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MAŁGORZATA DOŁOWY

Investigation of identity of dehydrocholic acid in selected pharmaceutical formulations with the use of NP- TLC method

Badanie tożsamości kwasu dehydrocholowego w wybranych preparatach farmaceutycznych metodą NP- TLC

One of the steps which is necessary in quality control of a drug is determination of its identity. Besides the colored reactions and different physicochemical properties to confirm the identity of drugs, chromatographic methods are applied such as: high performance liquid chromatography (HPLC) and thin–layer chromatography (TLC) [1, 2, 5, 6].

Not extensive literature concerning examination of dehydrocholic acid as an active ingredient in selected pharmaceutical formulations causes that the aim of this work was to describe the optimal NP-TLC conditions which allowed for effective and rapid identification of dehydrocholic acid in respective pharmaceuticals, e.g. Raphacholin C and Raphacholin Forte containing dehydrocholic acid in the quantity of 40 mg/tablet and 250 mg/tablet, respectively. Both commercial products of dehydrocholic acid are usually used in treating the liver and bile diseases [3]. The elaborated NP-TLC method can be helpful in routine quality control of dehydrocholic acid preparations.

MATERIALS AND REAGENTS

Chemicals. The following solvents: n-hexane, ethyl acetate, acetic acid, chloroform, acetone (POCh, Gliwice, Poland) were used as the components of the mobile phases used. Methanol (POCh, Gliwice, Poland) was applied for the preparation of stock solution of dehydrocholic acid. Sulfuric acid, 95% (POCh, Gliwice, Poland) was used to prepare a visualizing agent. Pure substance: dehydrocholic acid was from Sigma–Aldrich (St. Louis, MO, USA, No. D3750-25G). Pharmaceutical preparations containing dehydrocholic acid: Raphacholin Forte 250 mg, Raphacholin C 40 mg were from Herbapol (Wrocław, Poland). All reagents used were analytical grade.

TLC plates. TLC analysis was performed on chromatographic plates from E. Merck (Germany) precoated with silica gel $60F_{254}$ and silica gel 60 without concentrating zone: Art. 1. 05715, Art. 1.05554, Art. 1.05553 and also on chromatographic plates precoated with silica gel $60F_{254}$ with concentrating zone Art. 1.05583.

METHODS

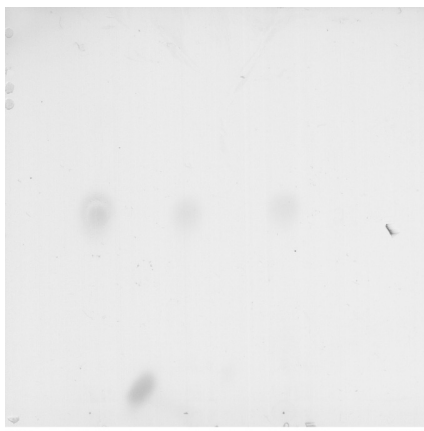
Preparation of standard solution. Stock solution of dehydrocholic acid (at concentration 5 mg/mL) was prepared by diluting accurately weighed 50 mg of dehydrocholic acid in 10 mL of methanol.

Preparation of samples. To prepare the extracts of dehydrocholic acid from drugs such as Raphacholin Forte and Raphacholin C, the tablets of both preparations were powdered. Then dehydrocholic acid from one powdered tablet of Raphacholin Forte was extracted with the use of 20 mL of methanol. In the case of Raphacholin C powder from two tablets was extracted using 10 mL of the solvent. The time of extraction was 10 minutes and allowed for complete dilution of all ingredients presented in tablets. Next the obtained extracts containing dehydrocholic acid were centrifuged and filtered. After filtration the samples of extracts were diluted to the concentration of 5 mg/mL of dehydrocholic acid in both extracts.

TLC analysis. TLC was performed on chromatographic plates 10cm×10cm. Before use, the plates were activated for 30 minutes at 120°C. Standard solution of dehydrocholic acid and extracts were applied on chromatographic plates in the quantity of 2 μL with the use of micropipettes. The chromatograms were developed at temperature 18±1°C in a classical chamber 20cm×20cm (Camag) using 50 mL of mobile phase. In each case, the chromatographic chamber was saturated with the solvent for 30 minutes before analysis. The development distance was 8 cm. Next, the plates were dried at room temperature using a fume cupboard. The examined dehydrocholic acid was detected by spraying the plates with the use of 10% sulfuric acid and heating them at 120°C for 15 minutes. On the basis of chromatograms, the R_F values (retention factor) of the spots were calculated. To confirm the identity of dehydrocholic acid in extracts of Raphacholin Forte and Raphacholin C, the R_F values of extract spots were compared with R_F value of dehydrocholic acid standard solution.

RESULTS AND DISCUSSION

After development of the chromatograms and visualization of spots of dehydrocholic acid with the use of 10% sulfuric acid as described above, the R_F values of dehydrocholic acid spots from standard solution and extracts of two examined drugs were measured. The R_F values obtained for standard solution of dehydrocholic acid and extracts of its drugs are evidently identical regardless of chromatographic conditions used. Table 1 and 2 present R_F values of dehydrocholic acid obtained under all chromatographic conditions. The excipients in both drugs did not interfere with spots of examined dehydrocholic acid (Fig. 1).



DH – dehydrocholic acid (standard solution), RPC – extract from Raphacholin C, RPF – extract from Raphacholin Forte

Fig. 1. TLC chromatogram of dehydrocholic acid examined on glass plates precoated with silica gel $60F_{254}$ (Art. 1.05715) with the use of mixture n-hexane –ethyl acetate –acetic acid in volume composition 22:22:6 as mobile phase at 18°C

Table 1 shows the results of identification of dehydrocholic acid on aluminum plates precoated with silica gel $60F_{254}$ and silica gel 60 (E. Merck, Art. 1.05554, Art. 1.05553) and on glass plates precoated with silica gel $60F_{254}$ (E. Merck, Art. 1.05715) developed with the use of mobile phase: n-hexane –ethyl acetate –acetic acid in the following volume compositions: 36:13.5:0.5, 25:25:0, 25:23:2, 25:20:5, 23:25:2, 22:22:6, 21:21:8, 20:25:5 and 20:20:10 (v/v). Table 2 presents the R_F values of dehydrocholic acid measured on the aluminum plates precoated with silica gel $60F_{254}$ and silica gel 60 without concentrating zone (E. Merck, Art. 1.05554 and Art. 1.05553) and also on aluminum plates precoated with silica gel $60F_{254}$ with concentrating zone (E. Merck, Art. 1.05583). A mixture of chloroform- acetone- acetic acid was used as a mobile phase in the volume compositions: 40:10:0, 40:8:2, 35:10:5, 25:23:2, 25:20:5, 20:20:10 and 20:25:5 (v/v). The R_F values measured in all cases are mean of five results.

Table 1. R_F values of dehydrocholic acids obtained from different chromatographic plates precoated with silica gel E. Merck (Art. 1.05715, Art. 1.05554 and Art. 1.05553) with the use of n-hexane – ethyl acetate –acetic acid in different volume compositions as mobile phases

N-hexane –ethyl acetate –acetic acid in volume composition (v/v)	Art. 1.05715	Art. 1.05554	Art. 1.05553
36:13.5:0.5	0.05	0.04	0.04
25:25:0	0.10	0.05	0.08
25:23:2	0.29	0.35	0.30
25:20:5	0.34	0.43	0.36
23:25:2	0.32	0.48	0.38
22:22:6	0.48	0.60	0.53
21:21:8	0.54	0.69	0.62
20:25:5	0.53	0.55	0.53
20:20:10	0.64	0.62	0.64

Table 2. R_F values of dehydrocholic acids obtained from different chromatographic plates precoated with silica gel E. Merck (Art. 1.05715, Art. 1.05554 and Art. 1.05583) with the use of chloroform –acetone –acetic acid in different volume compositions as mobile phases

Chloroform – acetone –acetic acid in volume composition (v/v)	Art. 1.05715	Art. 1.05554	Art. 1.05583
40:10:0	0.26	0.19	0.17
40:8:2	0.57	0.67	0.89
35:10:5	0.80	0.88	0.99
25:23:2	0.81	0.89	0.99
25:20:5	0.91	0.99	0.99
20:20:10	0.95	0.98	0.99
20:25:5	0.92	0.92	0.98

For determination of optimum chromatographic condition which allowed to detect dehydrocholic acid in extracts from the both pharmaceutical preparations Raphacholin Forte and Raphacholin C, the R_F values obtained with the use of n-hexane – ethyl acetate – acetic acid and chloroform-acetone –acetic acid on all applied plates were performed in the form of a relationship type: $R_F = f(\varphi)$, where φ is the volume composition of mobile phase used (Fig. 2 and Fig. 3).

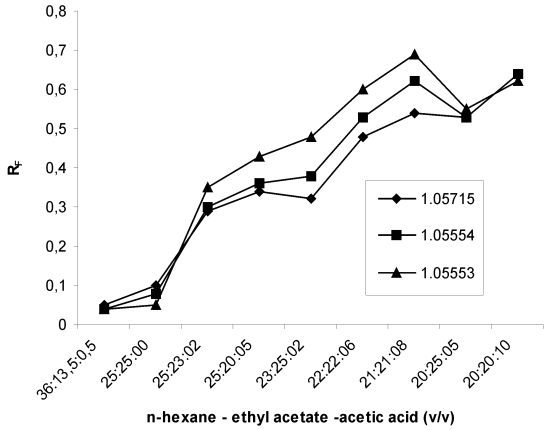


Fig. 2. The relationship between R_F values of dehydrocholic acid determined on different chromatographic plates (Art. 1.05715, Art. 1.05554, Art. 1.05553) and volume composition of mobile phase used n-hexane –ethyl acetate –acetic acid

On the basis of Fig. 2 it can be observed that of nine volume compositions of mobile phase used: n-hexane – ethyl acetate – acetic acid, the mixture in volume compositions: 25:20:5, 23:25:2 and 22:22:6 are optimal because they allowed to obtain R_F of examined dehydrocholic acid in the range 0.3-0.6 [4] on all applied chromatographic plates. In the case of the relationship between R_F values measured on the plates: Art. 1.05554, Art. 1.05553 and Art. 1.05583 and volume compositions of mobile phase: chloroform – acetone – acetic acid (Fig. 3.) it was stated that the best mobile phase for identification of dehydrocholic acid on chromatographic plates without concentrating zone (Art. 1.05554 and Art. 1.05553) is chloroform – acetone – acetic acid mixture in volume composition 40:8:2. The chromatographic plates with concentrating zone (Art. 1.05583) are not effective for this analysis because regardless of the applied volume composition of the mobile phase used, the R_F value is near 1 or below 0.2 like in the case of mobile phase 40:10:0. The spots obtained on this type of plates are elongates and difficult to identify.

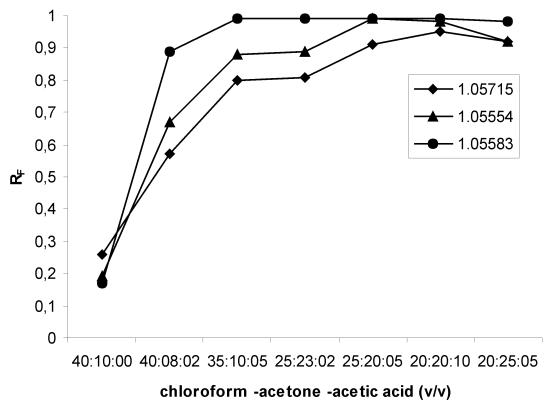


Fig. 3. The relationship between R_F values of dehydrocholic acid determined on different chromatographic plates (Art. 1.05715, Art. 1.05554, Art. 1.05583) and volume composition of mobile phase used chloroform –acetone –acetic acid

All R_F values were compared by means of cluster analysis (CA) – Fig. 4 and Fig. 5. Both dendrograms indicate the biggest similarity between R_F values obtained on the plates precoted with silica gel $60F_{254}$ such as Art. 1.05715 and Art. 1.05554 regardless the mobile phase applied.

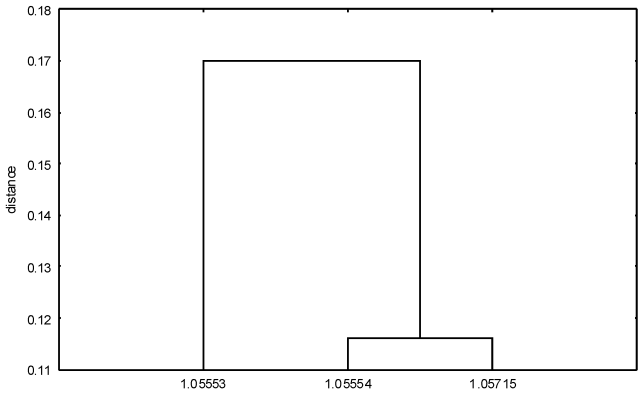


Fig. 4. Dendrogram of cluster analysis of R_F values of dehydrocholic acid examined on different chromatographic plates (Art. 1.05715, Art. 1.05554, Art. 1.05553) developed with the mixture n-hexane –ethyl acetate –acetic acid in various volume compositions

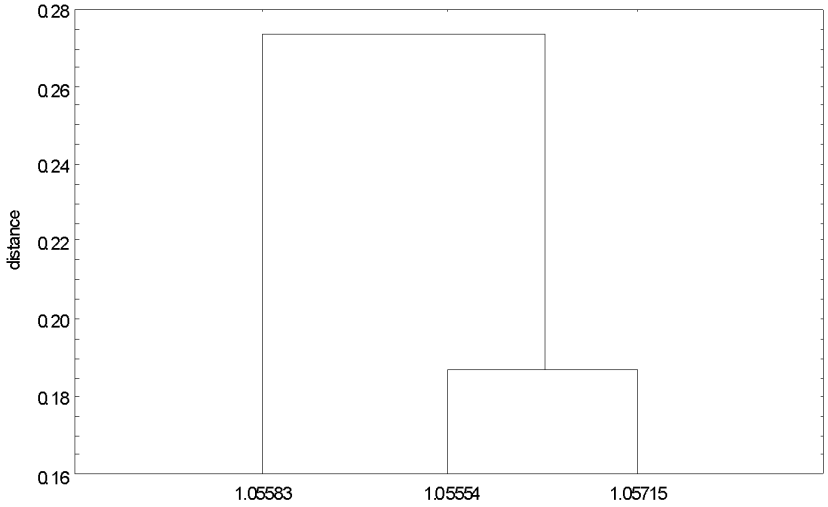


Fig. 5. Dendrogram of cluster analysis of R_F values of dehydrocholic acid examined on different chromatographic plates (Art. 1.05715, Art. 1.05554, Art. 1.05583) developed with the mixture chloroform –acetone –acetic acid in various volume compositions

The results of cluster analysis show that dendrograms are useful in predicting the effect of TLC analysis of dehydrocholic acids in its preparations.

CONCLUSIONS

On the basis of the obtained results it can be concluded that:

1. Thin–layer chromatography in normal phase system (NP-TLC) can be used to identity dehydrocholic acid as an active ingredient in selected pharmaceutical formulations such as Raphacholin C and Raphacholin Forte.

2. Of all applied chromatographic conditions, the best of them which allow for qualitative analysis of dehydrocholic acids in pharmaceutical formulations are • the mixture of n-hexane – ethyl acetate –acetic acid in the following volume compositions: 25:20:5, 23:25:2, 22:22:6 (v/v) as mobile phases and chromatographic plates precoated with silica gel $60F_{254}$ and silica gel 60 (Art. 1.05715, Art. 1.05554 and 1.05553) • the mixture of chloroform-acetone–acetic acid in volume compositions 40:8:2 (v/v) and chromatographic plates precoated with silica gel $60F_{254}$ (Art. 1.05715 and Art. 1.05554).

Under these chromatographic conditions, the optimal R_F values of dehydrocholic acid were observed.

3. The excipients presented in both pharmaceutical formulations did not influence the NP- TLC analysis of dehydrocholic acid in pharmaceutical formulations.

4. Cluster analysis is useful in prediction the effect of TLC analysis of dehydrocholic acid in its pharmaceuticals.

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SUMMARY

In the present work, application of NP-TLC method for determination of the identity of dehydrocholic acid in selected pharmaceuticals containing dehydrocholic acid such as: Raphacholic C and Raphacholin Forte was estimated. Dehydrocholic acid was extracted from pharmaceutical formulations using methanol. Of all applied chromatographic conditions the best which allowed for obtaining optimal R_F values of dehydrocholic acid are: the mixture of n-hexane–ethyl acetate–acetic acid in the following volume compositions: 25:20:5, 23:25:2, 22:22:6 as the mobile phases used and

the chromatographic plates (E. Merck, Art. 1.05715, Art. 1.05554, Art.1.05553) and also chloroform-acetone –acetic acid in volume composition 40:8:2 and chromatographic plates (E. Merck, Art. 1.05715 and Art. 1.05554). On the basis of the obtained results it was stated that the elaborated NP-TLC method can be used in routine control of the quality of dehydrocholic acid pharmaceuticals.

STRESZCZENIE

W pracy oceniono możliwość zastosowania metody NP-TLC do badania tożsamości kwasu dehydrocholowego w wybranych preparatach farmaceutycznych, takich jak Raphacholin C i Raphacholin Forte. Kwas dehydrocholowy był ekstrahowany z preparatów za pomocą metanolu. Spośród zastosowanych warunków chromatograficznych za najlepsze, pozwalające uzyskać optymalne wartości R_F dla kwasu dehydrocholowego, uznano: mieszaninę n-heksan–octan etylu–kwas octowy w następujących stosunkach objętościowych: 25:20:5, 23:25:2, 22:22:6 jako fazę ruchomą i płytki chromatograficzne (E. Merck, Art. 1.05715, Art. 1.05554, Art.1.05553) oraz chloroform–aceton–kwas octowy w stosunku objętościowym 40:8:2 i płytki chromatograficzne (E. Merck, Art. 1.05715 i Art. 1.05554). Na podstawie otrzymanych wyników stwierdzono, że opracowana metoda NP -TLC może być użyta w rutynowej kontroli jakości preparatów kwasu dehydrocholowego.