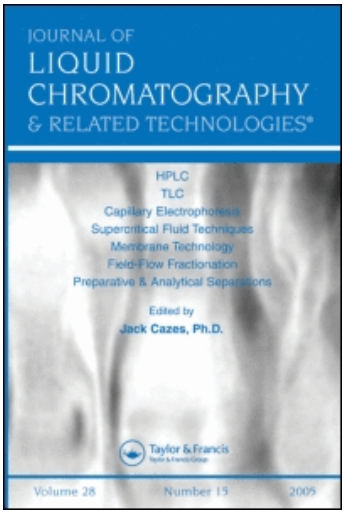


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# Thin Layer Chromatography with Post-Chromatographic Iodine-Azide Reaction for Thiuram Analysis in Food Samples

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# Thin Layer Chromatography with Post-Chromatographic Iodine-Azide Reaction for Thiuram Analysis in Food Samples

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**Abstract:** A simple, sensitive, precise, and rapid planar chromatographic method for thiuram analysis was elaborated. Reaction between iodine and azide ions induced by thiuram was applied as a detection system. The developed plates were sprayed with freshly prepared mixtures of sodium azide and starch solution adjusted to pH 6.0, and exposed to iodine vapor. The spots became visible as white spots on a violet-grey background. The iodine-azide detection system was proved to be the most favorable and enabled detection of quantities at the level 0.5 pmol per spot. The iodine-azide test was compared with other visualizing techniques commonly used in planar chromatography: iodine vapor, $UV_{254}$, Cu(II). Certain factors that influence detection with iodine-azide reaction as a detection system were checked. The developed method was linear over the concentration range of 2–9 pmol per spot. The proposed technique was applied to detect thiuram in food samples.

**Keywords:** Iodine-azide reaction, Thin-layer chromatography, Thiuram

## INTRODUCTION

Tetramethylthiuram disulphide (thiuram) is a common protective fungicide of the N,N′-dialkylthiocarbamate family, a group of compounds

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used for control of a variety of diseases on field crops, fruits, vegetables, and ornamentals. The latest review of analysis methods was reported.[1] One of the thiuram analysis methods is planar chromatography.[2] Mainly, silica gel is used as a stationary phase but also aluminum oxide is applied.[3] The most common method utilized for detection in TLC is visualization under 254 nm[4,5] and with a TLC/HPTLC scanner.[6,7] Most of the procedures cited in literature employed general (such as fast blue B,[5] 2,6-dibromoquinone chlorimide,[5] iodine vapor,[8] 2,6-dichloroquinone chlorimide,[9] Dragendorff reagent,[9] dithizone,[9,10] copper(II) ions[9,10] and enzymatic[11] procedures) rather than a specific detection reagent for thiuram. A great number of analyses require application of a selective and sensitive reagent for post-chromatographic detection.

One characteristic of thiuram is its ability to induce a reaction between iodine and azide ions. This feature led to employing this reaction as a method of thiuram determination. The mechanism of the iodine-azide reaction was discussed elsewhere.[12,13] Iodine consumption in the iodine-azide reaction is measured by volumetric titration[14] as well as spectrophotometric[15,16] measurements. The change in its quantity after the iodine-azide reaction is proportional to the amount of a sulfur(II) compound in a sample. In all methods based on the iodine-azide reaction, it is impossible to determine a thiol in a complex matrix, which contains also other thiols (e.g., pesticides in a food staff, drugs in biological fluids). To overcome this disadvantage, thiols must be separated and then the iodine-azide procedure can be applied. TLC technique of a sulfur(II) compound detection is based on the consumption of iodine in the inductor spot on a TLC plate treated with the iodine-azide detection procedure.[10,17,18]

In this paper, we have focused on employing the iodine-azide reaction as the proposed method of thiuram detection in food samples in order to improve its detection sensitivity at a pmol per spot level. For the first time we have proposed quantitative analysis for thiol determination by means of the iodine-azide procedure.

## EXPERIMENTAL

### Reagents, Solutions, and Apparatus

Thiuram, sodium azide, potassium iodide, iodine, starch, calcium nitrate(V), lead(II) nitrate (V), cobalt(II) nitrate (V), zinc nitrate (V), copper(II) nitrate(V), and all organic solvents were obtained from Sigma-Aldrich (Steinheim, Germany), LABSCAN Analytical Science (Dublin, Ireland) or POCH (Gliwice, Poland).

The stock solution: 1 mmol of thiuram, was dissolved in acetonitrile and diluted to 100 mL with acetonitrile to obtain 0.01 mol $L^{-1}$ concentration of thiuram solution. Standard thiuram solution: specific volumes of stock thiuram solution were diluted to 10 mL with acetonitrile.

To prepare the acetate buffer, appropriate quantities of sodium acetate was diluted in water and adjusted to pH 5.5 with glacial acetic acid. The pH of the buffer was adjusted by potentiometric titration. The titration system was calibrated with standard pH solutions. All solutions were prepared fresh daily.

The plates (TLC silica gel 60 $F_{254}$ aluminum sheets, Merck, Darmstadt, Germany; $10 \times 5$ cm, 0.2 mm thick layer) or HPTLC (silica gel 60 $F_{254}$ aluminum sheets, Merck, Darmstadt, Germany; $5 \times 5$ cm, 0.2 mm thick layer), or TLC (silica gel 60 $F_{254}$ glass plates, Merck, Darmstadt, Germany; $10 \times 10$ cm, 0.2 mm thick layer) were spotted with 0.1–1 μL pipette (Brand, Wertheim, Germany) or with TLC applicator Linomat 5 (Camag, Muttenz, Switzerland). They were developed in a horizontal DS-chamber ($10 \times 10$ cm; Chromdes, Lublin, Poland) or twin trough glass chamber ($20 \times 10$ cm; Camag, Muttenz, Switzerland) and sprayed with a TLC-sprayer (Merck, Darmstadt, Germany). Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance mode at 483 nm, and operated by winCATS Planar Chromatography version 1.2.1 (Camag, Muttenz, Switzerland). The source of radiation utilized was a halogen tungsten lamp.

The PC-scanner used was a PRIMAX Colorado 1200p. The plates were scanned at 150 dpi and stored in the form of 24-bit-True Color images.



## Planar Chromatography

### LOD Analysis

TLC silica gel or HPTLC silica gel aluminum plates were used for the determination of detection limits of thiuram. The plates were manually spotted, 1 cm (in TLC) or 0.5 cm (in HPTLC) from the edge of the plate with 1 μL of appropriate thiol solution (deposition area $\approx 0.1$ $cm^2$) using a pipette. Distance between spots was 1 cm. The plates were developed using a horizontal DS-chamber, which was ready for use 15 min after the solvent was poured into it (ambient temperature). The developing distances were: 8 cm (for TLC) and 4 cm (HPTLC). Methanol or dichloromethane was used as a mobile phase. Then the plates were air dried with a hair dryer. Spots were located by visualization with improved and non-improved iodine-azide, iodine, $UV_{254}$, and Cu(II) procedures. Spotted thiol concentration decreased in a following experiment like a calibration

function. The detection limits were obtained in six experiments and were considered when the spot corresponding to the smallest amount of the thiol appeared six times.

Quantitative Analysis (Procedure 1)

HPTLC silica gel aluminum plates were used for the determination of thiuram. The plates were manually spotted 0.5 cm from the edge of the plate with 0.2 μL of appropriate thiuram solution or thiuram sample (deposition area $\approx 0.1\,cm^2$) using a pipette. Distance between spots was 1 cm. The plates were developed using a horizontal DS-chamber, which was ready for use 15 min after the solvent was poured into it (ambient temperature). The developing distance was 4 cm. Methanol (thiuram as a standard) or dichloromethane (thiuram in food samples) was used as a mobile phase. Then plates were air dried with a hair dryer. Spots were located by visualization with improved iodine-azide procedures. Scanning was performed on a PC scanner and analyzed by a program written in Delphi (www.phys.uni.lodz.pl/zai/Analyzer.exe) and was then transported to Excel. Concentration of the chromatographed compound was determined from the dimension of spots as a number of pixels. Evaluation was via the number of pixels with linear regression.



Quantitative Analysis (Procedure 2)

TLC silica gel glass plates were used for the determination of thiuram. The samples, in the form of bands of length 2 mm, were spotted 1.5 cm from the edge of the plate, 1.5 cm from the left margin of the plate, and 1 cm apart, at a constant application rate of $150\,nL\,s^{-1}$ using a nitrogen aspirator. The plates were developed using methanol as a mobile phase. Linear ascending development was carried out in 10 cm × 20 cm twin trough glass chamber without chamber saturation (ambient temperature). The developing distance was 5 cm. Then, plates were air dried with a hair dryer. Spots were located by visualization with improved iodine-azide procedures. Scanning was performed on PC scanner and analyzed by a program written in Delphi and was then transported to Excel. Concentrations of the chromatographed compound were determined from the dimension of spots as a number of pixels. Evaluation was via number of pixels with linear regression.

Densitometric Analysis

TLC silica gel glass plates were used for the determination of thiuram. The samples, in the form of bands of length 2 mm, were spotted 1.5 cm

from the edge of plate, 1.5 cm from the left margin of the plate, and 1 cm apart, at a constant application rate 150 nL $s^{-1}$ using a nitrogen aspirator. The plates were developed using methanol as a mobile phase. Linear ascending development was carried out in 10 cm × 20 cm twin trough glass chamber without chamber saturation. The developing distance was 5 cm. Then, the plates were air dried with a hair dryer. Spots were located by visualization with improved iodine-azide procedures and scanned with a TLC densitometer at 483 nm. Concentrations of the chromatographed compound were determined from the difference in $R_F$ value at the end and start of the spot and recalculated to spot area in $mm^2$. Evaluation was via spot area with linear regression.

## Spray Reagent and Detection

All spray reagents were prepared fresh daily.



### Spray Reagents for Improved Iodine-azide Procedure

Aqueous sodium azide solution (5 mL of 10% (w/v)) and 12.5 mL of 2% aqueous starch solution were mixed and adjusted to pH 6.0 with 0.1 mol $L^{-1}$ hydrochloric acid solution. The solution was diluted to 25 mL with water. When the dependence of detection limits of thiuram was investigated, the specific amount of potassium iodide (1, 10, 100, 500, and 1000 mmol $L^{-1}$) was added into the spray solution.

### Spray Reagent for Non-improved Iodine-azide Procedure

Sodium azide solution (20 mL of 1 mol $L^{-1}$) adjusted to pH 6.0 with 0.1 mol $L^{-1}$ hydrochloric acid solution and 20 mL of 1 mol $L^{-1}$ iodine solution in 1 mol $L^{-1}$ potassium iodide solution were mixed.

### Spray Reagent for Copper(II) Ions Detection

Copper(II) sulphate(VI) pentahydrate, 0.25 g, was dissolved in acetate buffer (pH 5.5) in 100 mL.

### Detection with the Improved Iodine-azide Procedure (Improved $I_2 - N_3^-$)

After drying, the developed plates were sprayed with a freshly prepared mixture of sodium azide and starch solution adjusted to pH 6.0 and were exposed to iodine vapor for 5 s. Due to the catalytic effect of the C=S bound, the spots became visible as white spots on a violet-grey

background and they were stable for several minutes. The spots were read after two minutes since they were removed from the iodine chamber. When the concentration of potassium iodide into a spray solution was 0.5 or 1 mol $L^{-1}$, the spots were white on a brown background. They were stable for few hours.

Detection with the Non-improved Iodine-azide Procedure (Non-improved $I_2 - N_3^-$)[19]

After drying, the developed plates were sprayed with a freshly prepared mixture of sodium azide, iodine, and potassium iodide solution. Due to the catalytic effect of the C=S bound, the spots which were read after two minutes, became visible as white spots on a yellow background and they were stable for several minutes.

Detection with the Iodine Procedure ($I_2$)

After drying, the developed plates were exposed to iodine vapor for 10 min. The spots became visible as brown spots on a yellow background and they were stable for several minutes.

Detection with the $UV_{254}$ Procedure ($UV_{254}$)

After drying, substances were visualized under a UV lamp (254 nm) using TLC and HPTLC plates with a fluorescent indicator.

Detection with Copper(II) Procedure (Cu(II))

After drying, the developed plates were sprayed with copper(II) sulphate(VI) in acetate buffer (pH 5.5). Due to copper(II) complex formation, the spots which were read after ten minutes, became visible as yellow-green spots on a pale blue background. Spots were stable for several minutes.

## Analytical Application of Developed Procedure

Food Samples

Apples and carrots were colleted at a local market and were homogenized or squeezed. The homogenized apple of 2 g, 4 mL of apple juice, 800 μL of carrot juice samples were spiked with an appropriate amount of thiuram (at the level of μmol $L^{-1}$) and diluted with methanol to 5 mL. The solutions were mixed for 2 minutes, placed in an ultrasonic bath containing distilled water for 5 minutes at 20°C, and centrifuged at

900 rpm for 4 minutes. The 0.5 μL of supernatant, standard and blank solutions were spotted on HPTLC plates. Then, the plates were developed with dichloromethane as a mobile phase and the spots were visualized with improved iodine-azide reaction as a detection system (2% sodium azide, 1% starch, $c(KI) = 0.1\ mol\ L^{-1}$, pH 6.0).

### Quantitative Analysis of Thiuram in Carrot Juice

Carrots were collected at a local market and squeezed. The carrot juice (800 μL) with an appropriate amount of thiuram (25–40 nmol) was placed in a conical flask and fixed up to 1 mL with methanol. The solution was centrifuged at 9000 rpm for 4 minutes. The 0.2 μL of the supernatant, standard, and blank solutions were spotted on the HPTLC plates with pipettes. Then, the plates were developed with dichloromethane as a mobile phase and the spots were visualized with improved iodine-azide reaction as a detection system. Scanning was performed on a PC scanner and analyzed by a program written in Delphi and was then transported to Excel. Concentrations of the chromatographed compound was determined from the dimension of spots as a number of pixels. Evaluation was via number of pixels with linear regression.



## RESULTS AND DISCUSSION

### Separation

The $R_F$ value data (TLC and HPTLC) in methanol or dichloromethane are summarized in Table 1 and 2. There was no need to find new chromatographic systems because several different ones had been already proposed for standards.[1–11] It was necessary to establish a good resolution of thiuram in food samples since the iodine-azide detection system is sensitive towards other thiols present in the samples (e.g., cysteine, glutathione) or substances, which react with iodine in an acid conditions (e.g., ascorbic acid). It appeared that dichloromethane was sufficient enough as a mobile phase to separate thiuram ($R_F = 0.29$) from other sulfur(II) compounds in apples as well as in apple ($R_F = 0.20$) and carrot ($R_F = 0.0$) juice samples. Since the $R_F$ values were different from the case of thiuram the presence of compounds, which were visible with the iodine-azide detection system did not interfere with detection of thiuram in food samples.

***Table 1.*** Detection limits of thiuram in HPTLC (development distance 4 cm) and TLC (development distance 8 cm) on silica gel with methanol as a mobile; $R_F = 0.81$

| Detection method | | Iodide ions concentration (mol $L^{-1}$) | Detection limit (pmol per spot) |
|---|---|---|---|
| HPTLC | $I_2 - N_3$ | 0 | 2 |
| | | $1{\cdot}10^{-3}$ | 1 |
| | | $1{\cdot}10^{-2}$ | 0.7 |
| | | $1{\cdot}10^{-1}$ | 0.5 |
| | | 0.5 | 2 |
| | | 1 | 4 |
| | $I_2$ | | 7 |
| | $UV_{254}$ | | 100 |
| | Cu(II) | | 60 |
| TLC | $I_2 - N_3$ | $1{\cdot}10^{-1}$ | 3 |
| | $I_2$ | | 20 |
| | $UV_{254}$ | | 200 |
| | Cu(II) | | 200 |
| | Non-improved $I_2 - N_3$ | | 600 |



## Detection

The proposed method was checked to find optimum conditions of improved iodine-azide spray reagent for thiuram detection. That was achieved with 2% sodium azide solution and 1% starch solution at pH 6.0. The plates were exposed to iodine vapor for 5 s. This is the time within which iodine is adsorbed on the TLC plates. Such short exposure time is the consequence of iodine adsorption on the plate and vanishing of white spots because of iodine excess. The spots became visible as white dots on a violet-grey background within 2 minutes due to the catalytic effect of the C=S bound. The spots were stable for several minutes as sublimation of iodine from the plate proceeded. Appearance of white spots could take some time (especially when potassium iodide is present within the range of 0.1–1 mol $L^{-1}$), due to the induction time which depends on induction properties of thiuram. This is the time within which the induction reaction is completed. It is less than 2 minutes.

These optimum conditions of the improved method of detection using iodine-azide reaction were checked in comparison with the non-improved detection method. The non-improved method involving the iodine-azide reaction was based on spraying the plates with a mixture of sodium azide and iodine solution (in potassium iodide solution).[10,17–19]

As a result, white spots on a yellow background appeared. However, it was observed that the excess of iodine made the white spots vanish and consequently, it was the reason for the greatly increased detection limits. The general rule was observed, the lower iodine solution concentration, the lower detection limit: 600 pmol per spot (see Table 1) and 1 nmol per spot (paper chromatography[17]) obtained with 0.5 mol $L^{-1}$ iodine; 40 pmol per spot obtained with 0.05 mol $L^{-1}$ iodine.[10] This phenomenon does not generally occur when other visualization techniques of TLC plates are applied. The excess of a visualization solution does not make detection higher. Thus, it is important to spray the plates with a finely divided spray solution for optimum staining of the TLC plates. At the same time, it may prove to be difficult as well. The improved procedure overcomes it by applying iodine vapor in the proposed procedure.

The present potassium iodide solution in the spraying mixture adversely affected the detection limits. Hence, the introduced improvements to the method made it more favorable. The hampering effect of the iodide ions in the iodine-azide reaction induced by many thiols or organothiophosphorus compounds is intensified with increasing iodide ions concentration.[19–22] An increase of potassium iodide concentration stimulates an increase of iodine consumption in the iodine-azide reaction induced by many thiols and an increase of induction time. We investigated the influence of iodine ions on the detection limits obtained with the improved iodine-azide procedure (Table 1). We sprayed TLC plates with a solution containing a proper amount of potassium iodide (1, 10, 100, 500, and 1000 mmol $L^{-1}$). When the influence of potassium iodide concentration into a spray solution on detection limits was investigated, the background changed from violet-grey into brown with an increase of potassium iodide concentration. The higher concentration of iodide ions into a spray solution, the more stable background is obtained. In volumetric titration, the presence of iodide ions in a reagent solution slows down the course of iodine-azide reaction.[14] In planar chromatography, the appearance of spots takes more time in the case of iodide ions present in the spray solution compared with their absence in spray solution. The presence of high iodide ion concentrations (which correspond to the iodide ions concentration in spray solution applied in non-improved iodine-azide procedure), makes detection limits higher (600 pmol per spot). However, the specific amount of iodide ions (0.1 mol $L^{-1}$) in a spray solution in an improved iodine-azide procedure lowered the detection limit. We chose potassium iodide concentration equal to 0.1 mol $L^{-1}$ as the optimal one for detection of the studied compounds in TLC and HPTLC methods. Because of the difficulty with spraying plates with the finely divided spray solution, excess of iodine in sprayed plates, and the presence of iodide ions in spray solution, the non-improved procedure cannot

be proposed for detection of thiuram since relatively high detection limits were obtained with the procedure.

The comparison of the detection limits of thiuram achieved by using different detection systems (iodine-azide procedure, iodine vapor, UV, and copper(II) ions) used routinely in laboratories is presented in Table 1. The demonstrated outcome indicates that iodine-azide method is the most favorable one. In general, the detection limits were established at 3 and 0.5 pmol per spot using a iodine-azide detection system in TLC and HPTLC, respectively.

The methods utilized for detection in TLC, such as visualization under 254 nm[4,5] with a TLC/HPTLC scanner,[6,7] iodine vapor,[8] fast blue B,[5] 2,6-dibromoquinone chlorimide[5] 2,6-dichloroquinone chlorimide,[9] Dragendorff reagent,[9] dithizone[9,10] copper(II) ions[9,10] procedures, gave a detection limit at the level of 0.2–20 nmol per spot. Only the enzymatic technique[11] enables lowering the detection limit to 80 pmol per spot. Iodine-azide detection system remains one of the most sensitive of all chromatographic reagents for the qualitative evaluation of bivalent sulfur compounds.

Another advantage of iodine-azide reaction as a detection system in planar chromatography over other examined methods of detection was the quality of obtained chromatograms. Spots of thiuram detected with the proposed method were compact with sharp edges against the violet-grey background of the plate and provided an accurate measurement of $R_F$ value.

Application of the iodine-azide procedure in detection of thiuram in food samples are shown in Table 2. The detection limits of thiuram in spiked apples, apple, and carrot juice samples are 10 nmol $g^{-1}$, 1.25, and 10 nmol $mL^{-1}$, respectively. The detection limits in spiked samples correspond to the detection limits for standards only for apple juice. The detection limits for apples and carrot juice correspond to 2 pmol

***Table 2.*** Detection limits of thiuram in food samples by use of HPTLC on silica gel with dichloromethane as a mobile phase (development distance 4 cm) with improved iodine-azide procedure

| | Detection limit | | |
|---|---|---|---|
| Sample | (pmol per spot) | (nmol per 1 mL juice sample or nmol per 1 g apples sample) | $R_F$ |
| Apples | 2 | 10 | 0.29 |
| Apple juice | 0.5 | 1.25 | 0.29 |
| Carrot juice | 2 | 10 | 0.29 |

per spot value. The lower detection limits might be obtained by increasing the amount of biological material and with special pretreatment of samples (e.g., SPE method).

## Quantitative Analysis with Improved Iodine-Azide Procedure

The optimum conditions of the improved detection method using iodine-azide reaction for thiouracils,[20] mercaptopyridines, mercaptopyrimidines,[21] and heterocyclic thiols[22] detection were presented in previous reports. In this report, for the first time, we have expanded the application of the method to thiuram with quantitative analysis.

When the iodine-azide procedure was applied, due to the catalytic effect of C=S bound, the spots became visible as white spots on a violet-grey background and they were stable for several minutes. Background disappearance was the result of two processes: iodine sublimation from a plate area and uninduced iodine-azide reaction proceeding outside a spot. Iodine volatility was overcome by adding potassium iodine to the spray solution. The higher iodine ions concentration, the more stable background is obtained. At higher concentration of iodide ions, the background is stable because the non-volatile triiodide ions concentration increases as a result of a further shift in the equilibrium iodine-iodide ions ($I_2 + I^- \rightleftarrows I_3^-$) to the right. High potassium iodide concentration presence in a spray solution results also in hampering the uninduced iodine-azide reaction[23] and additionally, iodine amount is constant on a plate area.

The background color changes from violet-grey (corresponding to iodine-starch complex) to brown (iodine-iodide-starch complex) with an iodide ions concentration increase. This color transformation is the consequence of hipsochromic shift of background maximum absorption wavelength from 550 nm to 483 nm. Plates were covered with sodium azide (in a spray solution) and iodine (plates were placed in a iodine chamber). Thanks to starch and iodide ions presence, plates change color from violet-grey into brown. In the place of TLC plate where thiuram is present, iodine-azide reaction induced by thiuram takes place and a white spot appears.

Having examined the following range of induction time (as a function of spots expanding) 2–30 minutes, it was discovered that 15 minutes occurred to be enough to complete the iodine-azide reaction. This means that each plate was scanned 15 minutes after the end of the detection manipulation.

Separate standard solutions equivalent to 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 pmol per spot thiuram were spotted on TLC plates (Figure 1). A minimum of six determinations of each concentration were performed. The plates were spotted, developed, dried, visualized with improved

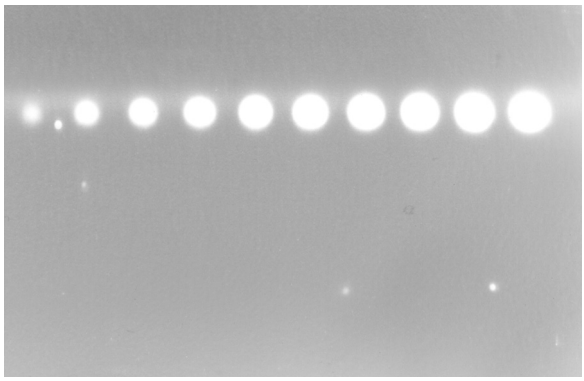

***Figure 1.*** Detection of thiuram with iodine-azide reaction method; TLC plate; methanol as a mobile phase; development distance 5 cm.



iodine-azide detection procedure, and scanned with a TLC densitometer. Generally, quantitative evaluation of a TLC/HPTLC plate is always performed densitometrically, either in absorption or fluorescence mode. The signal of each substance zone is compared to the substance free plate background. Classical densitometry uses monochromatic light and a slit of selectable length and width to scan the tracks of a chromatogram, measuring the diffusely reflected light. The measurement of the signal provides the signal for quantitative measurement. In the improved iodine-azide procedure detection, the plate is scanned at 483 nm, which corresponds to maximum absorption of iodine-iodide-starch complex (Figure 1). Since the plate is covered with iodine, a constant absorbance is maintained and recorded as a background from the iodine-iodide-starch complex. When the light passes a white dot, the signal decreases due to consumption of iodine in the iodine-azide reaction induced by thiuram within its spot. Outside the thiuram spot, the absorbance signal increases till up its initial value and a negative peak is recorded (Figure 2). The obtained chromatogram is the relationship between absorbance and $R_F$ value. From the chart, the $R_F$ of the negative peak end and start is determined which corresponds to the $R_F$ of spot end and start. The difference of the $R_F$ values is transformed into diameter and then into area (in $mm^2$) of a spot, which is treated as an ideal circle (Figure 1). Calibration plots were constructed by plotting area spot against the corresponding amount (pmol) of thiuram. The results are listed in Table 3. The linearity of the response was assessed in the range 2–8 pmol per spot by determination of slope, intercept, and correlation coefficient. The correlation coefficient, $r^2$ was 0.987, the slope was $0.0308 \pm 0.0045$ ($n = 6$) and the intercept was $0.1847 \pm 0.0033$ over this concentration range. In our research, we applied a mass product (PC scanner) as an analytical

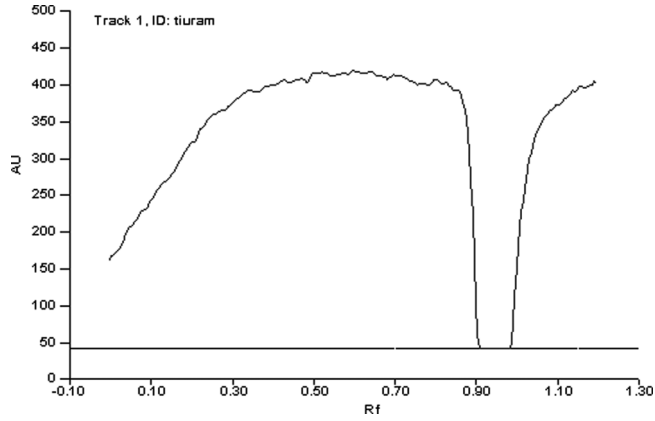


*Figure 2.* Scan of TLC plate for the dot corresponding 5 pmol/spot with TLC densitometer at 483 nm.



instrument.[24–26] The plates, scanned with commercially available TLC-densitometer, were also scanned with PC scanner. The chromatograms were stored in the form of 24-bit True Color Image. The pixels of white chromatographic bands were counted with a program written in Delphi. The quantitative analyses are shown in Table 3. A linear relationship was obtained between response (number of pixels) and amount of thiuram in the range 2–8 pmol per spot; the correlation coefficient $r^2$ was 0.9981, the slope was $431 \pm 34$ ($n = 6$), and the intercept was $1201 \pm 155$ over this concentration range. Accuracy of $R_F$ value determination defines the spot dimension (as difference in $R_F$ value between the end and start of the spot). Slight $R_F$ value change results in a huge scatter of found amount of thiuram in a spot. Hence, RSD values were enormous in the densitometric procedure. Such phenomena of spots established as a number of pixels did not occur, and RSD values were smaller. Results obtained with manual sample application with micropipettes (improved iodine-azide detection procedure; PC scanner, spot dimension as a number of pixels) are similar to those obtained with TLC sample applicator (improved iodine-azide detection procedure; PC scanner, spot dimension as a number of pixels).

The proposed procedure was applied to determine spiked thiuram in carrot juice. The results are given in Table 2. The relationship between spot dimension (as a number of pixels) and spotted amount of thiuram (pmol per spot) was demonstrated using five point calibration curves, which were linear in the range 5–9 pmol per spot. The equations for linear regression line and correlation coefficient were $y = 547 \times -1351$ and $r^2 = 0.9752$, respectively.

*Table 3.* Determination of thiuram with iodine-azide procedure detection; n = 6

| Sample | Scan method | Sample applicator | Taken (pmol) | Found $\bar{x} \pm t_{0,95} \cdot \bar{s}$ (pmol) | RSD (%) | Recovery (%) |
|---|---|---|---|---|---|---|
| Standard | PC scaner | 0.1–1 μL pipette | 5.00 | 4.9 ± 0.4 | 9.8 | 98 |
| | | | 6.00 | 5.9 ± 0.7 | 13.4 | 99 |
| | | | 7.00 | 7.2 ± 0.2 | 2 | 103 |
| | | | 8.00 | 8.1 ± 0.8 | 7.1 | 101 |
| | | | 9.00 | 8.8 ± 0.9 | 7.2 | 98 |
| Carrot juice | | 0.1–1 μL pipette | 5.0 | 4.9 ± 0.4 | 8.1 | 97 |
| | | | 6.0 | 6.2 ± 0.1 | 0.8 | 104 |
| | | | 7.0 | 7.1 ± 0.1 | 1.6 | 101 |
| | | | 8.0 | 8.0 ± 0.8 | 9.6 | 100 |
| | | | 9.0 | 9.1 ± 0.8 | 8.8 | 101 |
| Standard | | Linomat 5 | 2.0 | 2.1 ± 0.2 | 7.1 | 105 |
| | | | 3.0 | 3.0 ± 0.4 | 9.8 | 99 |
| | | | 4.0 | 3.9 ± 0.5 | 9.9 | 97 |
| | | | 5.0 | 5.1 ± 0.4 | 5.8 | 102 |
| | | | 6.0 | 6.0 ± 0.2 | 2.8 | 101 |
| | | | 7.0 | 7.1 ± 0.7 | 6.9 | 102 |
| | | | 8.0 | 7.9 ± 0.8 | 7.1 | 98 |
| | Densitometry | | 2.0 | 2.1 ± 1.0 | 25.2 | 104 |
| | | | 3.0 | 3.2 ± 1.7 | 28.9 | 107 |
| | | | 4.0 | 4.0 ± 1.3 | 23.1 | 99 |
| | | | 5.0 | 4.8 ± 1.9 | 28.2 | 96 |
| | | | 6.0 | 5.6 ± 1.4 | 17.6 | 94 |
| | | | 7.0 | 7.0 ± 1.3 | 13.3 | 99 |
| | | | 8.0 | 8.4 ± 2.2 | 18.6 | 105 |



## CONCLUSION

The discussed results confirm the potential and beneficial effects of iodine-azide reaction as a detection system in planar chromatography. The proposed detection system allows selective and the most sensitive detection for thiuram at pmol per spot level. The other applied detection methods routinely used in TLC- iodine vapor, UV, and Cu(II) ions gave a positive but less sensitive test. Iodine-azide detection system for detection of thiuram in food samples, is inexpensive, readily available chemicals, and with short analysis time. The non-improved iodine-azide method has not been widely applied since relatively high detection limits are obtained with the procedure.

It was found that the results that were obtained by use of a PC scanner were as good as more conventional measurements using a TLC densitometer. In fact, with modern PC scanners, the error from the

measurements are smaller than is intrinsic to TLC densitometry; thus, the use of PC scanners does not limit the accuracy of the technique.

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