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DEVELOPMENT AND UNIFICATION OF A THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF PHENSULKAL IN SOFT DOSAGE FORMS.

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ABSTRACT

In order to study the improvement and unification of quality control of phensulkal in soft dosage forms (suppositories, gels), the technic of thin layer chromatography (TLC) was used. On the basis of the experiments, the solvent selection, elution conditions, the frequency of extraction of the active substance from the dosage form and the composition of the extraction fluid, the volume of the sample, the type of plate for the identification of phensulkal by the TLC technic were substantiated. In the course of carrying out a qualitative analysis of phensulkal in suppositories according to the developed method, the Rf values of the studied samples were consistent with the chromatographic mobility of working standard (WS). No additional patches were found, therefore, the interaction products between the medication components are not formed, phensulkal is compatible with the ingredients of the base material and does not undergo destruction during the manufacturing process. On chromatograms obtained during the research of extracts from placebo suppositories, it was found that in experimental samples of dosage forms, unidentified patches were not found, which indicates the correct choice of the conditions for the extraction of the active factor. The developed TLC technic was used to determine the quality indicators of vaginal suppositories and gels with phensulkal and introduced into new draft regulatory documents.

Key words: standardization, phensulkal, thin layer chromatography, specificity, sensitivity, reproducibility.

抽象的

为了研究软剂型（栓剂、凝胶剂）中苯舒卡的质量控制的改进和统一，采用薄层色谱（TLC）技术。在实验的基础上，溶剂的选择、洗脱条件、从剂型中提取活性物质的频率和提取液的组成、样品的体积、用于鉴定苯酚的平板类型TLC 技术得到证实。在根据开发的方法对栓剂中的苯舒卡进行定性分析的过程中，研究样品的 Rf 值与工作标准 (WS) 的色谱迁移率一致。没有发现额外的贴剂，因此没有形成药物成分之间的相互作用产物，phensulkal 与基础材料的成分相容，并且在制造过程中不会受到破坏。在安慰剂栓剂提取物研究过程中得到的色谱图发现，在剂型的实验样品中，未发现不明贴剂，说明活性因子提取条件选择正确。开发的 TLC 技术用于确定阴道栓剂和含有苯舒卡的凝胶的质量指标，并被引入新的监管文件草案。

关键词：标准化，phensulkal，薄层色谱，特异性，灵敏度，重现性。

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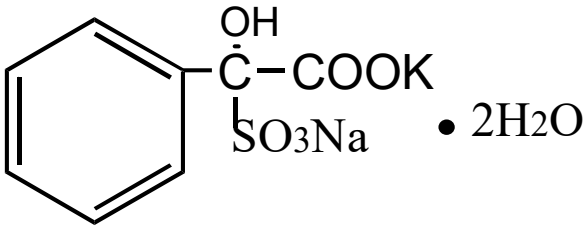
INTRODUCTION

The standardization and quality control of medicines is of great importance in providing the population with effective and proof medicinal drugs. Along with the search for new medication of anti-inflammatory diseases, it is necessary to address the development of methods for their analysis and ensure their quality.

Phensulkal-bisulfite derivative of phenyl-glyoxylic acid.



This substance is characterized by mild toxicity and has pronounced anti-inflammatory and antimicrobial properties, which cumulatively are very important for the pathogenetically oriented treatment of many gynecological diseases (1,2). The results of patent research indicated that the potassium salt of sodium bisulfite derivative of phenylglyoxylic acid (phensulkal) is a new, previously not synthesized and not reported compound (3). Phensulkal is recommended in the form of tablets (0.1 g of active substance WFS 42 Uz-0663-2003 and ointments (0.5% and 3% Manufacturer's monograph 42 Uz-0187-2007). Vaginal suppositories are allowed for medical use in practice (Manufacturer's monograph 42 Uz-1992-2008).

It is of current interest to conduct research for finding methods and developing methods for the analysis of phensulkal, necessary for standardization and pharmacokinetic study of the specified medication.

The aim of this research is to improve and unify the quality control of phensulkal in soft dosage forms (suppositories, gels) by thin layer chromatography (TLC).

MATERIAL AND METHODS

Vaginal suppositories 10 mg and 3% phensulkal gel, chromatography on a thin layer of coating substance.

In the course of developing a chromatography method in a thin layer of a coating substance, we used the pharmacopoeial substance phensulkal, which meets the requirements of PS 42 Uz - 0185-2017, organic solvents of different polarity of "AR grade" qualification. We tested production samples of the developed medication - vaginal suppositories based on witepsol with a dosage of 10 mg phensulkal. The quality indicators of all excipients used for obtaining prototypes met regulatory requirements. Chromatographic researches were carried out in a glass N-cabinet of rectangular cross-section along the height, which was previously saturated with vapors of the moving phase for 30 minutes at a constant temperature. Chromatography was carried out by ascending technic on SilufolUV254 (Merck) (Rf = 0.65); Sorbfil UV Russia (Rf = 0.60); SilufolUV254 (Czech Republic) (Rf = 0.66); Silica gel KSK (Rf = 0.60) plates with the addition of a luminescent indicator. The height of the rising of the eluent front is 80 mm.

Experiential part.

For extracting phensulkal from suppositories, a double exhaustion method was used, which was carried out in the following way: for suppositories, one candle weighing 2.5 g was thoroughly crushed, a test portion of a crushed

suppository weighing 0.3 g was placed in a measuring bottle with a capacity of 50 ml, 15 ml of purified water was added and heated in a water bath until complete fusion, shaking vigorously for 5 min, then cooled until the solidification of the base, and the liquid part was poured into a 50 ml measuring bottle. The exhaustion was carried out three times, the water extracts were combined, filtered and the volume was brought up to the mark with purified water.

The extraction of phensulkal from the aqueous phase into the organic phase was carried out by three times using a separating funnel and each time consuming 10 ml of chloroform, upon which the extracts were combined, filtered through a paper filter "blue ribbon" or a filter of the "Millipore" type with a pore diameter of 0.45 μg, discarding the first portions of the filtrate, and evaporated to a volume of 10 ml (test solution). In parallel, by successive dilutions, solutions of the working standard (WS) of phensulkal were prepared with an exposure of 0.1% (solution A), 0.05% (solution B), 0.02% (solution C). Only freshly prepared solutions were used. Taking into account the requirements for sample preparation and the solubility of the analyte, purified water was chosen as a solvent [2,4]. On the spotting line, located at a distance of 10 mm from the lower plate margin, using a microsyringe, 10 μg of the test solution of phensulkal extracted from dosage forms, solutions A, B and C, respectively equivalent to 10, 5 and 2 μg of WS of phensulkal. The plate with the applied samples was dried and chromatographed by the ascending technic. The macules on the obtained chromatograms were opened by viewing in UV light at a wavelength of 254 nm, while comparing the Rf values of the studied samples and WS. The eligibility of the chromatographic system was assessed by the following parameters: a macule is clearly visible on the chromatogram of the C phensulkal solution.

Statistical processing of the research results was carried out according to the NP XI ed. [4] using the Microsoft Excel 2002 software package (product number 54521-701-3227086-17559). The significance of the disparities was assessed using the Student's t-test at a significance level of $p < 0.05$ [5]. Phensulkal is insoluble in non-polar and universal solvents (chloroform, acetonitrile, spirit 96%), freely soluble in water, i.e. has pronounced hydrophilic properties. In order to select the optimal composition of the moving phase (MF), the chromatographic mobility of the phensulkal substance was studied in individual and combined solvents of different polarity, as well as in systems with different contents of acid and alkaline modifiers (acetic acid and ammonia solution concentrated 25%). On the spotting line of the chromatographic plate using a microsyringe, 10 μl of 0.5% phensulkal solution (50 μg) was applied at one marking. Elution was carried out in an ascending technic (6,7). The resulting chromatograms were dried in air stream at room temperature, developed under UV light, and the Rf values were calculated.

1. Preparation of the test solution. 5.0 mg of phensulkal substance was placed in a 10 ml measuring bottle, and 9 ml of methylene chloride R, one drop of triethypamide R were added, stirred until dissolved, the volume of the solution was brought to the mark with methylene chloride R and stirred (0.5 mg / ml phensulkal).

Preparation of the Comparison solution. 5.0 mg of phensulkal (CO company) is placed in a measuring bottle with a capacity of 10 ml, added 9 ml of methylene chloride R and one drop of triethypamine R, stirred until dissolved, the

volume of the solution is adjusted to the mark with methyl chloride R and stirred (0.5 mg / ml of phensulkal).

On the spotting line of the TLC plate with a layer of silica gel F 254 R with a solution of 10 x 20 cm with 0.25 mm layer thickness is applied twice by 15 µl of the test solution and the comparison solution. The plate with the applied samples was dried in air, placed in a chamber lined with blotting paper and saturated for 10 min with a solvent system chloroform R- methanol - R - ammonia (25%) (6,95+2,25+0,8) and chromatographed in ascending technic. When the solvent front passed about 10 cm from the spotting line, the plate was removed from the chamber, dried in air for 20 min, and viewed in UV light at a wavelength of 254 nm.

One main macular should be revealed on the chromatogram of the test solution, corresponding in Rf value (about 0.45) to the phensulkal macular on the chromatogram of the comparison solution.

2. Preparation of standard phensulkal solution. 5.0 mg of phensulkal substance was placed in a 10 ml measuring bottle, added 9 ml of methylene chloride R and one drop of triethypamide R, stirred until dissolved, the volume of the solution was brought to the mark with methylene chloride R and stirred (0.5 mg / ml phensulkal).

On the spotting line of the TLC plate with a layer of silica gel F 254 R with a solution of 10 × 20 cm with 0.25 mm layer thickness is applied twice by 15 µl of the test solution and the comparison solution. The plate with the applied samples was dried in air, placed in a chamber lined with blotting paper and saturated for 10 min with a solvent system chloroform R- methanol - R - ammonia (25%) (6,95+2,25+0,8) and chromatographed in ascending technic. When the solvent front passed about 10 cm from the spotting line, the plate was removed from the chamber, dried in air for 20 min, and viewed in UV light at a wavelength of 254 nm.

On the chromatogram of the test solution a main macular was revealed corresponding in terms of the Rf value (about 0.45 phensulkal) to the phensulkal macular on the chromatogram of the comparison solution.

RESULTS AND DISCUSSION

Determination of the sensitivity of the TLC technic consisted in the preparation of aqueous solutions of phensulkal in various concentrations and in chromatography system chloroform P-methanol -P - ammonia (25%) system (6,95+2,25+0,8). Determining sensitivity results and detection limit of phensulkal are shown in Table 1.

Table 1

Determination of the sensitivity and detection limit of phensulkal by various developing reagents

The amount of substance taken for analysis, µg	UV-254	Iodine vapor	$CuSO_4$	$FeCL_3$
100	+	+	+	+
50	+	+	+	+
10	+	+	+	-
5	+	+	-	-
1	_	+	_	_
0,1	_	_	_	_

One can see from the table 1 that when analyzing phensulkal, the detection limit is in the range from 100 to 5 µg, and the most optimal developing reagents are viewing in UV light and iodine vapor.

An important stage is the study of validation parameters (5) and characteristics as a confirming element of up-to-date requirements and proving the correct choice of methodology.

The next stage was to determine the specificity, selectivity, sensitivity and reproducibility of the technic (6,7).

The specificity of the TLC technic was studied by chromatography of the working and standard phensulkal samples. In order to determine the specificity of the developed chromatographic conditions, we carried out a series of chromatographic determinations. On the spotting line, plates "SilufolUV-254" were applied using graduated capillaries or an MSh-10 microsyringe, 0.01 ml of aqueous solutions of phensulkal, as well as a standard sample of the "testifier" substance in the same amount. After drying at room temperature, the plates with the samples were placed in a chromatographic chamber pre-saturated with vapors of the organic system. When the solvent front reached 10 cm from the spotting line, the plate was removed from the chamber and dried at room temperature until the vapors (odor) of organic solvents disappeared. The chromatogram was then viewed under UV light. When viewed, purple macules were found. The capillary was calibrated using 0.01 ml of purified water.

The chromatogram of the specificity of the developed TLC technique is shown in Fig. 1.

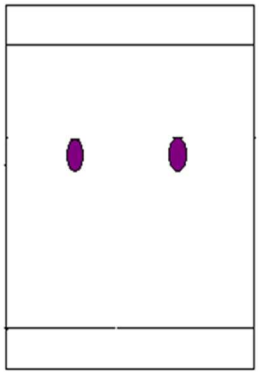


Fig: 1. Chromatogram of the test solution of phensulkal and the standard sample of "testifier" substance

Moving phase - chloroform R-methanol -R - ammonia (25%) (6,95+2,25+0,8) A-SSST - standard sample of substance- "testifier" of phensulkal "Rf = 0.45"
B - test sample of phensulkal.

The reproducibility of the developed TLC technic was studied on various chromatographic plates, and its reliability was assessed by the obtained Rf values.

The results of studying the reproducibility of TLC technic of phensulkal are shown in Table 2.

Table 2
Phensulkal Reproducibility Study Results

Plates	Rf
1. Silica gel KSK	0,60
2."SilufolUV254" (Czechia)	0,45
3."SilufolUV254" (Merck)	0,45
4. "Sorbfil UV" Russia	0,60

The obtained Rf indices meet the requirements of the State Pharmacopoeia XI and acknowledge the reproducibility of the developed TLC technic. Today "SilufolUV-254"

plates are more accessible and it does not take much time to prepare the chromatographic plate, which can significantly reduce the analysis time.

The results of the validation of the TLC technic made it possible to reveal its sensitivity, selectivity, and reproducibility, which will be very important for the analysis of the finished dosage form of phensulkal.

On the spotting line of the TLC plate with a layer of silica gel F 254 R with a size of 10 × 20 cm with 0.25 mm layer thickness is applied twice by 15 μl of the test solution and the comparison solution. The plate with the applied samples was air-cured, placed in a chamber lined with blotting paper and saturated for 10 min with a solvent system chloroform R- methanol - R - ammonia (25%) (6,95+2,25+0,8) and chromatographed in ascending technic. When the solvent front passed about 10 cm from the spotting line, the plate was removed from the chamber, air-cured for 20 min, and viewed in UV light at a wavelength of 254 nm.

On the chromatogram of the test solution a main macular was revealed corresponding in terms of the Rf value (about 0.45 phensulkal) to the phensulkal macular on the chromatogram of the comparison solution.

The results of chromatography of phensulkal in patient-specific solvent is shown in Table 3.

Table 3

Phensulkal movability during chromatography in patient-specific solvents on plates "SilufolUV254" (Merck)

R-methanol -R - ammonia (25%) (6,95+2,25+0,8)	R-methanol -R - ammonia (25%) (6,95+2,25+0,1)	R-methanol -R - ammonia (25%) (6,95+2,5+0,8)	R-methanol -R - ammonia (25%) (6,95+2,25+0,9)
0,45	0,40	0,38	0,53

During research of studying the chromatographic movability of the phensulkal substance in individual water-repellent and hydrophilic organic solvents on plates SilufolUV254 (Merck), the following consistent pattern was revealed: with an increase in the polarity of the eluent, the chromatographic movability of phensulkal increases. This may be due to stable bonds between the active centers of silica gel and the adsorption layer formed by polar solvents in comparison with nonpolar ones, as a result of which the ability of phensulkal molecules to displace adsorbed polar MP molecules from the adsorbent surface decreases. Subsequently, the composition of the moving phase was selected by mixing solvents from the beginning and end of the eluotropic series in various quotient. It was determined that the optimal movability of phensulkal is observed in the system P-methanol -P - ammonia (25%) (6,95+2,25+0,8)–Rf = 0,45**.**

In a further experiment, the effect of alkalotic (25% concentrated ammonia solution) and acidic (acetic acid) reagents on the change in the Rf value of phensulkal in indicated systems was researched. It was determined that in the existence of an ammonia solution, the chromatographic movability of phensulkal increases, and the introduction of acetic acid into the MP composition leads to a decrease for both systems. The results of chromatography in systems with different contents of acidic and alkalotic reagents, as an average of five parallel determinations, are shown in Table 4.

Table 4

Phensulkal movability during chromatography in combined eluent flow on

plates SilufolUV254 (Merck) in the presence of an acid type modifier

Eluent	Rf
Acetic acid 10%	0,60
Acetic acid 20%	0,50
Acetic acid 30%	0,48
Acetic acid 40%	0,45

During research of the chromatographic movability of the phensulkal substance in individual water-repellent and hydrophilic organic solvents on SilufolUV254 (Merck) plates, the following consistent pattern was revealed: with an increase in the polarity of the eluent, the chromatographic movability of phensulkal increases. This may be due to stronger bonds between the active centers of silica gel and the adsorption layer formed by polar solvents in comparison with nonpolar ones, as a result of which the ability of phensulkal molecules to displace adsorbed polar MP molecules from the adsorbent surface decreases. Next, the composition of the moving phase was selected by mixing solvents from the beginning and end of the eluotropic series in various ratios. The polarity of the combined eluents was evaluated by the value of the dielectric constant and the volume fraction of the individual solvents included in the PP. It was found that the optimal movability of phensulkal is observed in the binary system: P-methanol -P - ammonia (25%) (6.95 + 2.25 + 0.8) –Rf = 0.45. In a further experiment, the effect of alkalotic (25% concentrated ammonia solution) and acidic (acetic acid) reagents on the change in the Rf value of phensulkal in pointed systems was studied. It was found that in the presence of an ammonia solution, the chromatographic movability of phensulkal increases, and the introduction of acetic acid into the MP composition leads to its decrease. The results of chromatography in systems with different contents of acidic and alkalotic reagents, as an average of five parallel determinations, are presented in Table 5.

Table 5

Phensulkal movability during chromatography in combined eluent flow on plates SilufolUV254 (Merck) of alkalotic type modifier

Eluent	Rf
Aqua ammonia solution 10%	0,32
Aqua ammonia solution 15%	0,40
Aqua ammonia solution 20%	0,41
Aqua ammonia solution 25%	0,45

Phensulkal contains two leading radicals and belongs to weak acids [1, 2]. But the presence of not only leading but also alkalotic centers in a molecule determines its ability to ionize both in acidic and alkalotic circumstance. The positive effect of the alkalotic modifier may be due to the simultaneous dissociation of the hydroxyl groups of the sorbate and the free OH groups of the silica gel, which leads to a decrease of the phensulkal containment. Considering the effectiveness parameter, the chloroform systems of P-methanol -P - ammonia (25%) (6,95+2,25+0,8) were selected as MP. The values of the movability coefficients Rf of the phensulkal substance in the indicated systems were 0,45±0,02. The sensitivity of the determination method is 2 μg of phensulkal.

CONCLUSION

On the basis of the experiments, the solvents selection, elution conditions, the frequency of extraction of the active substance from the dosage form and the composition of the extraction agents, the sample volume, the type of plate for the identification of phensulkal by TLC method were substantiated. Based on the results of background research, a method was developed for obtaining an extract from suppositories for TLC analysis using the double extraction method. In the course of carrying out a qualitative analysis of phensulkal in suppositories using the developed technic, the Rf values of the studied samples were consistent with the chromatographic movability of WS. No additional macules were found, therefore, the interaction products between the components of the agents are not formed, phensulkal is compatible with the ingredients of the bases and does not undergo destruction during the manufacturing process. On chromatograms obtained in the research of extracts from placebo suppositories containing no active ingredient and were made on the same agents as the experimental samples of dosage forms, no unidentified macules were found, which indicates the correct choice of the conditions for the extraction of the active substance. The developed TLC technic was used to determine the quality indicators of vaginal suppositories and gels with phensulkal and entered into new DI projects.

CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article and report on the contribution of each author.

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