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Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)

ISSN 2760-4969 (Online) Volume 2, Issue 2, February 2026

https://eurekaoa.com/index.php/7

VALIDATION OF QUALITY CONTROL METHODS OF INJECTION SOLUTION "DALEN"

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Abstract

In recent years, the demand for hemostatic medicinal products has increased significantly. This trend is associated with a number of medical and social factors. Bleeding conditions are frequently encountered in surgical practice, traumatology, obstetrics and gynecology, as well as in diseases accompanied by disorders of the blood coagulation system. Therefore, the need for effective and safe hemostatic agents remains high.

The global increase in the number of surgical interventions, road traffic accidents, and various injuries further intensifies the demand for hemostatic drugs. In addition, the growing number of patients suffering from oncological diseases, cardiovascular disorders, and pathologies of the coagulation system also contributes to the increased need for this group of medicinal products [2].

Among hemostatic agents, antifibrinolytic drugs occupy a special place. In particular, tranexamic acid possesses a strong hemostatic effect and is effectively used in bleeding of various etiologies. Such preparations are of particular importance in emergency medical care, the postoperative period, and obstetric practice.

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The increasing demand for medicinal products necessitates strict quality control. This is especially important for injectable hemostatic preparations, where safety, sterility, and precise dosing are critical. Therefore, improving analytical control methods and developing procedures that meet modern validation requirements remains a pressing issue.

Tranexamic acid (trans-4-(aminomethyl) cyclohexanecarboxylic acid) is considered an effective antifibrinolytic agent [1–3].

Validation of an analytical method is the experimental confirmation that the method is suitable for its intended purpose. The validation process involves evaluating the method's compliance with its application conditions and established requirements. According to general pharmacopoeial standards, methods for quantitative determination, impurity testing, and limit testing are subject to mandatory validation. Identification methods are validated when confirmation of their specificity is required.

During validation, the following main characteristics are assessed: specificity, accuracy, repeatability, linearity, and range [4,5]. These parameters ensure the reliability and reproducibility of the analytical method.

Quality control is of particular importance for injectable dosage forms, as they are administered directly into the bloodstream. Tranexamic acid, being an effective antifibrinolytic agent, is widely used in the treatment of bleeding conditions. Therefore, the development and validation of a quantitative determination method for medicinal products containing this substance are of significant scientific and practical importance.

Based on the above, the aim of the present study was to investigate the validation characteristics of the developed analytical method for determining the active pharmaceutical ingredient in an injectable dosage form and to experimentally substantiate its suitability for practical application.

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Materials and Methods

The object of the study was "Dalen" injection solution manufactured by Mediofarm LLC. Quantitative determination of the active substance — tranexamic acid (TA) — in the preparation was carried out using the developed High-Performance Liquid Chromatography (HPLC) method [7,9].

The validation characteristics of the method were evaluated according to the following parameters: specificity, accuracy, repeatability, linearity, and range [4,5].

All validation characteristics of the identification and quantitative determination methods were established in accordance with the requirements of relevant regulatory recommendations [1,2].

The study was performed using an Agilent 1260 3D LC System (Germany) high-performance liquid chromatograph, Class A volumetric glassware, and analytical balances manufactured by Ohaus (AS-220/X, serial No. B635963283, Germany). Pharmacopoeial-grade reagents meeting State Pharmacopoeia requirements were used in the study: sodium phosphate, sodium lauryl sulfate, phosphoric acid, triethylamine, sodium chloride, disodium edetate, and benzyl alcohol.

A tranexamic acid reference standard substance (India) with a purity of 99.9% was used as the standard sample.

Chromatographic analysis was carried out on a liquid chromatograph with a UV detector, using a Zorbax Eclipse Plus C18 column (250×4.6 mm, particle size 5.0 µm, Hypersil). To prepare the mobile phase, anhydrous sodium phosphate and sodium lauryl sulfate were dissolved in water, triethylamine was added, and the pH of the prepared solution was adjusted to 2.5±0.05 with phosphoric acid. The resulting solution was made up to 600 ml, 400 ml of methanol was added, mixed, and filtered through a 0.45 µm membrane filter.

Chromatography was carried out under the following optimal conditions: mobile phase flow rate — 1 ml/min; injection volume — 20 µl; column thermostat temperature — 20ºC; detector wavelength — 220 nm. Chromatographic system

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eligibility condition: column efficiency for tranexamic acid peak — at least 1000 theoretical plates; peak symmetry coefficient — no more than 2.0; The relative standard deviation of the peak surface on 6 consecutive chromatograms should not exceed 2.0%.

The procedure for preparing standard sample (SN), test sample (TN), model solutions (ME) and placebo is as follows:

SN solution: Approximately 25.0 mg (accurately weighed) of standard tranexamic acid SO was placed in a 25.0 ml volumetric flask, dissolved in water, and made up to the mark with water and mixed (TC concentration ≈ 1 mg/ml).

TN solution: Approximately 2.0 ml of the drug was placed in a 50.0 ml volumetric flask, made up to the mark with water and mixed (Solution A). 5 ml of solution A was placed in a 25 ml volumetric flask, water was added and mixed (TC concentration ≈ 1 mg/ml).

ME solutions (No. 1–5): Tranexamic acid was prepared in amounts of 80, 90, 100, 110 and 120% of the amount in the Dalen injection solution (analytical range), as well as a placebo that did not contain the TC substance.

The SN and ME solutions were analyzed chromatographically in turn, ensuring that the number of parallel chromatograms was not less than or equal to the number used to check the suitability of the chromatographic system. The amounts of TC (mg) were calculated according to the following formula:

$$X = \frac{S_1 \text{ x } a_0 \text{ x } 50 \text{ x } 25 \text{ x } P}{S_0 \text{ x } 25 \text{ x } 5 \text{ x } 5 \text{ x } 100} = \frac{S_1 \text{ x } a_0 \text{ x } P \text{ x } 0{,}02}{S_0}$$

Here:

S1 – the area of the TC peak in the chromatogram of the test solution;

S0 – the area of the TC peak in the chromatogram of the standard SN TC solution;

a0 – the amount of CT obtained during the preparation of the standard SN TC solution, mg;

P – the amount of the main substance in the standard SN TC solution, %.

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The linearity parameters of the obtained results were estimated by the method of least squares in the normalized coordinate system, as well as the values of accuracy and repeatability.

Results and Discussion

The average values of the retention time of the TC peak for TN and SN solutions are calculated, respectively, Sm TN and Sm SN (in minutes).

Table 1. Peak retention time

Sample name	1	2	3	Mean value	tmSS-tmTS	δ, %
	t_1	t_2	t_3	Tm		
SS	11,037	11,147	11,084	11,089	-	-
TS	11,114	11,156	11,304	11,191	0,102	0,22

The specificity of the method was established by confirming the identity of tranexamic acid (TA) in “Dalen” 1 mg/mL injection solution and in the TA standard solution (analyzed in triplicate), followed by comparison with the placebo solution.

The relative deviation of the test solution (TS) results compared to the standard solution (SS) results was 0.22%, which is below the acceptable limit of 2.0% (analytical error), thereby confirming the specificity of the method.

As an evaluation criterion for identification, the retention times of the main TA peak in the chromatograms of the test and standard solutions were compared. The retention times in both chromatograms were required to coincide.

The results of the conducted studies are presented in Table 2 and Figures 1–3.

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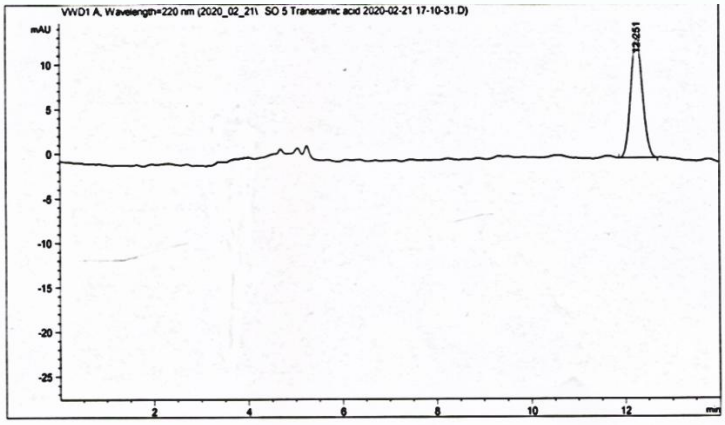

Figure 1. Tranexamic acid standard sample

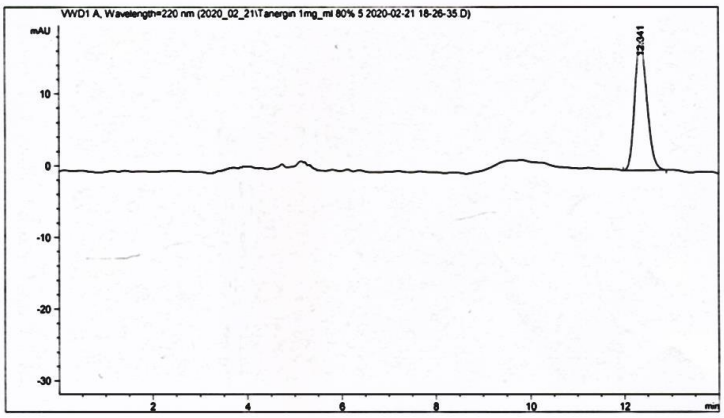

Figure 2. Chromatogram of drug "Dalen"

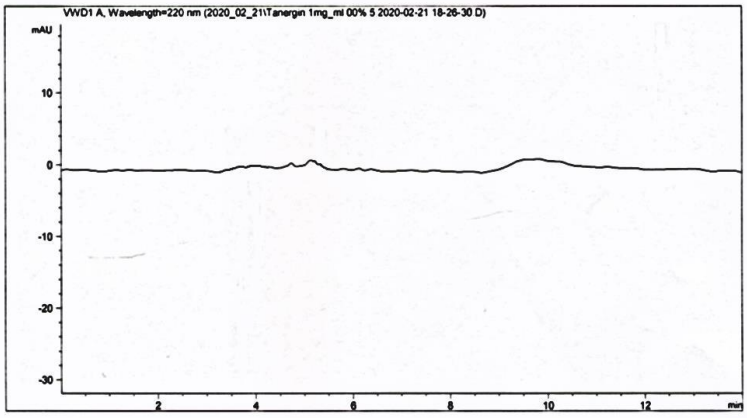

Figure 3. Chromatogram of "Placebo" solution

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The accuracy of the method was tested using model solutions. Three samples were prepared for each concentration. The amount of tranexamic acid in the model solutions was determined. The accuracy of the method is evaluated based on how close the obtained average test results are to the established reference value.

Acceptance criterion: the mean recovery should be between 98.0% and 102.0%.

The recovery (Recovery) was calculated using the following formula:

$$Recovery = 100 - \frac{(m_0 - m_1)}{m_0} \cdot 100\%$$

Here:

m_0 – the theoretical amount of the declared active substance;

m_1 – the determined amount of the active substance.

The results of the study are presented in Table 2.

Table 2 Results of the method accuracy determination

Sample name	№	Added amount (mg) and declared theoretical value (%)		Determined amount, mg	Recovery, %
Dalen	1	40,5104	80%	40,4220	99,78
	2	39,7051	80%	41,1559	103,65
	3	39,9992	80%	39,6634	99,16
	4	45,0011	90%	45,0344	100,07
	5	44,9663	90%	44,9701	100,01
	6	44,1313	90%	44,3572	100,51
	7	50,0031	100%	50,1149	100,22
	8	50,0012	100%	49,9997	100,00
	9	49,9969	100%	50,0111	100,03
	10	54,9451	110%	54,9293	99,97
	11	54,9412	110%	54,9855	100,08
	12	54,9711	110%	54,9673	99,99
	13	59,9551	120%	59,9923	100,06
	14	59,4812	120%	59,5737	100,16
	15	60,1220	120%	60,0004	99,80
Mean recovery [%]					100,23
95% confidence interval					99,89±1,02
RSD [%]					0,96

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The method under validation was found to be accurate, since the experimentally determined values fell within the confidence interval around the mean results of the analysis. The calculated t-values were found to be lower than the tabular value ($t_{ta}b$ = 2.36 at P = 95%, f = 8), indicating that there is no significant systematic error at the 95% confidence level.

The linearity of the method was established by chromatographing model solutions within 80.0–120.0% of the working concentration, with the drug fully dissolved. The linear regression parameters for the peak area were calculated using the least squares method.

The results of the study are presented in Table 3 and Figure 4**.**

Table 3 Results of the method linearity assessment

№	Model solution	
	Concentration [mg/50 mL]	**Peak height [mAU·s]**
1	40,510	190,4
2	39,999	195,3
3	45,001	212,1
4	44,966	211,9
5	50,003	236,0
6	50,001	235,5
7	54,945	258,9
8	54,941	259,2
9	59,955	282,7
10	59,481	283,0
y=a+b∗x		
Intercept, b		4,642879108
A segment on the ordinate axis, a		4,453291481
RSD		6,91562327
Correlation coefficient, r		0,997974051

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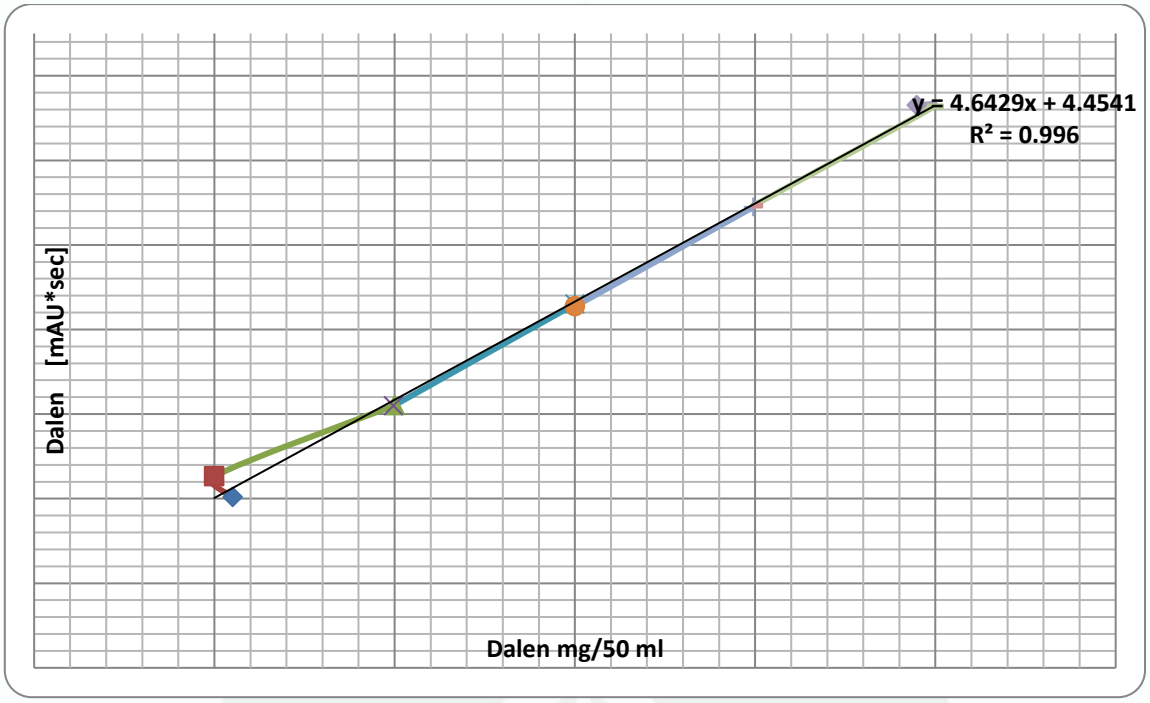

Figure 3. Linear relationship between the peak area and concentration of tranexamic acid in model solutions

The linearity parameters b, Sb, a, Sa and RSD_0 (relative standard deviation of residuals) were calculated using the least squares method. The regression equation **(**y = bx + a**)** was constructed in normalized coordinates (Xi and Yi).
The following criteria were applied for evaluating the linearity of the method:
Statistical and practical insignificance of the intercept (a) of the direct line;
Requirement for residual standard deviation (RSD_0);
Requirement for the correlation coefficient (r).
The calculated correlation coefficient must be at least 0.998.
The repeatability of the method was assessed by analyzing six samples of the model preparation: “Dalen” 1 mg/mL injection solutions, each containing 100% of the test concentration. The results obtained by two chemists over two days are presented in Table 4.

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Table 4 Results of tranexamic acid repeatability tests

Name	№	Amount of model preparation (relative to theoretical value, 100%)	
		Day 1 –Chemist 1	Day 2 – Chemist 2
Dalen	1	100,22	100,00
	2	100,00	99,83
	3	100,03	99,27
	4	100,65	99,44
	5	100,21	99,66
	6	100,30	99,73
Mean		100,24	99,66
RSD,%		0,234	0,264
95% Confidence Interval		100,21±1,81%	99,68±1,68%

The data presented in Table 4 show that the relative standard deviation (RSD) was 1.68% and 1.81%, respectively. This confirms that the repeatability of the developed high-performance liquid chromatography (HPLC) method can be considered acceptable.

Conclusion

The developed HPLC method for the quantitative determination of tranexamic acid in injection solutions has been validated. The method's validation characteristics, including specificity, accuracy, repeatability, and linearity, were confirmed by the obtained results.

Through the validation process, the analytical method developed for the "Dalen" injection solution was fully confirmed in terms of reliability, accuracy, repeatability, and linearity. The results demonstrated that this method allows precise and reliable assessment of the drug's quantitative parameters. Therefore, these methods are considered suitable for inclusion in regulatory and technical documentation to ensure effective quality control, safety, and stability of the drug being developed.

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Moreover, the developed method is suitable for practical use, as its simplicity and reproducibility of results allow for wide application in both industrial settings and laboratory practice.

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