Normal and Reversed Phases Thin-Layer Chromatography of Arylidene 2-Thiohydantoin Derivatives

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Abstract: In this paper, the retention behavior of thirteen newly synthesized arylidene derivatives of 2-hydamthione was investigated by normal and reversed phases thin-layer chromatography. In normal phase chromatography, thin layers of silica gel and cyano-propyl (CN) silica gel with fluorescent indicator F_{254} were used as the stationary phase. As the mobile phase, binary systems of non-aqueous solvents benzene-ethyl acetate and hexane-ethyl acetate were used, 8:2 (*v*/*v*). Reversed phase chromatography was performed using a thin layer of octadecyl (C-18) silica gel with fluorescent indicator F_{254} as the stationary phase and a binary solvent mixture of acetonitrile-water in the ratio 7.5:2.5 (*v*/*v*) as the mobile phase. Observing under a UV lamp, the spots of all tested derivatives were clearly visible. The R_F value was determined for each spot. Based on the obtained results the HPLC technique is recommended for further chromatographic examination of the newly synthesized arylidene derivatives of 2-hydamthione, using commercial columns with polar chemically bound phases and both non-aqueous and aqueous eluents.

Keywords: arylidene 2-thiohidantoin derivatives, TLC, normal phases, reversed phases, retention behaviour

1. Introduction

2-Thiohydantoin (2-thioxoimidazoline-4-one) is a non-aromatic five-membered heterocyclic compound with a cyclic ureid core [1]. The series of 13 arylidene 2-thiohydantoin derivatives were obtained through a condensation reaction with thiosemicarbazide, utilizing a previously published two-step protocol [2]. The structures of synthesized thiohydantoin derivatives **1-13** are given in Figure 1.

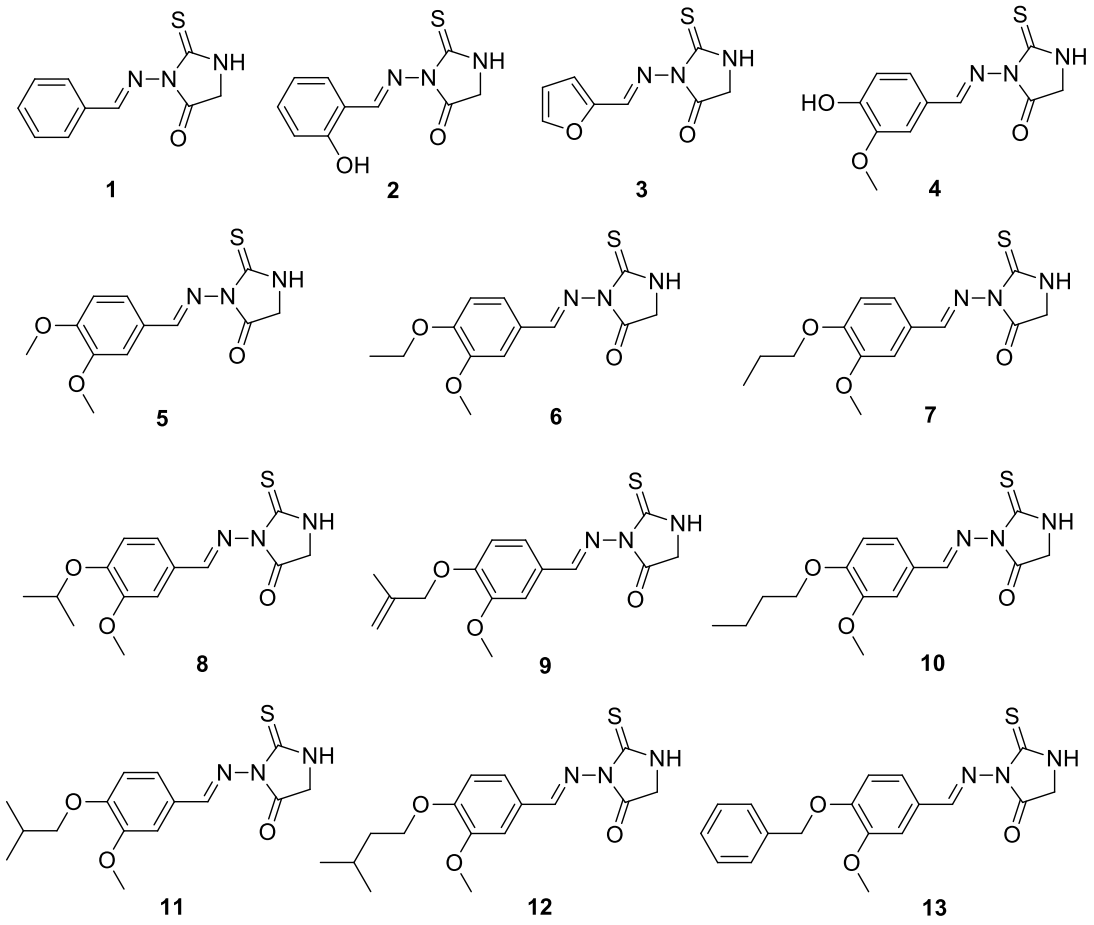

Figure 1. Structures of studied thiohydantoin derivatives **1**-**13.**

The aim of this work was to examine the possibility of chromatographic separation of newly synthesized 2-thiohydantoin derivatives, considering the different character of the functional groups that are introduced into the arylidene part of the molecule. Due to the presence of both polar and non-polar functional groups attached to the benzene ring in the molecule, the retention behavior of the newly synthesized derivatives will be examined by thin-layer chromatography on both normal and reversed chemically bonded phases.

2. Instructions

HPTLC was performed on 10x10 cm plates coated with silica gel 60, cyano propyl (CN) and octadecyl (C-18) silica gel, all with fluorescence indicator (Merck). The compounds were dissolved in acetonitrile (1 mg/ml) and 1μl volumes of the solutions spotted randomly on the plates. Silica gel and bonded CN silica gel plates were developed with non-polar mobile phases benzene-ethyl acetate and hexane-ethyl acetate, both in the ratio 8:2 (*v*/*v*).

Bonded C-18 silica gel plates with fluorescence indicator (Merck) were developed with acetonitrile-water 7.5:2.5 (*v*/*v*).
Spots were observed under UV light at λ = 254 nm and R_F values were determined for all spots , presented in Table 1.

Table 1. R_F values of investigated compounds designated as in Figure 1.

Compound	Silica gel (Bz-EtAc)	Silica gel (Hx-EtAc)	CN (Bz-EtAc)	C-18 (ACN-W)
1	0.43	0.15	0.68	front
2	0.47	0.03	0.71	front
3	0.40	0.14	0.65	front
4	0.24	0.05	0.56	front
5	0.28	0.04	0.63	front
6	0.33	0.06	0.65	front
7	0.38	0.11	0.65	front
8	0.36	0.12	0.67	front
9	0.38	0.11	0.82	front
10	0.40	0.13	0.68	front
11	0.39	0.13	0.70	front
12	0.38	0.13	0.71	front
13	0.35	0.07	0.70	front

Retention of the compounds examined on silica gel using the eluent benzene-ethyl acetate, decreases in the following order:

4 > 5 > 6 > 13 > 8 > 7 = 9 = 12 > 11 > 3 = 10 > 1 > 2;

while using the eluent hexane-ethyl acetate, decreases in the following order:

2 > 5 > 4 > 6 > 13 > 7 = 9 > 8 > 10 = 11 = 12 > 15 > 1.

By c omparing these two mobile phases it is noticeable that the retention of all examined derivatives on the silica gel is significantly lower if benzene is used as a diluent. Due to the presence of the benzene ring in the molecules, all examined derivatives are more soluble in benzene than in hexane, so the retention is logically lower.
If compared to silica gel, the retention of all examined compounds on CN silica gel is significantly lower using benzene-ethyl acetate as a mobile phase. The retention of the compounds decreases in the following order:

4 > 5 > 3 = 6 = 7 > 8 > 1 = 10 > 11 = 13 > 2 = 12 > 9.

Based on the obtained results it can be concluded that complete separation of the examined derivatives was not achieved on silica gel nor CN silica gel stationary phases. These results are in accordance with previously published results in normal phase chromatography [3,4].
Although the reversed phase (on C-18 and/or C-8 silica gels) is used in 80% of chromatographic analysis, it is not applicable in this case. Namely, the compounds were examined on C-18 silica gel with acetonitrile-water as an eluent, all compounds were eluting with the front of the mobile phase. This is a consequence of the high polarity of

these newly synthesized compounds, so the application of reverse phase chromatography is not recommended for further chromatographic behaviour examination of arylidene derivatives of 2-hydamthione. These results are also in accordance with previously published results [3,4].

3. Conclusions

Based on the obtained results the HPLC technique is recommended for further chromatographic examination of the newly synthesized arylidene 2-thiohydantione derivatives, using commercial columns with polar chemically bound phases and both non-aqueous and aqueous eluents [5,6]. By choosing the appropriate column and mobile phase, it is expected to achieve a complete separation of the newly synthesized arylidene 2-thiohydantione derivatives. Followed by successful chromatographic separation, the corresponding retention constants will be determined and applied in further interpretations of the retention behavior of the examined arylidene 2-thiohydantione derivatives and their biological activity.

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References

[1] M.A. Metwally, E. Abdel-Latif, *Thiohydantoins: synthetic strategies and chemical reactions*, Journal of Sulfur Chemistry 33 (2012) 229-257.

[2] B. Šmit, R.Z. Pavlović, A. Radosavljević-Mihailović, A. Došen, M.G. Ćurčić, D.S. Šeklić, M.N. Živanović, *Synthesis, characterization and cytotoxicity of palladium(II) complex of 3-[(2-hydroxy-benzyilydene)-amino]-2-thioxo-imidazolidin-4-one*, Journal of the Serbian Chemical Society 78 (2013) 217-227.

[3] M.M. Ačanski, *Tečna hromatografija derivata estradiola i estrona-monografija*, Tehnološki fakultet, Univerzitet u Novom Sadu, Novi Sad (2007).

[4] M.M. Ačanski, *Tečna hromatografija derivata benzimidazola*, Tehnološki fakultet, Univerzitet u Novom Sadu, Novi Sad (2010).

[5] M. Ilić, M. Ačanski, K. Pastor, Lj. Popović, S. Jovanović-Šanta, *New challenge in the lipophilicity determination and separation of biologically active 16,17-secoesterone derivatives by HPLC – Use of pentafluorophenyl-propyl column*, Journal of Liquid Chromatography and Related Technologies 43 (2020) 106-117.

[6] M. Ilić, K. Pastor, J. Marković, Lj. Grbović, S. Jovanović-Šanta, I. Mitrović, Z. Trivunović, M. Ačanski, *Feasibility study of separation and purification of bile acid derivatives by HPLC on C18 and F5 columns*, Steroids 186 (2022) 109074.