DOI 10.1007/s11094-022-02722-y
Pharmaceutical Chemistry Journal, Vol. 56, No. 6, September, 2022 (Russian Original Vol. 56, No. 6, June, 2022)

DEVELOPMENT OF A QUANTITATIVE DETERMINATION METHOD FOR THE ACTIVE SUBSTANCES IN TIMOGEL COMBINED EXTERNAL PREPARATION

A. V. Filatova[1,*]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 56, No. 6, pp. 59 – 64, June, 2022.

Original article submitted January 13, 2022.

A quantitative determination method for metronidazole, chlorhexidine, and methylparaben in commercial Timogel gel for the treatment of wounds and burns is presented. The analyses were carried out using an HPLC method on a Hewlett Packard chromatograph with a spectrophotometric detector. The conditions for the maximum sensitivity for determining the active substances in the medical product were selected. The proposed technique could be used to estimate the quantitative content of metronidazole, chlorhexidine, and methylparaben in combined external drugs. All procedures tested on laboratory batches of gel showed reproducible results and could be recommended for inclusion in regulations on the technology and quality control of the wound-healing gel.

Keywords: standardization, wound-healing gel, metronidazole, wounds, burns, chlorhexidine, HPLC.

Combined drugs for topical application that exhibit synergism of the active ingredients and are based on various dosage forms (aerosols, ointments, pastes, gels, liniments, solutions) are used in most cases to treat wounds and burns [1 – 5].

Gels are known from the literature to be preferred for treating wounds and burns because they have several advantages over other dosage forms [6 – 8].

Qualitative and quantitative analysis of the active ingredients in a drug are the most important parameters of gel compositions according to pharmacopoeial requirements. High-performance liquid chromatography (HPLC) is the most common (effective) method for developing qualitative and quantitative analytical methods for drugs [9 – 11].

The Timogel gel composition for treating wounds and burns was developed at the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan. Preclinical and clinical trials were conducted [12 – 14]. Timogel is a combined drug of macromolecular nature in gel form that is produced by mixing active ingredients and excipients. The active ingredients in Timogel are metronidazole and chlorhexidine with methylparaben as a preservative. Therefore, the drug was standardized for contents of these gel components.

[1] Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, 83 Mirzo Ulugbeka St., Tashkent, 100125 Uzbekistan.
[*] e-mail: albfil@mail.ru

EXPERIMENTAL PART

Five batches of Timogel wound-healing gel were studied. The gel composition included active ingredients Metronidazole (BP, Eur. Ph.; CAS Reg. No. 443-48-1) and Chlorhexidine (BP, Eur. Ph.; Reg. No. 55-56-1). The excipients (gel base of the drug) were gel-former Carbopol Ultrez 21 (Belgium, BP 516-93; Noveon Inc.); methylparaben preservative (CAS Reg. No. 99-76-3); plasticizer glycerin (PM 42-2202-99); and purified water (PM 42-0324-09).

The wound-healing gel Timogel was developed at the Institute of Bioorganic Chemistry, AS RUz, under a licensed contract with the manufacturer OOO Torimed Pharm. The following regulatory documentation for the drug was registered: Registration Certificate No. 05-11 DV/MO 1574/06/17, Pat. No. IAP 04608, IPM 42 Uz-24474240-3110-2017.

Quantitative determination of the active ingredients in the drug used a Hewlett Packard liquid chromatograph with a UV detector. The chromatography conditions were:

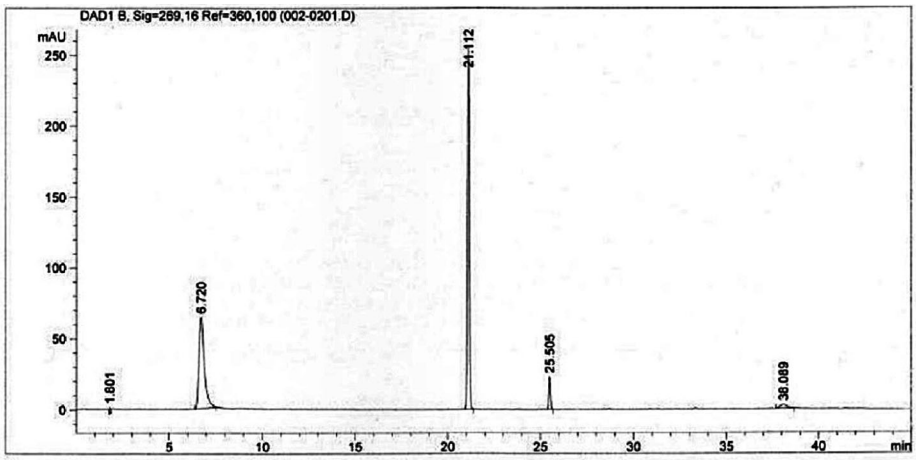

Fig. 1. Chromatogram of Timogel Gel.

ECLIPSE XDB column (4.6 × 150 mm) packed with C18 sorbent, particle size 5 μm or analogous; mobile phase solution A (0.1% trifluoroacetic acid solution), solution B (MeCN). The solutions were degassed using He or another available method. The mobile-phase flow rate was 1 mL/min. The mobile phase flow rate was 1 mL/min. Detection was made at 269 nm. The column was thermostatted at 30°C. The signal integration time was 0.32 sec.

The gradient conditions (MeCN concentration) were:

1) to 5 min, 0% B;

2) from 5 min, linear gradient of B from 0 to 60%;

3) from 35 min, 60% B;

4) from 40 min, reverse linear gradient of B from 60 to 0% in 5 min.

Preparation of test solution

Timogel (~1.00 g, accurate weight) was placed into a 50-mL volumetric flask, treated with aqueous trifluoroacetic acid (TFA) solution (25 mL, 0.1%), and heated gently on a water bath at 40 – 50°C with stirring. The mixture was cooled. The contents of the flask were adjusted to the mark with the same solvent and stirred. An aliquot of 20 μL was taken from the solution for chromatography.

TABLE 1. Measured Metronidazole Concentration in Five Batches of Timogel

Sample	Amount of metronidazole (mg/100 g of gel)
Timogel I	240
Timogel II	245
Timogel III	250
Timogel IV	260
Timogel V	250

Preparation of metronidazole reference standard (RS) working solution

Metronidazole (~2.5 mg) was placed into a 50-mL volumetric flask. The contents of the flask were adjusted to the mark with H_2O and stirred. An aliquot of 20 μL was taken from the solution for chromatography.

Preparation of chlorhexidine RS working solution

Chlorhexidine (~250 mg) was transferred into a 100-mL volumetric flask. The contents of the flask were adjusted to the mark with H_2O and stirred. An aliquot of 1 mL was taken from the solution and again transferred to a 100-mL volumetric flask, adjusted to the mark, and stirred. An aliquot of 20 μL was taken from the solution for chromatography.

Preparation of methylparaben RS working solution

Methylparaben (~1.2 mg) was transferred to a 50-mL flask, treated with EtOH to the mark, and stirred. An aliquot of 20 μL was taken from the solution for chromatography.

Freshly prepared RS solutions were used. The test solution and solutions of metronidazole, chlorhexidine, and methylparaben RS were alternately injected into the chromatograph. The amounts of metronidazole, chlorhexidine, and methylparaben per 100 g of drug (g) were calculated using the formula:

$$x = \frac{S_1 \cdot C_0 \cdot P \cdot 100}{S_0 \cdot C_1 \cdot 100} = \frac{S_1 \cdot C_0 \cdot P}{S_0 \cdot C_1},$$

where S_0 is the peak area of the solutions of metronidazole, chlorhexidine, or methylparaben RS; S_1, peak area of metronidazole, chlorhexidine, or methylparaben of the studied gel sample; C_0, final concentration of metronidazole, chlorhexidine, or methylparaben in the working standard solution (g/mL); C_1, final concentration of test solution (g/mL); P, content of active ingredient in RS (%).

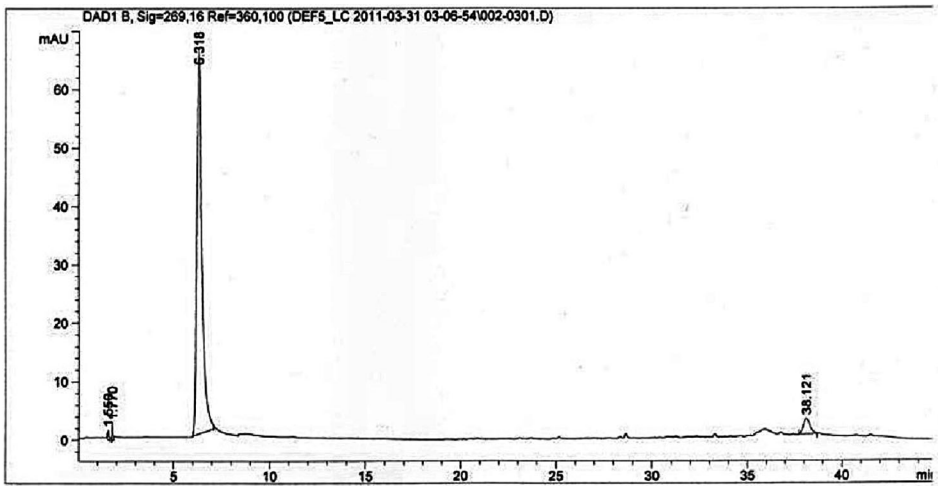

Fig. 2. Chromatogram of metronidazole.

The analytical results were considered reliable if the requirements of the Check of Chromatographic System Suitability were met.

Chromatographic system suitability

Chromatograms were run at least five times with peak maxima recorded for each separation. The chromatography system was considered suitable if the following conditions were met: The chromatography column efficiency calculated from metronidazole, chlorhexidine, and methylparaben RS peaks on chromatograms should be at least 2,000 theoretical plates for each [15]. The relative standard deviation should be ≤2%. The degree of peak separation should be ≥0.3. The peak asymmetry coefficient should be ≤1.5.

TABLE 2. Metrological Parameters for Chromatographic Determination of Metronidazole Concentration in Five Batches of Timogel

Parameter	Designation	X_1	X_2	X_3	X_4	X_5	Formula (n = number of experimental repetitions)
		240	245	250	260	250	
Arithmetic mean	$\bar{x}$	249					$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$
Deviation from arithmetic mean	d_i	9	4	1	11	1	$d_i = x_i - \bar{x}$
	d_i^2	81	16	1	121	1	
	Σd_i^2	220					
Scatter	S2	55					$S^2 = \frac{\Sigma d_i^2}{n-1}$
Mean square deviation	S	7.416					$s = \sqrt{s^2}$
Standard deviation from mean	$s_{\bar{x}}$	3.316					$s_{\bar{x}} = \frac{s}{\sqrt{n}}$
Number of degrees of freedom	f	4					$f = n - 1$
Confidence probability	p	0.01					0.01
Student coefficient	t	4.6041					Student coefficient table value
Confidence interval half-width	$\Delta\bar{x}$	15.27					$\Delta\bar{x} = t(p,, f) \cdot s_{\bar{x}}$
Boundary values of confidence interval of mean	$\bar{x} \pm \Delta\bar{x}$	$233.7 \le m \le 264.2$					$\bar{x} - \Delta\bar{x} \le \mu \le \bar{x} - \Delta\bar{x}$

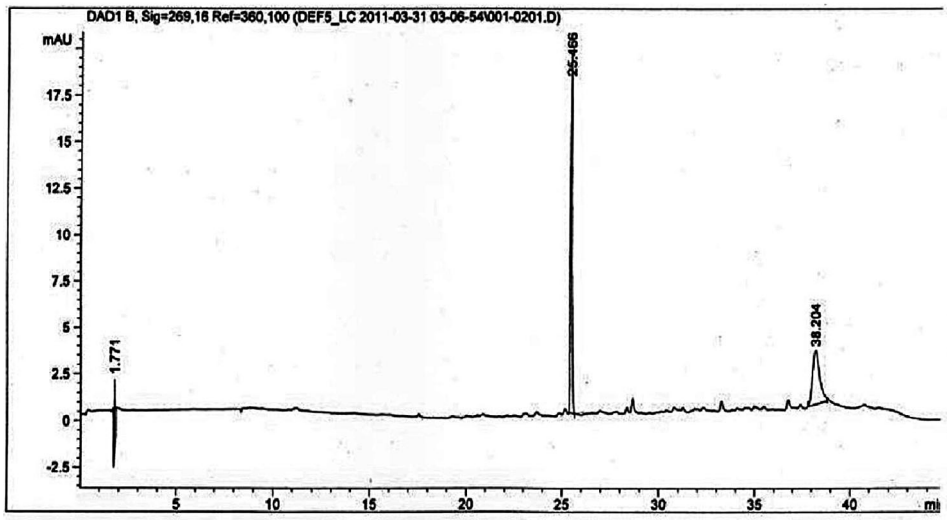

Fig. 3. Chromatogram of chlorhexidine.

Results were statistically processed using the Excel program embedded in Office XP (Microsoft, USA).

RESULTS AND DISCUSSION

A quantitative determination method for metronidazole, chlorhexidine, and methylparaben in a gel that combined identification and quantitative determination of the active ingredients was developed. The quality standards for the obtained combined gel were developed according to requirements of an OST [16].

Identity. The relative retention times of the main peaks in the chromatogram of the test solution prepared for quantitative determination of metronidazole, chlorhexidine, and methylparaben should agree with the relative retention times of the metronidazole, chlorhexidine, and methylparaben peaks in chromatograms of the RS. The identity of the drug was determined by chromatographing a solution of the drug (50 μL) in a Hewlett Packard liquid chromatograph with a UV detector under the conditions given in the Experimental part. Figure 1 shows a chromatogram of Timogel gel.

The test results detected peaks with retention times 6.0 – 7.0 min for metronidazole; 21.0 – 22.0 min for methylparaben; and 25.0 – 25.5 min for chlorhexidine. Peaks of metronidazole, chlorhexidine, and methylparaben were clearly visible in the chromatogram of the gel.

TABLE 3. Chlorhexidine Concentration in Five Batches of Timogel

Sample	Amount of chlorhexidine (mg/100 g of gel)
Timogel I	27
Timogel II	25
Timogel III	26
Timogel IV	23
Timogel V	24

Quantitative determination of metronidazole

Figure 2 shows a chromatogram of metronidazole with retention time 6.0 – 7.0 min. An identical peak with the retention time of metronidazole in the chromatogram of the RS also appeared in the chromatogram of the test solution.

The quantitative content of metronidazole should be 0.23 – 0.27 g/100 g of gel.

Quantitative determination of chlorhexidine

Figure 3 shows a chromatogram of chlorhexidine. An identical peak appeared in the chromatogram of the test solution with the retention time of the chlorhexidine RS (24.5 – 26.0 min).

The quantitative content of chlorhexidine should be 0.02 – 0.03 g/100 g of gel.

Figure 4 shows a chromatogram of methylparaben RS with retention time 20.5 – 21.5 min.

The quantitative content of methylparaben should be 0.099 – 0.15 g/100 g of gel.

A comparative analysis of the obtained chromatograms clearly demonstrated the accuracy of the determination of metronidazole, chlorhexidine, and methylparaben in the gel.

Metrological characteristics of Timogel

TABLEs 1 – 5 present experimental results and metrological parameters for chromatographic determination of the component concentrations in five batches of Timogel.

The quantitative content of metronidazole should be 0.25 ± 0.025 g/100 g of gel. The standard deviation, scatter, and confidence interval were calculated using the Microsoft Excel program for Windows using the following formulas, respectively:

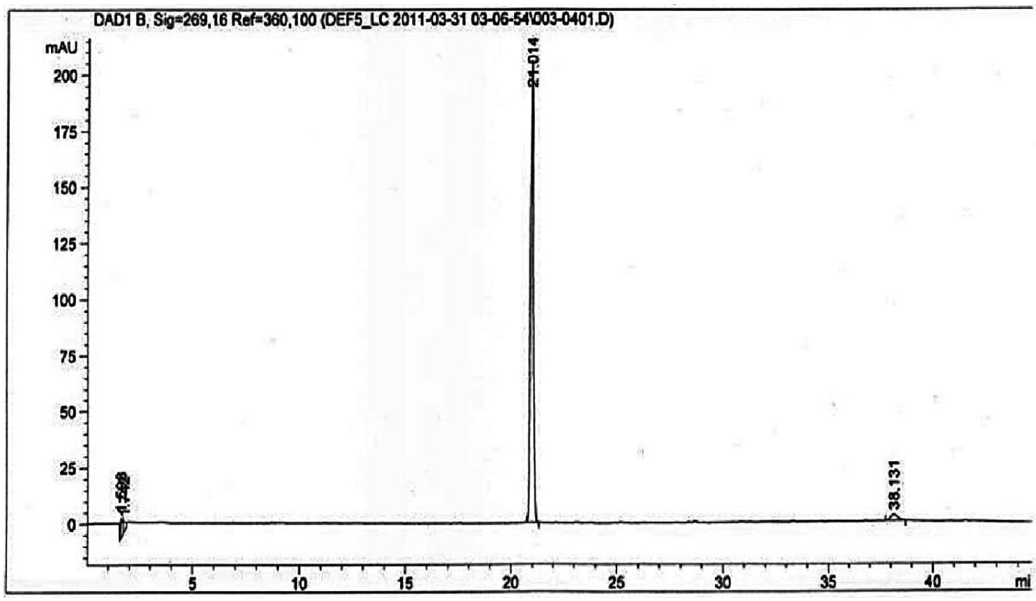

Fig. 4. Chromatogram of methylparaben.

$$\text{Standard deviation} = \sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}}; \quad (1)$$

$$\text{Scatter} = \frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}; \quad (2)$$

$$\text{Confidence interval} = \bar{x} \pm 1{,}96\left(\frac{\sigma}{\sqrt{n}}\right), \quad (3)$$

where n is the number of measurements (n = 5); x, experimental data; σ, standard deviation; and $\bar{x}$, the mean.

TABLE 4. Metrological Parameters for Chromatographic Determination of Chlorhexidine Concentration in Five Batches of Timogel

Parameter	Designation	X_1	X_2	X_3	X_4	X_5	Formula (n = number of experimental repetitions)
		27	25	26	23	24	
Arithmetic mean	$\bar{x}$	25					$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$
Deviation from arithmetic mean	d_i	2	0	1	2	1	$d_i = x_i - \bar{x}$
	d_i^2	4	0	1	4	1	
	Σd_i^2	10					
Scatter	S^2	2.5					$S^2 = \frac{\Sigma d_i^2}{n-1}$
Mean square deviation	S	1.581					$s = \sqrt{s^2}$
Standard deviation of mean	$s_{\bar{x}}$	0.707					$s_{\bar{x}} = \frac{s}{\sqrt{n}}$
Number of degrees of freedom	f	4					$f = n - 1$
Confidence probability	p	0.05					0.05
Student coefficient	t	2.77					Student coefficient table value
Confidence interval half-width	$\Delta\bar{x}$	1.96					$\Delta\bar{x} = t(p,,f) \cdot s_{\bar{x}}$
Boundary values of confidence interval of mean	$\bar{x} \pm \Delta\bar{x}$	$23.04 \le \mu \le 26.8$					$\bar{x} - \Delta\bar{x} \le \mu \le \bar{x} - \Delta\bar{x}$

TABLE 5. Measured Methylparaben Concentration in Five Gel Samples

Sample	Amount of methylparaben (mg/100 g of gel)
Timogel I	100
Timogel II	110
Timogel III	105
Timogel IV	99
Timogel V	113

The results were metronidazole mean concentration, 249 mg; standard deviation, 7.416; scatter, 55; confidence interval, 249 ± 15.27. The quantitative content of metronidazole should be 0.25 ± 0.025 g/100 g.

The quantitative content of chlorhexidine should be 0.02 – 0.27 g/100 g of gel.

The chlorhexidine mean concentration should be 25 mg; standard deviation, 1.581; scatter, 2.5; confidence interval, 25 ± 1.962. The quantitative content of chlorhexidine should be 0.025 ± 0.0025 g/100 g of gel.

The results were methylparaben mean concentration, 110 mg; standard deviation, 7.22; scatter, 52.25; confidence interval, 110 ± 8.97. The quantitative content of methylparaben should be 0.12 ± 0.012 g/100 g of gel.

Thus, the quantitative contents of main ingredients of Timogel dosage form were standardized and normalized by HPLC in the experiments. The proposed quantitative determination method for the main components of Timogel gel was specific and reproducible.

REFERENCES

1. L. A. Blatun, I. A. Chekmareva, V. A. Mitish, et al., *Consilium Med.*, No. 2, 53 – 62 (2019); doi: 10.26442 / 26583739.2019.2.190422.
2. V. N. Obolenskii, *Med. Sovet*, No. 10, 148 – 154 (2016); doi: 10.21518 / 2079-701X-2016-10-148-154.
3. S. M. Khoruk, *Khirurgiya*, No. 12, 47 – 50 (2008).
4. O. A. Baranova, N. I. Kuz'min, T. I. Samsonova, et al., *Khim. Volokna*, No. 1, 74 – 86 (2011).
5. V. V. Provol'nev, *Klin. Mikrobiol. Antimikrob. Khimioter.*, **13**(3), 214 – 222 (2011).
6. Yu. S. Vinnik, N. M. Markelova, and V. S. Tyuryumin, *Sib. Med. Obozr.*, No. 1 (79), 18 – 24 (2013).
7. V. L. Bagirova, *Farmatsiya*, No. 2, 24 – 26 (2002).
8. L. P. Larionov, T. G. Khonina, S. N. Kipper, et al., *Collection of Scientific Works* [in Russian], PSPA, Perm (2007), pp. 356 – 359.
9. A. Kh. Laipanov and V. E. Slanskii, *Farmatsiya*, **40**(1), 28 – 31 (1991).
10. A. Laban-Djurdjevic, M. Jelikic-Stankov, and P. Djurdjevic, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **844**(1), 104 – 111 (2006).
11. Ya. I. Yashin, *Zh. Ross. Khim. O'va im. D. I. Mendeleeva*, **47**(1), 64 – 79 (2003).
12. RUz Pat. I AP 20100248 (2010).
13. A. V. Filatova, A. S. Turaev, and D. T. Dzhurabaev, *Uzb. Khim. Zh.*, No. 1, 46 – 49 (2013).
14. A. V. Filatova, D. T. Dzhurabaev, and A. S. Turaev, *Uzb. Khim. Zh.*, No. 5, 34 – 38 (2014).
15. *State Pharmacopoeia*, SP XIth, No. 1, (1987), p. 105.
16. OST 91500.05.001.00. *Drug Quality Standards*, Approved Jan. 11, 2001, No. 388, Moscow (2001).