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DEVELOPMENT OF METHODS FOR QUALITY CONTROL OF DRY EXTRACT OF LEAVES AND ROOTS OF VOAD

***Tillaeva G.U. and Khakimzhanova Sh.O.**

Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan.

***Corresponding Author: Tillaeva G.U.**
Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan.

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ABSTRACT

Methods have been developed for the identification and quantitative determination of the active ingredients in dry extracts of the leaves and roots of Woad. Based on the studies carried out, a TLC technique was proposed for authenticity, and a choice of solvents, elution conditions and developers for identifying flavonoids was made. Validation characteristics for determining the sensitivity and detection limit of the prepared drugs are presented, proving the correctness, suitability, reproducibility and specificity of the developed method. To determine the quantitative content of active substances from the dry extract of leaves and roots of usma, a spectrophotometric method was used. The determination was carried out based on flavonoids in terms of luteolin as the dominant element contained in the extracts. The developed conditions will be included in the regulatory document for dry extract of leaves and roots of Woad.

KEYWORDS: Dry extract, Woad leaves and roots, flavonoids, validation of analytical method.

INTRODUCTION

Woad (Isatis) Tinctoria L.) is a biennial herbaceous plant whose growth is widespread throughout the world and in our region, and has various medicinal properties.[1,2] Woad is cultivated in China as a medicinal plant used in Chinese traditional medicine. Leaves (lat. Folium Isatidis) and roots (Radix Isatidis) of woad are used in official medicine of China in the form of decoctions and teas for various inflammatory and colds and are included in the state pharmacopoeia of China.[3,4] Currently, in order to introduce modern medicines (medicines), using domestic raw materials on the market of Uzbekistan, we have developed technologies for obtaining dry extracts from various parts of the Woad plant (leaves, roots). Work is underway to include this plant and dosage forms based on it in the state pharmacopoeia of Uzbekistan. The creation of extraction drugs based on plant raw materials, as well as the development of quality control methods, is one of the priority areas of pharmaceutical production. Promising is the production of solid dosage dosage forms based on them, giving them optimal technological properties.[5] The introduction of drugs into domestic pharmacy using available local raw materials in a rational dosage form is promising. For the introduction of drugs into production, an integral part is the development of specifications through quality control methods of both the dosage form itself and the dominant active ingredients contained in them in accordance with acceptable standards. According to the literature, it is known that Woad should contain flavonoids, and we were interested in extracting them.[6] Flavonoids are a large group of polyphenolic compounds that are genetically related to each other and have different pharmacological effects. They are widespread in higher plants and are much less common in microorganisms and insects. Flavonoids are involved in many processes occurring in the body - they have an antioxidant effect, reduce blood clotting, reduce capillary fragility and permeability, and improve metabolic processes. The maximum content of flavonoids is observed in the above-ground parts of plants. It is known from the literature that flavonoids are highly soluble in high concentration alcohol.[6]

Purpose of the study. Development of methods for quality control of active pharmaceutical ingredients in dry extracts from the roots and leaves of Woad.

MATERIALS AND METHODS

Dry extracts of Woad were obtained from roots and leaves by fractional extraction with various solvents and heating. Identity was determined by TLC. To determine the content of flavonoids, standard solutions were used as witnesses: rutin, luteolin and quercecin. For thin layer chromatography (TLC), silica gel plates of the Silufol brand were used. UV -254" (Czech Republic), organic

solvents of various polarities, analytical grade. Chromatographic studies were carried out in a glass N-chamber of rectangular cross-section in height, which was pre-saturated with mobile phase vapors for 30 min at a constant temperature. Chromatographed using the ascending method on "Silufol" plates UV 254" (Merck) (Rf =0.65); "Sorbfil UV" Russia; "Silufol UV 254" (Czech Republic); Silica gel KSK with the addition of a luminescent indicator. The height of the rise of the eluent front is 80 mm. For quantitative analysis, a UV spectrophotometer was used, brand Spectrophotometer UV-1800, from the company "SHIMADZU", Japan.

RESULTS AND DISCUSSION

The authenticity was determined by TLC based on the content of flavonoids: rutin, luteolin and quercecin. Chromatographic studies were carried out in a glass N-chamber of rectangular cross-section in height, which was pre-saturated with vapors of the mobile phase for 30 minutes at a constant temperature. Chromatographed using the ascending method on Sil ufol plates UV -254" 100x100 mm. Sorbent type – silica gel. The height of the eluent front rise is 80 mm. The plate with the applied samples was dried and chromatographed using the ascending method. Spots on the resulting chromatograms were opened when viewed in UV light at a wavelength of 254 nm, while comparing the $R_{f\ values}$ of the samples under study and RSO. The suitability of the chromatographic system was assessed by the following parameters: the corresponding spots are clearly visible on the chromatogram of the solutions: luteolin, rutin and quercetin. Statistical processing of research results was carried out in accordance with the Global Fund XI ed. 5.[8]

For the determination of flavonoids, the results using the system turned out to be the most sensitive:

N – butanol:	Ice. acetic acid	: Water
4	2	1
8	4	2

0.0507 g of dry extract +	2 ml 80% ethyl alcohol→	System
1. 0.0201 g standard sample of luteolin +	2 ml 80% ethyl alcohol→	System
2. 0.0202 g standard sample rutin +	2 ml 80% ethyl alcohol→	System
3. 0.0200 g standard sample of quercecin +	2 ml 80% ethyl alcohol→	System

According to TLC results: spots of luteolin, a faint trace of rutin and less noticeable quercecin were clearly detected.

Based on the experiments performed, the choice of solvents, elution conditions and manifestations for the identification of flavonoids by TLC was justified.

An important step is the study of validation parameters[5] and characteristics as a confirming element of modern requirements and proving the correct choice of methodology. To confirm the developed method, validation characteristics were determined according to the following indicators: sensitivity, specificity and reproducibility of the method. Determining the sensitivity of the TLC technique consisted of preparing aqueous solutions of drugs in various concentrations and chromatography in the system butanol: glacial acetic acid: water (4:2:1) and the same solvents in a ratio (8:4:2), and using various developing reagents. The results of the sensitivity limits and detection limits of the prepared drugs are presented in Table 1.

Table 1: Determination of sensitivity and detection limit of various developing reagents.

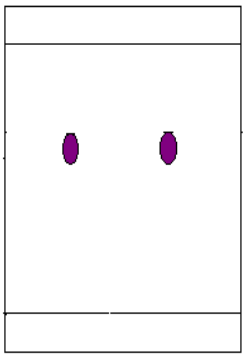
Amount of substance taken for analysis, mcg	UV-254	Iodine vapor	CuSO4	$FeCL_3$
100	+	+	+	+
50	+	+	-	+
10	+	+	-	-
5	+	+	-	-

From the presented table 1 it can be seen that in the analysis 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.

The suitability of the chromatographic system was assessed by the following parameters: spots were clearly visible in the chromatogram of solution B. Statistical processing of research results was carried out in accordance with the Global Fund XI ed. using the Microsoft Excel 2002 software package (product number 54521-701-3227086-17559). The significance of differences was assessed using Student's t-test at a significance level of $p < 0.05$.[8]

The next step was to determine the specificity and reproducibility of the technique.[7] Validation analytical techniques was carried out V Compliance with recommendations of ICH (International Council for Harmonisation) Topic Q 2 (R1) "Validation of Analytical Procedures Text and Methodology"

The specificity of the TLC technique was studied by chromatography of a working and standard witness sample of luthionine. To determine the specificity of the developed chromatographic conditions, we carried out a series of chromatographic determinations. The chromatogram was then examined under UV light. When viewed, purple spots were discovered. The capillary was calibrated using 0.01 ml of purified water. The specificity chromatogram of the developed TLC technique is presented in Fig. 1.



Rice. 1. Chromatogram of the test solution and a standard sample of the witness substance.
Mobile phase - butanol: glacial acetic acid: water (4:2:1); A-SOBC – standard sample of the "witness" substance luteolin " Rf = 0.65"; B - test sample.

The reproducibility of the developed TLC technique was studied on various chromatographic plates indicated above and its reliability was assessed based on the obtained Rf indicators. The results of studying the TLC reproducibility of the developed method are presented in Table 2.

Table 2: Results of studying the reproducibility of the technique.

Records	Rf
1. Silica gel KSK	0.60
2." Silufol UV 254" (Czech Republic)	0.65
3." Silufol UV 254" (Merck)	0.45
4. "Sorbfil UV" Russia	0.40

Rf values correspond to the requirements of the State Fund XI and confirm the reproducibility of the developed TLC procedure. Today, Silufol UV -254 plates are more affordable and do not require much time to prepare a chromatographic plate, which can significantly reduce the time of analysis.

The results of the validation of the TLC technique made it possible to identify its sensitivity, selectivity, and reproducibility, which will be very important for the analysis of the finished dosage form of the dry extract of Woad.

Quantitative determination of the extract was carried out based on the content of flavonoids in terms of luteolin. The analysis was carried out using a Spectrophotometer UV-1800 UV spectrophotometer from SHIMADZU . Method of determination: about 0,05 rthe drug (exactly weighed) was dissolved with stirring in 50 ml of 70% ethyl alcohol in a 100 ml volumetric flask. The volume of the solution was brought to the mark with the same alcohol and stirred (solution A). 1 ml of the resulting solution was added to a 50 ml volumetric flask, the volume of the solution was adjusted to the mark with 70% ethyl alcohol and mixed (solution B). When determining the optical density of the test solution, a reference solution of 70% ethyl alcohol was placed in a comparator cell. The optical density of the resulting solution was measured on a spectrophotometer in the range from 200 to 400 nm in a cuvette with a thickness of 10 мм. Figure 1 shows the UV spectrum of dry extract of Woad.

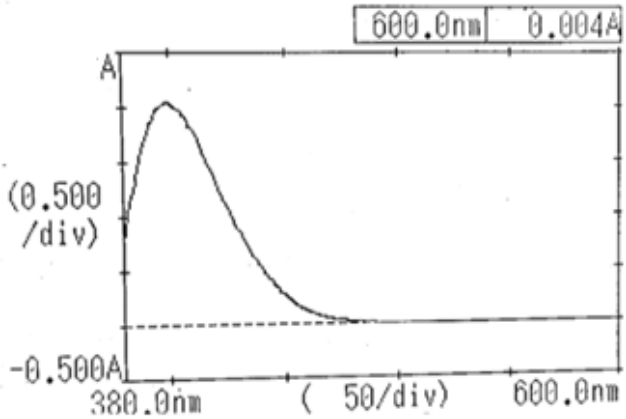


Figure 1: UV spectrum of dry extract of Woad.

In parallel, the optical density of a solution of a standard luteolin sample was measured. Under these conditions, the maximum light absorption by the test solution was observed at a wavelength of 330 nm.

Preparation of a solution of a standard sample of luteolin. About 0.025 g (exactly weighed) of a standard sample of luteolin (VFS 42 Uz-0194-97), previously dried for 2 hours at a temperature from 100 o to 105 o C, was dissolved with heating and vigorous shaking in 100 ml of 70% ethyl alcohol in volumetric flask with a capacity of 150 ml and, after cooling, bring the volume of the solution to the mark with 70% ethyl alcohol. Shelf life - one month from the date of preparation.

1 ml of the resulting solution was added to a 100 ml volumetric flask and the volume of the solution was adjusted to the mark with 70% ethyl alcohol. The solution was used freshly prepared.

The content of the total flavonoids in percent (X) in terms of luteolin was calculated using the formula:

$$X = \frac{D_1 * a_0 * 50 * 25 * 1 * 98 * 100}{D_0 * a * 1 * 100 * 50 (100 - W)} = \frac{D_1 * a_0 * 2500}{D_0 * a * (100 - W)}$$

Where:
D $_1$ – optical density of the test solution;
D $_o$ – optical density of a solution of a standard luteolin sample;
a – weight of the test drug in grams;
a $_o$ is the weight of the standard sample of luteolin in grams;
W – weight loss during drying of raw materials, in%.
Standard sample of luteolin = 0.0220 + 100 ml 70% ethyl. alcohol→
A) →1 ml + 1 ml AlCl $_3$ in alcohol + 50 ml 70% ethyl. alcohol

$D_{st(а)} = 0{,}116$ at α= 330 nm

B) →1 ml + 50 ml 70% ethyl. alcohol

$D_{st(б)} = 0{,}210$

Test sample = 0.0530 + 50 ml 70% ethyl. alcohol→
A) →1 ml + 1 ml AlCl $_3$ in alcohol + 50 ml 70% ethyl. alcohol

$D_{1(а)} = 0{,}159$

(AlCl3) = $X_а \frac{0{,}159*0{,}0220*50*1*25*98*100}{0{,}116*0{,}0530*100*1*25*(100-1{,}4)}$ 28.27 %

B) →1 ml + 25 ml 70% ethyl. alcohol

$D_{1(б)} = 0{,}155$

$X_б \frac{0{,}155*0{,}0220*50*1*25*98*100}{0{,}210*0{,}0530*100*1*25*(100-1{,}4)}$ = 15.22

Dry extract of usma roots (80% alcohol)
The numerical indicators of the dry extract of usma roots were determined using the same methods.
To confirm the authenticity, we used our previously developed end-to-end TLC method for determining the content of flavonoids: rutin, luteolin and quercecin.

To determine flavonoids, the following systems were used.

N – butanol:	Ice. acetic acid	: Water
4	2	1
8	4	2

0.0506 g of dry extract +	2 ml 80% ethyl alcohol→	System
1. 0.0200 g standard sample of luteolin +	2 ml 80% ethyl alcohol→	System
2. 0.0201 g standard sample rutin +	2 ml 80% ethyl alcohol→	System
3. 0.0200 g standard sample of quercecin +	2 ml 80% ethyl alcohol→	System

TLC result: clearly identified spots of luteolin, a weak trace of rutin and less noticeable quercecin.
The quantitative content of active substances from the dry extract of usma roots was determined by flavonoids in terms of luteolin as a dominating element.
Standard sample of luteolin = 0.0220 + 100 ml 70% ethyl. alcohol→
A) →1 ml + 1 ml AlCl $_3$ in alcohol + 50 ml 70% ethyl. alcohol

$\boldsymbol{D_{st(а)} = 0{,}116}$ at α= 330 nm

B) →1 ml + 50 ml 70% ethyl. alcohol

$\boldsymbol{D_{st(б)} = 0{,}210}$ at α= 330 nm

Test sample = 0.0524 + 50 ml 70% ethyl. alcohol→
A) →1 ml + 1 ml AlCl $_3$ in alcohol + 25 ml 70% ethyl. alcohol →SF at α= 330 nm: $D_{1(а)} = 0{,}064$

(AlCl $_3$) $X_а \frac{0{,}064*0{,}0220*50*1*25*98*100}{0{,}116*0{,}0530*100*1*25*(100-1{,}2)}$ = 11.35%

B) →1 ml + 25 ml 70% ethyl. alcohol →SF at α= 330 nm:

$D_{1(б)} = 0{,}055$

$X_б \frac{0{,}055*0{,}0220*50*1*25*98*100}{0{,}210*0{,}0530*100*1*25*(100-1{,}2)}$ = 5.39%

CONCLUSION

Thus, based on the conducted research, methods for identifying and quantifying the active ingredients of dry extracts of leaves and roots of Woad have been developed. For authenticity, a TLC technique was proposed, and a choice of solvents, elution conditions and developers for identifying flavonoids was made. Validation characteristics for determining the sensitivity and detection limit of prepared drugs are presented, proving the correctness, suitability, reproducibility and specificity of the developed method. To determine the quantitative content of active substances from the dry extract of leaves and roots of usma, a spectrophotometric method was used. The determination was carried out based on flavonoids in terms of luteolin as a dominating element contained in the extracts. The developed conditions will be included in the regulatory document for dry extract of leaves and roots of Woad.

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