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QUALIFICATION OF TLC-EQUIPMENT USED IN ANALYSIS OF THE COMBINED HERBAL MEDICINES

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Key words: quality control system; analysis; TLC-equipment; Klimased

On the example of Klimased drug with the purpose of choosing the analytical equipment for quality control of combined drugs of the plant origin the study on major functional and performance characteristics (design qualification) of the equipment used according to pharmacopoeial requirements(SPhU and USP) and the target analytical tasks has been conducted. It allows to select equipment for an enterprise. A comparative analysis of functional and technical specifications of the devices to perform analysis of the combined drugs from the medicinal plant raw material by the method of thin-layer chromatography has been conducted. It has been experimentally proven that the configuration of the chromatographic chamber does not affect the result of the analysis, chambers manufactured by Sorbfil are more affordable by economic indicators. When analysing the medicinal plant raw material and drugs from it the TLC and HPTLC plates on all substrates with an UV indicator can be used, it is advisable to make the final choice by economic components or their availability. Characteristics of the devices for applying solutions of chromatograms have revealed that when choosing microsyringes the best indicator is its volume. When comparing devices for detecting or quenching fluorescence manufactured by Sorbfil (Russia) and Spectroline (USA) it has been proven that each of the devices studied can be used to identify chromatographic zones. The manufacturer Spectroline has the quality certificate ISO 9001:2008 and is more affordable, therefore, it is superior to irradiator UVS-254/365 manufactured by Sorbfil. Among the devices for spraying chromatograms the sprayers of GGB (USA) have a comfortable design and a suitable volume, but by cost they are much more expensive than sprayers of Sorbfil (Russia). It is advisable to make the final choice based on the economic component.

A key element in organization of production and quality management is the quality control system, and one of its main objectives is to obtain reliable results during the analysis. Such results are only possible when using laboratory equipment with the required technical characteristics and correct functioning.

Equipment qualification is a necessary preliminary step for validation/verification of analytical methods [1, 4, 8].

The aim of our paper was to qualify the laboratory equipment used in the analysis of a multicomponent herbal drug Klimased by thin-layer chromatography (TLC).

Materials and Methods

When evaluating the quality of Klimased drug according to its Drug Master File (DMF) such physical and physicochemical methods of analysis as TLC, HPLC, GC, etc., were used.

On the example of Klimased drug with the purpose of choosing the analytical equipment for its quality control the study of major functional and performance characteristics (design qualification) of the equipment used according to pharmacopoeial requirements (SPhU and USP) and the target analytical tasks were conducted. It allows to select equipment for an enterprise [2, 3, 6, 7, 9].

In the process of our study the propositions of manufacturers that supply analytical equipment used to identify the active substances of the drug under research (TLC) to the pharmaceutical market of Ukraine were analyzed.

Results and Discussion

The results of analysis of the requirements of the SPhU and USP to the equipment used in the TLC method in the qualitative analysis of Klimased are given in Tab. 1.

A comparative analysis of functional and technical specifications of the devices to perform analysis by the method of thin-layer chromatography is given in Tab. 2.

As Tab. 2 shows, a comparative analysis of functional characteristics of the chromatographic chambers of different manufacturers (Sorbfil, Russia and GGB, USA) has found that the size of the plate affects the choice of chambers for conducting the experiment. The Latch-LID Chromatotanks model (27×7×26 cm) manufactured by GGB (USA) is optimal when using the TLC plates with the size of 20×10 (for simultaneous analysis of many samples). It has been experimentally proven that the configuration of the chromatographic chamber does not affect the result of the analysis [5]. Chambers manufactured by Sorbfil are more affordable by economic indicators.

Comparative characteristics of analytical and high-performance plates for TLC of such manufacturers as Sorbfil (Russia), Macherey-Nagel (Germany), Merck (Germany) have found that as a sorbent all plates contain silica gel, which is selective in separation of compounds containing various functional groups of the medicinal plant raw material (the medicinal plant raw material and drugs with the medicinal plant raw material) [5, 6, 8]. The plates under research contain aluminium, polyethylene terephthalate, glass as a substrate, each of them has advantages (inert glass material, aluminium and polyethylene terephthalate are easy to use) [5, 6]. When ana-

Table 1

Qualification of equipment for TLC analysis of Klimased drug (requirements of the SPhU and USP)

Equipment	Requirements of the SPhU	Requirements of the USP
TLC or HPTLC plates	The stationary phase consisting of a suitable material applied in the form of a standardized thin layer and fixed on the base (plate) made of glass, metal or plastic	
	To use plates made in industrial conditions is permitted if they comply with the requirements of section 4.1.1. «Reagents», as well as "System suitability test" described in a separate article. It is recommended to determine convergence of R_f. values	To use plates made in industrial conditions is permitted
	The substrate is made of glass, metal or plastic, coated with a layer of silica gel with a suitable thickness and particle size for HPTLC plates – 2-10 μm and for common TLC plates – 5-40 μm. If necessary, the particle size is indicated after the name of the sorbent in the tests where it is used. The sorbent may contain an organic binding substance	The average particle size for HPTLC plates – 5 μm, for TLC plates 10-15 μm
Micropipettes, microsyringes, calibrated capillaries	Devices suitable for applying solutions	Manual, semi-automatic or automatic devices for applying samples. A template for drawing zones manually at specific intervals, measuring the distance and assisting in labelling can be additionally used
Chromatographic chamber	The container with a tight fitting lid and a flat bottom or a bottom with two troughs made of an inert transparent material corresponding to the size of the plates used	
Developing devices or reagents	Suitable devices used to transfer reagents on the plate by spraying, vapour treatment or immersion that provide, if necessary, heating to detect the compounds separated	
Devices for detecting or quenching fluorescence	Requirements are not given	The UV light source for examining in the short-wave (254 nm) and long-wave (365 nm) light
Documentation	For example, photographs or computer files can be used for documenting the chromatograms detected	A suitable device to register the chromatograms detected

Table 2

A comparative analysis of functional and technical specifications of the devices for TLC analysis and their cost

Equipment	Name	Description	Cost
1	2	3	4
Chromatographic chamber			
Sorbfil, Russia	Chromatographic chamber with a dividing ledge for plates of 10×10 cm. The size – (150×120×80 mm)	Chambers are made of chemically resistant glass. They have a dividing ledge at the bottom for fixing plates and saving the eluent. Chambers are supplied with a ground lid	2 000 RUB
	Chromatographic chamber for plates of 15×15 cm. The size – (150×120×80 mm)		2 850 RUB
	Chromatographic chamber for plates of 15×15 cm (glued with silicone). The size – (190×195×65 mm)		1 600 RUB
GGB, USA	Chromatographic chamber with a flat bottom (Latch-LID Chromatotanks). The size – (27×7×26 cm)	Heavy glass of the design is suitable for regular use for many years. The design is sustainable against accidental contact due to the weight (about 15 feet) and the flat bottom. A clear glass allows to inspect easily the contents of the chamber from all sides. A flat lid provides reliable fit, it is made of milk glass with edges beveled inside and outside avoiding all sharp edges. The unique design of self-locking has the corresponding latches on the lid and the chamber and allows the structure when closing the chamber with the lid be firmly in position. Metal components of the mechanism-latch lid are made of stainless steel	156.28 $

Table 2 continued

1	2	3	4
	Chromatographic chamber with a flat bottom, 10 cm Thinline (Latch-Lid Chromatotank unit/Tank – Lid, intended for plates of 10×10 cm or 10×5 cm. The size – (12×6.4×11.5)	This model can place up to two plates and is suitable for storing small amounts of test solvents (when selecting the optimal solvent in the process of studying the unknown substances). A smaller cubic size of the chamber makes it faster and more evenly saturate the atmosphere with solvents and use a smaller volume of the solvent. A clear glass allows to inspect easily the contents of the chamber from all sides. A flat lid provides reliable fit, it is made of milk glass with edges beveled inside and outside avoiding all sharp edges. The unique design of self-locking has the corresponding latches on the lid and the chamber and allows the structure when closing the chamber with the lid be firmly in position. Metal components of the mechanism-latch lid are made of stainless steel	93.89 $
TLC or HPTLC plates			
Sorbfil, Russia, Krasnodar	Sorbfil PTLC-P-A (10x10), 50 ps	Analytical, without an UV indicator. Particle size: 5-17 μm. The substrate material – PET film (polyethylene terephthalate)	1480 RUB
	Sorbfil PTLC-P-A (10x15), 50 ps		2200 RUB
	Sorbfil PTLC-P-A (10x20), 50 ps		3350 RUB
	Sorbfil PTLC-P-A-UV (10x10), 50 ps	Analytical, with an UV indicator (254 nm). Particle size: 5-17 μm. The substrate material – PET film	1680 RUB
	Sorbfil PTLC-P-A-UV (10x15), 50 ps		2 580 RUB
	Sorbfil PTLC-P-A-UV (10x20), 50 ps		3 800 RUB
	Sorbfil PTLC-P-V (10x10), 50 ps	High-performance, without an UV indicator. Particle size: 8-12 μm. The substrate material – PET film	2100 RUB
	Sorbfil PTLC-P-V (10x15), 50 ps		3 300 RUB
	Sorbfil PTLC-P-V-UV (10×10), 50 ps	High-performance, with an UV indicator. PTLC-P-V-UV. Particle size: 8-12 μm. The substrate material – PET film	2 300 RUB
	Sorbfil PTLC-P-V-UV (10x15), 50 ps		3 590 RUB
	Sorbfil PTLC-AF-A (10×10), 50 ps	Analytical, without an UV indicator. Particle size: 5-17 μm. The substrate material – aluminium foil	1480 RUB
	Sorbfil PTLC-AF-A (10×15), 50 ps		2200 RUB
	Sorbfil PTLC-AF-A (10×20), 50 ps		3 350 RUB
	Sorbfil PTLC-AF-A-UV (10×10), 50 ps	Analytical, with an UV indicator (254 nm). Particle size: 5-17 μm. The substrate material – aluminium foil	1680 RUB
	Sorbfil PTLC-AF-A-UV (10×15), 50 ps		2 580 RUB
	Sorbfil, PTLC-AF-A-UV (10×20), 50 ps		3 800 RUB
	Sorbfil, PTLC-AF-V (10x10), 50 ps	High-performance, without an UV indicator. Particle size: 8-12 μm. The substrate material – aluminium foil	2100 RUB
	Sorbfil, PTLC-AF-V (10x15), 50 ps		3 300 RUB
	Sorbfil, PTLC-AF-V-UV (10×10), 50 ps	High-performance, with an UV indicator. Particle size: 8-12 μm. The substrate material – aluminium foil	2 300 RUB
	Sorbfil, PTLC-AF-V-UV (10×15), 50 ps		3 590 RUB
Macherey-Nagel, Germany	DC-Fertigfolien ALUGRAM Sil G/UV$_{254}$ (20×20) , 25 ps	Analytical, with an UV indicator (254 nm). Particle size: 5-17 μm. The substrate material – aluminium foil	1300 UAH
Merck, Germany	Silica gel 60 F$_{254}$ (10×10), 25 ps	Analytical, with an UV indicator (254 nm). Particle size: 10-12 μm. The substrate material – glass	1868.0 UAH
	Silica gel 60 F$_{254}$ (10×20), 50 ps	Analytical, with an UV indicator (254 nm). Particle size: 10-12 μm. The substrate material – glass	2347.0 UAH
	Silica gel 60 F$_{254}$ (20×10), 50 ps	High-performance, with an UV indicator (254 nm). Particle size: 5-6 μm. The substrate material – glass	4585 UAH

Table 2 continued

1	2	3	4
Device for applying			
Sorbfil, Russia	M-1, volume – 1 µl, without guiding (1 ps)	Calibration microsyringes are designed for metered applying standard solutions and dosed analytical samples on the plates. The needle has a brushed straight cut	2 750 RUB
	M-1H, volume – 1 µl, with guiding (1 ps)		3 570 RUB
	M-10, volume – 10 µl, without guiding (2 ps)		3 740 RUB
	M-10H, volume – 10 µl with guiding (2 ps)		5 170 RUB
	M-50, volume – 50 µl, without guiding (1 ps)		4 000 RUB
Hamilton, Switzerland	705N, volume – 50 µl	A microsyringe with a fixed needle, caliber 22s (22s/51/2)	574.50 UAH
	701N, volume – 10 µl,	A microsyringe with a fixed needle, caliber 26s (26s/51/2)	4 000 RUB
	702N, volume – 25 µl,	A microsyringe with a fixed needle, caliber 22s (22s/51/2)	470.50 UAH
Devices for detecting or quenching fluorescence			
Spectroline, USA	CM-10, a cabinet with 4W combined lamp (365 nm with 300µW/cm^2, 254 nm with 310 µW/cm^2). The size: 22.9×30.5×16.5 cm. The weight: 3.2 kg	The multifunctional cabinet that is specifically designed for use with Spectroline UV lamps. It is made of aluminium coated with polyurethane lacquer. A protective coating against UV-radiation is built in the vinyl window for looking at chromatograms. The vinyl window increases contrast between fluorescence area and the background, reducing eye fatigue. The cabinet can be ordered separately or with 4-/ 6-cotton lamp, that absorbs in the short-wave and long-wave regions	450 $
	CM-10 a cabinet with 6W combined lamp (365 nm with 350µW/cm^2, 254 nm with 390 µW/cm^2). The size: 22.9×30.5×16.5 cm. The weight: 3.4 kg		510 $
Sorbfil, Russia	Irradiator UVL -254/365 The supply voltage: 220 V Power consumption, VA: not more than 30 The size: 32×20×27 cm. The weight: 5 kg	The emission source: Luminescent ultraviolet lamp KLC 9/UV 365 nm – 1 ps Mercury germicidal lamp DKB 9 254 nm –1 ps The size of TLC plates is not more than – 15×15 cm	13023.00 UAH / 38800 RUB
Developing devices			
Sorbfil, Russia	Sprayer with a bulb, Sorbfil. The sizes: height – 170 mm, diameter of the sprayer – 26-30 mm.	A sprayer is intended for applying a developing reagent on chromatographic plates. A glass atomizer of the sprayer contains system and container for solution in the same body and placed on a PVC bulb	280.80 UAH / 1200 RUB
GGB, USA	Sprayer unit, volume – 10 ml	A screw design prevents «reverse spraying»; made of borosilicate glass. Changeable sprayer. The opportunity of choosing a sprayer of the appropriate volume. The device operates at low air pressure	117.75 $
	Sprayer unit, volume – 50 ml		117.75 $
	Sprayer unit, volume –125 ml		117.75 $

lysing the medicinal plant raw material both analytical (TLC) and high-performance (HPTLC) plates can be used, they differ mainly in the size of the particles. During routine analysis the use of TLC plates with the particle size of 5-17 µm is enough. The presence of an UV indicator is important when choosing plates since in the pharmaceutical analysis of various classes of biologically active substances (BAS) of the medicinal plant raw material detection of many compounds occurs in UV light. Therefore, when analysing the medicinal plant raw material and drugs from it the TLC and HPTLC plates on all substrates with a UV indicator can be used. It is advisable to make the final choice based on the economic component or availability of plates.

Characteristics of the devices for applying solutions of chromatograms have revealed that when choosing microsyringes the best indicator is its volume. The volume of 10-50 µl is optimal for the analysis of the medicinal plant raw material.

When comparing devices manufactured by Sorbfil (Russia) and Spectroline (USA) for detecting or quenching fluorescence it has been shown that each of the devices studied can be used to identify chromatographic zones. The manufacturer Spectroline has the quality certificate

ISO 9001:2008 and is more affordable, therefore, it is superior to an UVS-254/365 irradiator manufactured by Sorbfil.

Comparative characteristics of the devices for spraying chromatograms (Tab. 2) have found that sprayers of GGB (USA) have a comfortable design and a suitable volume, but by cost they are much more expensive than sprayers of Sorbfil (Russia). It is advisable to make the final choice based on the economic component or availability of devices.

CONCLUSIONS

On the example of Klimased drug with the purpose of choosing the analytical equipment for quality control of combined drugs of the plant origin the major functional and performance characteristics (design qualification) of the equipment used according to pharmacopoeial requirements (SPhU and USP) and the target analytical tasks has been studied. It allows to select equipment for an enterprise.

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КВАЛІФІКАЦІЯ ТШХ-ОБЛАДНАННЯ, ЩО ЗАСТОСОВУЄТЬСЯ В АНАЛІЗІ КОМБІНОВАНИХ РОСЛИННИХ ПРЕПАРАТІВ

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Ключові слова: *система контролю якості; фармакопейний аналіз; ТШХ-обладнання; Клімасед*

На прикладі препарату Клімасед з метою вибору аналітичного обладнання для проведення контролю якості комбінованих лікарських засобів рослинного походження досліджені головні функціональні і експлуатаційні характеристики (кваліфікація дизайну) обладнання, яке використовується відповідно до фармакопейних вимог (ДФУ і USP) і поставлених аналітичних завдань, що дозволяє провести вибір обладнання для підприємства. Проведено порівняльний аналіз функціональних і технічних характеристик приладів для виконання аналізу комбінованих препаратів з лікарської рослинної сировини методом тонкошарової хроматографії. Експериментально доведено, що конфігурація хроматографічної камери не впливає на результат аналізу, за економічним показником більш доступними є камери виробництва Сорбфіл. При аналізі лікарської рослинної сировини (ЛРС) і препаратів з ЛРС можуть бути використані ТШХ- і ВЕТШХ-пластинки на всіх підложках з УФ-індикатором, остаточний вибір доцільно проводити за економічною складовою чи їх доступністю. Характеристика пристроїв для нанесення розчинів хроматограм виявила, що оптимальним показником при виборі мікрошприців є його об'єм. При порівнянні пристроїв для виявлення або гасіння флуоресценції виробництва Сорбфіл (Росія) і Spectroline (США) встановлено, що будь-який з досліджуваних приладів може бути використаний для виявлення хроматографічних зон. Виробник Spectroline має сертифікат якості ISO 9001:2008 і за ціною більш доступний, що є його перевагою над опромінювачем УФС-254/365 виробництва Сорбфіл. Серед пристроїв для обприскування хроматограм зручну конструкцію і потрібний об'єм мають пульверизатори GGB (США), але за вартістю вони є значно дорожчими за пульверизатор Сорбфіл (Росія). Остаточний вибір доцільно проводити за економічною складовою.

КВАЛИФИКАЦИЯ ТСХ-ОБОРУДОВАНИЯ, ПРИМЕНЯЕМОГО В АНАЛИЗЕ КОМБИНИРОВАННЫХ РАСТИТЕЛЬНЫХ ПРЕПАРАТОВ

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Ключевые слова: *система контроля качества; анализ; ТСХ-оборудование; Климасед*

На примере препарата Климасед с целью выбора аналитического оборудования для контроля качества комбинированных лекарственных препаратов растительного происхождения

проведены исследования по изучению основных функциональных и эксплуатационных характеристик (квалификация дизайна) оборудования, которое используется в соответствии с требованиями фармакопей (ДФУ и USP) и поставленным аналитическим заданиям, что позволяет провести выбор оборудования для предприятия. Проведен сравнительный анализ функциональных и технических характеристик приборов для выполнения анализа комбинированных препаратов из лекарственного растительного сырья методом тонкослойной хроматографии. Экспериментально доказано, что конфигурация хроматографической камеры не влияет на результат анализа, по экономическим показателям более доступными являются камеры производства Сорбфил. При анализе ЛРС и препаратов из ЛРС могут быть использованы ТСХ- и ВЭТСХ-пластинки на всех подложках с УФ-индикатором, окончательный выбор целесообразно проводить по экономической составляющей или их доступности. Характеристика приборов для нанесения растворов хроматограмм показала, что оптимальным показателем при выборе микрошприца является его объем. При сравнении приборов для обнаружения или гашения флуоресценции производства Сорбфил (Россия) и Spectroline (США) показано, что каждый из исследуемых приборов может быть использован для выявления хроматографических зон. Производитель Spectroline имеет сертификат качества ISO 9001:2008 и по цене более доступен, что является его преимуществом перед облучателем УФС-254/365 производства Сорбфил. Среди приборов для опрыскивания хроматограмм удобную конструкцию и подходящий объем имеют пульверизаторы GGB (США), но по стоимости они значительно дороже пульверизаторов Сорбфил (Россия). Окончательный выбор целесообразно проводить по экономической составляющей.