

Chromatographic separation of oleanolic, ursolic and betulinic acid in plant extracts on aminopropyl silica using aqueous mobile phases

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ABSTRACT

Triterpenic acids are important group of plant secondary metabolites, which affect the biological activity of many medicinal plants and herbs. In the present paper, the applicability of aminopropyl silica using aqueous mobile phases for the separation of oleanolic, ursolic and betulinic acid was investigated. The mixtures of water with: acetone, methanol, acetonitrile, isopropanol and tetrahydrofuran in various ratios, were tested as eluents. The best results were obtained for mixture of water in acetone (10:90, v/v) with addition of 0.05% ammonia solution. The chromatographic system was used for qualitative analysis of triterpenic acids in *Salvia officinalis* L., *Origanum vulgare* L. and *Syzygium aromaticum* (L.) Merrill.

Keywords: oleanolic acid, ursolic acid, betulinic acid, aminopropyl silica

INTRODUCTION

Triterpenic acids are important group of plant secondary metabolites. They are widespread constituents of many medicinal herbs and affect their biological activity. There are many plants containing ursolic (UA), oleanolic (OA) and betulinic (BA) acid commonly employed in folk medicine, e.g. *Salvia officinalis* L. (*Lamiaceae*). Owing to high content of ursolic acid it is traditionally used as an anti-inflammatory remedy [2,3].

Nowadays, these triterpenes are the subject of many scientific research studies due to the wide range of pharmacological activities. There are numerous data on their anti-inflammatory, hepatoprotective, antitumor, antimicrobial, antifungal, anti-ulcer and gastroprotective, properties [5,8].

Chromatographic methods such as: high performance liquid chromatography (HPLC) [7,10,12,14,15] or gas chromatography [4,6] are the most often used for triterpenes investigations, both in plants and herbal preparations. Thin layer chromatography (TLC) on silica or on silica modified of alkyl groups with the use of organic and aqueous mobile phases was also employed [1,9,11,13,18].

Owing to the closeness of chemical structures of oleanolic and ursolic acid, their direct TLC separation is difficult. However, modern TLC is especially useful technique to analyse plant material because no sample pretreatment is required.

In the present work, the usefulness of TLC on aminopropyl silica to the separation of oleanolic, ursolic and betulinic acid was studied.

MATERIALS AND METHODS

Chemicals and standards. All solvents and reagents were pro analysis grade from Polish Reagents (POCh, Gliwice, Poland). Triterpenic acids standards were purchased from Sigma (St. Louis, MO, USA). Chromatographic HPTLC NH₂F₂₅₄ plates were from Merck (Darmstadt, Germany). *Salvia officinalis* L. (Synoptis Pharma, Poland), *Syzygium aromaticum* (L.) Merrill. (Prymat, Poland) and *Origanum vulgare* L. (Prymat, Poland) were obtained from local market.

Sample and standard preparation. Standard solutions of oleanolic, ursolic and betulinic acid at concentration 0.1 mg/ml were prepared in methanol.

Plant material was pulverized, accurately weighted (2.00 g) and extracted with methanol (2 x 50 mL) in ultrasonic bath (2 x 15 min) at 30°C. Next, each obtained

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extract was filtered, concentrated by evaporation of solvent under vacuum and transferred into a volumetric flask. Finally, the volume was made up with the extraction solvent to 100 mL for *S. officinalis*, 20 mL for *S. aromaticum* and *O. vulgare*.

Chromatography. TLC was performed on HPTLC NH_2 F₂₅₄ plates. Chromatograms were developed in horizontal Teflon DS chambers (Chromdes, Lublin, Poland). In our research, the retention of oleanolic, ursolic and betulinic acids was investigated with the use of aqueous eluents. The mixtures of water in concentrations 5%, 10%, 15% and 20% (v/v) with: acetone, methanol, acetonitrile, isopropanol or tetrahydrofuran were tested. The plates were sprayed with 10% sulfuric acid in methanol, dried and then heated to 120°C for 3 min. After derivatization the chromatograms were observed in daylight or in UV light at $\lambda = 366$ nm.

RESULTS AND DISCUSSION

Oleanolic, ursolic and betulinic acids are important, natural compounds with confirmed biological activity. They often occur together in many medicinal plants and herbs [6].

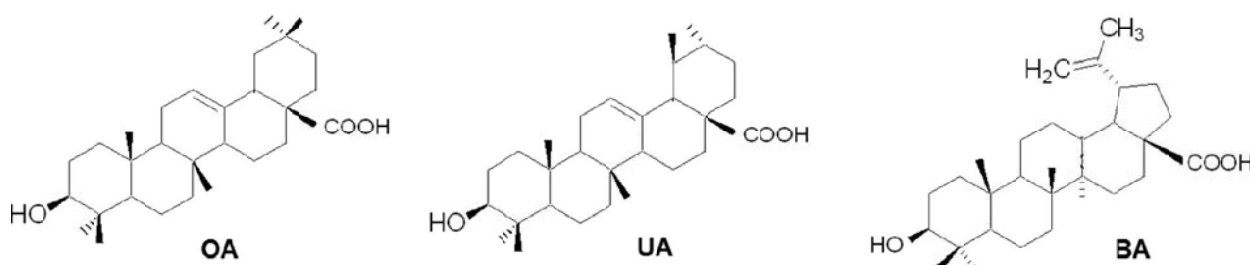


Fig. 1. Chemical structure of oleanolic, ursolic and betulinic acid.

Their separation is still a challenge in thin-layer chromatography, especially oleanolic and ursolic acid because they are closely related isomers differing in the position of one methyl group (Fig.1). In TLC different adsorbents including silica gel F₂₅₄ [1,13,16,17], two types of oktadecyl silica C18 RP and RP-18s and RP-2 plates [11,18] were employed to investigation of these triterpenes. However, none of chromatographic systems enables satisfactory resolution of this critical pair of isomers without prechromatographic derivatization [11,16].

Usually, in chromatography of acidic compounds, the addition of small amount of weak organic acids to the mobile phases is necessary to improve the shape of chromatographic bands. However, formic and acetic acids cause the strong demixtion effect on this type of adsorbent; thus in our experiments we used ammonia as a component of all eluents to obtain more compact spots. The examples of obtained results for different mobile phase compositions are presented in Table 1. In most investigated chromatographic system, betulinic acid was

well separated and had the highest hR_F values but hR_F values of oleanolic and ursolic acids are similar. In some cases, small differences useful to qualitative investigation are observed.

Table 1. The examples of chromatographic systems

Mobile phase composition	UA (hR_F)	OA (hR_F)	BA (hR_F)
Acetone/water (90:10 v/v)	28.0	30.5	36.6
Acetone/water (85:15 v/v)	61.6	60.5	67.4
Methanol/water (90:10 v/v)	71.8	73.0	73.0
Isopropanol/water (90:10 v/v)	4.5	5.7	6.9
Tetrahydrofuran/water (95:5 v/v)	18.4	16.1	20.7
Acetonitrile/water (90:10 v/v)	36.6	39.3	47.2

* All mobile phases contained 0.05% of ammonia solution.

The best results were obtained for mobile phases containing acetone. Figure 2 presents the relationships between hR_F values of investigated triterpenes and concentration of water in acetone.

The best chromatographic system (10% water in acetone with addition of 0.05% ammonia) was applied to qualitative analysis of triterpenic acids in plant materials e.g.: *S. officinalis*, *S. aromaticum* and *O. vulgare* (Fig. 3). The presence of oleanolic and ursolic acid was observed in all investigated extracts however betulinic acid was found only in *S. aromaticum*. Although the proposed

chromatographic conditions are not suitable for quantitative analysis of oleanolic and ursolic acids because their resolution is insufficient but presented method can be useful for their identification.

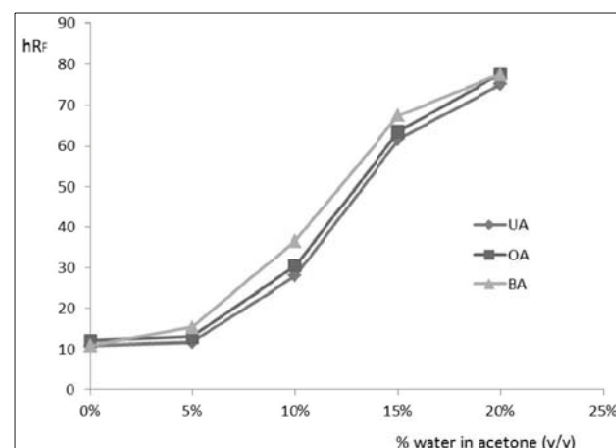


Fig. 2. Relationships between hR_F values and concentration of water in acetone

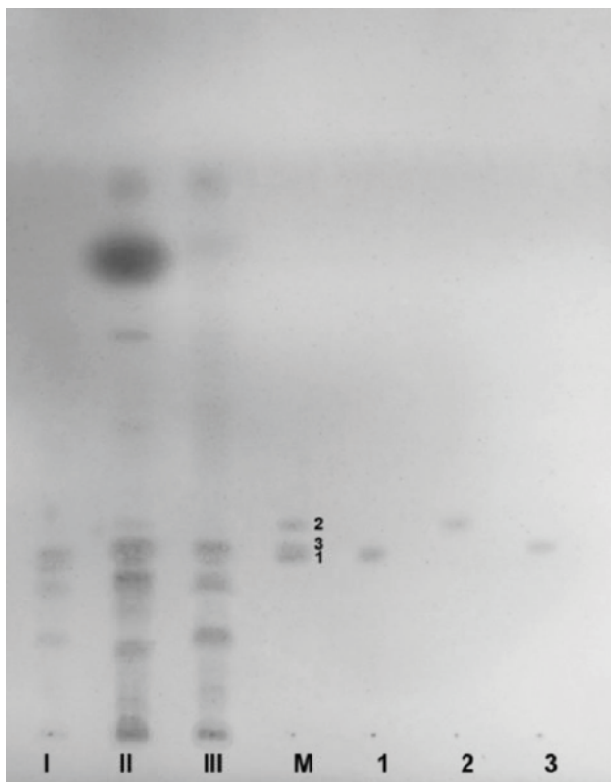


Fig. 3. The photograph of HPTLC plate: I – *Salvia officinalis* L.; II – *Syzygium aromaticum* (L.) Merrill.; III – *Origanum vulgare* L. M – mixture of standards: 1 – ursolic acid, 2 – betulinic acid, 3 – oleanolic acid. Mobile phase composition: acetone/water/ammonia (90:10:0.05 v/v/v).

CONCLUSION

Silica modified with aminopropyl groups was tested for separation of oleanolic, ursolic and betulinic acid with the use of aqueous mobile phases. The addition of small amount of ammonia solution turned out to be necessary to improve the shape of chromatographic bands. The best results were obtained for eluents consisting of 10% or 15% water and acetone. Betulinic acid was well separated but hR_F values of oleanolic and ursolic acids differed only slightly.

However, the presented chromatographic system can be useful for screening analysis of triterpenic acids content in plant material.

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