Spectrophotometric determination of a substance trifusol in a veterinary suppository

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The purpose of the work was to develop a method for the quantitative determination of piperidinium 2-[5-(2-furyl)-4-phenyl-1,2,4-triazol-3-ylthio] acetate (trifusol) as part of a dosage form – an effervescent intrauterine suppository using spectrophotometry in the ultraviolet region spectrum and its validation according to State Pharmacopoeia of Ukraine.

Materials and methods. The study was used a working standard sample of trifusol, intrauterine effervescent veterinary suppositories, 1.0 g of trifusol, as a solvent – purified water. Analytical equipment: Specord 200 spectrophotometer, electronic scales ABT-120-5DM, ultrasonic bath ELMASONICE 60 H, class A measuring dishes. Method of spectrophotometric analysis was used.

Results. A spectrophotometric method has been developed and validated for the quantitative determination of trifusol in a dosage form – an intrauterine effervescent veterinary suppository, based on measuring the absorption of an aqueous solution of the drug at 278 nm.

The methodology tally the requirements of State Pharmacopoeia of Ukraine for such validation characteristics as specificity, linearity, precision, correctness, and robustness. The analysis of the predicted total uncertainty of the analysis was showed the reproducibility of the method and the possibility of its application in other laboratories.

Conclusions. A method for the quantitative determination of trifusol in the composition of the dosage form, an effervescent intrauterine suppository, according to the requirements of State Pharmacopoeia of Ukraine, was developed and validated. It was proved that according to such validation characteristics as linearity, specificity, precision, correctness, and robustness, the technique is correct.
Endometritis of different etiologies is the most common form of postnatal pathology in cows, which can take a mass character and cause significant economic damage to both the economy and the state as a whole. Against the background of the intensification of all branches of animal breeding, animal diseases involving damage to the sexual sphere prompted the search, creation, and improvement of existing medicines.

The use of the substance piperidinium 2-[5-(2-furyl)-4-phenyl-1,2,4-triazole-3-iltio]acetate (trifusol), as an active substance in the composition of the effervescent intrauterine suppository, provides a sufficiently high therapeutic efficacy in the treatment, particularly of purulent postpartum cattle endometritis. Its use can improve the course of the pathological process and individual blood parameters [5,6].

The development of new accurate and sensitive methods for quantifying trifusol in new dosage forms is an immediate need at the stage of ensuring proper quality control of drugs, in terms of safe, rational and effective therapy.

For other dosage forms, an express and easy-to-use method of trifusol quantitative determination have already been proposed [1]. Any analytical technique (including the technique of quality control of a particular drug) that may be proposed for inclusion in a normative document or by means of which official tasks will be performed (for example, official opinion on the quality of the object) should be validated [2–4]. Only a specific method for a specific dosage form can be validated.

Aim

Thus, the purpose of our work was to develop a method for quantitative determination of piperidinium 2-[5-(2-furyl)-4-phenyl-1,2,4-triazole-3-iltio] acetate in the dosage form of effervescent intrauterine suppository using spectrophotometry in the ultraviolet region of the spectrum and its validation, according to the State Pharmacopoeia of Ukraine.

Materials and methods

Study objects, solvents and equipment. The objects of the investigation were intrauterine effervescent veterinary suppositories, 1.0 g of trifusol substance. The indicated dosage form was prepared extemporally according to the prescription [5].

Purified water was used as a solvent. As working standards of trifusol were used.


General methodology for quantification of trifusol substance. Aliquot of trifusol (0.050 g) was placed in a measuring flask of 100.00 ml and bring to the mark with purified water, and stirred. 1.00 ml of the resulting solution was transferred to a measuring flask with a capacity of 25.00 ml, and diluted with a solvent to the mark. Optical density was measured on the background of a compensation solution (purified water) at an analytical wavelength of 278 nm.

Results

Method for quantitative determination of trifusol in a veterinary effervescent suppository for intrauterine use. 1 suppository was placed in a glass of 50 ml, added 25 ml of distilled water and heated in an ultrasonic bath until the suppository was completely melted, then cooled and decanted in a measuring flask of 100.00 ml. This operation was repeated twice. The content of the flask was diluted with water to the mark and stirred thoroughly. 5.00 ml of the resulting solution was transferred to a measuring flask with a capacity of 25.00 ml, and diluted with a solvent to the mark. Optical density was measured against a solvent background at wavelength 278 nm. At the same time, a 1.00 ml of 0.05 % trifusol comparison solution was determined. The calculation of the active substance content was done according to
Prediction of complete methodology uncertainty. To verify that the technique would be replicated in other laboratories, it was not enough just the results of the validation in one laboratory, the level of equipment which may be much higher than allowed by the State Pharmacopoeia of Ukraine. Calculations of the forecast of complete methodology uncertainty in accordance with the requirements of the State Pharmacopoeia of Ukraine were created specifically for this purpose. The total uncertainty of the analysis technique was based not only on the real total uncertainty of sample preparation but also on the maximum uncertainty for a specific equipment type [7,8].

According to the requirements of the State Pharmacopoeia of Ukraine, uncertainty calculations were performed to the maximum permissible errors for measuring dishes, scales, and spectrophotometer as a final analytical operation in analysis (∆FAO = 0,70 %) (Table 1).

It can be seen from Fig. 1 that the most significant uncertainty in sample preparation is introduced by operations 6 – taking a standard sample of trifusol, as well as 2, 4 and 8 – taking aliquots by pipettes 1.00 and 5.00 ml. This distribution of uncertainty in sample preparation is quite typical for the quantitative determination of drugs.

Thus, the projected total uncertainty of the analysis results (∆As = 1,53 %) does not exceed the critical value (∆max = 3.20), i.e. the technique will be reproducible and correct in other laboratories as well.

Discussion
The specificity of the technique was established by determining the effect of auxiliary substances (Table 2), which are part of the studied dosage form, on the results of trifusol quantitative determination. For this purpose, the placebo solution (Ablank) absorption in purified water was measured in the concentrations of the corresponding prescription [5], making 3 measurements with cuvette extraction. At the same time the optical density of the comparison solution (As) was measured. The following was found out: Ablank = 0.0031; As
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The placebo contribution to the total absorption was $\delta_{\text{exc}} = 100 \cdot \frac{0.0031}{0.9542} = 0.32\%$ and was insignificant, because there is a ratio: $\delta_{\text{exc}} = \frac{0.033 \cdot B}{0.32} \leq 0.33$, therefore, the method had a sufficient specificity [7,8].

Linearity for suppositories was determined within 73–127% of the nominal trifusol concentration. For this purpose was prepared a solution of suppository in purified water according to the method of quantitative determination of trifusol in veterinary effervescent suppository for intrauterine use, later it was used to obtain nine dilutions. The absorption of the obtained solutions at 278 nm was measured and a graph of the dependence of optical density on the concentration of the studied substance in the sample was drawn up (Fig. 2, Table 3).

So, it was calculated numerical indicators testify that all requirements of the State Pharmacopoeia of Ukraine on parameters of linear dependence are fulfilled and linearity of a technique was confirmed in the chosen range of concentration [2–4].

To establish the accuracy of the technique nine parallel definitions (three weighed quantities of the studied drug form, three repetitions) were performed. The absorption of the comparison solution was measured simultaneously. The gram content of trifusol in the dosage form was calculated using a standard formula.

Table 3. Main characteristics of linear dependence

<table>
<thead>
<tr>
<th>Data</th>
<th>Value</th>
<th>Criteria (For tolerances 90.0–110.0%, number of points 9)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b \pm (s_b)$</td>
<td>1.0122 ± (0.0073)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$a \pm (s_a)$</td>
<td>-1.1938 ± (0.7116)</td>
<td>$</td>
<td>a</td>
</tr>
<tr>
<td>$S_{\Delta a}$</td>
<td>0.3859</td>
<td>$\Delta_{\Delta b}(%) / t(95%, 7) = 1.690$</td>
<td>corresponds</td>
</tr>
<tr>
<td>$r$</td>
<td>0.9998</td>
<td>$\geq 0.9555$</td>
<td>corresponds</td>
</tr>
</tbody>
</table>

Table 4. Determination of the convergence of the quantitative determination results in intrauterine suppositories with trifusol

<table>
<thead>
<tr>
<th>$\bar{Z}$</th>
<th>$S_{\bar{Z}}$</th>
<th>$\Delta$</th>
<th>$\Delta_{As}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>109.4</td>
<td>0.464</td>
<td>0.863</td>
<td>3.20</td>
</tr>
</tbody>
</table>

Table 5. Determination of the correctness of the results of the quantitative determination of trifusol in intrauterine suppositories by the standard addition method

<table>
<thead>
<tr>
<th>Index</th>
<th>Criteria (For tolerances 90–110 %)</th>
<th>Value and conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg, $\bar{Z}$ %</td>
<td>–</td>
<td>99.88</td>
</tr>
<tr>
<td>Relative standard deviation, $S_{\bar{Z}}$ %</td>
<td>$\leq 1.69$</td>
<td>0.519 Corresponds</td>
</tr>
<tr>
<td>Relative confidence interval $\Delta% = t(95%, 8) \cdot S_{\bar{Z}}$</td>
<td>$\leq 3.20$</td>
<td>0.965 Corresponds</td>
</tr>
<tr>
<td>Systematic error $\delta_{\text{ss}} =</td>
<td>\bar{Z} - 100</td>
<td>$</td>
</tr>
<tr>
<td>Criterion of insignificance of systematic error $\delta_{\text{ss}} \leq \Delta% / 3$</td>
<td>$\leq 0.322$</td>
<td>Corresponds</td>
</tr>
</tbody>
</table>

$= 0.9542$. The placebo contribution to the total absorption was $\delta_{\text{exc}} = 100 \cdot 0.0031 / 0.9542 = 0.32\%$ and was insignificant, because there is a ratio: $\delta_{\text{exc}} = 0.033 \cdot B = 0.32 \leq 0.33$, therefore, the method had a sufficient specificity [7,8].

On the concentration of the studied substance in the sample was drawn up (Fig. 2, Table 3).

So, it was calculated numerical indicators testify that all requirements of the State Pharmacopoeia of Ukraine on parameters of linear dependence are fulfilled and linearity of a technique was confirmed in the chosen range of concentration [2–4].

To establish the accuracy of the technique nine parallel definitions (three weighed quantities of the studied drug form, three repetitions) were performed. The absorption of the comparison solution was measured simultaneously. The gram content of trifusol in the dosage form was calculated using a standard formula.
According to the requirements of the State Pharmacopoeia of Ukraine, the one-sided confidence interval (Δ %) should not exceed the maximum allowable uncertainty of the analysis (ΔAs %). According to the State Pharmacopoeia of Ukraine, the deviation of the active substance content from the declared amount in the pessary (vaginal suppositories) was 90–110 % [3]. Thus, the maximum allowable uncertainty of the analysis (ΔAs %) was 3.20 %. Metrological characteristics were calculated based on the results: average value Z, relative standard deviation (S, %), relative confidence interval (Δ %) (Table 4).

The proposed methodology was accurate at the convergence level, as the one-sided confidence interval Δ % did not exceed the maximum allowable uncertainty of the analysis (ΔAs %).

To establish the correctness of the method, the standard addition method was used, in which different amounts of standard working solution of trifusol were added to three equal samples of the drug form and analysed three times.

According to the State Pharmacopoeia of Ukraine, the results of the definitions are correct if they are not burdened with a significant systematic error (δ tot), i.e. the true value of the quantity to be determined falls within the specified confidence interval (Δ %). Thus, the proposed method is correct because, as can be seen from Table 5, deviations Z from 100 % do not exceed their confidence interval [9].

The stability of solutions in time was studied to verify the robustness of quantitative determination methods. The tested solution and the comparison solution are stable for at least 60 min (Fig. 3).

Conclusions

A new sensitive, economic and express spectrophotometric method for quantitative determination of piperidinium 2-[5-(2-furyl)-4-phenyl-1,2,4-triazol-3-ylthio]acetate in 1% and 2.5% solutions-[5-(2-furfuryl)-1,2,4-triazol-3-yl]acetate in its dosage form – veterinary intrauterine effervescent suppository with its own absorption has been developed. The proposed method was validated and its compliance with the requirements of the State Pharmacopoeia of Ukraine was proved for the main validation characteristics: linearity, convergence, correctness and robustness.

Conflicts of interest: authors have no conflict of interest to declare.

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