine. The relative standard deviation values for precision and recovery within the calibration range were from 0.3 to 3.2% and from 97 to 102%, respectively. Limits of detection (LOD) and quantitation (LOQ) were 1 and 2 nmol/mL of urine, respectively. The method was applied to the separation and determination of patient urine samples and the analytical results were satisfactory.

1. Introduction

Methimazole (2-mercapto-1-methylimidazole; Fig. 1.) as an antithyroid agent (similar to carbimazole, propylthiouracil, methylthiouracil, benzylthiouracil) is used to treat hyperthyroidism (overactivity of the thyroid gland). This overactivity leads to Graves' disease in which overproduction of thyroid hormones is triggered by hormone receptors within thyroid cells as a result of autoantibody attachment. The thyroid gland with the assistance of peroxidase enzyme is responsible for the production of two thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). The formation of these hormones is based on iodine combining with a protein called thyroglobulin. The interaction of iodine and peroxidase with thyroglobulin is inhibited by methimazole which consequently decreases thyroid hormone production. Additionally, methimazole interferes with the conversion of T_4 to T_3. Taking into account the less potent nature of T_4 to T_3, the activity of these thyroid hormones is hampered [1,2].

Methimazole detection and determination in urine [3–7], plasma [6,8–10], serum [11–13] and tissues [4,13–18] have already been evaluated using various separation methods including gas chromatography–mass spectrometry [5,6], high-performance liquid chromatography–mass spectrometry [4,5,14], high-performance liquid chromatography with ultraviolet [3,7–10,13,15] and electrochemical [12] detection, capillary zone electrophoresis with amperometric detection [11] and thin-layer chromatography [6,14,16–18]. In many cases the selective isolation of methimazole and the preconcentration steps such as extraction [7,8,10] have included solid-phase extraction (SPE) [4,13,15,17], derivatization [3] or both steps [5,6,9,14,16,18] to obtain successful results. Biological samples which are multicomponent mixtures of organic and inorganic compounds are highly complex and require detection methods which are selective and sensitive.

Sulphur(II) compounds induce a reaction between azide and iodine and allow the selective and sensitive detection of thiols. The proposed procedure relies on the separation of the analyte on a chromatographic column and on the subsequent measurement of the unreacted iodine in the iodine-azide reaction [19,20]. The selective induction of a sulphur(II) compound is observed. Thus in the absence of these compounds a constant absorbance from iodine is recorded when an HPLC system is supplied with azide ions from the mobile phase and iodine solution from post-column reagents. Moreover, a decrease in the signal appears when a sulphur(II) compound is present in the chromatographic band. This is attributed to iodine-azide consumption in the iodine-azide reaction and is detected as a negative peak at 350 nm. The peak is quantitatively dependent on the amount of the sulphur(II) compound.

The aim of this study was to apply a selective and sensitive RP-HPLC method of separation with post-column detection of

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doi:10.1016/j.jchromb.2008.05.021

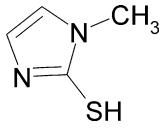

Fig. 1. Structure of 2-mercapto-1-methylimidazole (methimazole).

methimazole using the iodine-azide reaction. With this modification a significant improvement in detection sensitivity to the nmol/mL urine level was achieved. Due to the high separation efficiency and high selectivity of the iodine-azide reaction this method was applied to methimazole analysis in urine. The method is based on simple dilution without the necessity for extraction or derivatization and exhibits good reproducibility as well as practicality.

2. Experimental

2.1. Chemicals, reagents, standard solution preparation

All chemicals were of analytical or HPLC grade. Methimazole, sodium azide, hydrochloric acid, sodium hydroxide, iodine, potassium iodide, methanol and acetonitrile were obtained from Aldrich (Steinheim, Germany), LAB-SCAN Analytical Sciences (Dublin, Ireland) or POCH (Gliwice, Poland).

All the solutions were freshly prepared daily. Deionized water with subsequent 15 min helium sparging was used to prepare the solutions.

A stock methimazole solution: 100 μmol methimazole was dissolved in 1 mL 1 mol/mL sodium hydroxide solution and diluted to 100 mL with water. Appropriate serial dilutions of the stock solution with the mobile phase were performed to prepare working standard solutions of methimazole (10 μmol/mL).

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

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

The pH of the buffers was adjusted by potentiometric titrations. Calibration of the titration system was carried out with standard pH solutions. All reagents were tested and found to be stable for unattended analysis.

2.2. Calibration standards and sample preparation

Preparation of the urine calibration standards involved 1 mL portions of methimazole-free urine spiked with increasing amounts of the working standard solution of methimazole (prepared daily by dissolving the standard solution with water) to give methimazole concentrations of 2.0, 3.0, 4.0, 5.0, 7.0, 10.00 nmol/mL of urine which were then diluted with water to 10 mL. Recommended analytical procedures were applied to handle the calibration standards. Linear regression of peak versus concentration was used to fit the calibration curve.

Freshly collected methimazole-containing urine was diluted with water to 10 mL. This volume of urine was chosen to adjust the methimazole peak height to *ca.* 0.1 AU. A 20 μL of the solution was injected into the chromatographic system. 0.50, 0.75 and 1.0 nmol of methimazole were added to the urine sample aliquots. The calibration curve was a source of information on proper dilution and quantities of methimazole. The basis of this procedure was a standard addition technique.

2.3. Instruments

The chromatographic separation was performed on a Waters liquid chromatographic system equipped with Multisolvent Delivery System Model 600E, a Rheodyne 7725i injector, and 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 was a mixture of acetonitrile–2.5% sodium azide; pH 5.5–water (1:50:49, v/v/v). The flow-rate was maintained at 0.5 mL/min at ambient temperature. The iodine-azide post-column reaction was carried out on a Waters system provided with a Reagent Manager as a single-piston, a pulsedampened pumping system for the post-column reagent (mixture of 0.2 mmol/mL iodine solution in 20 mmol/mL potassium iodide) delivery at a flow-rate of 0.2 mL/min to the Post-column Reaction Module (the

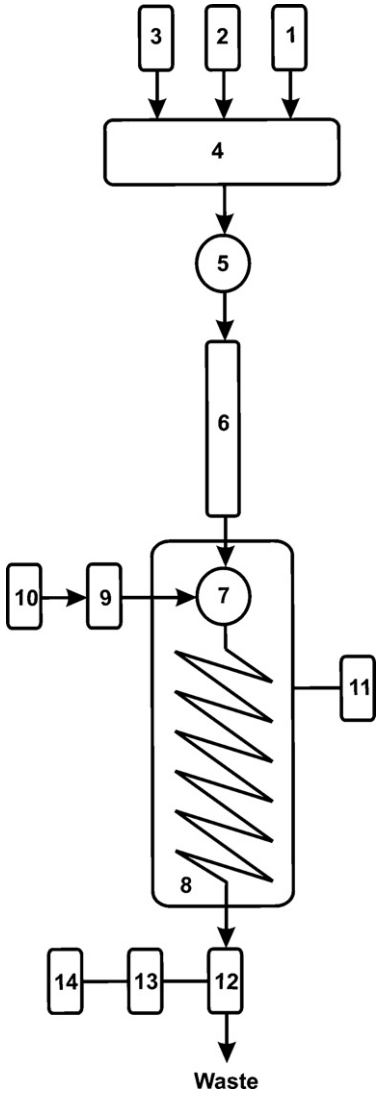

Fig. 2. Flow diagram system with iodine-azide procedure detection. (1) Sodium azide solution, (2) acetonitrile, (3) water, (4) pump, (5) injector valve, (6) analytical column, (7) mixing tee, (8) post-column reaction module, (9) pump, (10) iodine solution in potassium iodide solution, (11) temperature control system, (12) LC spectrophotometer, (13) busSaT/In module and (14) computer.

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 (50:49:1; v/v/v)
Flow-rate of the mobile phase [ml/min]	0.5
Sodium azide solution concentration [%]	2.5
Sodium azide solution pH	5.5
Iodine solution concentration c(I) [mM]	0.2
Potassium iodide solution concentration c(KI) [mM]	20
Post-column reaction solution flow-rate [ml/min]	0.2
Post-column reaction module temperature [°C]	25

reaction tube, 6 m × 0.46 mm i.d.) with a Temperature Control System (Waters). The chromatographic system and recorder were linked by busSaT/In Module (Waters). The Millennium^{32} software (Waters) was used to integrate the chromatograms. The manual injection volume was equipped with a loop of 20 μL and the detection was performed at a wavelength of 350 nm. A schematic diagram and the established optimal conditions for the separation of methimazole by HPLC, and for its quantitation by the post-column reaction are depicted in Fig. 2 and Table 1, respectively.

2.4. Assay validation

The methimazole peak area was referred to the standard curves of methimazole in drug-free urine in order to determine methimazole concentration in urine. Linearity was established on the basis of the equation $A = aC + b$ where A is the peak area (mV s) and C (nmol/mL of urine) is the concentration of methimazole in urine samples.

The selectivity of the method was evaluated by analysis of blank matrices and matrices spiked with methimazole standards. The analysis of limits of detection (LOD) (S/N = 3) and quantitation (LOQ) (S/N = 8) involved the injection of methimazole solutions of decreasing concentration. Any contamination of the analyte was excluded by processing water according to a given procedure before analyzing the samples.

Intra-day accuracy and precision were calculated by analyzing four replicates of quality control (QC) samples at different concentration levels (2, 3, 4, 5, 7 and 10 nmol/mL) during the same day. Inter-day accuracy and precision were determined by QC samples (2 and 10 nmol/mL) representing the low and high concentration of the linearity concentration range on three separate occasions using 4 replicates. The applied criterion for precision and accuracy evaluation required the relative standard deviation and mean value to be less than 10%. Recovery was calculated with the use of formula:

$$\text{recovery}\,(\%) = \frac{\text{measured amount}}{\text{added amount}} \times 100\%$$

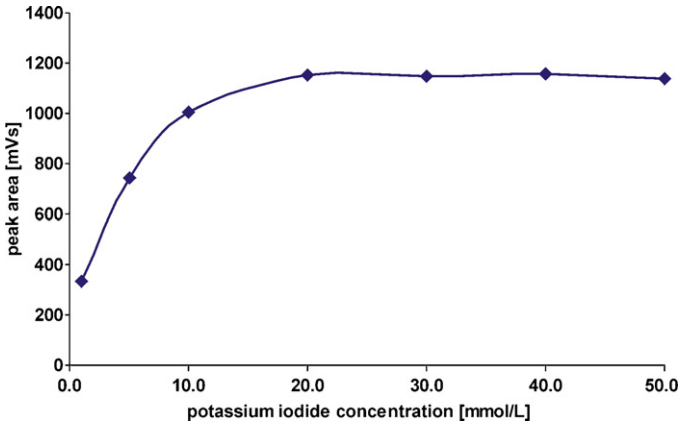

Fig. 3. The influence of potassium iodide concentration on the peak area; c_{met} = 10 nmol/mL urine and for analysis conditions see Table 1.

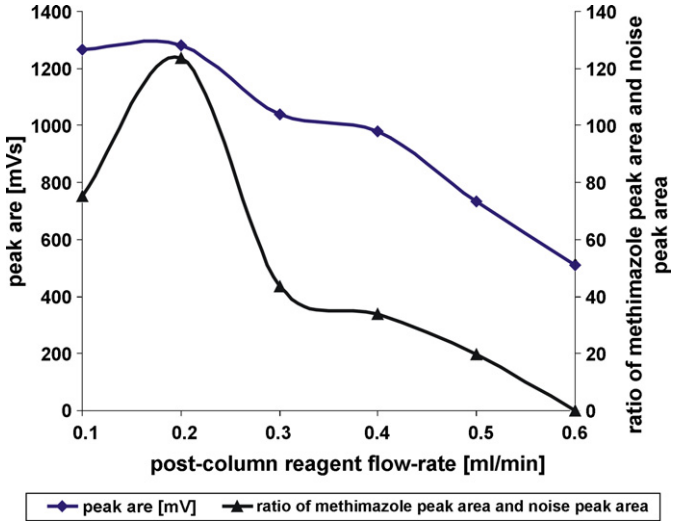

Fig. 4. The influence of flow-rate of iodine solution on the ratio of methimazole peak area and noise peak area as well as methimazole peak area; c_{met} = 10 nmol/mL urine and for analysis conditions see Table 1.

2.5. Stability of methimazole in urine and tested interference

The short-term stability of methimazole in urine was determined by assessing replicate QC samples at a concentration of 10 nmol/mL, which were kept at room temperature (25 °C) for 5 h. Freeze–thaw stability was studied after three cycles (−20 °C/room temperature) in the QC samples on three consecutive days.

To establish possible interference the following compounds were evaluated: cysteine, cystine, homocysteine, glutathione, methionine, thiocyanate, ascorbic acid and sulphur metabolites of methimazole (2-merkaptoimidazole and *N*-methylthiourea). The procedure involved the preparation of a 10 μmol/mL drug solution and a further 1 mL of urine was spiked with the appropriate amount of chosen substance to give a final concentration of 0.1 μmol/mL of urine for each compound.

3. Results and discussion

3.1. Method development

In the present study, we developed a straightforward and accurate HPLC method with a post-column iodine-azide reaction as a detection system for the determination of methimazole in urine samples. Based on the analytical column tests, the methimazole samples were successfully separated on the C_{18} analytical column. Several mobile phases consisting of different concentrations of organic modifiers (acetonitrile, methanol) and sodium azide (concentration, pH) with different volume ratios were tested. It was found that acetonitrile:2.5% sodium azide solution (pH 5.5):water (1:50:49, v/v/v) was an appropriate mobile phase for the separation

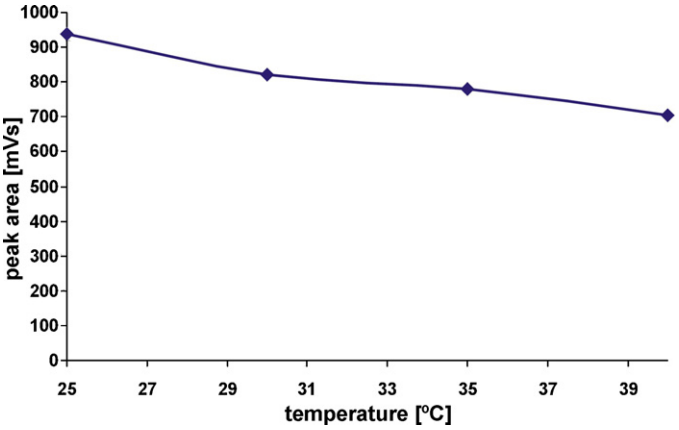

Fig. 5. The influence of post-column module temperature on the peak area; c_{met} = 10 nmol/mL urine and for analysis conditions see Table 1.

of the tested compound within a run-time of 12 min in isocratic mode. If the acetonitrile concentration was above 1%, the methimazole peak overlapped with the urine constituent peak. Several sources of urine samples were checked and in each case the methimazole peak was well separated from the blank urine peak. Under established conditions there were no interfering peaks which disturbed methimazole in the urine chromatograms.

3.2. Impact of various factors on methimazole detection

To establish optimal conditions for HPLC determination with the post-column iodine-azide reaction, a wide range of parameters which can lead to high iodine consumption need to be considered. These parameters include iodine in the potassium iodide solution and flow-rate as well as the post-column reaction module temperature. However, there are also a number of factors with an impact on the separation and detection process alike which include flow-rate of the mobile phase, pH and concentration of the sodium azide solution.

Potassium iodide solution concentration within the range 0.2–50 mmol/mL was applied to establish the influence of iodide concentration on the area of methimazole peak (Fig. 3). The experiments were performed under constant iodine concentration and flow-rate. An increase in the peak area was observed in the range 0.2–20 mmol/mL. This was attributed to a further shift in the equilibrium of iodine/iodide ions to the right, consequently leading to an increase in triiodide ion concentration. The recorded absorbance was higher with a constant iodine concentration. There was no change in the peak area within the range 20–50 mmol/mL. This proved that the course of the iodine-azide reaction was not affected by iodide ions in this concentration range. This is in contrast to the case of sulphide ion determination when high potassium iodine concentrations hampered the course of the iodine-azide reaction [19]. As a consequence, the value of 20 mmol/mL for potassium iodide was found to be optimal for further research.

The flow-rate of iodine solution in the range 0.1–0.6 mL/min was assessed with constant iodine and iodide ion concentrations (Fig. 4). The oxidation of methimazole in the iodimetric reaction was quicker at high iodine concentrations, the inductor then took part in the induced reaction for a shorter time with decreased iodine consumption (increased detection limit). A flow-rate of 0.2 mL/min was chosen, as iodine consumption in the iodine-azide reaction induced by methimazole was highest at this flow-rate (highest peak area).

The next factor studied was the iodine solution flow-rate, considered to be an indicator of LOQ (maximum consumption of iodine in the post-column reaction; maximum peak area) and LOD (maximum ratio of methimazole peak area and noise peak area). According to the results, the peak area decreased when the iodine solution flow-rate increased above 0.2 mL/min. This indicated that the time for methimazole to induce the iodine-azide reaction was insufficient and that the reaction was incomplete. The pump applied to the iodine solution was responsible for some background noise. In the case of a high iodine solution flow-rate, noise in the peak area was detected. As a consequence, it was vital to determine the relationship between detection limit and methimazole peak area as well as noise peak area (Fig. 4). An iodine solution flow-rate of 0.2 mL/min was selected as the optimal due to the highest signal-to-noise ratio at this flow-rate.

No impact of iodine solution concentration on the methimazole peak area in the range 0.1–0.3 mmol/mL was observed. It was noted that an iodine concentration 20–30% of the initial quantity gave the highest iodine consumption in the iodine-azide reaction. When lower iodine concentrations were evaluated, higher reaction rates were obtained which led to lower detection limits. However, very low concentrations of iodine resulted in complete consumption of iodine in the induced reaction. That caused the decrease absorbance to a level of *ca.* 0 AU. This resulted in no proportional relationship between the peak area and methimazole concentration. Since iodine concentration did not have an impact on methimazole peak area, then iodine solution concentration (applying 20 mmol/mL potassium iodide solution concentration and post-column reagent rate of 0.2 mL/min) should be maintained in order to achieve an absorbance that is within the range 0.4–0.6 AU. The optimal concentration of iodine solution was found to be $c(I) = 0.2$ mmol/mL.

The post-column reaction module temperature was investigated in relation to the methimazole peak area (Fig. 5). The chosen range was 25–40 °C. Increasing temperature led to a decrease in the methimazole peak area. This was due to an increase in the oxidation rate of an iodimetric reaction in which a non-inducing compound was created from methimazole. These results indicate that increased temperature influenced the reduction in iodine consumption. A fixed temperature of 25 °C was maintained during the analysis in the post-column reaction module. If a decrease in the temperature below the ambient was necessary, the commercial Post-column Reaction Module was applied.

A key parameter which influenced the course of the iodine-azide reaction was the reaction time. This reaction time can be maintained not only by the iodine solution flow-rate but by the flow-rate of the mobile phase which also has an impact on the separation step.

To conduct the iodine-azide reaction accurately, the contact time between the eluate (containing methimazole and azide ions)

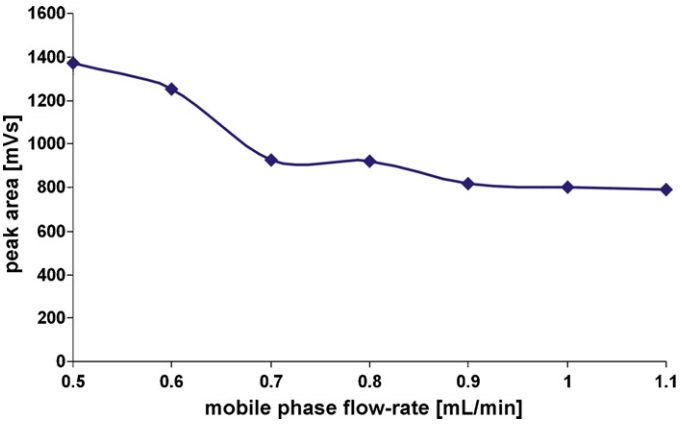

Fig. 6. The influence of mobile phase flow-rate on the peak area; c_{met} = 10 nmol/mL urine and for analysis conditions see Table 1.

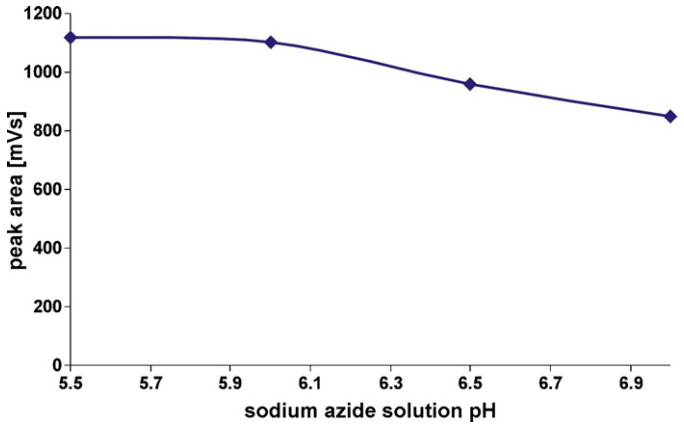

Fig. 7. The influence of sodium azide solution pH on the peak area; c_{met} = 10 nmol/mL urine and for analysis conditions see Table 1.

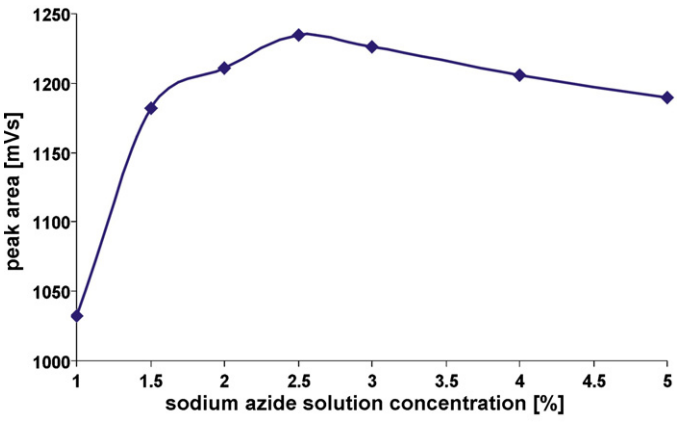

Fig. 8. The influence of concentration of sodium azide solution on the peak area; c_{met} = 10 nmol/mL urine and for analysis conditions see Table 1.

and the post-column reaction solution (containing iodine solution) should be sufficient. The reported findings from flow-injection analysis suggest that in the range of 0.5–1.1 mL/min of the mobile phase flow-rate there was a decrease in the peak area along with an increase in the flow-rate (Fig. 6). These results confirm the suggestion that the contact time was shorter than the time of the iodine-azide reaction. When the mobile phase was delivered at a comparatively slow rate, the maximum iodine consumption was recorded. At the same time it was widely recognized that a decreased flow-rate was responsible for a prolonged time and band spreading. To compromise the separation and detection procedure, a flow-rate of 0.5 mL/min was chosen and the retention time in relation to these conditions was 8 min with reasonable bandwidth (less than 0.5 min.).

Buffer sodium azide of neutral pH is an inhibitor of bacterial growth. In addition, it does not modify the chromatographic performance of proteins and does not exhibit any affinity with them. As a result of its activity towards anion exchangers and binding sites this compound is usually excluded from ion exchanger chromatography. In addition, buffer sodium azide has no application in the determination of sulphur anions as part of a mobile phase [19,20]. However, in this research, sodium azide was incorporated into the mobile phase as one of the reagent in the iodine-azide reaction and substantially simplified the methimazole determination procedure. To complete the post-column reaction, higher concentrations of sodium azide solution were introduced into the ion chromatographic technique. Due to the poisonous properties of sodium azide it is recommended that it should be used in small quantities. In the present study sodium azide was used as a buffer for pumping the separation column, its use was based on two main facts. Firstly, it was important to stop sodium azide dilution which could lead to a decrease in iodine consumption in the induced reaction. Secondly, using sodium azide in this way it was possible to use only one buffer solution for the separation process and the post-column detection system.

Both the separation process and the iodine-azide reaction are influenced by pH of the sodium azide solution. The effect of pH is mainly found in the level of iodine consumption. There are some restrictions applied to pH when it comes to sodium azide solution. At solutions of pH lower that 5.5, the emission of poisonous, volatile hydrazoic acid is detectable, and above pH 8.0 the formation of iodate(I) prevents the iodine-azide reaction. Consequently, a pH range of 5.5–7.0 was chosen for methimazole analysis. According to the results obtained, increased pH caused a reduction in peak area (Fig. 7). Thus, the value of 5.5 was selected as the optimal pH and gave the best results. It was shown that volumetric titration [21] and spectrophotometric [22] measurements of the course of the iodine-azide reaction in aqueous medium demonstrated similar results.

To establish the effect of azide ion solution concentration on the iodine-azide reaction, an azide ion solution in the range 0.1–5% was analyzed (Fig. 8). It was found that the peak area increased when the sodium azide concentration increased. In a further set of experiments an azide ion solution of 2.5% was chosen, however, additional increases in sodium azide concentrations did not significantly change the peak area.

3.3. Assay validation

Method validation was based on the following parameters: solution stability, linearity, detection and quantitation limits, precision, as well as accuracy.

For the assessment of methimazole stability, repeated injections of one sample were performed at ambient temperature over 5 h

Table 2
Stability results of methimazole (10 nmol/ml urine) under various storage conditions

Storage conditions	R.E. [%]
Room temperature (5 h)	−3.2
Three freeze–thaw cycles	−1.6

Table 3
Results of methimazole determination with iodine-azide post-column detection system (inter-day); $n = 4$

Taken [nmol/ml urine]	Found $\bar{x} \pm t_{0.95} \cdot \bar{s}$ [nmol/ml urine]	R.S.D. [%]	Recovery [%]
2.000	1.951 ± 0.088	3.2	97
3.000	2.943 ± 0.109	2.7	98
4.000	4.082 ± 0.024	0.4	102
5.000	5.088 ± 0.024	0.3	102
7.000	7.162 ± 0.074	0.7	102
10.00	9.950 ± 0.2	1.5	99

Table 4
Validation of intra-day assay; $n = 12$

Taken [nmol/ml urine]	Found $\bar{x} \pm t_{0.95} \cdot \bar{s}$ [nmol/ml urine]	R.S.D. [%]	Recovery [%]
2	1.853 ± 0.045	1.8	93
10	9.76 ± 0.12	0.9	98

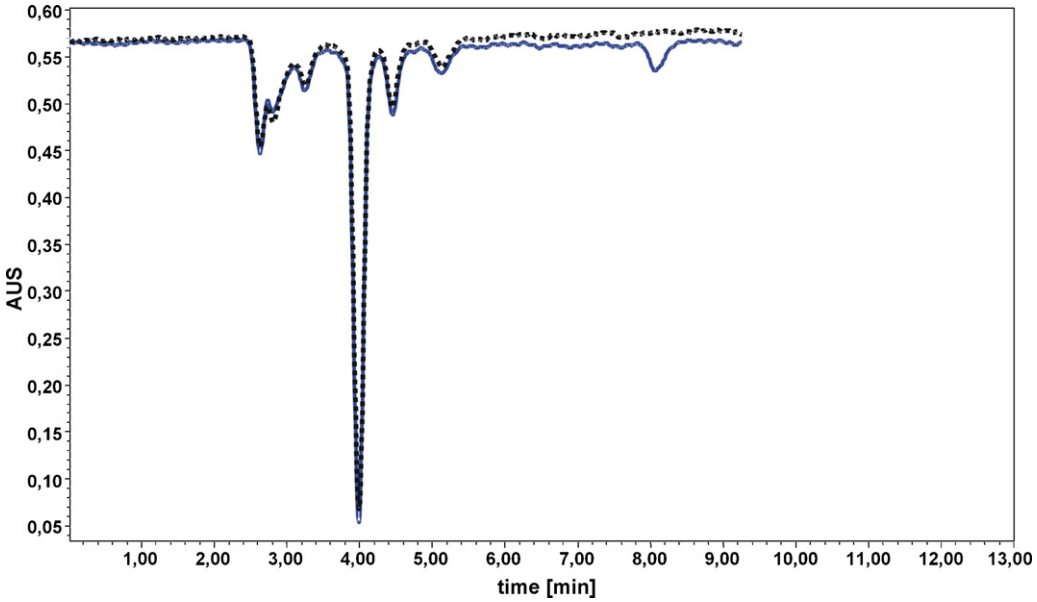

Fig. 9. Chromatogram obtained for urine (···) methimazole in spiked urine (quantification limit) (—) with iodine-azide reaction procedure detection (for chromatographic and post-column reaction conditions see Table 1).

(Table 2). Within this time the thiol solution proved to be stable. This time period was chosen since it was required to complete the entire HPLC analysis of urine. It was observed that the addition of EDTA or sodium citrate (750 μL, 0.1 mol/mL) did not affect the results. Methimazole in urine was also found to be stable after three freeze–thaw cycles (Table 2).

To establish detector response linearity, six standard samples within the range 2–10 nmol/mL of urine were fixed, injected and replicated four times. Standard curves consistently gave r^2 values above 0.999 within a calibration range of the analyte. The equation obtained by least squared regression was $A = 121.7c + 0.2$ where A is the peak area [mV s] and c is the concentration of methimazole (nmol/mL of urine). The calibration range can easily be extended upwards if required.

The value of 1 nmol/mL of urine was found to be the estimated limit. The limit of quantitation was established as 2 nmol/mL of urine at relative standard deviation (R.S.D.) of 3.2% ($n = 4$). Fig. 9 shows representative chromatograms obtained from each blank matrix and the matrix spiked with the LOQ standard (2 nmol/mL of urine).

The sensitivity of this method was comparable to, or better than those of HPLC methods with some clean-up procedures for urine described by others: 1.3 nmol/mL of urine (with derivatization procedure) [3], 0.2 nmol/mL (with SPE) [4], and 44 nmol/mL (with extraction) [7]. The proposed procedure did not require sample preparation. This is in contrast to the case of the procedures mentioned above.

Inter and intra assay precision and accuracy based on peak area ratios are presented in Tables 3 and 4, respectively. The procedure was very accurate, with a minimum and maximum recovery of 97 and 102%, respectively. The intra-day coefficient of variation (R.S.D.; $n = 4$), and inter-day coefficient of variation (4 days, R.S.D.; $n = 12$) were found to be lower that 3.2%.

3.4. Interferences

It was possible to eliminate matrix interferences by shifting the detection wavelength from the UV region (corresponding to methimazole absorption) to the vis region ($\lambda = 350$ nm, iodine adsorption). In general, applying the iodine-azide detection system allows only sulfur(II) compounds to be visible on chromatograms. However, three groups of additional peaks may be found due to certain compounds: (1) iodine-azide reaction inductors (e.g. cysteine or cystine) which generate a negative peak, (2) compounds which react with iodine under experimental conditions (e.g. ascorbic acid) which generate negative peaks, (3) compounds which react with iodide ion (e.g. bromate(V), iodate(V), nitrate(III)) which generate positive peaks [20] and (4) compounds which absorb at 350 nm (e.g. sulfasalazine).

Although there are particular sulphur(II) compounds which can be found in urine samples such as cysteine, cystine, homocysteine, glutathione, methionine, thiocyanate, ascorbic acid as well as sulphur metabolites of methimazole (2-merkaptoimidazole and *N*-methylthiourea [15]), these compounds eluate in the front of the

Table 5
Results of methimazole determination in patient's urine samples

Dosage [mg/day]	Age [year]	Sex	Drug name	Found $\bar{x} \pm t_{0.95} \cdot \bar{s}$ [nmol/ml]	R.S.D. [%]
10	76	Female	Thyrozol	0.97 ± 0.08	4.9
20	69	Female	Favistan	65.1 ± 3.9	3.7
40	11	Female	Metizol	20.2 ± 2.0	6.2
60	56	Male	Metizol	35.3 ± 4.6	8.1
	75	Female		11.0 ± 0.5	3.4
	29			17.2 ± 0.2	0.7
80	37	Female	Thyrozol	26.0 ± 1.0	2.6
	28			19.3 ± 0.4	4.7

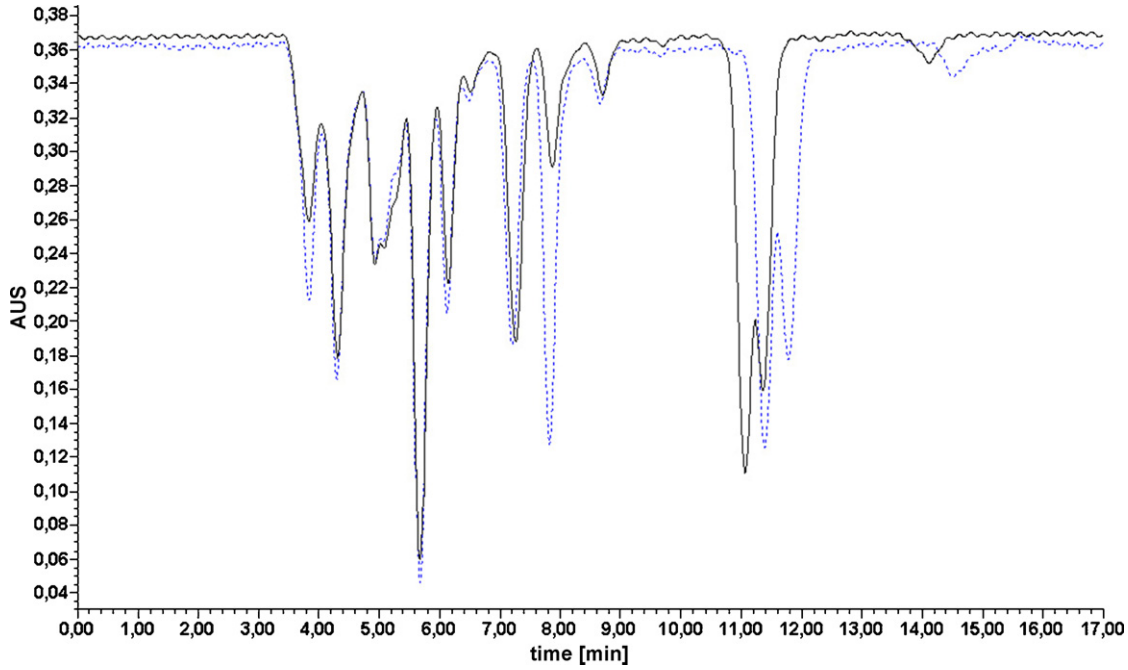

Fig. 10. Chromatogram obtained for methimazole in patient's urine (—) and spiked patient's urine (2 nmol methimazole) (···) with iodine-azide reaction procedure detection (for chromatographic and post-column reaction conditions see Table 1).

mobile phase in RP-HPLC mode. Possible interference may appear only if the retention times are similar, which is not the case in this procedure.

3.5. Assay of methimazole from urine samples

The application of the elaborate HPLC method for methimazole determination using the iodine-azide reaction detection system was extended to include patient urine samples (Table 5). The collection of urine samples from patients for method development was approved by an Institutional Review Board (University of Łódź). The results of chromatographic analysis of patients' urine samples are shown in Fig. 10. According to these results, parameters such as dose value, age, drug dose, and patient weight are related to methimazole concentration in urine samples. However, the effects of these parameters on methimazole urine concentration were not included in the aims of the current study.

4. Conclusions

In this study, a method based on reversed-phase HPLC with post-column detection using the iodine-azide reaction induced by methimazole has been developed for the determination of methimazole in urine. This method allowed the detection of methimazole in urine samples and offered a wide linear range (2–10 nmol/mL of urine), a lower detection limit (LOQ, 2 nmol/mL of urine) and shorter analysis time (retention time 8 min.). The sensitivity of this method (LOD, 1 nmol/mL of urine) is comparable to, or better than those of LC separation techniques for urine described by others: 1.3 nmol/mL of urine (with derivatization procedure) [3], 0.2 nmol/mL (with SPE) [4], and 44 nmol/mL (with extraction) [7].

The technique overcomes the limitations and obstacles of conventional methods such as the use of expensive and frequently contaminated organic solvents and of tedious and time-consuming sample preparation (e.g. derivatization procedure, SPE, extraction) with satisfactory sensitivity.

Moreover, the post-column reaction was compatible with the mobile phase of HPLC. The results from this study indicate that the proposed HPLC with the iodine-azide post-column reaction could potentially be used for routine clinical monitoring and in pharmacokinetic studies of methimazole.

Acknowledgements

The presented study was conducted with the support of 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 from the Department of Instrumental Analysis, University of Łódź and Dr. A. Zygmunt and Prof. A. Lewiński from the Department of Endocrinology and Metabolic Diseases, Medical University of Łódź for their contributions during discussions.

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