Development of Spectrophotometric Method for Analysis of Framicetine Sulphate in a Medicinal Product and its Validation

Gapparov Dilshodbek Davronbekovich[1], Smanova Zulaykho Asanalievna[2]

[1]Associate Professor, National University of Uzbekistan, PhD Student in Chemistry,
[2]Professor, National University of Uzbekistan, Doctor of Chemistry

Abstract

A procedure for the quantitative determination of framycetin sulfate in the drug 'Framex' (nasal spray) 8000 IU/ml has been developed, using spectrophotometry (SF) method. The developed method has been validated using the following indicators: specificity, correctness, linearity, repeatability and intralaboratory accuracy. It has been found that the developed methodology is valid for all characteristics and can be used for the quantitative determination of framycetin sulfate in the substances and composition of medicinal products.

Keywords: *Framycetin sulfate, validation, spectrophotometry, framex, antibiotic, aminoglycosides.*

Introduction

On the current pharmaceutical market, there are very few drugs with antibacterial activity, that can be used for children under 1 year old. These include the drug 'Framex' (produced by Aseptica, Uzbekistan)[1]. The nasal spray composition is based on the antibiotic of the aminoglycoside series of the topical bactericidal action of framycetin. 1 ml of the drug contains 8000 units of framycetin[2].

It is a common fact that aminoglycosides are highly active against most gram-negative and gram-positive microorganisms - pathogens of upper respiratory tract infections. However, systemic antibacterial drugs of these groups have a side ototoxic effect, which makes it impossible to use their antimicrobial potential in bacterial infections of the nasal cavity, nasopharynx and paranasal sinuses in children. The indicated side effects can not be expected with the local administration of these drugs, since, according to the results of clinical studies, the level of their systemic absorption during topical use is extremely low[1].

One of the urgent problems of pharmaceutical chemistry is the development and validation of theroutine analysis method for the quantitative determination of the active pharmaceutical ingredient in substances and finished medicinal products. The developed method are designed to minimize the consumption of solvents and reagents, the duration of the analysis, and to obtain the most accurate result. Validation of an analytical method is an experimental proof that the method is suitable for solving the proposed problems[2].

In our work, we used the drug 'Framex' (nasal spray) containing 8000 IU/ml of framycetin sulfate. The pharmacologically active ingredient of the drug is framycetin sulfate[3]. In its chemical structure, it is 2-deoxy-4-O-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine sulfate. The chemical structure of framycetin sulfate allows its staining using basic conditions (pH=9-10)[4].

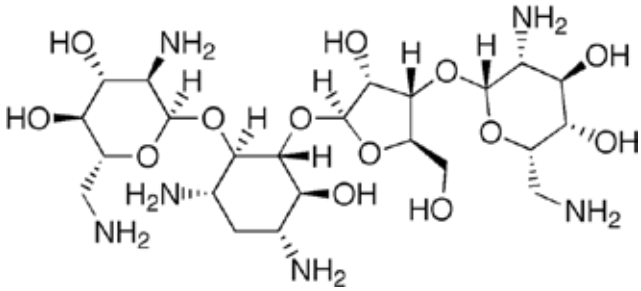

Fig. 1. Structural formula of framycetin

The aim of this work is to develop and validate the SF method for the quantitative determination of framycetin sulfate in substances and the drug 'Framex' (nasal spray).

Materials and Method. In our study, we used class A measuring glassware, an CY 224 CITIZEN analytical balance (India), a 'UV-1800' UV spectrophotometer (manufactured by Shimadzu Corporation, Japan) (wavelength range from 190 to 1100 nm, relative accuracy less than 0.1%)

The specificity of the method is determined by comparing the values of the active ingredient in the analysis of the solvent (form), the active ingredient and the pharmaceutical product.

The linearity of the method is determined by at least 5 different dilutions of the test solution in the range of application of at least 80-120% of the concentration of the analyte in the test solution[5].

Repeatability is carried out using tests of at least 6 prepared samples at 100% concentration of the active ingredient in the test solution.

The correctness of the method determines the response of the active ingredient in placebo[6]. The placebo concentration is 100% of the weigh indicated in the control method. The active ingredient is added to the model mixture in accordance with the required concentration level (lower limit - max. 80, 100%; upper limit - min. 120%)[7].

The object of the study was the drug 'Framex' (the content of framycetin sulfate - 12.5 mg/ml), the corresponding MPhM (manufacturer's pharmacopoeial monograph)is 42 Уз-26491354-2019. Solutions of 80% (0.40 mg/ml), 90% (0.45 mg/ml), 100% (0.50 mg/ml), 110% (0.55 mg/ml), 120% (0.60 mg/ml) were prepared. The substance of framycetin sulfate 'Suzhou Pharmaceutical Technology CO., LTD' (China) (content of active ingredientis 654 IU/mg) was used as a working standard sample (WSS). A WSS solution of framycetin sulfate with concentrations of 0.5 mg/ml was prepared. We prepared WSS, test and blank solutions. All three flasks were simultaneously placed in a water bath for 15 minutes at a temperature of 70-80 ^{0}C. Then, the flasks were removed, cooled to room temperature and water was added, in order to bring the total volume of solution to the mark. The optical density of the obtained solutions was measured on a spectrophotometer at a wavelength of 564 nm in a cuvette with a layer thickness of 10 mm, using a blank solution as a compensation solution[8].

Results and Discussion

The obtained solutions were measured using a spectrophotometer under the optimized conditions given in the article. The obtained spectra confirm that the measurement conditions are optimized and the absorption spectrum of the test solution prepared for quantitative determination in the region from 200 to 700 nm corresponds to the spectrum of the WSS solution of framycetin sulfate and has maxima at a wavelength of 252 nm (3), 401 nm (2) and 564 nm (1).

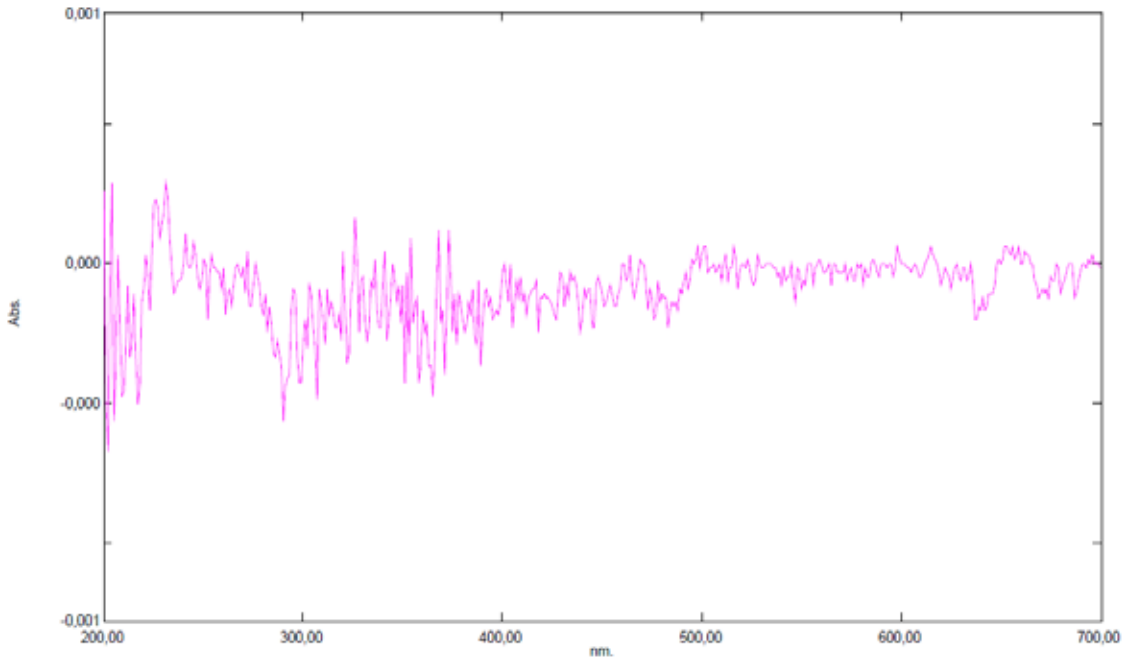

Fig. 2. Absorption spectrum of the blank solution

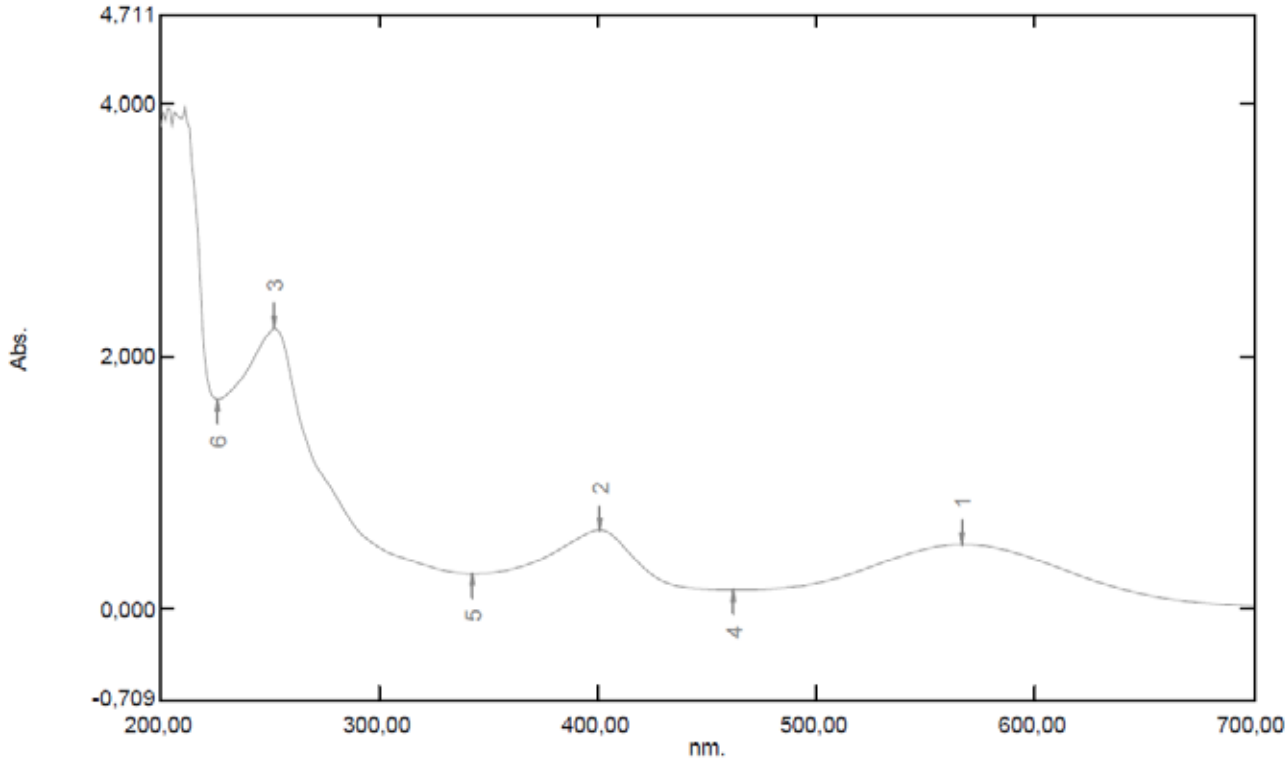

Fig. 3. WSS absorption spectrum of framycetin sulfate (C=0.50 mg/ml).

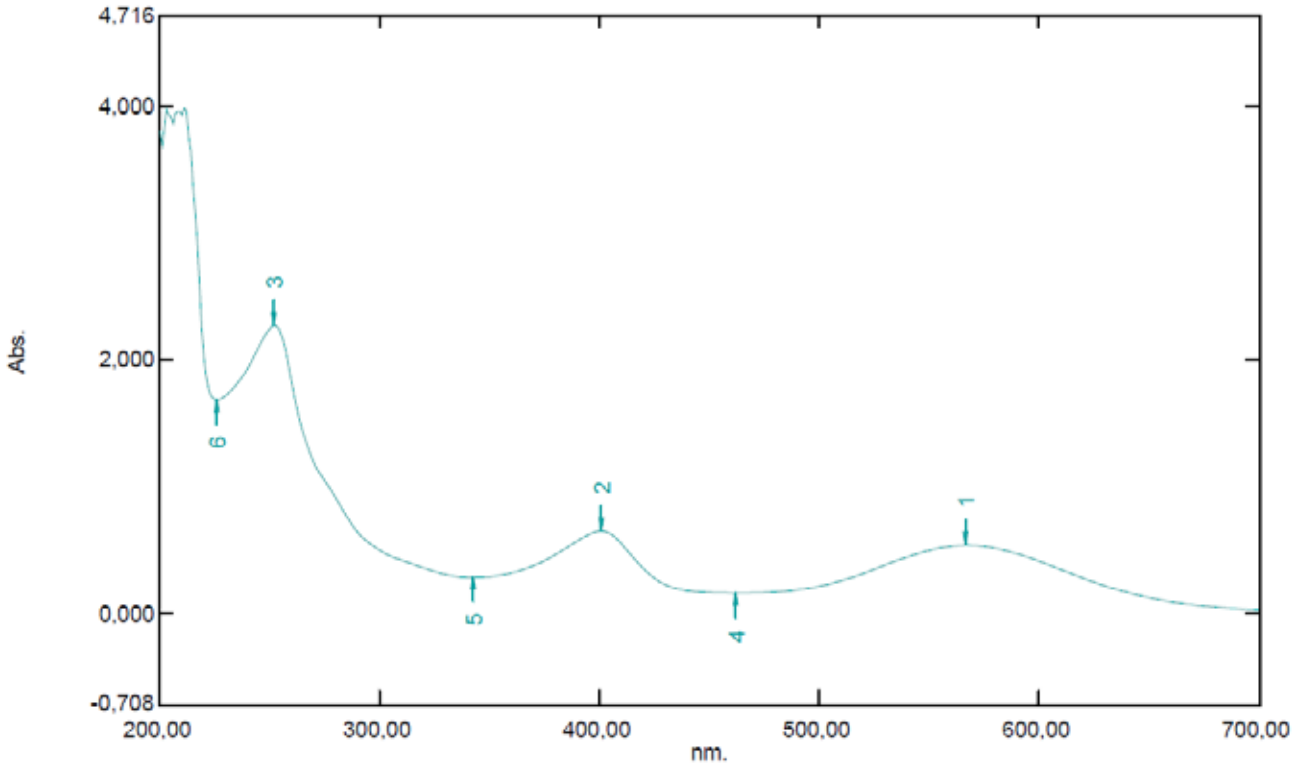

Fig. 4. Absorption spectrum of the test solution (C=0.50 mg/ml).

Fig. 1-3 shows that, under optimized conditions, the maximum values of the WSS and test solutions are identical. The developed technique indicates that the conditions for preparing samples and measuring spectra for determining the quantitative content of framycetin sulfate are specific.

Specificity. The blank solution, standard solution and pharmaceutical product were measured. The optical density of the samples was measured at a wavelength of 564 nm.

Assessment criteria: neither solvents nor reagents used for sample preparation should distort the result.

Linearity. We prepared and measured solutions of the drug with an active ingredient concentration in the range from 80 to 120%: 2 solutions with an active ingredient concentration of 80%, 2 solutions with an active ingredient concentration of 90%, 2 solutions with an active ingredient concentration of 100%, 2 solutions with a concentration active ingredient of 110% and 2 solutions with an active ingredient concentration of 120%. The linearity results are shown in Table 2.

Based on the obtained data, a dependence of optical density on the concentration of framycetin sulfate in solution can be found. The program also draws a trend line and determines the regression equation.

Table 1: Results of linear dependence of optical density on the concentration of framycetin sulfate

No.	Concentration level, %	X (Weigh of drug in mg/ml)	Y (Optical density, A.U.)
1	80	0.4012	0.283
2	80	0.4007	0.281
3	90	0.4501	0.315
4	90	0.4509	0.316
5	100	0.5006	0.352
6	100	0.5000	0.350
7	110	0.5499	0.386
8	110	0.5504	0.388
9	120	0.5993	0.421
10	120	0.6001	0.423
Average		0.5003	0.352

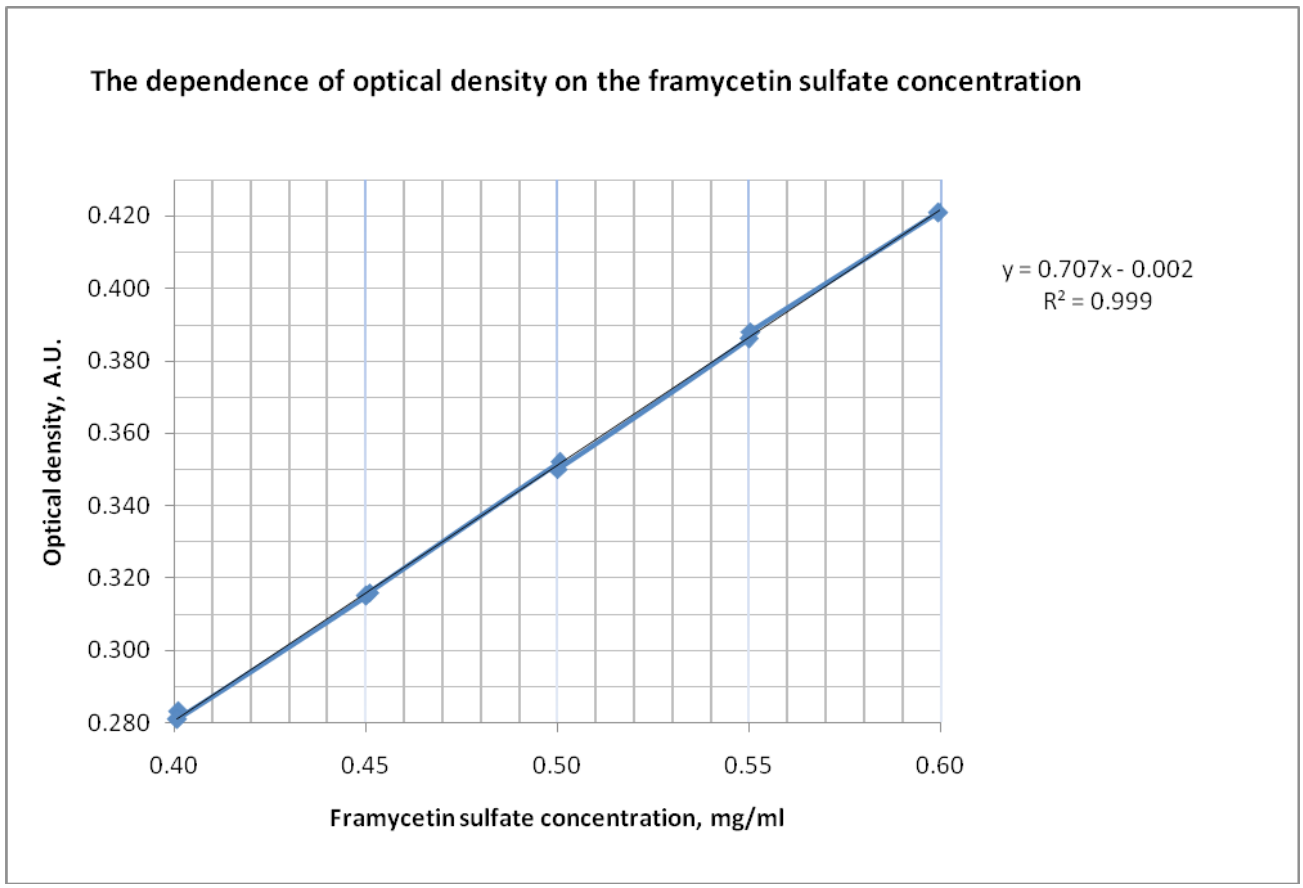

Fig. 5. Graph of the linear dependence of the optical density on the concentration of framycetin sulfate.

Criteria for assessing the linearity: correlation coefficient ≥ 0.99.

Correctness. We prepared and measured drug solutions with an active ingredient concentration in the range from 80 to 120%: 3 solutions with an active ingredient concentration of 80%, 3 solutions with an active ingredient concentration of 90%, 3 solutions with an active ingredient concentration of 100%, 3 solutions with a concentration active ingredient of 110% and 3 solutions with an active ingredient concentration of 120%. The correctness results are shown in Table 2.

Table 2: Results of determining correctness for the developed methodology

Concentration level, %	Optical density, A.U.	Specific content of framycetin sulfate, IU/ml	Specified content of framycetin sulfate, IU/ml	Response, %
80%	0.283	6493.4	6400.0	101.46
80%	0.281	6447.5	6400.0	100.74
80%	0.280	6424.6	6400.0	100.38
90%	0.315	7227.7	7200.0	100.38
90%	0.316	7250.6	7200.0	100.70
90%	0.314	7204.7	7200.0	100.07
100%	0.350	8030.7	8000.0	100.38
100%	0.352	8076.6	8000.0	100.96
100%	0.353	8099.6	8000.0	101.24
110%	0.386	8856.8	8800.0	100.64
110%	0.388	8902.6	8800.0	101.17
110%	0.386	8856.8	8800.0	100.64
120%	0.421	9659.8	9600.0	100.62
120%	0.423	9705.7	9600.0	101.10
120%	0.422	9682.8	9600.0	100.86
Average				100.76

We performed statistical processing of the obtained t_{kp} data, calculated the average value, the common standard deviation, the coefficient of variation, and the relative confidence interval. The results are shown in Table 3.

Table 3. Results of static processing of correctness determination

Statistical characteristic, %	Results
Average value	100.76
Standard deviation	0.3789
Coefficient of variation	0.3761
Lower limit of the confidence interval (P=95%)	100.55
Upper limit of the confidence interval (P=95%)	100.97

Criteria foraccessing correctness: average value 97.5-102.5%, coefficient of variation ≤ 2.0 %, confidence interval should include 100%.

Repeatability. For the study, 6 solutions of the drug at 100% concentration of the active ingredient were prepared. The analysis was carried out on an interday basis for each sample, by two different analysts, using different measuring glassware. The repeatability results are shown in Tables 5 and 6:

Table 4. The results of the determination of repeatability obtained by the chemist-1 (n≥6; P=0,95)

Sample No.	Optical density, A.U.	Specific content of framycetin sulfate, IU/ml	X_i-X_{cp}	$(X_i-X_{cp})^2$
1	0.352	8076.6	7.6483	58.4968
2	0.352	8076.6	7.6483	58.4968
3	0.351	8053.7	-15.2966	233.9872
4	0.352	8076.6	7.6483	58.4968
5	0.351	8053.7	-15.2966	233.9872
6	0.352	8076.6	7.6483	58.4968
Average		8069.0		

Table 5. The results of the determination of repeatability obtained by the chemist-2 (n≥6; P=0,95)

Sample No.	Optical density, A.U.	Specific content of framycetin sulfate, IU/ml	X_i-X_{cp}	$(X_i-X_{cp})^2$
1	0.350	8030.7	-15.2966	233.9872
2	0.351	8053.7	7.6483	58.4968
3	0.351	8053.7	7.6483	58.4968
4	0.350	8030.7	-15.2966	233.9872
5	0.351	8053.7	7.6483	58.4968
6	0.351	8053.7	7.6483	58.4968
Average		8046.0		

We performed statistical processing of the obtained t_{kp} data, calculated a single standard deviation relative confidence interval. The results are shown in Table 6.

Table 6. The results of static processing of the definition of repeatability

Statistical characteristic, %	Results of Chemist-1	Results of Chemist-2
Lowest value, IU/ml	8053.7	8030.7
Highest value, IU/ml	8076.6	8053.7
Average value	8069.0	8046.0
Standard deviation	90.6228	90.6228
Coefficient of variation	1.1231	1.1263
Lower limit of the confidence interval (P=95%)	99.67	100.58
Upper limit of the confidence interval (P=95%)	102.05	100.58
t_{kp} (95% and N1+N2-k=10) 2.228	1.43	
F(95% f1=N1-1;f2=N2-1) 5.05	1.00	

Criteria for accessing repeatability and ruggedness: coefficient of variation ≤1.5% (n≥6), confidence interval F(5%,5,5): ≤ 5,05, t(5%,10): ≤ 2,228.

Conclusions

The method of quantitative determination of framycetin sulfate in the drug 'Framex' has been developed and validated using SF method. Based on the results of the intra-laboratory experiment, it has been found that the metrological characteristics of validation parameters as correctness, specificity, repeatability, linearity and ruggedness do not exceed the validation criteria. The stability of the method can be reproduced in the laboratory. The deviation of a single value is 100.7% with a confidence probability of 95%.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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