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# Thin-layer chromatographic detection of new azaphenothiazines

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ABSTRACT

Many new phenothiazines exhibit promising anticancer and antibacterial activities, reversal of multidrug resistance and potential treatment in Alzheimer's, Creutzfeldt-Jakob and AIDS diseases. Their synthesis may proceed through a stage of the Smiles rearrangement and may lead to different products: cyclic phenothiazines (rearranged or not), cyclic side-products of isosteric structures and non-cyclic products when the ring-closure processes did not occur. The TLC method was found suitable for detection of new modified phenothiazines (being tri-, tetra- and pentacyclic azaphenothiazines with hydrogen, alkyl, dialkylaminoalkyl, aryl and heteroaryl substituents at the thiazine nitrogen atom and in a few cases additional substituents in the benzene ring) during their synthesis. The natural fluorescence of phenothiazines and azaphenothiazines under irradiation of UV light of 365 nm is very characteristic and becomes useful additional analytical information of these compounds. The spots of azaphenothiazines were also distinguished from the spots of substrates and side-products by giving color reactions with the visualizing reagents: sulfuric acid in ethanol, concentrated nitric acid and citric acid in acetic anhydride. The combination of the separation (the $R_F$ values) and the spot color detection (as the native fluorescence and the results of the usage of visualizing reagents) facilitated the identifications of new azaphenothiazines in the reaction mixtures containing also other compounds. This paper is the first attempt of the determination of new azaphenothiazines by TLC method preceding the identification by spectroscopic methods. It facilitates the separation of the proper fraction in column chromatography and preparative TLC.



## 1. Introduction

Phenothiazines (dibenzo-1,4-thiazines) are an important class of heterocyclic compounds consisting of a three-ring fused system with an >NR group and a sulfur atom in the central six-membered ring. They possess not only widely recognized pharmaceutical activity such as neuroleptic activity, antihistaminic, antitussive and antiemetic but also interesting chemical properties [1,2]. Many recent reports deal with promising anticancer, antiplasmid and antibacterial activities, reversal of multidrug resistance (MDR) and potential treatment in Alzheimer's, Creutzfeldt-Jakob and AIDS diseases of classical and newly synthesized phenothiazines [2–5]. Due to the presence of chemically active sulfur and nitrogen atoms and the dialkylaminoalkyl substituents located at the 10(N)-position, phenothiazines also exhibit several interesting analytical properties [2]. For a number of years, several analytical methods have been proposed for the determination of phenothiazine and its derivatives in pharmaceutical formulations and biological fluids, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), spectrofluorimetry, either direct or with derivatization, flow-injection analysis (FIA) and other techniques [2]. Thin-layer chromatography (TLC) is still the most used chromatographic analytical method in the pharmaceutical industry, using from the first test in the synthetic research laboratory to follow the synthesis of a new entity, to the quality control of commercial finished products and the control in the therapeutic practice and the diagnostics of poisonings [6]. The separation and identification of neuroleptic phenothiazines by TLC present a certain difficulty because of closeness of their psychochemical properties and, consequently their retention properties and the character of interaction with the mobile and stationary phases [7]. However, TLC is regarded to be the best suited technique for control of neuroleptic phenothiazines in the therapeutic practice and the diagnostics of poisonings [7–12].

New phenothiazine derivatives were obtained by introduction of new pharmacophoric substituents (other than the dialkylaminoalkyl groups) at the thiazine nitrogen atom and by substitution of the benzene ring with the heteroaromatic azine rings to form various azaphenothiazines. As the modification of the phenothiazine structure with the benzene ring exchange is most perspective, one can expect syntheses of many new types of azaphenothiazines now and in the future. Similar to the synthesis of phenothiazines, the synthesis of azaphenothiazines may proceed through a stage of the Smiles rearrangement. These reactions

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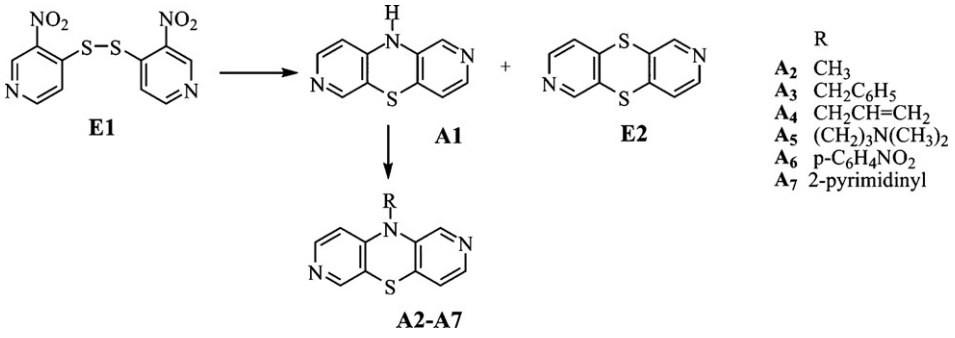
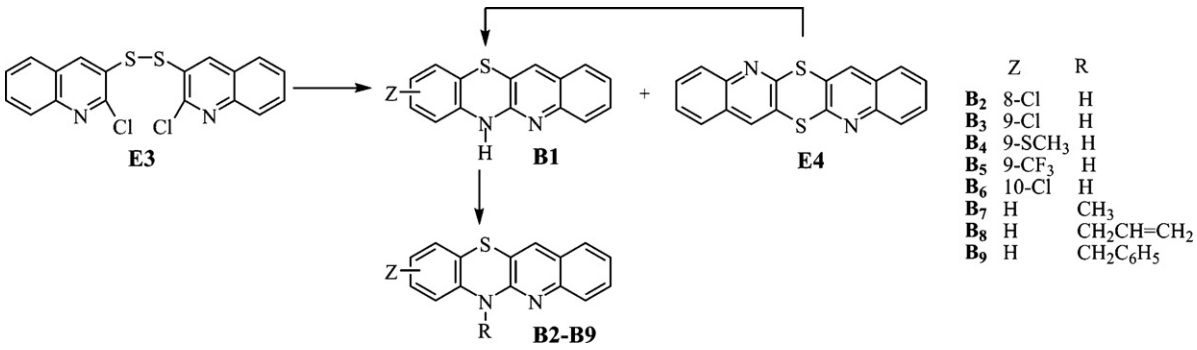
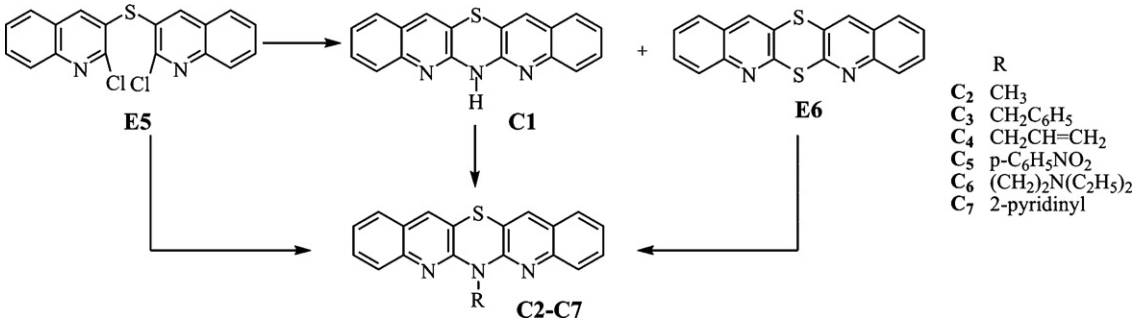


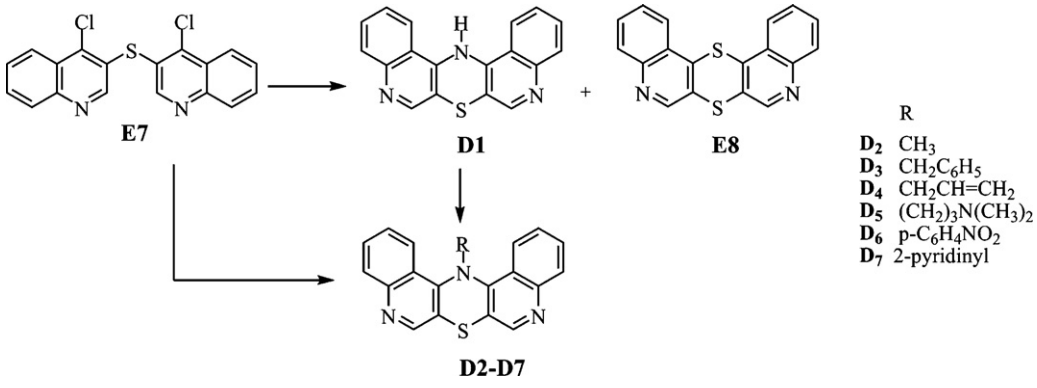

**Scheme 1.** The syntheses and the structures of azaphenothiazines **A**.




**Scheme 2.** The synthesis and the structures of azaphenothiazines **B**.




**Scheme 3.** The synthesis and the structures of azaphenothiazines **C**.




**Scheme 4.** The synthesis and the structures of azaphenothiazines **D**.

may lead in the end to different products: the cyclic compounds as the result of the ring closure processes, most of them of the azaphenothiazine structure (rearranged or not) and the non-cyclic products when the ring-closure processes did not occur [1,13]. As the cyclic side-products in the synthesis of azaphenothiazines were detected isosteric dithiin and pyrazine compounds, possessing two sulfur or two nitrogen atoms in the central ring [14–21]. An introduction of the alkyl, aryl, heteroaryl and aminoalkyl substituents at the thiazine nitrogen atom (instead of the hydrogen atom) and further transformations of the aminoalkyl

**Table 1**
The chromatographic and separation factors of azaphenothiazines **A**, **B**, **C**, **D** and substrates and side-products **E**.

| TLC system | Compounds/$R_F$ | $\Delta R_F$ | $R_S$ | $\alpha$ | TLC system | Compounds/$R_F$ | $\Delta R_F$ | $R_S$ | $\alpha$ |
|---|---|---|---|---|---|---|---|---|---|
| I | **E1**/**A1** 0.68/0.30 | 0.38 | 9.77 | 5.05 | II | **E3**/**B1** 0.74/0.32 | 0.42 | 9.45 | 6.05 |
| | **E1**/**A2** 0.68/0.50 | 0.18 | 4.05 | 2.15 | | **E3**/**B2** 0.74/0.33 | 0.41 | 10.54 | 5.78 |
| | **E1**/**A3** 0.68/0.56 | 0.12 | 3.09 | 1.67 | | **E3**/**B3** 0.74/0.29 | 0.45 | 11.57 | 6.97 |
| | **E1**/**A4** 0.68/0.60 | 0.08 | 2.06 | 1.42 | | **E3**/**B4** 0.74/0.24 | 0.50 | 12.86 | 9.01 |
| | **E1**/**A5** 0.68/0.17 | 0.51 | 13.11 | 10.54 | | **E3**/**B5** 0.74/0.34 | 0.40 | 9.00 | 5.52 |
| | **E1**/**A6** 0.68/0.07 | 0.61 | 18.30 | 28.83 | | **E3**/**B6** 0.74/0.32 | 0.42 | 10.80 | 6.05 |
| | **E1**/**A7** 0.68/0.24 | 0.44 | 13.20 | 6.73 | | | | | |
| | **E2**/**A1** 0.68/0.30 | 0.38 | 11.40 | 5.05 | | **E4**/**B1** 0.64/0.32 | 0.32 | 7.20 | 3.78 |
| | **E2**/**A2** 0.68/0.50 | 0.18 | 4.63 | 2.15 | | **E4**/**B2** 0.64/0.33 | 0.31 | 7.97 | 3.61 |
| | **E2**/**A3** 0.68/0.56 | 0.12 | 3.60 | 1.67 | | **E4**/**B3** 0.64/0.29 | 0.35 | 9.00 | 4.35 |
| | **E2**/**A4** 0.68/0.60 | 0.08 | 2.40 | 1.42 | | **E4**/**B4** 0.64/0.24 | 0.40 | 10.29 | 5.63 |
| | **E2**/**A5** 0.68/0.17 | 0.51 | 15.30 | 10.54 | | **E4**/**B5** 0.64/0.34 | 0.30 | 6.75 | 3.45 |
| | **E2**/**A6** 0.68/0.07 | 0.61 | 21.96 | 28.83 | | **E4**/**B6** 0.64/0.32 | 0.32 | 8.23 | 3.78 |
| | **E2**/**A7** 0.68/0.24 | 0.44 | 15.84 | 6.73 | | | | | |
| | **A1**/**A2** 0.30/0.50 | 0.20 | 5.14 | 2.35 | | **B1**/**B7** 0.32/0.83 | 0.51 | 11.48 | 10.38 |
| | **A1**/**A3** 0.30/0.56 | 0.26 | 7.80 | 3.03 | | **B1**/**B8** 0.32/0.87 | 0.55 | 11.00 | 14.22 |
| | **A1**/**A4** 0.30/0.60 | 0.30 | 9.00 | 3.57 | | **B1**/**B9** 0.32/0.89 | 0.57 | 12.83 | 17.19 |
| | **A1**/**A5** 0.30/0.17 | 0.13 | 3.90 | 2.09 | | | | | |
| | **A1**/**A6** 0.30/0.07 | 0.23 | 8.28 | 5.70 | | | | | |
| | **A1**/**A7** 0.30/0.50 | 0.06 | 2.16 | 1.33 | | | | | |
| II | **E5**/**C1** 0.67/0.48 | 0.19 | 5.70 | 2.20 | I | **E7**/**D1** 0.84/0.25 | 0.59 | 17.70 | 15.79 |
| | **E5**/**C2** 0.67/0.78 | 0.11 | 3.30 | 1.75 | | **E7**/**D2** 0.84/0.73 | 0.11 | 2.83 | 1.95 |
| | **E5**/**C3** 0.67/0.81 | 0.14 | 4.20 | 2.13 | | **E7**/**D3** 0.84/0.78 | 0.06 | 1.80 | 1.47 |
| | **E5**/**C4** 0.67/0.79 | 0.12 | 3.60 | 1.81 | | **E7**/**D4** 0.84/0.75 | 0.09 | 2.70 | 1.74 |
| | **E5**/**C5** 0.67/0.79 | 0.12 | 3.60 | 1.81 | | **E7**/**D5** 0.84/0.78 | 0.06 | 1.54 | 1.47 |
| | **E5**/**C6** 0.67/0.08 | 0.59 | 17.70 | 23.47 | | **E7**/**D6** 0.84/0.63 | 0.21 | 5.40 | 3.11 |
| | **E5**/**C7** 0.67/0.57 | 0.10 | 3.00 | 1.53 | | **E7**/**D7** 0.84/0.72 | 0.12 | 3.09 | 2.05 |
| | **E5**/**C1** 0.60/0.48 | 0.12 | 3.60 | 1.03 | | **E8**/**D1** 0.67/0.25 | 0.42 | 10.80 | 6.12 |
| | **E5**/**C2** 0.60/0.78 | 0.18 | 5.40 | 2.39 | | **E8**/**D2** 0.67/0.73 | 0.06 | 1.35 | 1.32 |
| | **E5**/**C3** 0.60/0.81 | 0.21 | 6.30 | 2.91 | | **E8**/**D3** 0.67/0.78 | 0.11 | 2.83 | 1.75 |
| | **E5**/**C4** 0.60/0.79 | 0.19 | 5.70 | 2.48 | | **E8**/**D4** 0.67/0.75 | 0.08 | 2.06 | 1.48 |
| | **E5**/**C5** 0.60/0.79 | 0.19 | 5.70 | 2.48 | | **E8**/**D5** 0.67/0.78 | 0.11 | 2.48 | 1.75 |
| | **E5**/**C6** 0.60/0.08 | 0.52 | 15.60 | 16.67 | | **E8**/**D6** 0.67/0.63 | 0.04 | 0.90 | 1.20 |
| | **E5**/**C7** 0.60/0.57 | 0.03 | 0.90 | 1.12 | | **E8**/**D7** 0.67/0.72 | 0.05 | 1.13 | 1.26 |
| | **C1**/**C2** 0.48/0.78 | 0.30 | 9.00 | 3.86 | | **D1**/**D2** 0.25/0.73 | 0.48 | 12.34 | 8.10 |
| | **C1**/**C3** 0.48/0.81 | 0.33 | 9.90 | 4.70 | | **D1**/**D3** 0.25/0.78 | 0.53 | 15.90 | 10.71 |
| | **C1**/**C4** 0.48/0.79 | 0.31 | 9.30 | 4.00 | | **D1**/**D4** 0.25/0.75 | 0.50 | 15.00 | 9.09 |
| | **C1**/**C5** 0.48/0.79 | 0.31 | 9.30 | 4.00 | | **D1**/**D5** 0.25/0.78 | 0.53 | 13.63 | 10.71 |
| | **C1**/**C6** 0.48/0.08 | 0.40 | 12.00 | 10.65 | | **D1**/**D6** 0.25/0.63 | 0.38 | 9.77 | 5.08 |
| | **C1**/**C7** 0.48/0.57 | 0.09 | 2.70 | 1.44 | | **D1**/**D7** 0.25/0.72 | 0.47 | 12.09 | 7.69 |

derivatives not always run smoothly and give the desired product.

The aim of this study is to find a simple and rapid TLC method to follow the synthesis of different azaphenothiazines from various substrates, to separate and to detect the azaphenothiazine products from other products and to separate N-substituted azaphenothiazines with different substituents from NH-azaphenothiazines.

## 2. Materials and methods

Azaphenothiazines **A**–**D** (30 compounds), substrates and side-products **E** (8 compounds) were obtained according to the described procedures [14–21]. Phenothiazines **F** (3 compounds) were commercial (Aldrich and Sigma).

TLC was performed on the 10 cm × 10 cm plastic TLC plates precoated with silica gel 60 $F_{254}$ (Merck, #1.05735) and aluminum oxide 60 $F_{254}$ (type E, Merck, #1.05581). Solutions of four compounds: two phenothiazines (**A1** and **A*n*** or **C1** and **C*n*** or **D1** and **D*n***, ***n*** = 2–7) and two substrates/side-products (**E1** and **E2** or **E5** and **E6** or **E7** and **E8**, respectively) were prepared by mixing (1:1:1:1, v/v/v/v) single solutions of concentrations of 2.0 mg/mL in chloroform. In the case of azaphenothiazines **B**, solutions of four compounds: two phenothiazines (**B1** and **B*n***, ***n*** = 7–9) and two substrates/side-products (**E3** and **E4**) or of three compounds (**B*n***, ***n*** = 2–6, **E3** and **E4**) were prepared in the same manner. Such a mixed solutions were results of the synthetic routes (Schemes 1–4). The solutions were spotted on a plate in 1 μL volume onto the starting line 0.5 cm from the bottom edge of the plate (6 lanes for series with compounds **A/E**, **C/E** and **D/E**, and 8 lanes for series with compounds **B/E**). Before development of plates, chromatographic chamber (Desaga No. 120212) was saturated with the mobile phase for 0.5 h using filter paper lining. The chromatograms were developed at room temperature by ascending technique up to 0.5 cm below the top edge of the plate using one of two TLC systems as the mobile phases: **I** $SiO_2$/$CHCl_3$:EtOH (5:1, v/v) or **II** $Al_2O_3$/$CHCl_3$–EtOH (10:0.5, v/v). The development distance was 9 cm. After development, the plates were dried at room temperature in a fume cupboard and the resulting spots were observed in the daylight, under UV light at λ = 254 and 365 nm. The resulting spots were circular with diameters of 2–4 mm. At least three chromatograms were developed for each group of used compounds and the $R_F$ values were averaged.

The separation factors $\Delta R_F$, $R_S$ and $\alpha$ (Table 1) were calculated by use of the equations:

$$\Delta R_F = R_{F1} - R_{F2} \quad (1)$$

$$R_S = \frac{d_1 - d_2}{0.5(w_1 + w_2)} \quad (2)$$

$$\alpha = \frac{[(1/R_{F1}) - 1]}{[(1/R_{F2}) - 1]} \quad (3)$$

where $d_1$ and $d_2$ are the distances between the centers of the spots and the staring points, $w_1$ and $w_2$ are the widths of the spots.

In the end, the spots were underwent the reactions with various visualizing reagents by spraying with them to produce characteristic color zones. Out of 26 examined visualizing

**Table 2**
The spot colors of phenothiazines **A**, **B**, **C**, **D** and **F**.

| | VIS | UV (365 nm) | UV (254 nm) | $H_2SO_4$/EtOH | $HNO_3$ | Citric acid |
|---|---|---|---|---|---|---|
| **A1** | Light-yellow | Green-yellow | Violet | Light-yellow | Yellow | Canary |
| **A2** | Colorless | Green-yellow | Violet | Light-yellow | Light-yellow | Yellow |
| **A3** | Colorless | Green-yellow | Violet | Light-yellow | Light-yellow | Yellow |
| **A4** | Colorless | Green-yellow | Violet | Light-yellow | Light-yellow | Yellow |
| **A5** | Light-yellow | Celadon-green | Violet | Light-yellow | Canary | Canary |
| **A6** | Yellow | Intense orange | Yellow | Light-yellow | Yellow | Light-yellow |
| **A7** | Orange | Intense orange | Brown | Light-yellow | Yellow-orange | Light-orange |
| **E1** | Beige | Dark-beige | Violet | Colorless | Beige | Beige |
| **E2** | Colorless | White | Violet | Colorless | Light-yellow | Colorless |
| **B1** | Green | Yellow | Violet | Canary | Brown | Cannary |
| **B2** | Green-beige | Yellow | Violet | Canary | Green-grey | Light-yellow |
| **B3** | Green-beige | Yellow | Violet | Canary | Green-grey | Cannary |
| **B4** | Beige | Yellow | Violet | Canary | Navy blue | Light-orange |
| **B5** | Beige | Yellow | Violet | Canary | Light-yellow | Light-yellow |
| **B6** | Beige | Yellow | Violet | Canary | Green | Light-yellow |
| **B7** | Green | Blue-yellow | Violet | Canary | Light-yellow | Light-yellow |
| **B8** | Green | Blue-yellow | Violet | Canary | Light-yellow | Celadon |
| **B9** | Beige | Blue-yellow | Violet | Canary | Light-yellow | Light-yellow |
| **E3** | Grey-yellow | Beige | Violet | Grey-yellow | Colorless | Grey-yellow |
| **E4** | Grey-yellow | Blue | Violet | Grey-yellow | Colorless | Grey-yellow |
| **C1** | Orange | Orange | Yellow-orange | Vivid orange | Light-yellow | Vivid orange-brown |
| **C2** | Lemon | Yellow | Yellow | Canary | Orange-brown | Canary |
| **C3** | Lemon | Yellow | Yellow | Vivid yellow-orange | Vivid yellow-orange | Canary |
| **C4** | Lemon | Orange | Yellow | Vivid yellow-orange | Vivid orange | Yellow |
| **C5** | Lemon | Orange | Yellow-orange | Yellow | Vivid orange | Yellow |
| **C6** | Yellow-orange | Yellow | Orange | Orange | Orange | Orange |
| **C7** | Orange-brown | Yellow | Violet-yellow | Orange | Orange | Orange |
| **E5** | Colorless | Violet | Violet | Colorless | Colorless | Colorless |
| **E6** | Colorless | Blue | Violet | Colorless | Colorless | Colorless |
| **D1** | Lemon | Green | Lemon | Orange | Yellow | Orange |
| **D2** | Light-yellow | Yellow | Violet | Vivid orange-red | Pink-orange | Yellow-green |
| **D3** | Light-yellow | Green | Violet | Vivid orange | Red-orange | Orange |
| **D4** | Light-yellow | Green | Violet | Orange | Vivid orange | Yellow |
| **D5** | Light-yellow | Grey-green | Violet | Orange-brown | Orange-yellow | Yellow |
| **D6** | Green | Green | Green | Yellow | Yellow-brown | Green |
| **D7** | Light-yellow | Violet | Violet | Light-yellow | Yellow | Yellow |
| **E7** | Colorless | Violet | Violet | Colorless | Colorless | Colorless |
| **E8** | Colorless | Orange | Violet | Colorless | Colorless | Colorless |
| **F1** | Green | Grey-green | Violet | Brown | Pink | Yellow-green |
| **F2** | Light-orange | Celadon | Violet | Raspberry red | Light-pink | Pink-violet |
| **F3** | Orange | Celadon | Violet | Raspberry red | Light-pink | Pink-violet |

reagents, three best ones (the most selective) were described in Table 2:

(1) 20% solution of $H_2SO_4$ in EtOH; (2) conc. $HNO_3$ and (3) solution of citric acid (0.1 g) in acetic anhydride (5 mL).

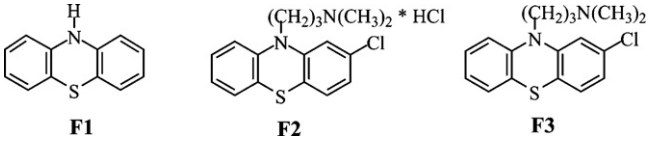
## 3. Results and discussion

The neuroleptic phenothiazines were analyzed by TLC method both as ammonium salts i.e. (dialkylammonio)alkylphenothiazine [di(alkyl)phenothiazinoalkylammonium] salts (most often as hydrochlorides) and as free amines i.e. dialkylaminoalkylphenothiazines using neutral or basic silica gels and neutral or basic solvent systems [7–12,22]. This TLC study was performed using new tri-, tetra- and pentacyclic types of azaphenothiazines **A**–**D**, the substrates and side-products **E** as free amines on neutral aluminum oxide and silica gel with neutral solvent systems. These new azaphenothiazines, 10-substituted 2,7-diazaphenothiazines **A1**–**A7**, 6-, 8-, 9- and 10-substituted quino[3,2-b]benzothiazines **B1**–**B9**, 6-substituted diquino[3,2-b;2′,3′-e]thiazines **C1**–**C7** and 14-substituted diquino[3,4-b;4′,3′-e]thiazines **D1**–**D7** possess different azaphenothiazine ring systems, different types of substituents at the thiazine nitrogen atom (hydrogen, alkyl, dialkylaminoalkyl, aryl and heteroaryl) and in a few cases additional substituents in the benzene ring (chlorine, trifluoromethyl and methylthio) [14–20]. It worth noting that some azaphenothiazines **A** and **C** exhibited significant anticancer and immunosuppressive activities [23,24].

Azaphenothiazines **A**–**D** were separated from sulfides, disulfides and dithiins **E1**–**E8** as the substrates or side-products in the synthesis of these azaphenothiazines. N-Substituted azaphenothiazines were also separated from NH-azaphenothiazines in order to follow their synthesis by the N-alkylation and N-arylation reactions (Schemes 1–4).

For each type of azaphenothiazines one simple TLC system was found which enabled desired separations (Table 1). In many cases high quality separations were observed with the factors $\Delta R_F \geq 0.50$, $R_S > 10$ and $\alpha > 10$ but sometimes the separation was insufficient. Although there is the great similarity in chemical structures between NH-azaphenothiazines **A1**, **B1**, **C1** and **D1** and heteroaromatic dithiins **E2**, **E4**, **E6** and **E8**, good separations were achieved. Good separation conditions were also found to distinguish NH-azaphenothiazines from N-substituted azaphenothiazines.

The resulting chromatographic spots were observed in the daylight, in the UV light at $\lambda = 254$ nm and 365 nm, and after spraying with visualizing reagents. Most of these spots were visible in the daylight being beige, yellow, lemon, canary, orange, green, green-beige and orange-brown (Table 2). As the precoated layers included a fluorescence indicator $F_{254}$, the spots were detected under UV light of 254 nm. In most cases violet and in some cases also yellow, yellow-orange, orange, green and brown spots were observed on the yellow-green background. The most valuable was the observation of the color change of the spots during irradiation with UV




**Scheme 5.** The structures of phenothiazines **F**.

light of 365 nm. No procedure was used to stabilize or intensify this fluorescence. As can be seen from Table 2, the color change is characteristic only for azaphenothiazines **A**–**D** but not for the substrates and side-products **E**. To widen the scope of this study, classical phenothiazines: 10*H*-phenothiazine **F1**, chlorpromazine (10-dimethylaminopropyl-2-chlorophenothiazine) as hydrochloride **F2** and free amine **F3** were also checked (Scheme 5). All compounds **F** gave color spots in the daylight, violet spots under UV light of 254 nm and the color change under UV light of 365 nm (Table 2).

The natural fluorescence of phenothiazines was rather neglected, rarely described and limited to neuroleptic drugs, i.e. phenothiazines with the dialkylaminoalkyl substituents at position 10 [8,22]. All azaphenothiazines **A**–**D** exhibit native fluorescence, despite the nature of the substituents and kind of the phenothiazine ring system, and selected classical phenothiazines **F1**–**F3**. The natural fluorescence of phenothiazines during UV irradiation can serve to distinguish phenothiazines and azaphenothiazines from other compounds.

In order to support the detection of phenothiazines and azaphenothiazines by native fluorescence, the detection of these compounds was also examined using visualizing reagents. There are not known specific visualizing reagents only for the detection of phenothiazines, containing also other groups than dialkylaminoalkyl (for example alkyl, aryl and heteroaryl). Over 20 visualizing reagents were examined including those used for neuroleptic phenothiazine (sulfuric acid, formaldehyde in sulfuric acid, 2,6-dibromoquinone-4-chloroimide, hydrogen peroxide, iron chloride in sulfuric acid and Dragendorff reagent [7,9,22,25]) and those regarded as specific for the detection of the nitrogen compounds or amines (Dragendorff reagent, sodium nitroprusside, nitric acid, citric acid, ninhydrin, ammonium metavanadate, potassium hexacyanoferrate [25–28]). Table 2 contains only three best universal visualizing reagents which detect azaphenothiazines despite their substituents: sulfuric acid in ethanol, concentrated nitric acid and citric acid in acetic anhydride. These reagents gave color spots with all azaphenothiazines **A**–**D** but not with the substrates and side-products **E**. Some specific visualizing reagents for the detection of the nitrogen compounds and amines (Dragendorff reagent, sodium nitroprusside, ninhydrin, ammonium metavanadate and potassium hexacyanoferrate) turned out unexpectedly to be nonreactive with all types of azaphenothiazines. To compare, the visualizing reagents were used also for the spots of classical phenothiazines **F1**–**F3**, giving color spots with most of visualizing reagents.

In this study, the combination of the separation (the $R_F$ values) and the spot color detection (as the native fluorescence and the results of the usage of the visualizing reagents) facilitated the identifications of new azaphenothiazines in the reaction mixtures containing also substrates and resulting side-products.

## 4. Conclusions

The simple, rapid and cheap TLC method is suitable for the detection of new modified phenothiazines (being tri-, tetra- and pentacyclic compounds with various substituents: a hydrogen atom, and alkyl, dialkylaminoalkyl, aryl and heteroaryl groups at the thiazine nitrogen atom and in a few cases with additional substituents in the benzene ring) during the synthesis. The natural fluorescence of phenothiazines and azaphenothiazines under irradiation of UV light of 365 nm is very characteristic and becomes useful additional analytical information of these compounds. This paper is the first attempt of the determination of new azaphenothiazines by TLC method preceding the identification by spectroscopic methods. It facilitates the separation of the proper fraction in column chromatography and preparative TLC and saves time and chemicals.

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