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ORIGINAL RESEARCH PAPER

Development of thin-layer chromatography–densitometry for the quantification of lecithin in dietary supplements

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Abstract
Lecithin is a mixture of phospholipids which naturally occurs in plants and animals cells. This is a component of drugs as well as dietary supplements, which are used to improve memory and concentration, likewise to decrease cholesterol level in blood, decrease risk of cardiovascular diseases and in a lot of other cases. In this paper the quantitative and qualitative analysis of phosphatidylcholine, which is the main component of lecithin, by thin-layer chromatography with densitometric detection, was described. First, the method was validated according to the International Council for Harmonisation (ICH); validation results have shown that the method was selective, precise, and accurate in the range of the linearity 0.23–3.21 mg mL^{-1}. Next, described method was used for the quantification of phosphatidylcholine in dietary supplements and drugs containing lecithin. The obtained results, both in terms of the quality of chromatographic separation and statistical evaluation are satisfactory, and the developed analytical procedure may be an alternative to other separation techniques, for the quick analysis of lecithin products.

Keywords Lecithin · Phosphatidylcholine · Dietary supplements · Quality control · Thin-layer chromatography–densitometry

1 Introduction

Lecithin is a naturally occurring mixture of phospholipids, including phosphatidylcholine, phosphatidylserine, and phosphatidylinositol. In medicine and biochemistry, the term "lecithin" is reserved for its main ingredient, which is 3-phosphatidylcholine [1]. Phosphatidylcholine (PC) is a phospho-O-glyceride containing choline connected with phosphoric acid by an ester bond (Fig. 1) [2, 3]. In the first and second positions of glycerol, there are residues of various saturated (palmitic, stearic, myristic) and/or unsaturated (oleic, linoleic, palmitoleic, arachidonic, eicosanoic) fatty acids in varying proportions. They are connected with the glycerol by ester bonds. Usually in the first position there are saturated fatty acids. In the third position of glycerol is phosphorylcholine [4]. The average molecular weight of the PC is 768 g mol^{-1} [5]. PC is soluble in methanol, ethanol, and chloroform, but it is insoluble in water. According to various sources the PC solution is neutral (pH 7) or slightly acidic (pH 6.6) [6]. Due to its properties, it has been used in the food, cosmetic, and pharmaceutical industries [7]. PC is classified by the FDA (US Food and Drug Administration) as GRAS (Generally Recognized as Safe). No adverse side effects were noted when people use it even in high doses [8–10].

Lecithin is a surfactant with a lipophilic (fatty acids) and hydrophilic (phosphorylcholine) part, which makes it an emulsifier. It is characterized by the ability to solubilize, wet the substances, stabilize suspensions, and when it is added to food, it prevents them from sticking to the surface of the dishes. The word lecithin comes from the Greek "lekithos," meaning the egg yolk, from which lecithin was first time isolated. A rich source of lecithin are: chicken egg yolks, wheat germ, soybean oil, butter, milk, seafood [3, 11].

In addition to the food industry, lecithin is also used for therapeutic purposes. Despite divergent reports on the benefits of its use, lecithin is supplemented mainly to prevent neurochemical and cardiovascular diseases [3]. PC is present in all cells of the body. It constitutes about 40% of the plants and animals cell membrane lipids [12, 13], and is located especially in the outer part of the lipid bilayer. PC also acts as a protective factor within the gastrointestinal barrier, and forms a hydrophobic layer in the lumen of the colon that acts as a protective barrier to prevent bacteria

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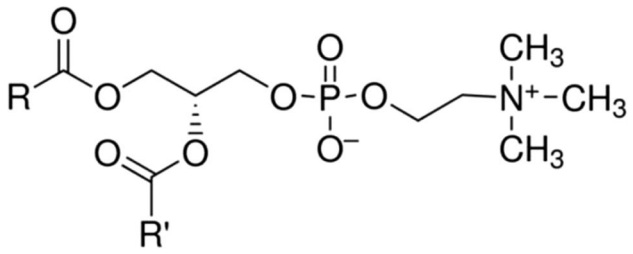

Fig. 1 Structure of phosphatidylcholine. Substituent R'- fatty acid residues (palmitic, stearic, oleic, linoleic, myristic, eicosanoic, or arachidonic acid) [5]

from adhering and invading [14]. PC builds up the surface of the alveoli, and is also part of the mitochondria [15, 16]. Research on PC has shown that, in addition to the building function, it takes part in metabolic processes, therefore it is difficult to predict the final role it plays in the human body. It was found that slightly different PCs have completely different biological activity [17]. 1-palmityl-2-oleylphosphatidylcholine is an endogenous ligand for nuclear PPARα receptors in hepatocytes, which influences the expression of genes regulating lipid metabolism [18]. 1-stearyl-2-oleyl PC reduces the triglyceride level after meals and, by affecting PPARα receptors, increases the consumption of fatty acids in muscle cells [19]. Some of the varieties of PC (dilauroyl-, dicapryl-, dipalmityl-, diundecanyl-PC) are agonists of the liver LRH-1 receptor (liver homolog-1), responsible for the production of bile [20]. Dilauryl PC affects the expression of genes encoding LRH-1, which reduces the level of triglycerides in the liver and the concentration of glucose in the blood [17]. PC is essential for cell differentiation, proliferation and regeneration, and for the transport of molecules across cell membranes. As the amount of dilinoleoyl-PC increases in cell membranes, their permeability increases, which makes them more functional. The PC controls the membrane-dependent metabolism and the processes between the interior of the cell and the intercellular space. It activates membrane-bound enzyme proteins (ATPase K^+/Na^+, lipoprotein lipase, lecithin:cholesterol acyltransferase, cytochrome oxidase) or receptor (insulin receptor) [9]. Dipalmitoyl lecithin is an essential component of the surfactant which reduces the surface tension between lung tissue and respiratory gases. This prevents the surfaces of the alveoli from sticking together [2]. From the ratio of lecithin to sphingomyelin in the mother's amniotic fluid, the maturity of the respiratory system of the fetus and its ability to breathe after delivery can be determined [21].

PC is a part of lipoproteins, especially HDL (high-density lipoprotein) and participates in the transport of cholesterol [22]. As one of the components of bile, it emulsifies fats in the digestive tract. It inhibits the aggregation of platelets and affects the immune processes at the cellular level [9, 13]. PC is a complex compound that releases molecules involved in various metabolic pathways. It contains polyunsaturated fatty acids which are used as precursors of cytoprotective prostaglandins and other eicosanoids. It may also be a donor of arachidonic acid, which is an important factor in the course of inflammatory processes. The PC is the source of the secondary transmitters in the cells (e.g., diacylglycerol). It contains phosphates which are essential for the synthesis of ATP in the cell. PC supplies choline, which is the precursor of the neurotransmitter acetylcholine. PC derived from bile is necessary for the absorption of cholesterol from the intestinal lumen [9]. PC is a good marker of age-related membrane degradation and memory loss. Decreased blood levels of PC announce a decline in cognitive function. Increased PC levels positively correlate with a thicker gray matter layer in the left hemisphere prefrontal cortex, and also improves cognitive abilities [23]. Lecithin in the blood comes from food; it is also produced in the human body by phosphatidylethanolamine N-methyltransferase. Lecithin, is not digested by lingual and gastric lipase. It is decomposed by pancreatic lipase in the large intestine [24]. It is absorbed in over 90% from the gastrointestinal tract [11]. 50% of PC is absorbed as lyso-lecithin, the rest is absorbed as glycerophosphocholine or phosphorylcholine [25]. Most PC is deposited in the liver and a small part in other organs: spleen, lungs, muscles, kidneys, and brain [9].

Due to the many functions of PC in the human body, this compound is being investigated for its possible use in the therapy of various diseases. There are many various medications and dietary supplements with lecithin available on the market. All these preparations are indicated in the case of memory problems, elevated blood cholesterol levels, concentration disorders, for learners and stressed people and as an auxiliary in atherosclerosis. The ability to reduce cholesterol levels by lecithin confirmed numerous studies [26–30]. It was also shown that lecithin derived from egg yolk is more effective in reducing cholesterol absorption than those derived from soybean. Lecithin is also more effective as it contains more saturated than unsaturated fatty acids [8]. It was proven that repeated intravascular injections of PC reduced cholesterol deposits present in atherosclerotic plaque [27]. Lecithin is used to improve memory and concentration by learners and the elderly. Supplementation in pregnant rats showed a positive effect on spatial memory in offspring in later life [31]. The fatty acids included in the PC molecule (e.g., eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) also have a positive effect on the functioning of the brain [32]. Studies of the individual components of lecithin have shown that dilinolylphosphatidylcholine and 1-oleyl-2-palmitylphosphatidylcholine also used separately have a beneficial effect on improving memory [33]. It is supposed that memory impairment in the elderly is due to a reduction in the amount of PC and/or PUFA (polyunsaturated

fatty acid) and a reduction in the fluidity of cell membranes in brain tissues. Therefore, the use of lecithin seems to be justified to prevent or to alleviate memory disorders in the elderly [11]. In addition, PC provides important building components for cell membranes thanks to which it has neuroprotective properties [23]. The pathomechanism of Alzheimer's disease is associated with a reduced amount of the enzyme converting choline into acetylcholine in the brain, therefore it is supposed that an additional supply of choline contained in lecithin, may reduce the symptoms of this disease. Similar hopes are associated with the administration of PC in Parkinson's disease. Despite the widespread opinion about the beneficial effects of lecithin in these diseases, a review of studies shows that lecithin supplementation has no clear benefit in both cases [34, 35]. Other studies have shown that lecithin has anti-inflammatory properties, and the benefits of its use are comparable to those associated with diclofenac treatment for arthritis [36]. In addition, PC protects the gastric mucosa and reduces its damage during administration of non-steroidal anti-inflammatory drugs, PC also has beneficial anti-inflammatory effects in ulcerative colitis after administration of the delayed release formulation [11]. Due to its beneficial effect on cell membranes, PC can be used in the case of liver diseases. Soy phospholipids improve the condition of patients in the case of fatty liver (both alcoholic and non-alcoholic), viral liver diseases, poisoning [9, 10]. Patients with gallstones have decreased levels of PC in the bile that can be increased by supplementation. However, research on the efficacy of lecithin in patients with gallstones show inconclusive results. Some of them point to a reduction in the level of cholesterol in the bile, and even a reduction of gallstones, according to other reduction is slight [37].

The wide public interest in lecithin, due to its multidirectional beneficial effects, is a starting point for scientific and research units that are taking steps to develop new analytical procedures necessary in the quality control of lecithin-containing products. Pharmacies offer both drugs and dietary supplements containing lecithin. While the quality of drugs does not raise doubts as to the safety of their use, but the real composition of supplements may sometimes be different from the declared one. Therefore, it is necessary to develop new, reliable and simple analytical methods for qualitative testing of products containing lecithin. Among the methods used to determine the content of PC are: chromatography, spectrofluorimetry, potentiometry, and spectrophotometry combined with enzymatic reactions [38–46]. A number of methods for determining PC by thin-layer chromatography (TLC) have been described in the literature in flax, soybean, and sunflower lecithin [47, 48], food additives [49, 50], and living organisms [51–53]. The TLC was also used as an intermediate step in the analysis of lecithin, to separate it from other phospholipids and further analysis for fatty acid content by gas chromatography, and in medical diagnostics, where lecithin and sphingomyelin are measured in the amniotic fluid [52, 54]. Wang et al. described several methods for the determination of PC in plasma by spectrofluorimetry using various types of detection [54]. One of these methods is based on the phenomenon of enhancing the fluorescence of the tetracycline europium complex by the PC molecule, which is directly proportional to the concentration of PC. Other methods are based on reducing the fluorescence of various complexes: norfloxacin-terbium, enoxacaine-terbium, ciprofloxacin-terbium, at a wavelength of 545 nm [55–57]. Lecithin may also be determined using infrared spectroscopy with Fourier transform by partial least squares (PLS-FTIR) [58]. Campanella et al. described a spectrophotometric method for the determination of PC in pharmaceutical preparations, eggs and food additives, by a three-step enzymatic reaction PC with: phospholipase D, choline oxidase, and peroxidase [7]. A potentiometric method for the determination of PC in pharmaceutical preparations with a gas electrode was also developed. It uses two enzymes: phospholipase D and choline oxidase. A correlation was found between the PC content in the sample and the amount of oxygen used for the enzymatic reaction [39]. PC content can also be determined by high performance liquid chromatography with different detection (Table S1; Supplementary Data). The described methods allow for the quantification of PC in various materials. In the literature, also can be found methods that permit the analysis of the PC molecule by identifying fatty acid residues in its structure. Among these methods are: gas chromatography and mass spectrometry [59].

The purpose of the presented study was to develop a new analytical method to confirm the identity and determination of lecithin (by the PC content) in dietary supplements and pharmaceutical preparations using the TLC technique with densitometric detection. PC is not a homogeneous compound, and its molecules include various fatty acids. The developed method allows for the qualitative determination of PC, without its division into individual varieties, as well as for its quantitative analysis taking into account the sum of the individual types of this phospholipid.

2 Experimental

2.1 Instrumentation

Linomat 5 sample applicator, densitometer TLC Scanner 3 with winCATS4 software, and UV lamp (254/366 nm) were manufactured by CAMAG (Muttenz, Switzerland). Laboratory drying oven Ecocell was produced by BMT Medical Technology (Brno, Czech Republic), and analytical balance

WPA 120/C/1 by Radwag (Radom, Poland). HPTLC silica gel $60F_{254}$, TLC silica gel $60F_{254}$ and HPTLC cellulose chromatographic plates were purchased from Merck (Darmstadt, Germany).

2.2 Reagents and standard substance

Ammonia, butylamine, ammonium molybdate tetrahydrate (No. A7302), ninhydrin (No. 151173), iodine (No. 207772) and L-α-phosphatyidylcholine (No. P3556) were purchased from Sigma-Aldrich (Poznań, Poland). Chloroform, ethanol, glacial acetic acid, sulfuric acid 95%, and methanol were purchased from Chempur (Piekary Śląskie, Poland).

2.3 Examined preparations

Thirteen products containing lecithin were tested. Two of them were registered as drugs, and eleven are a dietary supplements. All preparations and supplements were purchased at local pharmacies. Some of the preparations have exceeded the expiry date within the time of the study. The following preparations were analyzed: Products A−F, H, and K−N, swallow capsules containing 1200 mg of lecithin; Product G, chewable dragees containing 750 mg of lecithin; and Product I, liquid containing 10.4 g of lecithin per 100 mL.

2.4 Standard and sample solutions

Standard solution of L-α-phosphatidylcholine with a concentration of 0.1% (*m/V*) was prepared by dissolving the substance in methanol.

The contents of five capsules/dragees were weighed, and the average weight of a single capsule/dragee was calculated. The appropriate amount (to obtain solutions with a concentration of approx. 0.1%) of each preparation was weighed or the appropriate volume of the liquid was taken, assuming the lecithin content declared by the manufacturer. Each of the samples was dissolved in methanol in 25 mL volumetric flasks, that were left in the refrigerator overnight, to fully extract the PC from the preparation mass. Next, the solutions were filtered through cellulose filters; the obtained filtrate was used for further studies.

2.5 Staining reagent

Ammonium molybdate solution (0.5%, *m/V*) was prepared in a mixture of ethanol−25% aqueous) H_2SO_4 in a 1:9 volume ratio.

2.6 Optimization of the analysis conditions

In order to select the appropriate mobile phase, solvent mixtures of varying proportions were tested. In order to determine the optimal composition, after performing preliminary tests and analyzing the available literature, it was decided to use a mixture of chloroform and methanol with the addition of various amounts of ammonia or acetic acid. Each of the mobile phases was tested by developing plates with two bands from the standard solution and sample solution (PC concentration about 0.1%). In addition, different stationary phases (TLC silica gel $60F_{254}$, HPTLC silica gel $60F_{254}$, HPTLC cellulose) were tested. Detection of PC in the UV range turned out to be impossible, therefore it was necessary to carry out a color reaction with the components on the chromatographic plates. Several detection methods were tested, such as staining in iodine vapor (aqueous solution of iodine 0.05 mol/L), spraying with sulfuric acid, spraying with 5% (*m/V*) solution of ammonium molybdate in 25% aqueous sulfuric acid, spraying with 5% (*m/V*) solution of ammonium molybdate in ethanol with the addition of sulfuric acid, immersion in 5% (*m/V*) ethanolic solution of ammonium molybdate with the addition of sulfuric acid, spraying with 0.2% (*m/V*) solution of ammonium molybdate in ethanol with the addition of sulfuric acid, spraying with 0.5% (*m/V*) ammonium molybdate in ethanol with sulfuric acid, or spraying with 0.2% (*m/V*) ninhydrin solution [60–62]. The effects of some of the performed experiments are presented in Table S2.

Based on performed tests a mixture of chloroform−methanol−glacial acetic acid (3:6:0.4, *V/V*) was selected as the mobile phase. On cellulose plates after applying the solutions, developing and visualization, two blurred streaks were obtained, which made it impossible to identify. The use of TLC and HPTLC plates allowed to obtain the chromatograms with no differences in the position of the spots, and the time of analysis. Consequently, the cheaper TLC $60F_{254}$ plates were chosen. And finally, for visualization of the chromatograms, a 0.5% (*m/V*) ammonium molybdate solution in a mixture of ethanol and H_2SO_4 (1:9, *V/V*) was selected for staining by spraying on the plates, and then drying at 100 °C for 5 min. The reagent was prepared immediately before use. In effect, colored gray-blue bands were obtained on a white background. The plates were then densitometrically scanned at the selected wavelength.

2.7 Sample preparation

The effect of PC extraction time on its efficiency was investigated. For this purpose, one of the preparations (Product L) was weighed (approx. 0.02 g) into 25 mL flasks, dissolved

in methanol, and shaken for 5, 15, and 30 min, respectively. The solutions were filtered, and applied to the TLC $60F_{254}$ plates with the Linomat 5 applicator in various volumes. A PC standard solution in a volume of 5 µL was also applied. The plates were placed into a chromatographic chamber saturated with a chosen earlier mobile phase. After development, the chromatogram was drying and sprayed with a staining reagent. The plates were dried at 100 °C for 5 min, and then scanned densitometrically. Moreover, an analogous sample was prepared, which was left in the refrigerator overnight after being filled with methanol. After applying the obtained solution and developing the chromatogram, it was found that the registered peak areas were larger. Thus, it was found that the extraction time had a great influence on its effectiveness. Therefore, the following extraction time was selected for further analysis: 15 min of shaking with methanol, 24 h in the refrigerator, 5 min of shaking, and filtering.

2.8 Conditions of the TLC–densitometry assay

In order to analyze the PC content in pharmaceutical products and dietary supplements, methanol solutions of preparations were prepared with a concentration of about 0.1% (*m/V*) in relation to the amount of lecithin declared by the manufacturer. For this purpose, the appropriate mass or volume (in the case of liquids) of the preparations were weighed into 25 mL flasks. Methanolic suspensions were prepared as described above (Sect. 2.7) and then filtered through cellulose filters. For each preparation, three series of samples were made, prepared in the same way from three independent weights. The solutions were applied with a Linomat 5 applicator at a rate of 650 nL s^{-1} to TLC $60F_{254}$ silica gel chromatography plates, 15 × 10 cm in size. On each plate the preparation and standard solutions (in a volume of 15 and 5 µL) were placed. The bands were 0.7 cm wide, and were placed 1 cm from the bottom and left edges of the plate. Next, chromatograms were developed in the chamber saturated for approx. 15 min with the mobile phase containing chloroform−methanol−glacial acetic acid (3:6:0.4, *V/V*), on a 9 cm path, in approx. 30 min. After drying, the chromatogram was sprayed with 0.5% (*m/V*) ammonium molybdate in mixture of 25% aqueous sulfuric acid and ethanol (1:9, *V/V*), prepared immediately before use. The plates were dried for 5 min at 100 °C, and then scanned with a TLC Scanner 3 densitometer in absorption–reflection mode at $\lambda = 360$ nm. The densitometric scanning (winCATS 1.4.8v. software) conditions were as follows: slit dimensions 5.00 × 0.45 mm, scanning speed 20 mm s^{-1}, data resolution 100 µm per step. Based on the recorded densitograms, the retardation factor (R_F) for PC was designated to be 0.23. The values of the obtained peak areas for the sample and standard solutions were used for quantitative analysis.

2.9 Validation of the method

Method validation is an essential element in assessing the suitability of an analytical procedure to generate reliable results [63]. The validation process is carried out by checking the parameters of the method using a number of appropriate indicators, i.e., accuracy, precision, linearity, range, limit of detection (LOD), and limit of quantification (LOQ).

2.9.1 Linearity

The linearity of an analytical method is the ability to obtain results that are proportional to the content (or concentration) of the analyte in the sample. In order to determine the linearity of the developed method, standard substance solutions were prepared at the concentrations in the range from 0.23 to 3.21 mg mL^{-1}. Each of the solutions was applied to the TLC plate in the same volume. Based on the obtained results, the relationship P = f(c) was drawn, and the parameters of linear regression were determined. Then, residual analysis was performed, which allows to determine if the selected model was properly fitted.

2.9.2 Sensitivity

The limit of detection (LOD; the smallest amount of an analyte in a sample that can be detected) and the limit of quantification (LOQ; the smallest amount of an analyte in a sample that can be quantified with sufficient precision and accuracy) were determined according to the equations:

$$LOD = \frac{3.3 \times S_b}{\mathrm{a}}$$

and

$$LOQ = \frac{10 \times \mathrm{S_b}}{\mathrm{a}}$$

where: S_b = standard deviation of the intercept, a = the slope of the calibration curve.

2.9.3 Precision

The precision of an analytical method (repeatability, reproducibility, intermediate precision) is the degree of dispersion of the results obtained in a series of measurements on the same homogeneous sample, using the same method, under the described conditions. The evaluated parameters for precision are: standard deviation or relative standard deviation (RSD, %).

2.9.4 Accuracy

The accuracy of the analytical method determines the degree of agreement between the value considered to be true, and the content of the substance in the test sample obtained during the analysis. It should be determined at 3 concentration levels, based on a minimum of 9 test results. The calculation for accuracy (R, as % recovery) was calculated according to the formula:

$$R[\%] = 100 \cdot \frac{\left(x + s_i\right) - (x)}{s},$$

where: x = content in the sample without addition of standard, $x + s_i$ = content in the sample with addition of standard, s = added amount of standard.

3 Results and discussion

Lecithin is present in dietary supplements, medicinal preparations, and food additives. Customers are informed about a variety of nutritional supplements that will help improve health quickly, faster than any diet. No wonder they reach for such products. Unfortunately, most dietary supplements, not only those containing lecithin, are not subject to adequate quality control, which results in their very low effectiveness. In order to control the quality of these products, it is necessary to develop appropriate analytical methods that will allow the analysis of the composition of these products, which contain various other ingredients and different matrix.

Lecithin is a mixture of phospholipids in which PC accounts for about 1%, 23% for soybean lecithin (most commonly used) [6, 38]. Some dietary supplements contain sunflower lecithin (approx. 16.2% PC), which is preferred by a certain group of consumers [64] due to the current fear of genetically modified soybeans. Therefore, various studies of lecithin content in products available to consumers are carried out. The main goal of this study was to develop a method for the determination of lecithin in dietary supplements and pharmaceutical products. The technique of TLC with densitometric detection was used, which allows to obtain reliable results in a relatively short time and with low expenditure. Compared to other TLC methods available in the literature (e.g., HPTLC–electrospray ionization–mass spectrometry [$ESI^{(+)}$–MS]), the developed procedure is simple and allows for a quick analysis of the PC content in dietary supplements, which is very important from the point of view of quality control (confirmation of compliance with the actual content of ingredient in relation to the manufacturer's declaration on the product packaging).

The presented analysis was performed using TLC $60F_{254}$ silica gel plates as the stationary phase, and a mixture of chloroform–methanol–glacial acetic acid (3:6:0.4, *V/V*) as the mobile phase. Detection of the components was carried out densitometrically at $\lambda = 360$ nm, after the derivatization of the components on the plates, by spraying with 0.5% (*m/V*) ethanolic ammonium molybdate solution and heating at 100 °C. The peak areas and absorption spectra of the examined compound were recorded for quantitative and qualitative analysis.

On the recorded absorption spectra for PC (after derivatization with ammonium molybdate reagent) in the wavelength range from 200 to 600 nm several absorption maxima (230, 300, 360, 465 nm) were observed (Fig. 2). As a result wavelength 360 nm was chosen for further analysis of PC.

The obtained chromatograms showed that peaks were symmetrical, well-resolved, easy to identify and determine (Fig. 3). Based on the recorded densitograms, the R_F for PC was designated to be 0.23 (Fig. 4).

In the next step of the study, the correctness of the presented method was confirmed by validation, in accordance with the International Council for Harmonisation (ICH) guidelines [63]. The developed method was found to be specific for the test compound. There are no other peaks on densitogram, recorded for standard and sample solutions, where studied components occur. The observations made under process of the optimization of analysis conditions, indicate that the small changes in the method parameters, such as: composition of the mobile phase (±0.2 mL), developing distance (±0.5 cm), chamber saturation time (± 2 min), chamber size (18 × 16 × 6, 18 × 16 × 8, 20 × 12 × 6 cm), have no the influence on the chromatographic separation, which confirms the robustness of the presented procedure (Table 1).

In order to determine the linearity of the developed method, standard substance solutions were prepared at the

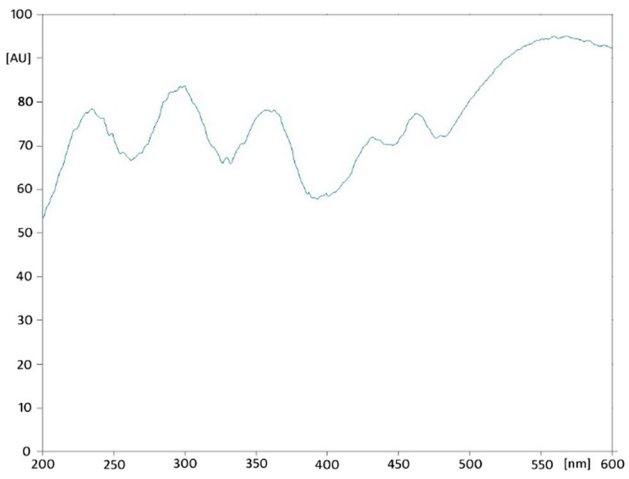

Fig. 2 Absorption spectrum for phosphatidylcholine (after derivatization with ammonium molybdate reagent) in the wavelength range of 200–600 nm

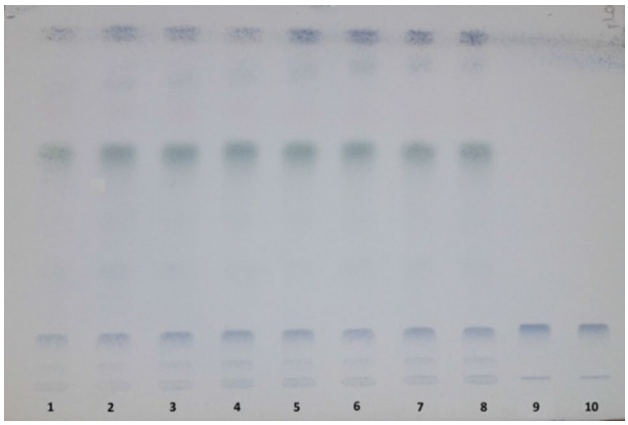

Fig. 3 An example of the chromatogram for Product G (1–8, preparation; 9–10, PC standard substance)

concentrations in the range from 0.23 to 3.21 mg mL^{-1}. The dependence of the peak area on the concentrations of the tested solutions was determined; a linear relationship was obtained in the tested concentration range (Fig. 5a).

The obtained equation $P = 3103.10 \bullet c + 638.87$ (P peak area, c concentration), correlation coefficients $r = 0.9983$, $r^2 = 0.9970$, and standard deviations of the slope, intercept, and estimation are $S_a = 0.03$, $S_b = 81.25$, and $S_e = 219.46$, respectively. The coefficient $r > 0.99$ indicates a strong, positive correlation between the values of the peak areas vs PC concentrations. Peak area values show a linear relationship with PC concentrations in the range of 0.26 to 3.21 mg mL^{-1}. In the presented method, the mean value of the eliminated residues was: −27.42. Irregular scattering of residuals confirms that the selected linear model is correct. Moreover, the correlation coefficient (r) of the raw residues to the values of the predicted concentrations was -0.4×10^{-6}, which means that there is no correlation between these variables (Fig. 5b). The sensitivity of the method is good; obtained LOD and LOQ values for PC in the developed method are 0.09 and 0.26 mg mL^{-1} (equivalent to 0.45 and 1.3 µg per spot), respectively. The precision of the developed method was tested by performing nine determinations in the method conditions. In order to test the recovery of the developed method, the standard PC solution, the solution of one of the preparations and previously prepared solutions of the preparation with the addition of the standard substance were applied to the chromatographic plates. According to the guidelines, the analysis was carried out at three concentration levels in relation to the PC content in the tested sample: 80, 100, and 120%. The results obtained for precision and accuracy assays are presented in Table 2.

Summing up, the range of the developed method, i.e., the range between the upper and lower levels of the concentration of the analyzed substance, which have been demonstrated with appropriate precision and accuracy, was defined to be: 0.26–3.21 mg mL^{-1}. The specified limits of quantification and detection are 0.26 and 0.09 mg mL^{-1}, respectively. The accuracy of the developed method (expressed as a percentage of recovery), determined by adding a known amount of the analyte to the tested product, and then determining it with the tested method, is in the range of 97–99% (RSD lower than 1%). And the precision, expressed as %RSD, not greater than 2%, confirms the agreement between the obtained results of the analysis. Since the obtained validation parameters indicate that the developed method meets the criteria for the analytical method intended for quantitative control of pharmaceutical preparations, it can be assumed that the developed method is suitable for the analysis of PC-containing products.

Next, the proposed method was successfully applied for the determination of PC in the commercially available pharmaceuticals and dietary supplements. Dietary supplements are not subject to thorough qualitative and quantitative research, therefore the research presented in the paper has a research and substantive aspect. Thirteen preparations containing lecithin were tested. Two of them are registered as drugs (Products G and I), and the rest are dietary supplements. For the qualitative and quantitative analysis of lecithin-containing preparations, the solutions of standard and preparations were applied to the chromatographic plates, and developed under conditions described previously.

The presence of PC in the samples was confirmed by the presence of a peak corresponding to the standards, based on the retardation factor (R_F 0.23) and absorption spectra. The PC content in the individual preparations was calculated based on the registered peak area. The obtained results with the statistical evaluation are shown in Table 3.

In our study, in each of the analyzed preparations, the presence of PC has been confirmed. The PC content in the tested lecithin-containing products ranged from 59.25 to 328.02 mg per capsule (22–119% in relation to the PC content declared by the manufacturer). In the case of medicinal preparations, the following were obtained: 2682.80 mg PC per 100 mL of Product I, and 201.15 mg PC per dragee of Product G (during the shelf-life). For the expired preparations, the determined PC content was lower, and ranged from 59.25 to 184.86 mg per capsule (22–67% in relation to the declared). This indicates that degradation of PC takes place over the storage time, and justifies discontinuation of preparations containing this substance after the expiry date. In the tested dietary supplements it was also observed that different

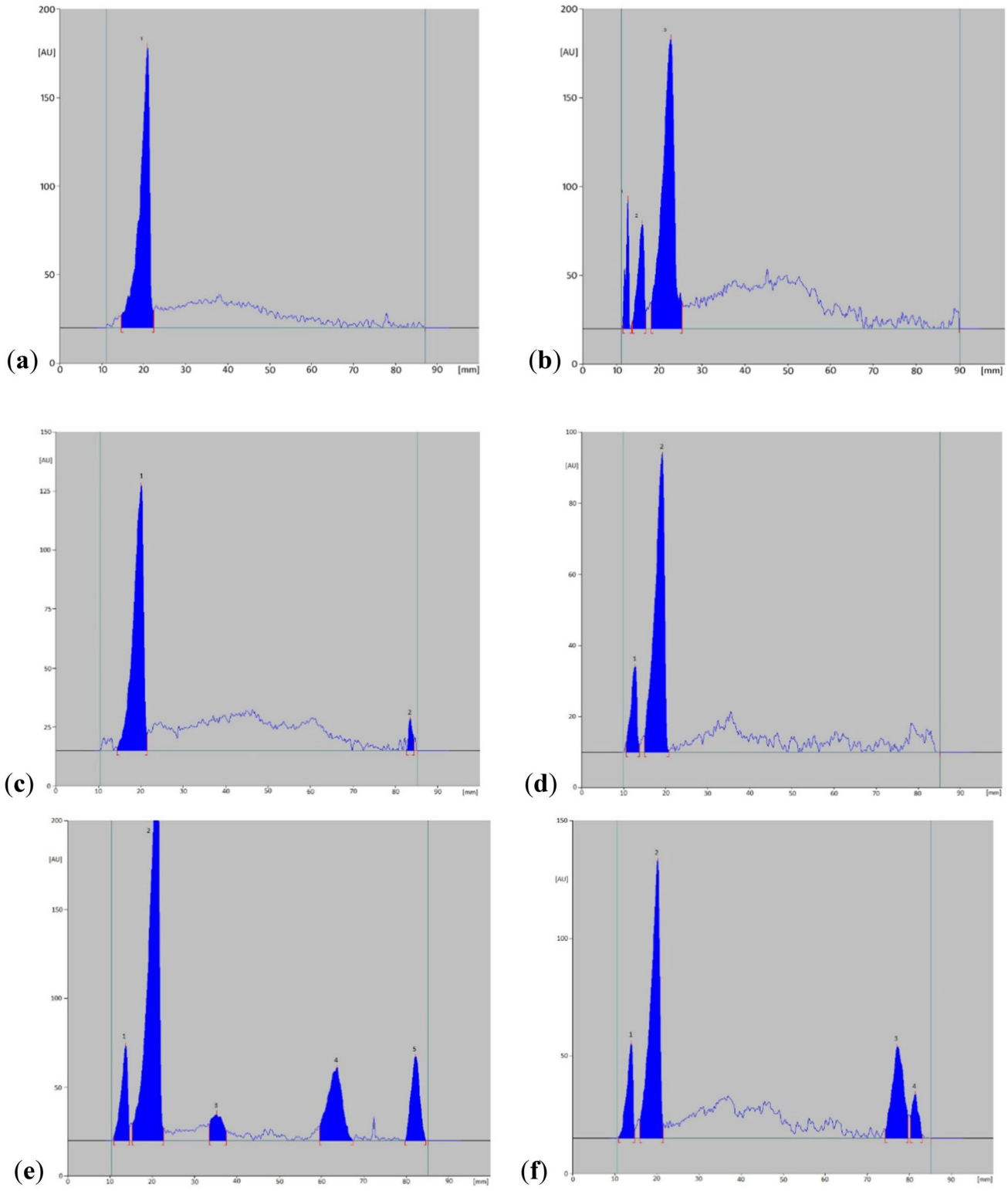

Fig. 4 Densitograms recorded (at 360 nm) for standard substance phosphatidylcholine (**a**), Product A **b**, 3, PC; 1−2, other components of the preparation), Product B **c**, 1, PC; 2, other component), Product C **d**, 2−PC; 1−other component), Product G **e**, 1, PC; 1, 3−5, other components), and Product K **f**, 2, PC; 1, 3, 4, other components

Table 1 Results of robustness study

Parameter	Peak area ± SD	%RSD	R_F
Mobile phase composition (chloroform−methanol−glacial acetic acid, *V/V*)			
2.8:6:0.4	2508.8 ± 30.82	1.09	0.22
3:5.8:0.4	2561.0 ± 26.91	1.15	0.21
3.2:6:0.4	2514.2 ± 28.29	0.88	0.21
3:6.2:0.4	2533.5 ± 36.01	1.18	0.23
Developing distance (cm)			
8.5	2487.1 ± 31.10	1.04	0.22
9.5	2462.3 ± 28.97	0.73	0.23
Chamber saturation time (min)			
13	2767.2 ± 39.09	0.88	0.23
17	2725.5 ± 38.71	0.97	0.23
Chamber size (cm)			
18 × 16 x 6	2860.7 ± 28.92	1.03	0.23
18 × 16 x 8	2841.0 ± 34.29	0.79	0.22
20 × 12 x 6	2853.1 ± 29.11	0.79	0.23

batches of the same preparation showed different PC content (203.97, 188.97, or 325.36 mg per caps.).

4 Conclusion

A new analytical method for assay of lecithin by PC content in dietary supplements and pharmaceutical preparations containing lecithin was developed. The presence of lecithin was found in all analyzed preparations, drugs and dietary supplements. Expired preparations were characterized by a much lower PC content compared to those in the shelf life. It has also been shown that individual series of the same dietary supplement have a different content of lecithin in a capsule. The conducted qualitative and quantitative analysis of lecithin content (via PC) can be of great help in terms of the quality of food products containing lecithin. Validation parameters (including recovery, LOD or LOQ) confirm its reliability in the concentration range corresponding to the content declared by supplement manufacturers. Then, this new developed TLC–densitometry procedure may be an alternative to other methods in the terms of quality control, which are mostly more time-consuming, labor intensive or expensive.

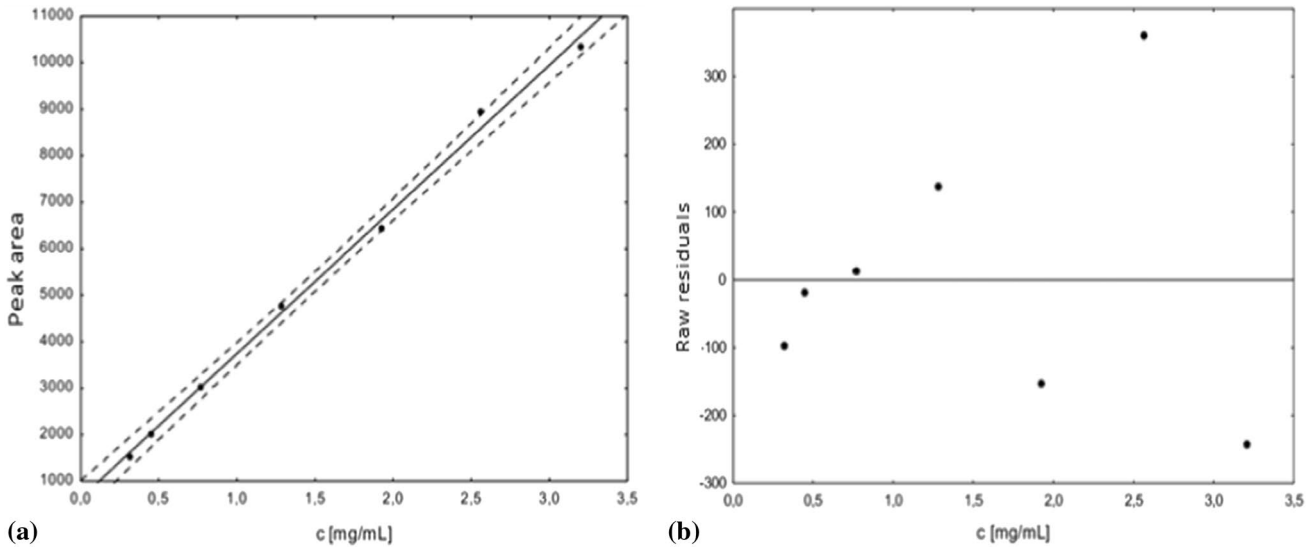

Fig. 5 A calibration curve (**a**), and the plot of residues from the predicted phosphatidylcholine concentration values (**b**)

Table 2 Results of precision and accuracy

Parameter	Results		Statistical data
Direct precision [P]	2308.9 2364.0 2326.5		$\bar{x}$= 2350.27
	2380.9 2286.0 2396.0		S$\bar{x}$= 13.67 SD = 41.02
	2395.1 2317.4 2377.6		RSD = 1.75
Intermediate precision [P]	3587.6 3648.1 3623.1		$\bar{x}$= 3588.78
	3621.2 3610.9 3512.9		S$\bar{x}$= 14.10 SD = 42.30
	3548.1 3583.6 3563.5		RSD = 1.18
Accuracy [%]	80%	98.22 97.73	$\bar{x}$=97.66
		97.93 96.77	S$\bar{x}$= 0.31 SD = 0.63
			RSD = 0.64
	100%	98.25 99.03	$\bar{x}$=98.94
		98.23 100.25	S$\bar{x}$= 0.47 SD = 0.95
			RSD = 0.96
	120%	97.89 96.60	$\bar{x}$=97.07
		96.17 97.62	S$\bar{x}$=0.41 SD = 0.82
			RSD = 0.84

P peak area; $\bar{x}$ arithmetic mean; *S*$\bar{x}$ standard deviation of the mean; *SD* standard deviation; *RSD* relative standard deviation [%]

Table 3 Content of phosphatidylcholine in the analyzed preparations

Preparation	Declared content	Determined content ($n = 10$)	Statistical evaluation
Product A	276.00 [mg per caps.]	300.50 [mg per caps.]	S$\bar{x}$=2.18 SD = 6.90 RSD = 2.30
Product B	192.00 [mg per caps.]	149.18 [mg per caps.]	S$\bar{x}$=1.05 SD = 3.34 RSD = 2.24
Product C	276.00 [mg per caps.]	217.63 [mg per caps.]	S$\bar{x}$=1.53 SD = 4.83 RSD = 2.22
Product D	276.00 [mg per caps.]	203.97 [mg per caps.]	S$\bar{x}$=1.58 SD = 4.99 RSD = 2.45
Product E	276.00 [mg per caps.]	188.97 [mg per caps.]	S$\bar{x}$=1.33 SD = 4.20 RSD = 2.23
Product F	276.00 [mg per caps.]	325.36 [mg per caps.]	S$\bar{x}$=1.99 SD = 6.28 RSD = 1.93
Product G	172.50 [mg per dragee]	201.15 [mg per dragee]	S$\bar{x}$=1.60 SD = 5.05 RSD = 2.17
Product H	276.00 [mg per caps.]	328.02 [mg per caps.]	S$\bar{x}$=2.49 SD = 7.86 RSD = 2.40
Product I	2392.00 [mg per 100 mL]	2682.80 [mg per 100 mL]	S$\bar{x}$=18.29 D = 57.83 RSD = 2.16
Product K	276.00 [mg per caps.]	59.25 [mg per caps.]	S$\bar{x}$=0.41 SD = 1.30 RSD = 2.20
Product L	276.00 [mg per caps.]	184.86 [mg per caps.]	S$\bar{x}$=1.59 SD = 5.02 RSD = 2.72
Product M	276.00 [mg per caps.]	80.44 [mg per caps.]	S$\bar{x}$=0.59 SD = 1.86 RSD = 2.32
Product N	276.00 [mg per caps.]	71.90 [mg per caps.]	S$\bar{x}$=0.39 SD = 1.22 RSD = 1.70

S $\bar{x}$ standard deviation of the mean; *SD* standard deviation; *RSD* relative standard deviation [%]

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Declarations

Conflict of interest The authors declare that they do not have any conflict of interest.

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