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Development of Theoretical Approaches to Determination of the Main Groups of Biologically Active Substances of Medicinal Plant Raw Materials by TLC Method

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Abstract

Introduction. As is known, the selection of optimal conditions for the analysis of extracts from medicinal plant raw materials (MPM), as well as medicinal herbal preparations and pharmaceutical substances of plant origin, characterized by a complex variable composition of biologically active substances (BAS) by TLC, presents certain difficulties. For the design of mobile phases for the separation of mixtures of BAS of plant origin, in a thin layer of sorbent, the following approaches are used: literary sources; standard mobile phases; spot elution method; the scheme proposed by the firm Camag (Switzerland); model "PRISMA"; variocameras and others. In foreign literature, there are publications on the generalization of the available experimental data on the determination of various natural groups of BAS in objects of plant origin. However, such reviews did not reveal the regularities of the chromatographic behavior of individual BAS in a thin layer, as well as the influence of various factors on the reproducibility of R_f values. The study of the possibility of a theoretical approach to the choice of optimal conditions for chromatography of groups of BAS of different polarity, allowing them to separate, identify and quantify by TLC is a relevant and poorly developed area of chromatography in general.

Aim. The aim of this work was to develop a theoretical approach to the choice of optimal conditions for the chromatographic separation of various groups of BAS of plant origin in a thin layer of sorbent.

Materials and methods. To study the regularities of chromatographic behavior in a thin layer of representatives of the main classes of BAS present in MPM (amino acids, flavonoids, tannins, simple sugars, ascorbic acid, fat-soluble vitamins), the value of the main factor affecting the parameters of the efficiency of the chromatographic process, the polarity of the eluent, was studied. As objects of research, we used ready-made chopped raw material of nettle leaves, produced by a domestic manufacturer, that meets the requirements of regulatory documents, as well as sea buckthorn fruits collected on the territory of the Voronezh region, according to the rules for harvesting MPM of various morphological groups in fresh and dried form.

Results and discussion. The regularities of elution and mathematical models describing the chromatographic behavior of plant BAS in a thin layer of sorbent have been established. Based on the totality of the results obtained, from the standpoint of the efficiency of the chromatographic process, the optimal conditions for their TLC analysis were selected and theoretically substantiated. To study the qualitative composition of BAS and to achieve a clear separation of zones on chromatograms, TLC methods were developed and tested on the studied MPM using simple, frontal or two-dimensional chromatography.

Conclusion. It is shown that the determination and separation in a thin layer of the sorbent of hydrophilic and lipophilic BAS of MPM in the presence of a joint requires different approaches and techniques. The paper proposes an algorithm for the selection of the mobile phase and methods of chromatography of BAS of medicinal products. The revealed mathematical models describing the chromatographic behavior of BAS will make it possible to select the conditions under which it is possible to determine the individual components of multicomponent mixtures without preliminary separation. The developed methods for the determination of BAS can also be used for standardization and quality assessment of other types of MPM, phytopreparations and pharmaceutical substances of plant origin.

Keywords: thin layer chromatography, eluent polarity, biologically active substances of plant origin

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Contribution of the authors. The author carried out a review of the literature, its systematization and analysis, and conducted experimental studies. The author wrote the text of the article, including the conclusion and discussion of the results.

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Разработка теоретических подходов к определению основных групп биологически активных веществ лекарственного растительного сырья методом ТСХ

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Резюме

Введение. Как известно, подбор оптимальных условий анализа извлечений из лекарственного растительного сырья (ЛРС), а также лекарственных растительных препаратов и фармацевтических субстанций растительного происхождения, характеризующихся сложным вариабельным составом биологически активных веществ (БАВ), методом ТСХ представляет определенные трудности. Для конструирования подвижных фаз для разделения смесей БАВ растительного происхождения в тонком слое сорбента применяются следующие подходы: литературные источники; стандартные подвижные фазы; метод «элюирования пятна»; схема, предложенная фирмой Camag (Швейцария); модель «ПРИЗМА»; вариокамеры и др. В зарубежной литературе встречаются публикации по обобщению имеющихся экспериментальных данных по определению различных природных групп БАВ в объектах растительного происхождения. Однако в подобных обзорах не выявлены закономерности хроматографического поведения отдельных БАВ в тонком слое, а также влияние различных факторов на воспроизводимость величин R_f. Изучение возможности теоретического подхода к выбору оптимальных условий хроматографирования групп БАВ различной полярности, позволяющих провести их разделение, идентификацию и количественное определение методом ТСХ является актуальным и мало разработанным направлением хроматографии в целом.

Цель. Целью настоящей работы являлась разработка теоретического подхода к выбору оптимальных условий хроматографического разделения различных групп БАВ растительного происхождения в тонком слое сорбента.

Материалы и методы. Для исследования закономерностей хроматографического поведения в тонком слое представителей основных классов БАВ, присутствующих в ЛРС (аминокислоты, флавоноиды, дубильные вещества, простые сахара, аскорбиновая кислота, жирорастворимые витамины), было изучено значение основного фактора, влияющего на параметры эффективности хроматографического процесса – полярности элюента. В качестве объектов исследования использовали готовое измельченное сырье листьев крапивы двудомной, выпускаемое отечественным производителем, соответствующее требованиям нормативной документации, а также плоды облепихи крушиновидной, собранные на территории Воронежской области, согласно правилам заготовки ЛРС различных морфологических групп в свежем и высушенном виде.

Результаты и обсуждение. Установлены закономерности элюирования и математические модели, описывающие хроматографическое поведение растительных БАВ в тонком слое сорбента. По совокупности полученных результатов с позиций эффективности хроматографического процесса были выбраны и теоретически обоснованы оптимальные условия их ТСХ-анализа. Для исследования качественного состава БАВ и достижения четкого разделения зон на хроматограммах разработаны и апробированы на изучаемом ЛРС ТСХ-методики с применением простого, фронтального или двумерного хроматографирования.

Заключение. Показано, что определение и разделение в тонком слое сорбента гидрофильных и липофильных БАВ ЛРС при совместном присутствии требует различных подходов и приемов. В работе предложен алгоритм выбора подвижной фазы и приемов хроматографирования БАВ ЛРС. Выявленные математические модели, описывающие хроматографическое поведение БАВ, позволят подбирать условия, в которых возможно определять отдельные составляющие многокомпонентных смесей без предварительного разделения. Разработанные методики определения БАВ могут быть также использованы для стандартизации и оценки качества других видов ЛРС, фитопрепаратов и фармацевтических субстанций растительного происхождения.

Ключевые слова: тонкослойная хроматография, полярность элюента, биологически активные вещества растительного происхождения

Конфликт интересов. Автор декларирует отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Автор осуществлял обзор литературы, его систематизацию и анализ, проводил экспериментальные исследования, а также написал текст статьи, в том числе заключение и обсуждение результатов.

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INTRODUCTION

In scientific literature, examples of a theoretical approach how to solve tasks of multicomponent mixture separations [1–4] with TLC method have been long known and described. The selection of optimal conditions for the analysis of extractions from medicinal plant raw materials (MPM), as well as, herbal medicinal products and pharmaceutical substances of plant origin which, as it is known, have a complex variable composition of biologically active substances (BAS). For formation of mobile phases for separation of BAS mixtures of plant origin in a thin sorbent layer, the following approaches are used: literature sources; standard mobile phases; "spot elution" method; the scheme offered by company Camag (Switzerland); model "PRISMA"; vario chambers [1–3], etc. In practical work, using mobile phases obtained from literature sources for separation of a definite group of compounds, the following should be considered: sorbent type; sorbent brand; thickness of sorbent layer; conditions for plate rinsing and activation prior to work; type of an elution chamber; chamber saturation; chamber saturation time, and in some cases, plate saturation time; air temperature and relative humidity of laboratory facility; presence and contents of stabilizers in diluents.

Only when all abovementioned factors are considered, separation with adequate resolution and selectivity may be performed. The scheme offered by company Camag and model "PRISMA" are most widely used [5].

The scheme for optimal selection of mobile phase composition offered by company Camag includes four stages: test mixture on the first stage is separated in 12 diluents with various strengths. On the second stage, the strength of the diluent which has led to separation of mixture components and water or n-hexan, is increased or decreased. On the third stage, if necessary, an acidic or base modifier is added. On the fourth stage; elution system with optimal selectivity is chosen based on variation of components of the mobile phase obtained on the third stage [5].

Model "PRISMA" was developed by Sh. Neyredy for selection and optimization of elution systems in direct phase and reversed phase planar chromatography. The model represents a three-dimensional variant of the Snyder triangle being the prism base. The model gives the principal opportunity, combining with the formulation – 2-5-component eluent, to separate test mixtures with a high resolution and selectivity. In practical work, mixture of diluents taken in ratio (1:1:1) is initially tested, and if Rf values of test mixture components are within the range above 0.7, then n-hexan is added to a four-component eluent, and if R_f is < 0.2, water is added to the eluent. Then selectivity is tested in points 8:1:1, 1:8:1 and 1:1:8. If obtained results of mixture separation are satisfactory, separation selectivity will be tested in points 7:2:1, 2:7:1 and 1:2:7, and in the neighboring areas, achieving the best selectivity and efficiency of the separation. After the optimal ratio of eluent components is found, an additional optimization (by selectivity and resolution, increasing or decreasing eluent's strength and/or amount of the modifier) may be performed [5].

Foreign literature also contains publications summarizing available experimental data on determination of various natural BAS groups in herbal objects [6–12]. However, the patterns of chromatographic behavior of separate BAS in a thin layer, as well as, influence of various factors on reproducibility of Rf values are not found.

The disadvantages of such approaches may include: the absence of gas phase accountability in a chromatographic chamber; difficulties in separation of strongly polar compounds which include most groups of plant BAS; divergence between estimated data and results obtained in practice [5], as well as duration and complexity.

The investigation of the possibility to use a theoretic approach to the selection of optimal conditions for chromatography of BAS groups of various polarity allowing to separate, identify and quantify them with TLC method is a relevant and poorly developed field in chromatography in general.

Due to the abovementioned, the development of a theoretic approach for the selection of optimal conditions of chromatographic separation of various groups of herbal BAS in a thin sorbent layer.

MATERIALS AND METHODS

To investigate patterns of chromatographic behavior in a thin sorbent layer of substances from the main classes of BAS present in MPM [amino acids, flavonoids, tannins, simple sugars, ascorbic acid, fat-soluble vitamins (FSV)], the value of the main factor influencing efficiency parameters of the chromatographic process – eluent polarity (*P*) was examined.

As reference samples for identification of patterns of chromatographic behavior of the main representatives of various BAS groups, commercially available individual substances were used: α-tocopherol (≥ 97 %, ICN Biomedical, USA), rutin (≥ 94 %, Sigma, USA), retinol acetate (FS 42-7811-97), ergocalciferol (FS 42-0008018000), β-carotene (VFS 42-0008018000), tanin, gallic acid, quercetin, simple sugars (rhamnose, glucose, xylose, fructose), ascorbic acid (AsA), amino acids (proline, glycine, glutamic acid, methionine, phenylalanine, arginine, valin, leucine) (≥ 98 %, CJSC "Vekton", Russia). As reference samples for identification of patterns of chromatographic behavior of the main representatives of various BAS groups, commercially available individual substances were used: α-tocopherol (≥ 97 %, ICN Biomedical, USA), rutin (≥ 94 %, Sigma, USA), retinol acetate (FS 42-7811-97), ergocalciferol (FS 42-0008018000), β-carotene (VFS 42-0008018000), tanin, gallic acid, quercetin, simple sugars (rhamnose, glucose, xylose, fructose), ascorbic acid (AsA), amino acids (proline, glycine, glutamic acid, methionine, phenylalanine, arginine, valin, leucine) (≥ 98 %, CJSC "Vekton", Russia). Analytical pure grade and chemically pure reagents and diluents (CJSC "Vekton", Russia) meeting the requirements of corresponding regulatory documents were used in the work. High performance chromatographic plates Sorbfil PTSH-AF-A and PTSH-AF-V 10 × 10 cm in dimensions (sorbent type: silicagel STH-1A, STH-1VE; granulation: 5–17, 8–12 μm; layer thickness: 90–120, 80–100 μm, respectively; binder: silicasol) were used in the

experiment. Samples were applied with microsyringes (MSh-1 and MSh-10, Russia).

The developer allowing to obtain a stable staining with BAS group separation was selected with regards to the analysis of literature sources [1–13]. BAS areas on chromatograms were found when chromatograms were spayed with a developing reagent solution with a sprayer.

The developed theoretical approaches to separation of studied BAS groups were tested, identified, quantified using medicinal plants materials of common nettle as a species – a source of hydrophilic BAS groups and MPM of sea buckthorn containing a vast range of lypophilic nature. As research objects, we used a finished granulated raw material of nettle leaves (*Folia Urticae*) produced by the domestic manufacturer that meets the requirements of regulatory documents, as well as sea buckthorn fruits (Fructus Hippophaae. rhamnoides) collected on the territory of the Voronezh region according to the rules for harvesting MPM of various morphological groups in fresh and dried form. Fruits were dried at temperature 60 °C up to residual moistness not above 14 %.

RESULTS AND DISCUSSION

The selection of diluents differing both by strength and selectivity is rather huge [1–3]. The work was performed on selection of eluents for determination of main natural BAS groups (on the example of main representatives) in a thin sorbent layer. In the experiment, over twenty types of eluting system were investigated for tested BAS in a wide range of polarity, and dependencies of between Rf value and system polarity established (figure 1, a–c). The eluents offered in the literature [13–18], as well as new chromatographic systems were examined.

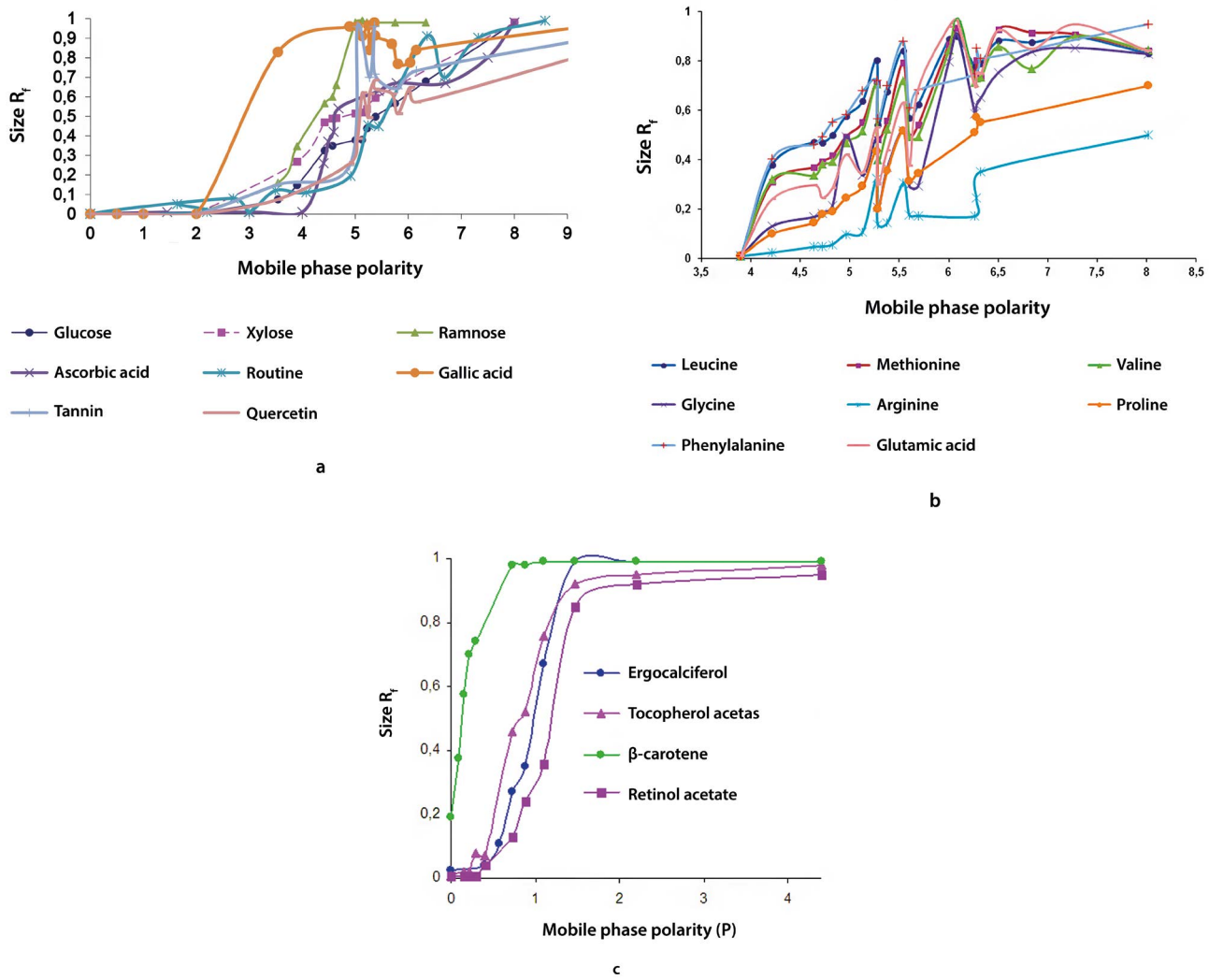

Figure 1. The type of dependence of the R_f value of BAS on the polarity of the eluent:
a – simple sugars, polyphenolic compounds, ascorbic acid; b – amino acids; c – fat-soluble vitamins

During the comprehensive investigation of system polarity effect on Rf value for each individual BAS, the range of eluent polarity values in which a linear dependence was found, was determined (figure 2, a–c). The summarized data is given in table 1.

Therefore, the pattern of elution and mathematical models describing chromatographic behavior of plant BAS in a thin sorbent layer were established (table 1). Using these dependencies, various systems may be selected for BAS determination so that R value falls within optimal values, as well as, to predict possibility of separation of complex multicomponent mixtures at assigned value of eluent polarity.

Based of the summary of obtained results, in the context of efficiency of a chromatographic process [height equivalent to a theoretical plate (H) and number of theoretical plates (N), distribution coefficient (K)] optimal conditions for chromatography of tested BAS were selected and theoretically justified in a thin sorbent layer: eluent, developer, sorbent, sample volume, sensitivity of determination, system polarity. The summarized data is given in table 2.

Table 1. Parameters of the established linear dependences of the R_f value of the BAS on the *P* system (where $y = R_f$, and $x = P$)

№	BAS	Linearity range	R^2	Linear equation	System *P* range for optimum R_f values
Lipophilic BAS					
1	β-carotene	0–2.0	0.9756	$y = 2.5383x + 0.1729$	0.05–0.17
2	Ergocalciferol	0.58–1.10	0.9701	$y = 1.0455x - 0.5100$	0.77–1.06
3	Retinol acetate	0.73–1.10	0.9907	$y = 0.3880x - 0.0847$	0.20–1.10
4	α-tocopherol	0.40–1.10	0.9830	$y = 0.9647x - 0.2976$	0.62–0.93
BAS of hydrophilic nature					
5	Routine	4.90–6.30	0.9740	$y = 0.4762x - 2.1095$	5.06–5.69
6	Ascorbic acid	4.0–4.70	0.9850	$y = 0.7524x - 3.0127$	4.40–4.80
7	Arginine	3.90–8.01	0.9545	$y = 0.1293x - 0.5408$	6.50–8.82
8	Glycine	3.90–5.54	0.9243	$y = 0.3074x - 1.2072$	4.90–5.88
9	Glutamic acid	5.13–6.00	0.9615	$y = 0.6191x - 2.7941$	5.00–5.48
10	Valin	3.90–5.27	0.9646	$y = 0.4669x - 1.8293$	4.56–5.20
11	Leucine	3.90–5.27	0.9789	$y = 0.5433x - 2.1007$	4.42–4.97
12	Methionine	3.90–5.27	0.9817	$y = 0.4770x - 1.8580$	4.52–5.15
13	Proline	3.90–5.54	0.9056	$y = 0.2982x - 1.1963$	5.02–6.02
14	Phenylalanine	3.90–5.27	0.9851	$y = 0.5243x - 2.0039$	4.39–4.97
15	Glucose	5.13–6.33	0.9616	$y = 0.2321x - 0.7789$	4.65–5.94
16	Xylosis	4.42–5.22	0.9727	$y = 0.0750x + 0.1409$	2.12–6.12
17	Rhamnose	3.54–4.64	0.9937	$y = 0.4367x - 1.3736$	3.83–4.52
18	Gallic acid	3.54–5.36	0.9777	$y = 0.0827x + 0.5407$	До 0.72
19	Quercetin	4.89–5.36	0.9529	$y = 0.8792x - 4.0492$	4.95–5.29

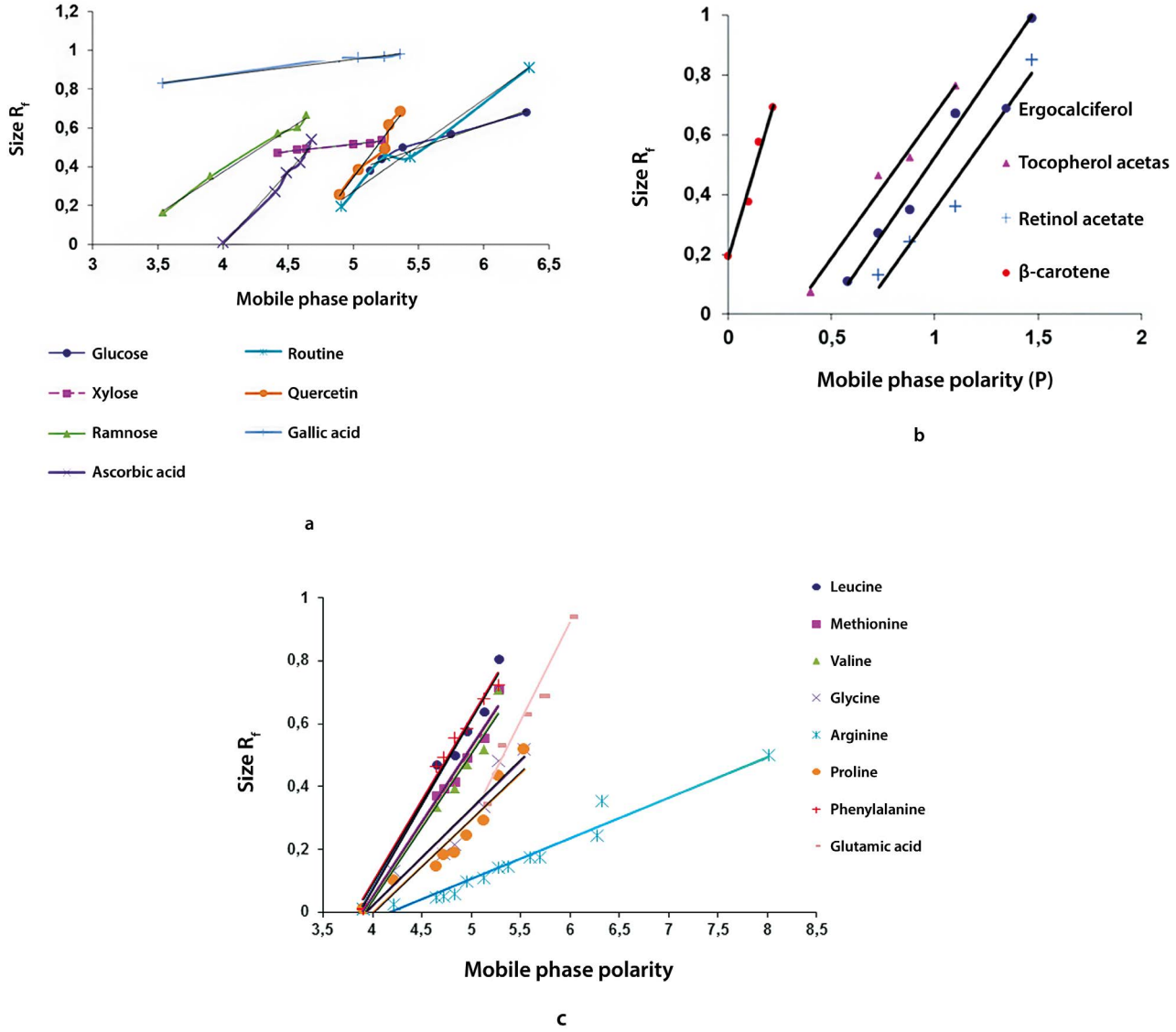

Figure 2. Linear dependences of the R_f value of BAS on the polarity of the eluent:
a – simple sugars, polyphenolic compounds, ascorbic acid; b – fat-soluble vitamins; c – amino acids

The main parameters reflecting efficiency of chromatography of BAS tested with TLC method in the systems described in table 2 are given in table 3.

Thus, the studies carried out have shown that the definition and separation in a thin layer sorbent hydrophilic and lipophilic BAS LRS at the joint presence requires different approaches and techniques. The proposed algorithm for the selection of the mobile phase and methods for the chromatographic analysis of biologically active substances of medicinal products is shown in figure 3.

To investigate a qualitative composition of BAS and achievement of a clear separation of areas on chromatograms, the developed TLC methods with a simple, frontal or two-compartmental chromatography have been tested on the tested MPM. The results are given in table 4.

For quantification of BAS in the tested MPM present together with various variations of the developed TLC methods used on the next stage immediately after development of chromatographic areas on calibration chromatograms with batches of reference solution, plates are scanned and treated with computer program Sorbfil. Videodensitometer (CJSC "Sorbpolymer", Russia). The linear dependencies were established between contents of BAS (x) and chromatographic area (y) in the range of test concentrations. The developed methods of TLC data quantification were tested on the tested MPM. The method characteristics and determination results are given in table 5.

Table 2. Characteristics of the developed methods of separation and identification of the studied BAS in a thin layer of sorbent

№	BAS	Analysis conditions				
		Eluent	Detection reagent	R_f	*P*	Detection limit, g
Lipophilic BAS						
1	β-carotene	hexane : benzene (29:1)	–	0.38 ± 0.01	0.10	$1 \cdot 10^{-5}$
2	Ergocalciferol	hexane : chloroform (4:1)	5 % alcohol solution of phosphoromolybdic acid	0.35 ± 0.01	0.88	$7 \cdot 10^{-9}$
3	Retinol acetate	hexane : chloroform (3:1)	10 % alcohol solution of phosphoromolybdic acid with concentrated hydrochloric acid (25:1)	0.36 ± 0.01	1.10	$1 \cdot 10^{-7}$
4	α-tocopherol	chloroform	concentrated nitric acid	0.59 ± 0.02	4.40	$1 \cdot 10^{-6}$
5	Fat solubile vitamins with joint presence	eluent 1 (run height 8 cm) – hexane : chloroform (19:1); eluent 2 (run height 6 cm) – hexane : chloroform (3:1)	5 % alcohol solution phosphoromolybdic acid	–	eluent 1 – 0.22; eluent 2 – 1.10	–
BAS of hydrophilic nature						
6	Routine	ethylacetate : glacial acetic acid : water (7.5:1.5:1.5)	5 % alcohol solution of NaOH	0.46 ± 0.01	5.24	$5 \cdot 10^{-7}$
7	Ascorbic acid	ethylacetate : glacial acetic acid (85:15)	5 % alcohol solution of phosphomolybdic acid or 0.2 % alcohol solution of 2.6-sodium dichlorophenolindophenolate	0.42 ± 0.01	4.59	$4 \cdot 10^{-7}$
8	Arginine	*n*-butanol : acetic acid : water (4:1:2)	1 % alcohol solution of ninhydrin	0.55 ± 0.02	5.13	$1 \cdot 10^{-8}$
9	Glycine			0.29 ± 0.02		$1 \cdot 10^{-8}$
10	Glutamic acid			0.68 ± 0.01		$1 \cdot 10^{-8}$
11	Valin			0.11 ± 0.01		$5 \cdot 10^{-8}$
12	Leucine			0.34 ± 0.01		$3 \cdot 10^{-8}$
13	Methionine			0.35 ± 0.01		$3 \cdot 10^{-8}$
14	Proline			0.52 ± 0.01		$10 \cdot 10^{-8}$
15	Phenylalanine			0.64 ± 0.02		$3 \cdot 10^{-8}$
16	Glucose	*n*-butanol : glacial acetic acid : water (4:1:2)	sulfanilamide and *o*-phthalic acid	0.29 ± 0.02	5.69	$2.5 \cdot 10^{-6}$
17	Xylosis			0.45 ± 0.01		$2.5 \cdot 10^{-6}$
18	Rhamnose			0.55 ± 0.02		$5 \cdot 10^{-6}$

Окончание таблицы 2

№	BAS	Analysis conditions				
		Eluent	Detection reagent	R_f	*P*	Detection limit, g
19	Gallic acid	eluent 1 (run height 9 cm) – diethyl ether : acetic acid : hexane : ethyl acetate (20:20:20:40); eluent 2 (run height 7 cm) – ethyl acetate : formic acid : acetic acid : water (67:7.5:7.5:18)	1 % alcoholic solution of ammonium iron alum	eluent 1 – 0.83 ± 0.02	eluent 1 – 3.54; eluent 2 – 9.68	$3 \cdot 10^{-7}$
20	Quercetin			eluent 1 – 0.08 ± 0.01; eluent 2 – 0.84 ± 0.02		$1 \cdot 10^{-6}$
21	Tannin			eluent 1 – 0.15 ± 0.01; eluent 2 – 0.91 ± 0.01		$5{,}5 \cdot 10^{-7}$

Note. *P* – polarity of the system, calculated according to L. Snyder; DL – detection limit.

Table 3. Parameters of the efficiency of chromatography of the studied BAS by TLC

№	BAS	H, мм	N	K
1	β-carotene	0.78	108.97	0.38 ± 0.01
2	α-tocopherol	0.55	156.00	0.69 ± 0.02
3	Routine	0.71	100.80	0.46 ± 0.01
4	Ascorbic acid	0.10	767.00	0.42 ± 0.01
5	Glucose	0.70	127.14	0.29 ± 0.02
6	Xylosis	0.42	211.90	0.45 ± 0.01
7	Rhamnose	0.35	254.29	0.55 ± 0.02
8	Arginine	0.44	179.55	0.11 ± 0.01
9	Glycine	0.89	87.64	0.34 ± 0.01
10	Glutamic acid	0.36	186.11	0.35 ± 0.01
11	Valine	0.37	210.81	0.52 ± 0.01
12	Leucine	0.17	458.82	0.64 ± 0.02
13	Methionine	0.35	222.86	0.55 ± 0.02
14	Proline	0.30	256.67	0.29 ± 0.02
15	Phenylalanine	0.65	116.92	0.68 ± 0.01

CONCLUSION

For quantification of BAS in the tested MPM present together with various variations of the developed TLC methods used on the next stage immediately after development of chromatographic areas on calibration chromatograms with batches of reference solution, plates are scanned and treated with computer program Sorbfil. Videodensitometer (CJSC "Sorbpolymer", Russia). The linear dependencies were established between contents of BAS (x) and chromatographic area (y) in the range of test concentrations. The developed methods of TLC data quantification were tested on the tested MPM. The method characteristics and determination results are given in table 5.

The developed new, easy to use, economically available, express methods for separation, identification and quantification of BAS with various variations of high performance thin layer chromatography can be also used for standartization and evaluation of other MPM types (tested on the example of common nettle and sea buckthorn frutis), herbal preparation and pharmaceutical substances of plant origin.

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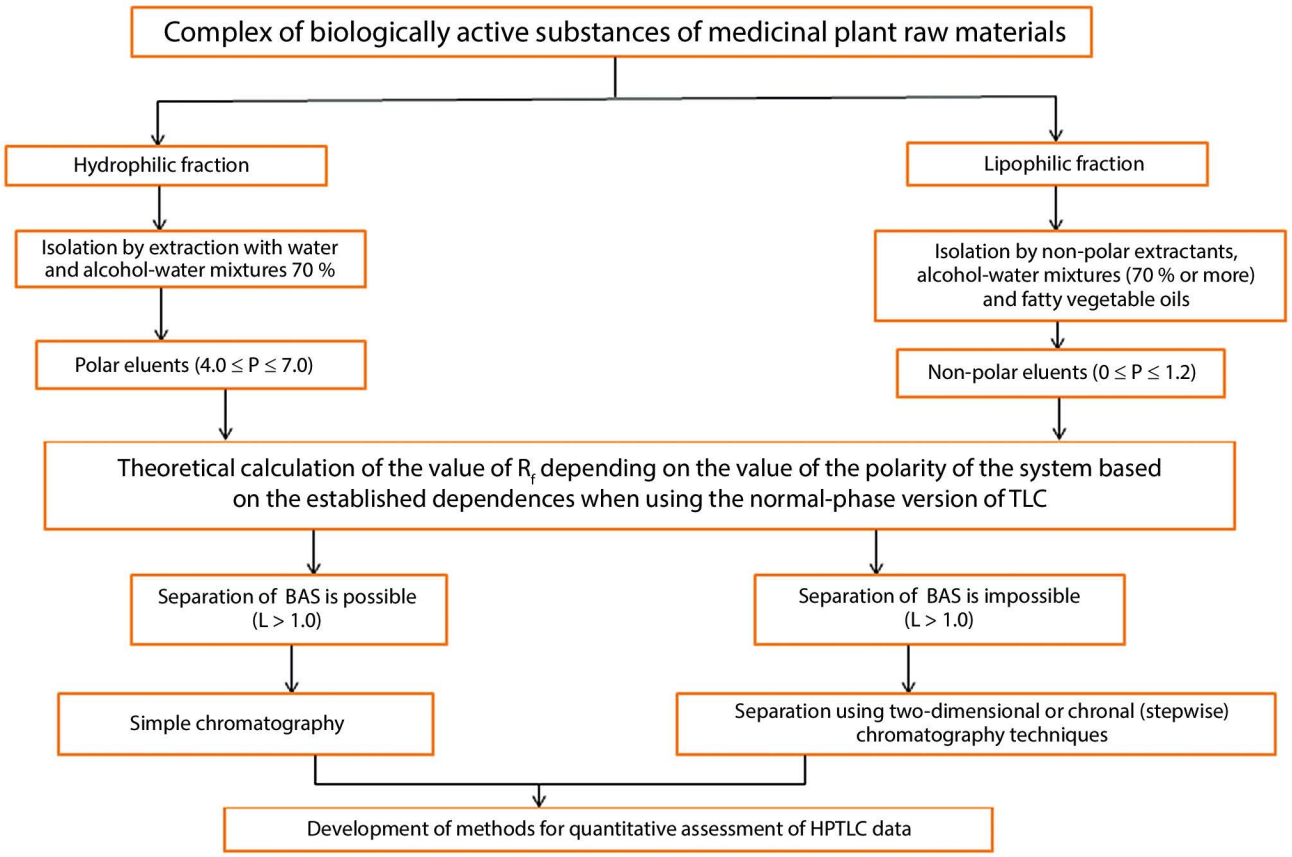

Figure 3. Algorithm for the selection of the mobile phase and methods for the chromatographic determination and separation of BAS of MPM (L is the coefficient of selectivity of sorption, showing the efficiency of separation of zones on chromatograms in TLC)

Table 4. Identification of BAS zones on chromatograms of extracts from MPM

№	BAS	Research objects		
		Nettle leaves	Dried sea buckthorn fruits	Fresh sea buckthorn fruits
1	Organic acids	Oxalic, tartaric, ascorbic acid	Oxalic, tartaric, citric, malic, ascorbic acid	Oxalic, tartaric, malic, ascorbic acid
2	Flavonoids	Rutin, quercetin	Rutin, hyperoside, quercetin	
3	Amino acids	Arginine, proline, glycine, glutamic acid, valine, leucine, phenylalanine	Arginine, proline, glycine, glutamic acid, leucine, phenylalanine	Proline, glycine, glutamic acid, leucine
4	Tannins	Tannin, gallic acid	Gallic acid	
5	Simple sugars	Glucose, xylose, rhamnose		

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Table 5. Parameters of the developed TLC methods for identification and quantitative determination of biologically active substances in medicinal product (in terms of absolutely dry raw materials)

№	BAS	Linearity range, mg/ml	R^2	Linear equation	BAS determination results, %	
					Nettle leaves	Dried sea buckthorn fruits
1	Ascorbic acid	1.5–4.5	0.9941	$y = 9233.6x - 1550.1$	0.053 ± 0.015	0.047 ± 0.001
2	Glutamic acid	0.5–3.5	0.9707	$y = 18190x + 13560$	0.011 ± 0.001	0.330 ± 0.036
3	Quercetin	1.0–6.0	0.9916	$y = 3.6734x + 1.1792$	0.643 ± 0.040	–
4	Tannin	0.5–4.5	0.9721	$y = 4.4805x + 0.7287$	0.724 ± 0.016	–
		5.0–7.0	0.9531	$y = 6.0083x - 0.1537$		
5	Gallic acid	0.3–1.5	0.9660	$y = 16.601x - 5.3268$	0.579 ± 0.056	0.041 ± 0.001
		1.5–4.0	0.9154	$y = 4.8778x + 13.128$		
6	Glucose	15.0–35.0	0.9947	$y = 16.327x - 158.66$	14.070 ± 1.263	35.569 ± 3.446
7	Xylose	15.0–30.0	0.9744	$y = 3.7854x + 23.337$	0.273 ± 0.056	Менее 0.05 %
8	Ramnose	10.0–30.0	0.9734	$y = 3.3692x + 8.744$	2.315 ± 0.131	1.705 ± 0.098

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