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DEVELOPMENT OF RP HPLC METHOD FOR AMINOCAPROIC ACID DETERMINATION IN A COMPLEX NASAL DRUG

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The aim *of this work was to develop a method of RP HPLC for the quantitative determination of aminocaproic acid in a complex nasal drug.*
Materials and methods*. A solution of aminocaproic acid and a solution of a complex model mixture containing aminocaproic acid were used for the study purposes. A method used for sample preparation was derivatization of aminocaproic acid with dansyl chloride. A method of quantitative determination was RP HPLC analysis with UV detection at 288 nm.*
Results*. The obtained data confirm the specificity, linearity, and correctness of the method proposed for quantitative analysis. Therewith, the correlation coefficient, limit of detection, limit of quantification and relative standard deviation (RSD) are R=0.9998, LOD=4.6·10^{-5} g/mL, LOQ=1.4·10^{-4} g/mL, and RSD=1.16 % respectively.*
Conclusions*. A method of RP HPLC for the quantitative determination of aminocaproic acid in a complex nasal drug has been developed and its validation assessment has been carried out according to the following validation parameters: specificity, accuracy, linearity, and precision (repeatability). Statistical processing of the obtained results shows that all the validation parameters studied are within the acceptance criteria*
Keywords*: RP HPLC, aminocaproic acid, dansyl chloride, complex nasal drug*

1. Introduction

Influenza and acute respiratory viral infections (ARVIs) are among the most common viral diseases with up to incidents rate 0.07–0.13/10,000 population in epidemic season [1–3], as it was found in following economically developed countries: Australia [4], USA [5], Canada [6, 7], Great Britain [8], Europe [9]. In addition, these diseases cause various complications [10, 11], especially in lugs [12, 13].

Today, medical science has a very limited number of drugs with antiviral activity against influenza / ARVI viruses (interferons, membrane protein M2 inhibitors, and neuraminidase inhibitors, etc.) [14]. Each of these drugs influences different stages of the virus life cycle in human cells (binding, copying, building), but all these drugs have very narrow antiviral activity and have no symptom relief effects.

One of the promising substances that exhibit anti-influenza virus activity is an aminocaproic acid (ACA). Aminocaproic acid has a number of important pharmacological activities: hemostatic, detoxifying, capillary strengthening, anti-allergic, and it also inhibits a number of enzymes and exhibits antiviral activity against influenza virus [14, 15]. This prompted the authors to conduct research on the development of a complex nasal drug with aminocaproic acid for the treatment and prevention of influenza and ARVIs. Other active substances of the nasal composition are para-aminobenzoic acid, decamethoxine, and oxymetazoline hydrochloride [16]. At the same time, in previous *in vitro* studies of anti-influenza activity, a synergistic effect of ACA and PABA mixture (at component ratio of 100:1) and high index of selectivity were determined [17, 18].

These facts prove the usefulness of the development of a complex nasal drug for symptom relief effects and etiotropic treatment or prevention of influenza / ARVI diseases based on these substances.

However, the multicomponent composition creates difficulties in its quantitative and qualitative analysis, and in view of the peculiarities of the physical and chemical properties of aminocaproic acid, its analysis in this mixture of active substances requires the development of a separate method for quantitative determination.

In this regard, **the aim** of this study was to develop a method of RP HPLC for analysis of aminocaproic acid in a complex nasal drug.

2. Planning (methodology) of research

The Quality by design conception was used to plan our studies so as to achieve the aim of research work [19]. Because in light of this conception the safety and quality of developed drug are directly depend on technological process, quality active pharmaceutical ingredients (API), excipients, and methods of analyses. On the Fig. 1 there is presented focus of our study.

Therein the analytical method of API determination is the essential key in quality control of drug in all its life cycle stages including development and design.

Aminocaproic acid is one of the among other API in developed nasal composition. In regard to its special physical and chemical properties, main tradition methods of its analysis like titration, spectrophotometry and even RP HPLC with DAD in developed composition cannot be used. Thus the authors were used the precolumn derivatization for this substance with RP HPLC method [20].

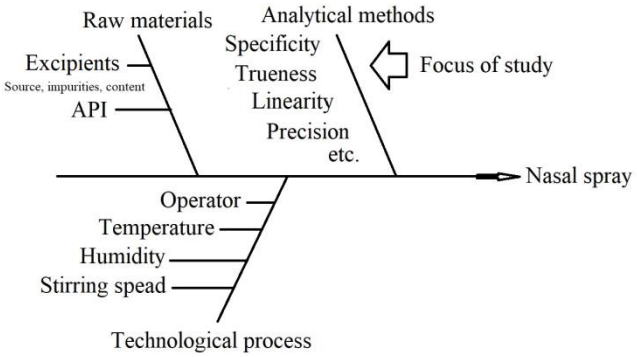


Fig. 1. Focus of study in this work

3. Materials and methods

Chemicals

For studies, aminocaproic acid was used, official reference standard of the State Pharmacopoeia of Ukraine, batch number A0016 and medicine "Aminocaproic acid" in granules (LLC "Zdorovya", Ukraine, serial number 10317, use before 03/2020); oxymetazoline hydrochloride was used, official reference standard of the State Pharmacopoeia of Ukraine, batch number O0310; decamethoxine, official reference standard of the State Pharmacopoeia of Ukraine, series number D0451 and decamethoxine, pure grade (Institute of Organic Chemistry of the National Academy of Ukraine, Ukraine, serial number C.210917, use before 09/2020); aminobenzoic acid had pure grade (Shanghai Synnad, China, serial number 20170722, use before 07/2020) and aminobenzoic acid was official reference standard of the State Pharmacopoeia of Ukraine, batch number A0015; dansyl chloride CAS Number 605-65-2 (Sigma-Aldrich, Germany); sodium tetraborate with high grade (Reachem, Russia).

Water quality was for analytical laboratory use, lithium perchlorate trihydrate, perchloric acid 65 % and methanol quality had high grade (Reachem, Russia), ethanol 95 % v/v quality had pharmaceutical grade (Medchemprom, Russia).

Reverse phase high-performance liquid chromatography

The analysis was carried out by using reverse phase high-performance liquid chromatography method on chromatograph *Agilent Technologies 1200 Infinity*, *Agilent Technologies*. The chromatography analysis with RP HPLC method was performed after preliminary derivatization of aminocaproic acid with dansyl chloride.

Chromatographic conditions: mobile phase (A): 1 % aqueous solution of formic acid, mobile phase (B): ethanol 95 % vol. in linear gradient feeding mode; chromatographic column: *Supelco Ascentis express* C18, particle size: 2.7 μm, column length: 100 mm, internal diameter: 4.6 mm; mobile phase velocity: 0.5 mL/min; chromatographic column temperature: +35.00±0.03°C; sample volume: 5 μL; analytical wavelength: 288 nm. Gradient conditions:

time 0 min (A – 100 %, and B – 0 %),

time 60 min (A – 0 %, and B – 100 %).

The method and conditions are described in more detail in [20].

Preparation of a model solution of a mixture of active substances (pure grade): transfer accurately weighed quantity of aminocaproic acid (1.25 g), para-aminobenzoic acid (0.012 g), decamethoxine (0.005 g), and oxymetazoline hydrochloride (0.012 g) into a 25.0 mL volumetric flask, dissolve in water, and make up the volume to the mark.

Preparation of the initial solution of aminocaproic acid (official reference standard of the State Pharmacopoeia of Ukraine): transfer accurately weighed quantity of the standard/substance (0.25 g) into a 5.0 mL volumetric flask, dissolve in water, and make up the volume to the mark (concentration 5.0 % w/v).

Preparation of dansyl chloride solution: transfer accurately weighed quantity of the substance (0.03 g) into a 10.0 mL volumetric flask, dissolve in methanol, and make up the volume to the mark (concentration 3.0 % w/v).

Preparation of sodium tetraborate solution: transfer accurately weighed quantity of the substance (19.07 g) into a 1,000.0-mL volumetric flask, dissolve in water, and make up the volume to the mark (concentration 0.05 % M).

A method used for derivatization of aminocaproic acid: transfer an aliquot of the initial solution of aminocaproic acid (volume 12.5, 25.0, 40.0, 50.0, 60.0, 75.0, 100.0, 150.0 and 200.0±0.5 μL) into a 10.0 mL volumetric flask, add a two-time larger volume of dansyl chloride solution, 5.0 mL of sodium tetraborate solution, stir and transfer into a thermostat for 30 minutes at a temperature of 60±1 °C. After that, cool the flask with the contents to room temperature, make the volume to the mark with sodium tetraborate solution.

Processing of experimental data was carried out using regression and statistical methods of analysis in MS Excel 2010 using add-in "Data Analysis" (regression function), according to general monographs "Statistical processing of chemical experiment results" and "Validation of analytical procedures" [21]. The LOD and LOQ were calculated from the linear regression equation by using the value of standard deviation of intercept and the value of the slope as it is described in general monograph "Validation of analytical procedures" in Ukrainian Pharmacopoeia [21].

Main parameters of method validation and suitability of RP HPLC system for determination of aminocaproic acid derivative with dansyl chloride are presented in Table 1.

Table 1

Main parameters of method validation and suitability of RP HPLC system for determination of aminocaproic acid derivative with dansyl chloride

Parameter	Pharmacopoeia condition [21]	Aminocaproic acid derivative
1. Retention time, min*	–	28.5±0.5
2. Distribution coefficient	≥1.5	96.5±3
3. Number of theoretical plates	≥1.000	241.485±7.245
4. Asymmetry coefficient	0.8–2.0	1.16±0.03
5. Relative standard deviation, RSD, %	≤2.0	1.50
6. LOD, g/mL	–	$4.6 \cdot 10^{-5}$
7. LOQ, g/mL	–	$1.4 \cdot 10^{-4}$
8. Correlation coefficient, *R*	≥0.99	0.9996
9. Linear regression equation	–	*y= 6831675x – 296*
10. The value of the intercept term of the linear equation	–	–296±303
11. The value of the slope ratio of the linear equation	–	$(6.83 \pm 0.25) \cdot 10^{6}$

Note: the mean value and its confidence interval (X±ΔX) were calculated for the number of repeats n=3 and the confidence level (probability) P=95.0 %

4. Results

In the first part of the studies, we investigated the specificity of the method suggested, which involved using the reaction of dansyl chloride with aminocaproic acid in a separate solution and in the model mixture of active substances. The result is shown in Fig. 2–5.

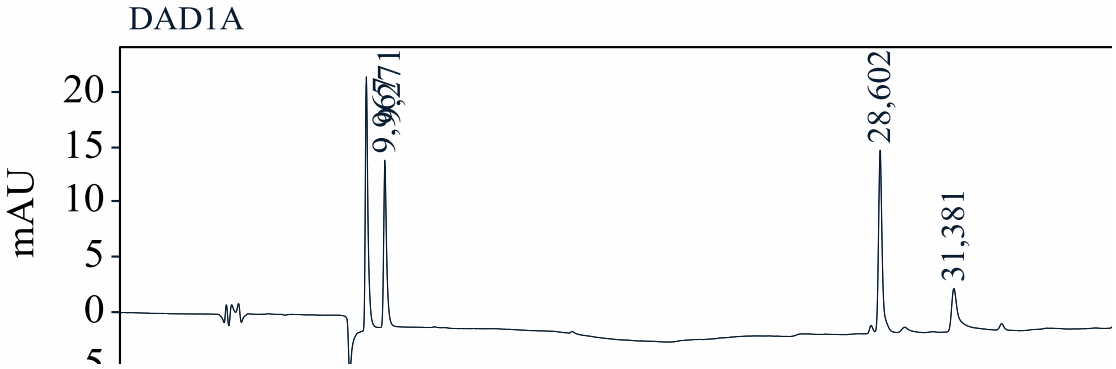


Fig. 2. RP HPLC chromatogram of a model mixture of active substances after derivatization. I is dansyl chloride (9.3 min); II is para-aminobenzoic acid (10.0 min); III is aminocaproic acid derivative (28.6 min). The analytical wavelength is 288 nm

As seen from Fig. 2–5, the peak of aminocaproic acid derivative with dansyl chloride in the model mixture does not intersect with other substances and has identical UV spectra, which indicates the compliance of this method with the principle of specificity. More detailed data on the numerical values of the distribution coefficient, the number of theoretical plates and tailing factor, as well as Pharmacopoeia requirements for these indicators are presented in Table 1.

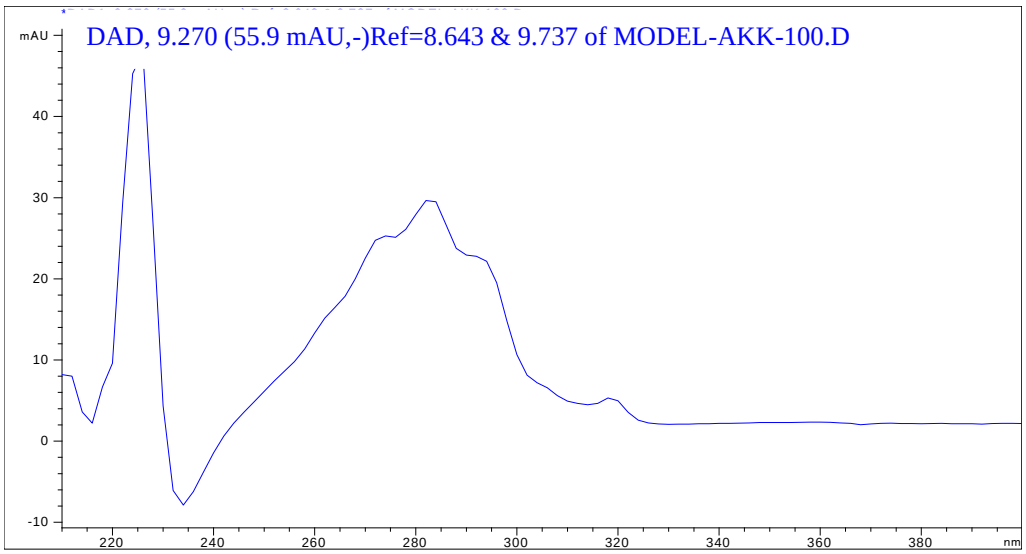
In the second part of the studies, the optimal amount of dansyl chloride solution was investigated to obtain the maximum area of aminocaproic acid derivative. For these purposes, a dependence of the area of aminocaproic acid derivative on the ratio of the mass of dansyl chloride and aminocaproic acid was constructed. The obtained results are presented in Fig. 6.

As seen from the experimental dependence that presented in Fig.5, the relatively stable value of area of aminocaproic acid derivative is observed when the ratio of the mass of dansyl chloride and aminocaproic acid in solution is ranged from 1.1 to 2.3.

In the final part of the study, we investigated the linear regression of the dependence of the area of aminocaproic acid derivative on its concentration was investigated in solution under the above conditions. The dependence obtained is shown in Fig. 7.

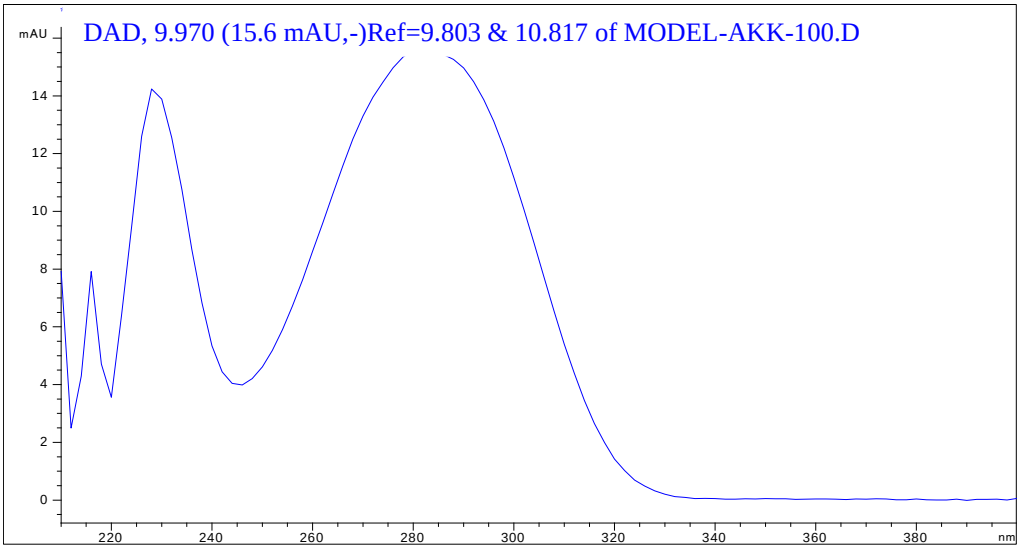
As seen from the data in Fig. 6, the linear dependence of the area of aminocaproic acid derivative on its concentration in the solution has a high correlation coefficient *R*=0.9996, which confirms the linearity of the method developed in the range of aminocaproic acid concentrations studied (0.26÷1.74 mg/ml). The regression equation and some additional parameters are presented in Table 1.

In order to evaluate accuracy of the method the experiment was provided of standard addition method. The results are listed in Table 2.



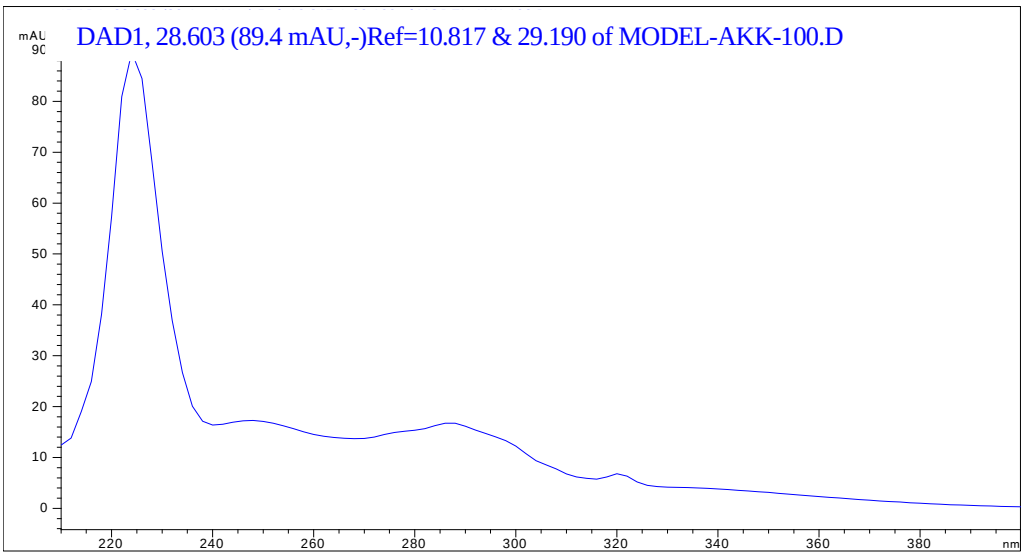
9,9,3 min

a



9,9 min

b



28,6 min

c

Fig. 3. UV spectra: *a* – dansyl chloride; *b* – para-aminobenzoic acid; *c* – aminocaproic acid derivative

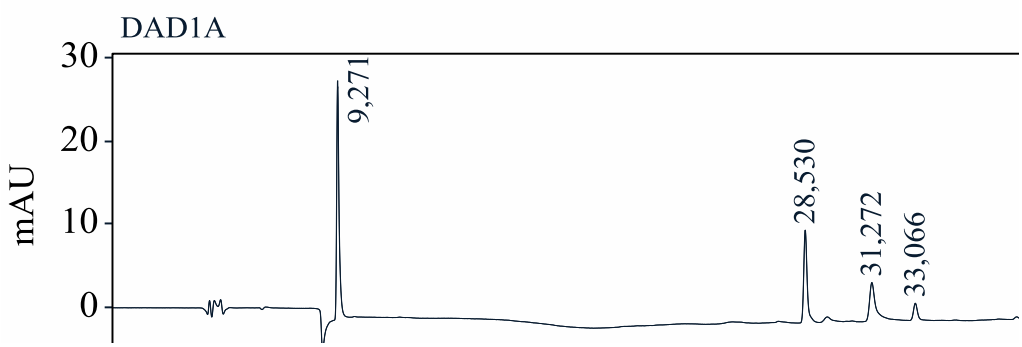
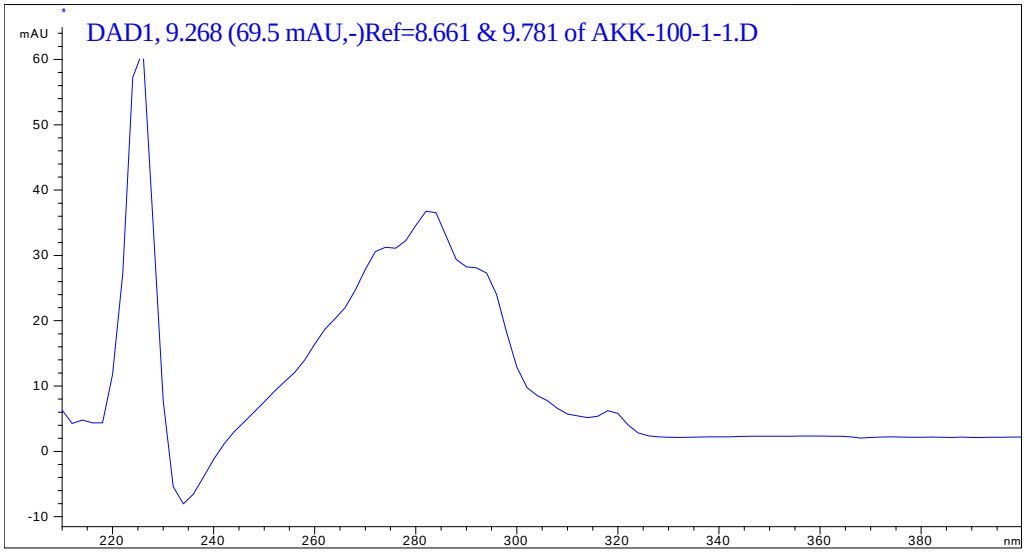
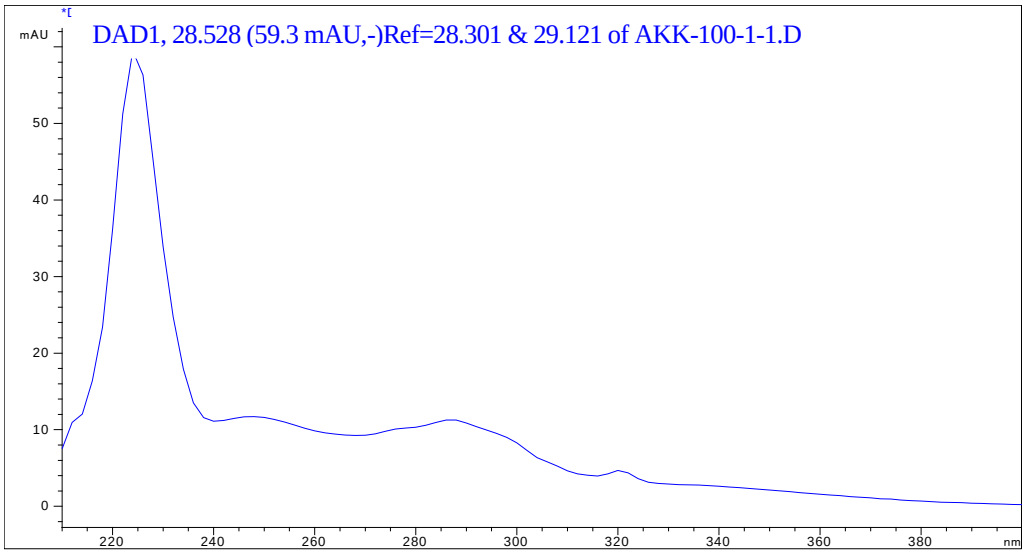


Fig. 4. RP HPLC chromatogram of aminocaproic acid official reference standard of the State Pharmacopoeia of Ukraine after derivatization. I is dansyl chloride (9,3 min); II is aminocaproic acid derivative (28,5 min). The analytical wavelength is 288 nm



9,3 min

a



28,5 min

b

Fig. 5. UV spectra: *a* – dansyl chloride; *b* – aminocaproic acid derivative

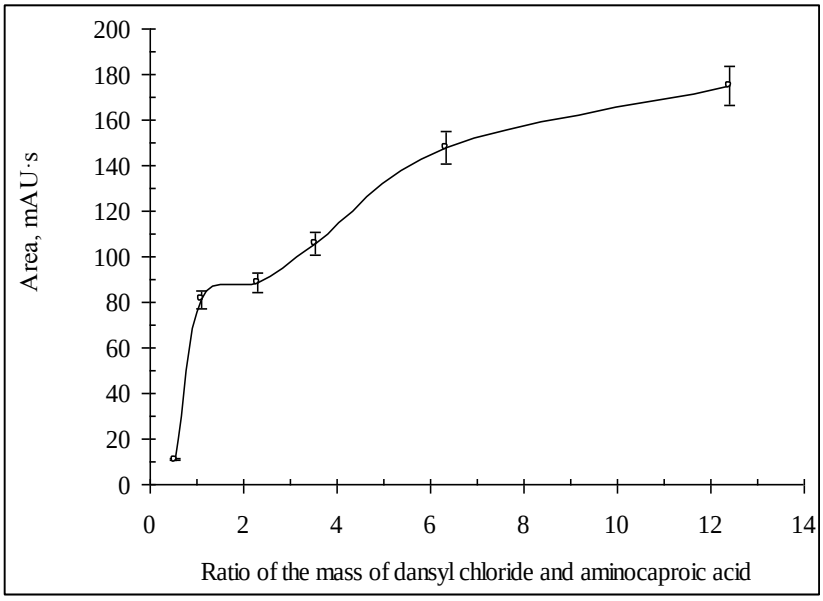


Fig. 6. Dependence of the area of aminocaproic acid derivative on the ratio of mass of dansyl chloride and aminocaproic acid

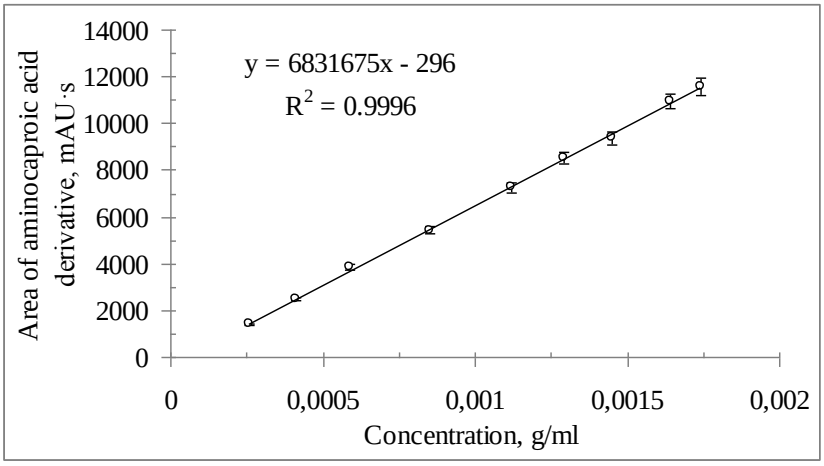


Fig. 7. Linear regression of the dependence of the area of aminocaproic acid derivative on its concentration in the solution

Table 2

Accuracy

No	Level of concentration, %	Added concentration (X), mg/ml*	Found concentration (Y), mg/ml	Recovery (Z=100·Y/X), %
1	80	40.3	41.2	102.2
2	80	40.3	40.8	101.2
3	80	40.3	40.6	100.7
4	100	50.1	51.1	102.0
5	100	50.1	49.5	98.8
6	100	50.1	50.8	101.4
7	120	60.2	61.3	101.8
8	120	60.2	59.4	98.7
9	120	60.2	60.5	100.5
10	Mean, %			100.8
11	Standard deviation (SD)			1.31
12	Relative standard deviation (RSD), %			1.30
13	Confidence interval (*P*=95.0 %)			1.01
14	Minimum			98.7
15	Maximum			102.2

Note: the aminocaproic acid solution was added into placebo solution that contents other ingredients

As seen from the data in Table 2, the accuracy is lead into acceptable range of recovery parameter (100.8±1.01 %).

Precision (repeatability) estimated by the following criteria: standard deviation (SD), relative standard deviation (RSD), and confidence interval are represented in the Table 3.

As seen from the data in Table 3, the RSD parameter is lead into acceptable level (1.50 % ≤ 2.0 %). The intermediate precision data are presented in Table 4.

Table 3

Precision (repeatability)

No	Peak area, mAU·s	Mean±SD, mAU·s	Mean±CI*, mAU·s	RSD, %
1	7256	7232±108	7232±114	1.50
2	7162			
3	7339			
4	7138			
5	7374			
6	7120			

Note: CI is confidence interval at confidence level (probability) P=95.0 %

Table 4

The intermediate precision

No	The value in first day	The value in second day
1	7256	7312
2	7162	7150
3	7339	7265
4	7138	7198
5	7374	7232
6	7120	7174
Mean	7232	7222
SD	108	60
RSD, %	1.50	0.83
Mean RSD, %	1.16	

As seen from the data in table 4, the intermediate precision is lead into acceptable level (1.16 % ≤ 2.0 %).

The results obtained confirm the possibility of quantitative determination of aminocaproic acid in a complex nasal drug using the method of RP HPLC analysis suggested.

5. Discussion

The obtained results demonstrate that developed method has better linearity (R^2=0.9996>0.994), larger analytical range of concentration (0.26÷1.74 mg/ml vs 40÷60 mg/ml), repeatability (RSD=1.16<1.6 %) and specificity (analytical signal of aminocaproic acid derivative is differed from other components of composition) vs spectrophotometric method with ninhydrin reactive that described in literature [22].

However, the developed method has the disadvantages that in general typical for RP HPLC method like necessity to use high cost equipment and time consuming.

The developed method has a possibility for quantitative control of aminocaproic acid among other API that is guarantee the quality and safety of developed nasal composition.

Study limitation. This method cannot be used for the determination of other APIs. That question needs additional studies.

Prospects for further research. The obtained results will be used in further research on the development of the composition and technology of a new complex nasal preparation for the etiotropic and symptomatic treatment of SARS and influenza.

6. Conclusions

A method of RP HPLC for quantitative determination of aminocaproic acid in a complex nasal drug has been developed and its validation assessment has been carried out according to the following validation parameters: specificity, accuracy, linearity, and precision (repeatability). Statistical processing of the results obtained shows that all the validation parameters studied are within the acceptance criteria. The optimal conditions for analysis have been found.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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