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Application of targeted 2D planar chromatography in the control of ginkgolic acids in some herbal drugs and dietary supplements

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Two-step targeted 2D planar chromatographic method (2D-TLC) was used in the determination of ginkgolic acids in pharmaceuticals and dietary supplements. The choice of the extraction method and the separation technique was guided by the formulation type (capsule, tablet, tincture) with expected low amounts of ginkgolic acids in the analyzed herbal samples. Separation of ginkgolic acids C15:1 and C17:1 on HPTLC RP18 WF_{254s} was preceded by its separation from the sample matrix on TLC Si60 F_{254s}. Mobile phases consisted of acetonitrile/water/formic acid (80:20:1, *V*/*V*/*V*) and *n*-heptane/ethyl acetate/formic acid (20:30:1, *V*/*V*/*V*), resp. Identification of separated compounds was based on 2D-TLC co-chromatography with reference substances and off-line 2D-TLC x HPLC-DAD-ESI-MS analysis. Quantification of ginkgolic acids C15:1 and C17:1 was conducted densitometrically. Among the analyzed products, the presence of ginkgolic acids was confirmed only in herbal drugs containing 60 % ethanolic tinctures of *Ginkgo biloba* leaves. The use of TLC in the quantification of ginkgolic acids C15:1 and C17:1 in ginkgo extracts was described for the first time.

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Alkylphenols present in *Ginkgo biloba* L. (also known as ginkgolic acids) belong to a group of 2-hydroxy-6-alkylbenzoic acid derivatives. Their structures differ in the length of the alkyl chain, the number and the location of double bonds. The main ginkgolic acids, C13:0, C15:1 and C17:2, have been determined both in nutshells and leaves of *Ginkgo biloba* (1–7). The content of alkylphenols in ginkgo leaves can reach up to 100,000 μg g^{-1}.

Ginkgolic acids are considered undesirable compounds of ginkgo leaves extract because of their proven cytotoxic, mutagenic, neurotoxic, hepatotoxic and nephrotoxic activities (8-15). Allergenic properties of *Ginkgo biloba* leaves extract are also ascribed to the

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presence of these compounds (16). However, studies conducted by Satyan *et al*. (16) pointed out that, as long as the carboxyl group is present in the structure of ginkgolic acid conjugates, these compounds have no allergenic properties and, on the contrary, exert anti--allergic activity. Moreover, studies conducted on rats had proven that some ginkgolic acid conjugates (namely, *n*-tridecyl-, *n*-pentadecyl-, *n*-heptadecyl-, *n*-pentadecenyl- and *n*-heptadecenylsalicylates) were responsible for anxiolytic activities of ginkgo leaves extracts (16). Other studies highlighted ginkgolic acids as candidates for antitumour drugs because of their ability to induce apoptosis and inhibit cell cycle progression, protein SUMOylation and metastasis (17–19).

In order to reduce the side-effects of ginkgolic acids, it has been established that the concentration in pharmaceuticals should not exceed 5 μg g^{-1} (20, 21). Qualitative and quantitative analyses of ginkgolic acids in extracts and products are mostly conducted with the use of high-performance liquid chromatography (HPLC) that is considered more sensitive and accurate than thin-layer chromatography (TLC) (22–25). However, it has been shown that the development of TLC analysis allows the use of TLC interchangeably with HPLC both in qualitative and quantitative surveys (26, 27).

Chromatographic conditions enabling TLC separation and the identification of the main ingredients responsible for therapeutic properties (flavonoids and terpene lactones) of ginkgo extract were described in *European Pharmacopoeia* (28), but also in several published articles (20, 21). Separation of ginkgolic acids with the use of TLC in herbal extracts has not been described so far.

The main obstacle in the determination of the composition of herbal extracts is the complex nature that hinders the separation and the determination of single components. Therefore, more and more sophisticated procedures, including multidimensional separation techniques, were employed (29, 30). Among them, targeted 2D planar chromatography (2D-TLC), focused on the separation of a single compound or a group of compounds, is the most selective (29). It enables the use of a wide range of adsorbents and also incorporates both concentrating and purification within a single separation of the analyzed sample (29). In this technique, a part of the plate containing the partly separated compound is cut off and the compound is transferred to the second stationary phase for subsequent analysis. The separation can be performed on one or different adsorbents (29–32). If the separation is carried out on different adsorbents, both bilayer plates (33) or graft TLC techniques (34) can be used. Unless the number of types of bilayer TLC plates available on the market is limited, graft TLC allows any types of adsorbents to be combined and thus appears to be the most useful (29–32, 35).

In the present study, qualitative and quantitative analyses of the main ginkgolic acids (C15:1 and C17:1) in four drugs and four dietary supplements were performed for the first time with the use of TLC. Targeted 2D planar chromatography, along with HPLC-DAD-ESI-MS analysis, was employed for the determination of ginkgolic acids in the analyzed products.

EXPERIMENTAL

Chemicals

Analytical grade solvents were purchased from POCh, Poland (methanol, acetone, ethyl acetate, and ethanol 96 %), Merck, Germany (*n*-heptane, acetonitrile, tetrahydrofuran, and formic acid). Deionized water was obtained using the Elix/Synergy system (Merck-

-Millipore, USA). The standards of ginkgolic acid C15:1 and ginkgolic acid C17:1 were purchased from Merck GmbH (Germany). Stock solutions (1 μg μL^{-1}) of reference substances were prepared in methanol and stored at –20 °C.

Plant material and herbal products

The analyzed group of herbal products included liquid (60 % ethanolic tinctures of *Ginkgo biloba* leaves) and solid formulations, tablets and hard capsules, containing dry extracts of ginkgo leaves (Table I). Herbal drugs and dietary supplements were purchased from the pharmacies.

Table I. The analyzed herbal products

Symbol	Drug form	Content of dry ginkgo extract (mg in a tablet or a capsule)	Status
D1	Hard caps*ule*	80	Drug
D2	Tablet	114	Drug
S1	Tablet	60	Supplement
S2	Tablet	80	Supplement
S3	Hard capsule	80	Supplement
S4	Tablet	40	Supplement
T1	Tincture	60 % ethanolic extract of *G. biloba* leaves	Drug
T2	Tincture	60 % ethanolic extract of *G. biloba* leaves	Drug

Sample preparation

Five mL of the analyzed tinctures (T1, T2), equaling to 4.675 g or 4.525 g of *Ginkgo* leaves, resp. (Table I), were partitioned with 20 mL hexane in an Erlenmeyer flask on a magnetic stirrer at room temperature for 15 min. Fifteen mL of the hexane layer was collected. The solvent was evaporated under reduced pressure at room temperature. The residue, corresponding to 3.506 g (T1) or 3.394 g (T2) of plant substance, was dissolved in 2 mL of methanol and stored in a freezer at –20 °C.

Five tablets or opened hard capsules (Table I) were weighed and dispersed in 20 mL of water on a magnetic stirrer for 30 minutes. The suspension was partitioned with 50 mL of hexane. Forty-five mL of the hexane layer was collected and the solvent was evaporated under reduced pressure at room temperature. The dry residue was dissolved in 2 mL of methanol. The samples were stored in a freezer at –20 °C.

Optimization of chromatographic conditions

Preliminary separations were performed on silica TLC glass plates (TLC Si60 F_{254S}, Merck, Germany) or silica gel modified with octadecylsilane groups (HPTLC RP18 WF_{254s}, Merck). Mobile phases consisting of ethyl acetate, *n*-heptane, acetone, formic acid, acetic acid and

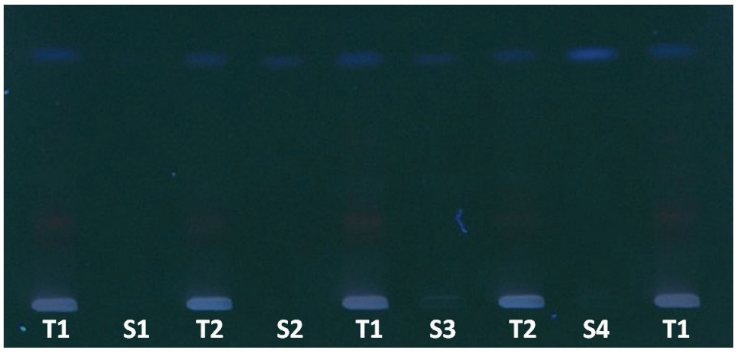
Table II. Mobile phases tested in the study

Code	Composition of mobile phases tested on the TLC Si60 F_{254s} plate[a]	Code	Composition of mobile phases tested on the HPTLC RP18 WF_{254s} plate
NP1	ethyl acetate/water/acetic acid/formic acid (67.5:17.5:7.5:7.5)	RP1	acetonitrile/formic acid (100:1)
NP2	*n*-heptane	RP2	acetonitrile/water/formic acid (90:10:1)
NP3	*n*-heptane/formic acid (10:0.1)	RP3	acetonitrile/water/formic acid (80:20:1)
NP4	*n*-heptane/etyl acetate/formic acid (9:1:0.1)	RP4	acetonitrile/water/formic acid (70:30:1)
NP5	*n*-heptane/ethyl acetate/formic acid (8:2:0.1)	RP5	acetonitrile:water/formic acid (60:40:1)
NP6	*n*-heptane/ethyl acetate/acetone/formic acid (20:10:10:1.2)	RP6	acetonitrile:water/formic acid (80:20:0.5)
NP7	*n*-heptane/ethyl acetate/formic acid (20:20:1.2)	RP7	tetrahydrofuran/water/formic acid (90:10:2)
NP8	*n*-heptane/acetone/formic acid (20:20:1.2)	RP8	tetrahydrofuran/water/formic acid (80:20:2)
NP9	*n*-heptane/ethyl acetate:formic acid (30:20:1.2)	RP9	tetrahydrofuran/water/formic acid (60:40:2)
NP10	*n*-heptane/ethyl acetate/formic acid (20:30:1.2)	RP10	tetrahydrofuran/water/formic acid (70:30:2)
NP11	*n*-heptane/ethyl acetate/formic acid (25:25:1)	RP11	tetrahydrofuran/water/acetonitrile/ formic acid (40:40:20:2)
NP12	*n*-heptane/ethyl acetate/formic acid (20:30:1)		
NP13	*n*-heptane/ethyl acetate/formic acid (15:35:1)		

[a] *V/V*

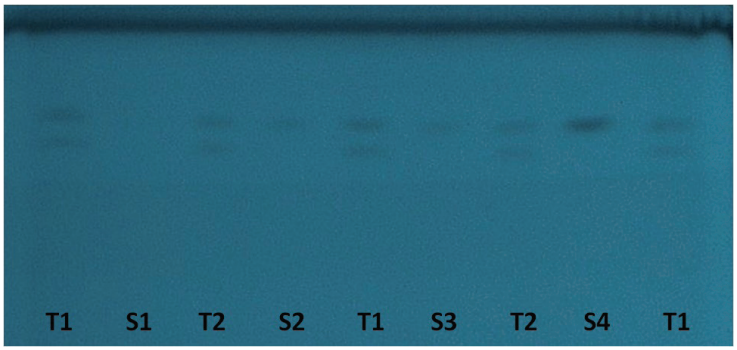
water were tested on TLC Si60 F_{254S} plates (mobile phases NP1-NP13, Table II). Moreover, the samples were separated with the use of mobile phases consisting of acetonitrile, tetrahydrofuran, water and formic acid on HPTLC RP18 WF_{254s} plates (mobile phases RP1-RP11, Table II). The hexane-partitioned ginkgo tinctures T1 or T2 were applied next to ginkgolic acid C15:1 and ginkgolic acid C17:1 standard with the use of the Hamilton syringe (10 μL). Separations were conducted in a horizontal TLC chamber (Camag, Switzerland) at a distance of 40 mm (Table II). Chromatograms were analyzed under UV light (λ = 254 and 366 nm) (Fig. 1).

I step



ginkgolic acids zone

II step



ginkgolic acid 15:1
ginkgolic acid 17:1

Fig. 1. Targeted 2D planar chromatography separation of ginkgolic acids C15:1 and C17:1 (T1, T2 – samples of tinctures; S1-4 – increasing amount of ginkgolic acid 15:1).

TLC conditions: I step: adsorbent – TLC Si60 F_{254s} glass plate 100 mm × 50 mm, UV detection: λ = 366 nm, mobile phase – *n*-heptane/ethyl acetate/formic acid (20:30:1, *V*/*V*/*V*); distance: 40 mm.

II step: HPTLC RP18 WF_{254S} glass plate 100 × 50 mm, UV detection: λ = 254 nm, acetonitrile:water/formic acid (80:20:1, *V*/*V*/*V*), distance: 40 mm.

Targeted 2D-TLC separation

Nine samples were applied as 5-mm bands using Desaga HPTLC-AS 30 applicator (Desaga, Germany) [syringe volume – 10 μL, sample volume – 4 μL (tinctures) or 30 μL (solid forms), application speed – 0.33 μL s^{-1}, carrier gas – nitrogen] 10 mm from the low edge and 7 mm from the side of TLC Si60 F_{254S} glass plate cut to the proper size (10 × 5 cm) with the use of the TLC-cutter (OM Laboratory, Japan).

The separation in the first dimension was conducted on TLC Si60 F_{254S} glass plate in the twin-through chamber (Camag) conditioned for 15 min with the mobile phase vapor. The separation was carried out with the use of *n*-heptane/ethyl acetate/formic acid (20:30:1, *V*/*V*/*V*) mixture as a mobile phase at a distance of 40 mm. The developed plate was dried

at room temperature for 10 min and analyzed under UV light (λ = 366 nm and 254 nm). The zone of ginkgolic acids (a complex visible as a blue band under UV light) was excised by cutting the plate with a TLC-cutter. The band of ginkgolic acids was transferred to the second glass plate, HPTLC RP18 WF_{254s} (10 × 5 cm, prewashed with methanol and activated at 120 °C for 30 min), in a vertical chamber. Methanol was used as a mobile phase to transfer the band of alkylphenols at a distance of approximately 10 mm. During the transferring time, TLC and HPTLC plates were combined with the use of the device described by Łuczkiewicz *et al*. (31). TLC plate was then disconnected and HPTLC plate was dried. The separation was continued in the second dimension on HPTLC plate in the horizontal developing chamber (Camag) using acetonitrile/water/formic acid (80:20:1, *V*/*V*/*V*) as a mobile phase at a distance of 40 mm (Fig. 1). The chromatogram was analyzed densitometrically (λ = 320 nm).

Identification of ginkgolic acids

Preliminary identification of the separated compounds was based on co-chromatography with reference substances. Next, the zones referring to ginkgolic acids C15:1 and C17:1 were scraped off from the plate and extracted with methanol. Qualitative analysis was conducted in the modified HPLC-DAD-ESI-MS system described by Xian-guo *et al*. (24). The HPLC system (Shimadzu, Japan) consisted of chromatographic pumps (LC 20AD), injector (autosampler SIL 20ACXL), DAD detector (SPD M20A), degasser (DGU 20AS), column oven (CTO 20AC) and interface (CBM 20A). The system was controlled by Labsolutions (Shimadzu) software. The isocratic separation was carried out on a Discovery C18 (150 mm × 2.1 mm, 5 μm, Supelco, USA) column at 45 °C with the use of a mobile phase of acetonitrile:water/acetic acid (92:7:1, *V*/*V*/*V*; flow rate 0.5 mL min^{-1}). The separated compounds were applied directly to LC-ESI-MS detector (LC-MS 2020, Shimadzu). The spectra

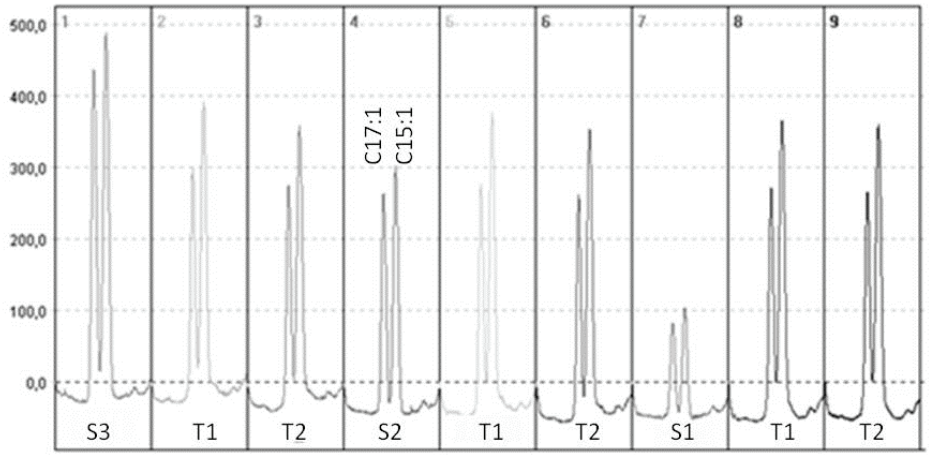


Fig. 2. Densitograms of analyzed samples and ginkgolic acid C15:1 and C17:1 standards separated by the use of targeted 2D planar chromatography (T1, T2 – samples of tinctures; S1-S3 – increasing amount of ginkgolic acid 15:1). For TLC conditions for step I and step II see Fig. 1.

were acquired in the negative-ion mode: mass range measured: 300–600 *m*/z, block temperature: 200 °C, desolvation line (DL) temperature: 250 °C, detector voltage: 1 kV, interface voltage: 4.5 kV (Fig. 2).

Quantitative analysis

Increasing amounts of standard (200, 500 and 1000 ng) and two samples in triplicate were separated on each plate. The chromatograms were analyzed densitometrically (CD 60 apparatus controlled by ProQuant 1.03.300 software (Desaga) and every track was scanned in linear mode (slit height – 0.02 mm, slit width – 6 mm, wavelength – 320 nm, scanning mode – absorbance, the average peak area of four measurements in the spot was recorded).

Preliminary validation

It included the assessment of detection limit (*LOD*), quantification limit (*LOQ*), linearity, instrument precision, repeatability of measurement, inter-day and intra-day precision and recovery (Table III). The limits of detection (*LOD*) and quantification (*LOQ*) were determined by analysis of ginkgolic acids standards (50–2000 ng per spot) by using signal-to-noise ratio 3:1 and 10:1, resp. (Table III). The precision of the measurement was calcu-

Table III. Validation parameters for quantitation of ginkgolic acids in herbal products

Parameter[a]	Ginkgolic acid C15:1	Ginkgolic acid C17:1
Linearity (ng per point)[b]	90–1500	80–1400
LOD (ng per point)[c]	50	50
LOQ (ng per point)[c]	170	160
R^2 [d]	0.996–0.999	
Repeatability of measurement (RSD, %)[e]	0.7	1.3
Repeatability of sample application (RSD, %)[f]	1.7	2.1
Intra-day precision (RSD, %)[g]	2.8	4.6
Inter-day precision (RSD, %)[h]	4.0	6.9
Recovery (%)[i]	98–105	97–108

[a] Parameters recorded densitometrically under UV light (λ = 320 nm).

[b] Analysis of increasing amounts of reference compounds applied in duplicates (4 increasing concentrations).

[c] Analysis of increasing amounts of reference compounds at levels corresponding to the *S*/*N* ratio of 3 and 10 (determination of *LOD* and *LOQ*, resp.).

[d] Average R^2 calculated for 3-point calibration curves on different plates.

[e] Repeated (n = 9) scanning of a single band of the reference compound.

[f] Repeated (n = 9) application of the same amount of the reference compound on a single TLC plate.

[g] Analyses of the same sample within one day (3 independent analyses in triplicate).

[h] Analyses of the same sample on 3 subsequent days (3 independent analyses in triplicate).

[i] Analyses of samples spiked with a known amount of ginkgolic acid (3 independent analyses in triplicate).

lated basing on the repeated scanning of standards spots (n = 9) at 320 nm (Table III). The assigned range of method linearity showed that it enabled the quantification of ginkgolic acids C15:1 and C17:1 in the range of 90–1500 ng per point and 80–1400 ng per point, resp., with the correlation coefficient of $R^2 > 0.996$ (Table III). Intra-day precision was studied by the analysis of the sample in triplicate on three TLC plates for one day; for inter-day precision, the same analyst repeated analyses on three consecutive days (Table III).

Recovery study based on the standards addition method was used in the assessment of the method accuracy. Ginkgolic acid 15:1 and 17:1 standards were added to the samples at two levels, 25 and 50 % of the analyzed content in the sample, and each was analyzed as described above (Table III). The tinctures (T1 and T2) were used for validation of the developed method.

RESULTS AND DISCUSSION

Optimization of experimental conditions

Tinctures and water-dispersed tablets or capsules were partitioned with hexane. The previously described selectivity of hexane towards alkylphenols (despite high levels of polyphenols in ginkgo extracts) (14) and its low boiling point which enabled rapid concentrating of samples at room temperature, made it a useful choice for preparing samples for analyses. Separation of the main ginkgolic acids C15:1 and C17:1 with the use of planar chromatography has not been described so far. In the present study, attempts to separate the compounds were carried out both under normal phase and reverse phase conditions. Glass plates covered with silica gel (TLC Si60 F_{254s}) or silica gel modified with octadecylsilane groups (HPTLC RP18 WF_{254s}) were used for this purpose.

Separations of ginkgolic acids on TLC glass plates covered with silica gel (TLC Si60 F_{254s}) were conducted under normal phase conditions with the use of solvent mixtures consisting of variable proportions of *n*-heptane, ethyl acetate, acetone and formic acid (Table II). It has been noted that the greater the concentration of ethyl acetate or acetone in the mobile phase, the worse the separation of analyzed compounds from interfering substances. For this reason, the addition of *n*-heptane was needed to slow down the migration of the ginkgolic acids zone. However, zones of the separated compounds were tailing during the development which impeded their identification. It has been observed that the addition of formic acid improves the shape of the separated zones with ginkgolic acids. Separation of ginkgolic acids zone from the interfering compounds was achieved under normal phase conditions. The well-separated ginkgolic acids zone was visible (λ = 366 nm) near the front of the mobile phase on TLC Si60 F_{254s} plate developed at a distance of 40 mm with the use of *n*-heptane/ethyl acetate/formic acid (20:30:1, *V*/*V*/*V*). Nonetheless, none of the thirteen mobile phases tested in the study has enabled the separation of ginkgolic acids C15:1 and C17:1 from one another.

Attempts of ginkgolic acids C15:1 and C17:1 separation were continued under the reverse phase conditions on HPTLC RP18 WF_{254s} plates at a distance of 40 mm. Mobile phases consisting of variable amounts of acetonitrile, tetrahydrofuran, water and formic acid were tested (Table II). It was noted, that the addition of formic acid in mobile phase improved the shape of ginkgolic acids zones. Ginkgolic acids' zones migrated together in all of the used mobile phases containing tetrahydrofuran (phases RP7-RP11, Table II). In turn,

increasing the concentration of acetonitrile in the mobile phase enhanced the retention factor of ginkgolic acids zones and hindered the separation. Among the analyzed mobile phases containing acetonitrile (RP1 – RP6, Table II), the mobile phase consisting of acetonitrile/water/formic acid (80:20:1, *V*/*V*/*V*, mobile phase RP6) was chosen for further studies. However, the separation of ginkgolic acids was impeded by the accompanied constituents that made the quantitative analysis impossible.

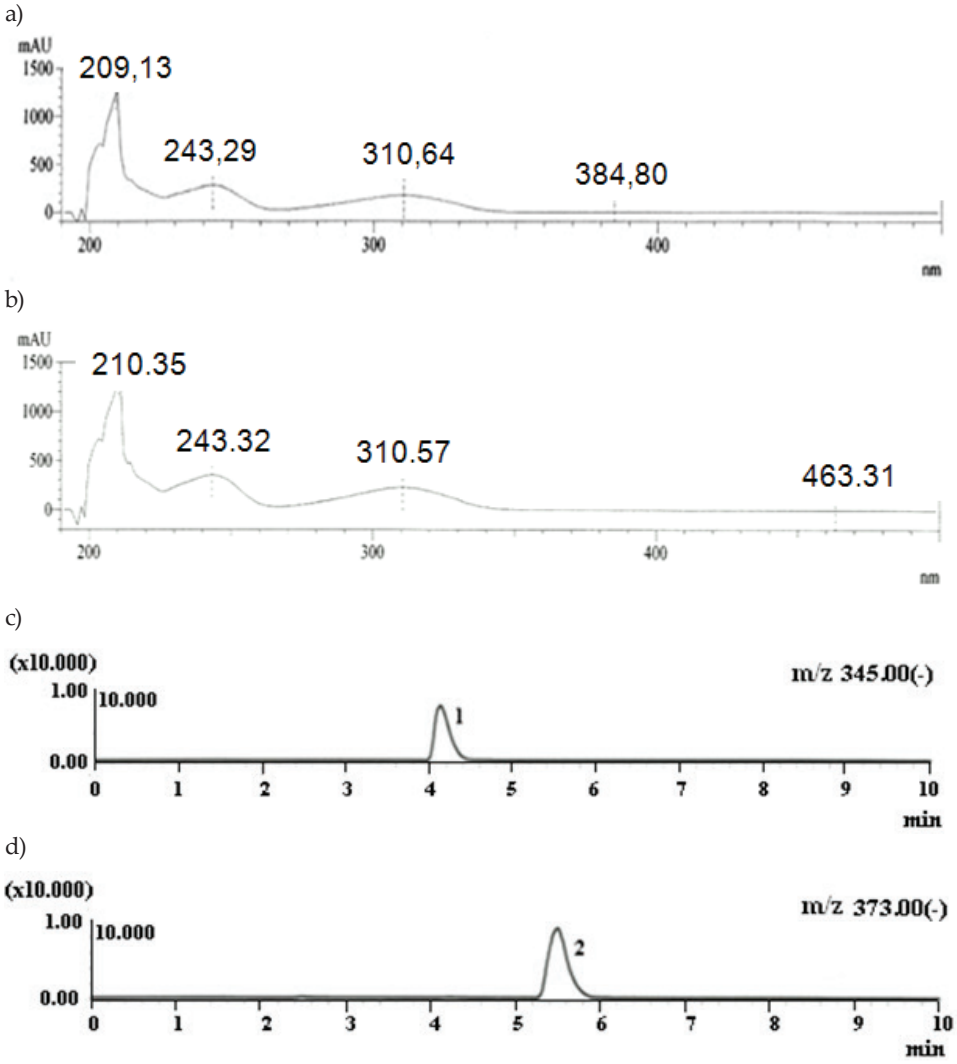


Fig. 3. UV spectra and ion chromatograms of the TLC-separated ginkgolic acids C15:1 and C17:1 recorded by HPLC-DAD-ESI-MS analysis: a) UV spectrum of ginkgolic acid C15:1, b) UV spectrum of ginkgolic acid C17:1, c) ion chromatogram at *m*/*z* 345 and *m*/*z* 373 of ginkgolic acid C15:1, d) ion chromatogram at *m*/*z* 345 and *m*/*z* 373 of ginkgolic acid C17:1.

For this reason, based on the literature data and our own experiences (29, 30), targeted 2D planar chromatography technique combining concentrating, purification, and separation of sample's constituents was used in further studies. Maintaining orthogonal rules, separation of ginkgolic acids was conducted consecutively on TLC Si60 F_{254s} and HPTLC RP18 WF_{254s} glass plates with the use of the best mobile phases described above (Figs. 1 and 2, Table II). Separation of ginkgolic acids from the accompanying less lipophilic constituents was obtained in the first dimension using *n*-heptane/ethyl acetate/formic acid (20:30:1, *V*/*V*/*V*) on TLC Si60 F_{254s} plate. Basing on chromatogram analysis under UV light, the fragment of TLC plate containing alkylphenols band was cut off. The final separation of ginkgolic acids was preceded by transferring of this band from TLC Si60 F_{254S} glass plate on HPTLC RP18 WF_{254s} glass plate with methanol. Ginkgolic acids C17:1 and C15:1 were separated by the use of the mobile phase consisting of acetonitrile/water/formic acid (80:20:1, *V*/*V*/*V*) to an extent that allowed their qualitative and quantitative analysis (Figs. 1, 2).

Analytical parameters

The assigned range of method linearity showed that it enabled the quantification of ginkgolic acids C15:1 and C17:1 in the range of 90–1500 ng per point and 80–1400 ng per point, resp., with the correlation coefficient of $R^2 > 0.996$ (Table III). However, the presence of accompanied compounds made the quantification of less than 170 ng per spot of C15:1 and less than 160 ng per spot of C17:1 impossible (Table III).

Meanwhile, the accompanying substances have limited the application of greater volumes of analyzed samples due to the adsorbent overloading and tailing of constituent zones during the TLC chromatogram development. It was found out, that up to 30 μL of extract from tablets or capsules could be applied on the TLC plate with no influence on the separation of ginkgolic acids. This volume limitation enabled the quantification of no less than 10 μg g^{-1} of ginkgolic acid C15:1 or C17:1 in the analyzed products.

Analytical application

The identification of ginkgolic acids bands was based on 2D-TLC co-chromatography along with HPLC-ESI-MS analysis in the presence of reference substances (25). It was found out that ginkgolic acids C15:1 and C17:1 were dominant alkylphenols in the analyzed tinctures T1 and T2 of ginkgo leaves (Figs. 1–3). Ginkgolic acids were not detected in tablets or capsules.

The quantitative analysis was conducted densitometrically with the use of the developed targeted 2D planar chromatography method. The analyzed samples were applied on each plate next to increasing amounts of the reference substances in order to reduce the influence of variable chromatographic conditions (*e.g.*, humidity, temperature, atmospheric pressure) on the separation of the compounds. The procedure of ginkgolic acids' quantification had been preliminary validated (Table III). Due to the fact that the developed 2D-TLC method combined the purification and the separation of the analyzed samples, the procedure of sample preparation was simplified and therefore shortened. As in any TLC, the solvent consumption analysis was significantly lower in comparison with HPLC as two samples were determined during a single separation.

Ginkgolic acids C15:1 and C17:1 were determined in the analyzed tinctures T1 and T2; these products contained similar quantities of ginkgolic acids, ranging 209 to 283 mg g^{-1}.

Table IV. Content of ginkgolic acids C15:1 and C17:1 in the analyzed tinctures

Sample	Content (μg g^{-1})[a]	
	Ginkgolic acid C15:1	Ginkgolic acid C17:1
T1	257.33 ± 6.20	281.68 ± 5.46
T2	283.83 ± 7.35	209.33 ± 9.67

[a] Mean ± SD (analysis in triplicate).

Based on the quantitative data, the concentration of ginkgolic acid C15:1 and C17:1 in 60 % ethanolic extracts of ginkgo leaves was significantly greater than the acceptable level of 5 μg g^{-1}, specified in the *European Pharmacopoeia* for ginkgo dry extract (Table IV) (28). Our results are in accordance with Schwabe's patent (14) and are pointing out that the concentration of ginkgolic acids in commercial extracts may reach 320 μg g^{-1} (14).

CONCLUSIONS

The aim of this study was to quantitatively and qualitatively analyze the ginkgolic acids present in herbal products with the use of the planar chromatography technique. The developed targeted 2D planar chromatography combined concentration, purification, and separation of the main ginkgolic acids C15:1 and C17:1. This study has proven that planar chromatography is useful, not only for qualitative, but also for quantitative analysis of ginkgolic acids in mono- and multi-component herbal products.

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