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THE FIRST VALIDATION HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF BECLOMETHASONE DIPROPIONATE AND FUSIDIC ACID IN PURE AND PHARMACEUTICAL DOSAGE FORM

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The present work is concerned with the novel, accurate, and precise quantification of beclomethasone dipropionate and fusidic acid in combination (cream), which was performed using high-performance thin-layer chromatography (HPTLC), and validation was performed based on International Council for Harmonization guidelines (Q2 R1). A series of HPTLC tests was conducted on pre-coated silica gel G60 F_{254} plates as the stationary phase and *n*-hexane:ethyl acetate:toluene:diethyl ether (4.5:5.5:1:0.2, v/v/v/v) as the mobile phase. Chamber saturation time was 30 min to attain the desired results. The R_f of beclomethasone dipropionate and fusidic acid was determined to be 0.52 and 0.36 respectively. The densitometric estimation was performed in reflectance mode at 238 nm. A linear relationship was seen in the range of 0.4–2.0 μg/band for beclomethasone dipropionate and 0.8–4.0 μg/band for fusidic acid with R^2 of 0.9914 and 0.9927 for beclomethasone dipropionate and fusidic acid respectively. The limit of detection was found to be 0.2653 and 0.27040 for beclomethasone dipropionate and fusidic acid respectively, and the limit of quantification was found to be 0.42065 and 0.81940 for beclomethasone dipropionate and fusidic acid respectively. The percentage recovery was found to be within the range 98–102% for both beclomethasone dipropionate and fusidic acid. The method proposed was determined to be novel, error-free, robust, and reproducible.

Keywords: fusidic acid; beclomethasone dipropionate; TLC; HPTLC; densitometric; analytical validation.

INTRODUCTION

Beclomethasone dipropionate belongs to the class of corticosteroids. It is chemically (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*S*,17*R*)-9-chloro-11-hydroxy-10,13,16-trimethyl-3-oxo-17-[2(propionyloxy)acetyl]-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-17-ylpropionate (Figure 1a) [1]. It is used as an anti-inflammatory and anti-asthmatic medication, as well as a stimulant. Its hydrophilic 17-hydroxyl group forms the valerate ester with it, increasing its lipophilicity and making it more appropriate for topical application. Corticosteroids are frequently utilized in topical preparations. They are used for asthmatics and people with seasonal allergies, with varying stages that respond to corticosteroids. The active monoester, 17-monopropionate (17-BMP), acts as a mediator of anti-inflammatory actions [2]. The binding affinity of 7-BMP in BD is 25 times that of dexamethasone and 13 times that of cortisol [3].

Fusidic acid is an anti-staphylococcal antibiotic recommended for treating skin infections. Chemically, it is (2*Z*)-2-[(3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)]-16-acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1*H*-cyclopenta[*a*][phenanthren-17-ylidene]-6-methylhept-5-enoic acid (Figure 1b) [4]. Fusidic acid is used to treat bacterial infections and interacts with the elongation factor G (EF-G). In the 50S subunit of the ribosome, elongation factor G hydrolyzes guanosine triphosphate (GTP) and guanosine diphosphate (GDP) to generate energy for the translocation of peptidyl-transfer ribonucleic acid (tRNA) from the A to P site. After GTP hydrolysis, EF-G stays bound to the ribosome, preventing the next stage of protein synthesis. It works by inhibiting peptidyl tRNA from translocating [5]. It is employed to treat bacterial infections such as cellulitis, impetigo, and conjunctivitis in the eyes and on the epidermis (red, itchy eyes). Skin infection caused by susceptible strains of *S. aureus*, *Streptococci* species, and *C. minutissimum* are treated with fusidic acid, which acts as a bacteriostatic antibiotic [6]. FA helps to prevent the growth

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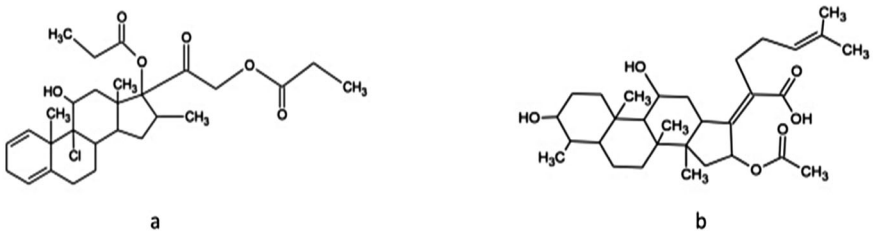

Fig. 1. Structure of beclomethasone dipropionate (a) and fusidic acid (b).

of bacteria while the immune system clears the infection. Its working mechanism is to stop the growth of bacteria after it is applied to the skin.

In this research, beclomethasone dipropionate and fusidic acid were accurately and simultaneously analyzed using high-performance thin-layer chromatography (HPTLC) for the first time. The suggested approach was straightforward, and it can be used in an analysis laboratory. The estimation of beclomethasone dipropionate and fusidic acid alone or in combination with other medications in pharmaceutical formulations by spectroscopic methods has been documented using a variety of techniques: high-performance liquid chromatography [2–16] and HPTLC [17–22]. There is no method reported for the simultaneous estimation of beclomethasone dipropionate and fusidic acid in combined dose form, according to the literature review conducted by the authors. This method was developed and validated for simultaneous estimation of beclomethasone dipropionate and fusidic acid in bulk and mixed dose form using simple, fast, selective, and inexpensive HPTLC technology. It was verified in accordance with International Council for Harmonization (ICH) guidelines.

EXPERIMENTAL

Instruments

CAMAG Linomat V automatic sample applicator (CAMAG), CAMAG 100-μL sample syringe (Hamilton), CAMAG twin trough chamber 10 × 10 cm (CAMAG), UV chamber (CAMAG), micro syringe (Linomat syringe), precoated silica gel 60 F_{254} glass plates (10 × 10 cm with 200 μm thickness HPTLC; Merck), TLC scanner III (CAMAG), and winCATS version 1.4.0 software (CAMAG) were used in this study.

Chemicals and reagents

Gift samples of pure beclomethasone dipropionate and fusidic acid was received from KLM Lab. Pvt. Ltd. (Vadodara, India). Chromatography grade methanol (purity, 99.8%), *n*-hexane (purity, 99.8%), ethyl acetate (purity, 99.8%), toluene (purity ≥ 96%), diethyl ether (purity 99–100%). Fusidic acid and beclomethasone dipropionate are in combination (labeled amount IP 0.20 % w/w FA and IP 0.10 % w/w BD), excipients as cream base (q.s.), manufactured by Cadila Healthcare Ltd (Zydus, Ahmedabad, India) (generics) in formulation (cream) purchase from the local market.

Preparation of standard stock solutions

Standard stock solution of beclomethasone dipropionate and fusidic acid was prepared by dissolving 10 mg of the drug in 10 mL of volumetric flask with methanol, sonicating for 10 min, and the final volume of the solution was made up to 10 mL with methanol to obtain the stock solution containing 1000 μL/mL.

Preparation of standard working solutions

From stock solution take 1 mL in a 10-mL volumetric flak and make up to the volume 100 μL/ml as standard working solution.

Chromatographic conditions

Bands of the standard and sample solutions (5 μL each of 1000 μL/mL) were applied to the plate 1 cm from the bottom of the 8-mm bandwidth using a CAMAG 100-μL syringe connected to a nitrogen tank, an aluminum plate precoated with silica gel G 60F_{254} using a CAMAG Linomat V applicator. The optimized mobile phase consisting of *n*-hexane:ethyl acetate:toluene:diethyl ether (4.5:5.5:1:0.2 v/v/v/v) was employed in a chromatographic run with a saturation time of 30 min at ambient temperature. Twin trough chambers were used to grow in an ascending manner. The plates were dried using a dryer. This was done using the TLC scanner to scan the bands in the absorbance-reflectance mode at optimal wavelength. Aiming to achieve certain objectives, the results were assessed by observing R_f values of drugs within the range 0.2–0.8.

Method validation parameters

According to the ICH guidelines Q2(R1) [23, 24] all of the method validation parameters, including accuracy, linearity, precision, limit of detection, limit of quantification, and robustness, were verified.

Linearity and calibration standards of the pure bulk powder

In methanol, beclomethasone dipropionate and fusidic acid were dissolved in a standard stock solution for use as a working standard. For beclomethasone dipropionate and

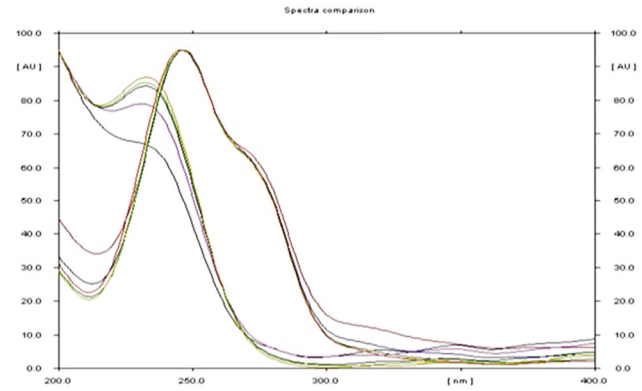

Fig. 2. Spectra of beclomethasone dipropionate and fusidic acid.

fusidic acid, five measurements were made at five different concentration levels, resulting in a linear relationship between peak area and concentration. Regression equations were then developed, and correlation values were calculated.

Specificity

Five microliters of standard and sample solutions (1000 µg/mL) were accurately measured and put on an HPTLC plate in order to test the specificity of the improved procedure. Using a wavelength of 238 nm, a plate was created and scanned at three distinct levels of the band (peak start, peak apex, and peak end). In order to establish the presence of beclomethasone dipropionate and fusidic acid in the sample, R_f and spectra were compared with those of the standard. Spectra at three distinct levels (peak start, peak apex, and peak end locations) of the band at wavelength 238 nm were used to test the purity of beclomethasone dipropionate and fusidic acid.

Accuracy

The accuracy of the results was tested by determining three concentration levels (low, middle, and high quality control) of beclomethasone dipropionate and fusidic acid in triplicate using the proposed procedures. The concentrations were estimated using the respective regression equations, and the mean recovery percentage was calculated.

Precision

Although inter-assay precision represents the variation in findings from multiple tests, intra-assay precision describes the variation of results within a data set produced from a single experiment. To gauge the accuracy of the suggested method, the relative standard deviations were determined.

Repeatability. Under the same experimental settings, three intra-daily concentrations of beclomethasone dipropionate and fusidic acid were analyzed using the suggested approach.

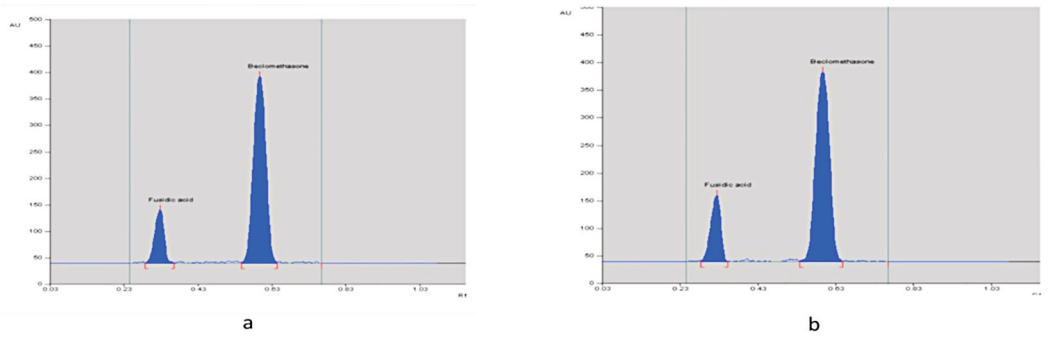

Fig. 3. Representative chromatogram of beclomethasone dipropionate 100 µL/mL and FA 200 µL/mL, with retention time 0.52 and 0.26 R_f (a) and beclomethasone dipropionate 100 µL/mL and fusidic acid 200 µL/mL, with retention time 0.54 and 0.25 R_f (b).

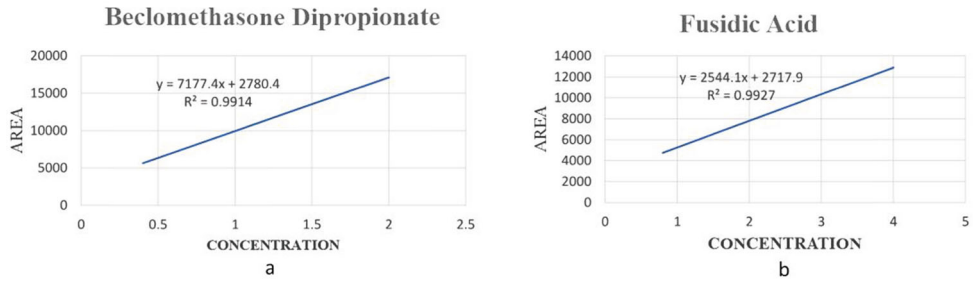

Fig. 4. Calibration curve of beclomethasone dipropionate (a) and fusidic acid (b).

TABLE 1. Peak purity results for standard and cream sample

Drug	R_f of standard	R_f of sample	Peak purity
Beclomethasone dipropionate	0.52	0.54	More than 0.99
Fusidic acid	0.26	0.25	More than 0.99

Intermediate precision. For the analysis of three different concentrations, the prior process was performed 3 days in a row, inter-daily.

Sensitivity

The standard deviation of the response and the slope of the calibration curve were calculated for the lower limit of detection 0.2653 and 0.27040 for beclomethasone dipropionate and fusidic acid and the lower limit of quantification 0.42065 and 0.81940 for beclomethasone dipropionate and fusidic acid.

Robustness

A variety of technique parameters, including saturation time (±5 min) 30 min and 40 min, analysis at different wavelength (±2 nm) as 236 and 340 nm and two different mobile phase conditions, (*n*-hexane:ethyl acetate (EA):toluene (T):diethyl ether (DE) 4.4:5.4:0.9: 0.1 v/v/v/v 4.6:5.3:1.1:0.3 v/v/v/v). On average, just one parameter changed at a time, whereas the others remained consistent. It was carried out by analyzing standard concentrations of both drugs in triplicate. %RSD of the area for each altered condition was evaluated.

Application to the market products

Sample stock solution (cream) was prepared by dissolving 1 g of cream in a 10-mL volumetric flask with methanol, sonicating for 30 min, and filtering using a 0.2-micron filter. Resulting solution concentrations are 100 µL/mL of beclomethasone dipropionate and 200 µL/mL of fusidic acid.

TABLE 2. Results of the linearity of beclomethasone dipropionate and fusidic acid

Parameters	Beclomethasone dipropionate	Fusidic acid
Calibration range (µg/band)	0.4–2.0	0.8–4.0
Regression equation	$y = 7177.4x + 2780.4$	$y = 2544.1x + 2717.9$
Correlation coefficient	0.9914	0.9927
Standard deviation of intercept	117.7678	83.71298
Standard deviation of slope	121.2555	19.26344
Limit of detection (µg/band)	0.13881	0.27040
Limit of quantification (µg/band)	0.42065	0.81940

RESULTS AND DISCUSSION

Detection of wavelength

The densitogram of beclomethasone dipropionate and fusidic acid was determined using a CAMAG TLC scanner IV. It was found that beclomethasone dipropionate and fusidic acid showed highest intensity at 245 nm and 233 nm and the isosbestic point was 238 nm (Figure 2).

Specificity

The chromatogram of the cream sample was obtained using the developed method. It was compared with the chromatogram of standard drugs. Beclomethasone dipropionate eluted with the R_f value of 0.52 and fusidic acid eluted with the R_f values of 0.26 peak of standard and for the sample peak eluted is 0.54 and 0.25 (Figure 3). Peak purity for beclomethasone dipropionate and fusidic acid was assessed by comparing spectra acquired at the start (S), apex

TABLE 3. Accuracy of results

Level		Conc. (µg/band) (standard)	Conc. (µg/band) (sample)	SD	%RSD	% Recovery
LQC		0.5		199.4785	1.55593	99.67
MQC	Beclomethasone dipropionate	1.0	0.5	158.7525	0.953642	98.38
HQC		1.5		110.791	0.576051	99.13
LQC		1.0		53.97419	0.515943	100.56
MQC	Fusidic acid	2.0	1.0	34.55812	0.239237	99.3
HQC		3.0		105.1282	0.635966	99.01

LQC = low quality control, MQC = medium quality control, HQC = high quality control.

TABLE 4. Results of precision

Conc. (µg/band)	Beclomethasone dipropionate				Fusidic acid			
	Intra-day		Inter-day		Intra-day		Inter-day	
	SD	%RSD	SD	%RSD	SD	%RSD	SD	%RSD
1.0	43.44621	0.784251	40.54212	0.584295	43.57205	0.65934	57.14858	0.826432
2.0	136.0261	1.709457	43.66452	0.532682	46.62557	0.585106	52.58175	0.678247
3.0	49.0286	0.450907	40.54212	0.584295	60.84656	0.667409	34.50454	0.393074
0.5	123.8694	1.819958	61.43829	0.908694	116.4504	1.75423	125.6051	1.950501
1.0	158.2931	1.471599	36.87113	0.34668	78.20801	0.71706	68.88065	0.65331
1.5	181.3346	1.31386	64.06692	0.486343	84.51917	0.624878	135.6512	1.030946

(M), and end (E) of the peak. It was found that for both beclomethasone dipropionate and fusidic acid r (S, M) and r (M, E) were more than 0.99, as shown in Table 1.

Linearity and calibration curve

Beclomethasone dipropionate and fusidic acid respectively showed a good correlation over a concentration range of 0.4–2.0 µg/band and 0.8–4.0 µg/band respectively, with respect to peak area (Figure 4). %RSD for the area at each concentration for both drugs was found to be less than 2.0% (Table 2).

Accuracy

In accuracy studies percentage recovery was calculated for both drugs at each low, medium, and high level. Percentage recovery for both drugs was found to be between 98 and 102%, as shown in Table 3.

Precision

The analysis of marketed samples was performed intra-day and inter-day for 2 days and the precise method is shown in Table 4.

Robustness

The optimized method was reliable, according to the %RSD of this test under various altered circumstances (such as mobile phase ratio, wavelength, and saturation time) that were calculated. The findings showed that the method was reliable, as shown in Table 5.

DISCUSSION

Following that, the ionization, sensitivity, and separation effectiveness have all been taken into account when choosing

TABLE 5. Results of robustness for beclomethasone dipropionate and fusidic acid

Beclomethasone dipropionate				Fusidic acid			
Parameter	Mean area	SD	%RSD	Parameter	Mean area	SD	%RSD
Change in saturation time				**Change in saturation time**			
25 min	11,826.83	37.16535	0.314246	**25 min**	9196.5	41.82906	0.454837
30 min	5123.333	86.74044	1.693047	**30 min**	10,817.97	44.73079	0.413486
35 min	13,826.13	63.52719	0.459472	**35 min**	12,808.9	78.24295	0.610848
Change in wavelength				**Change in wavelength**			
236 nm	12,280.4	33.92271	0.276235	**236 nm**	87,55.233	35.20019	0.402047
238 nm	7920.9	28.80903	0.363709	**238 nm**	9241.167	65.57944	0.709645
240 nm	11,482.1	61.47251	0.535377	**240 nm**	8766.6	25.06531	0.285918
Change in mobile phase *n*-hexane:EA:T:DE ratio (v/v/v/v)				Change in mobile phase ***n*-hexane:EA:T:DE ratio (v/v/v/v)**			
4.4:5.4:0.9:0.1	4095.63	39.12	0.95DE 4.4:5.4:0.9:	4.4:5.4:0.9:0.	5082.4	62.01	1.22.9:0.1 v/v/v/v)
4.5:5.5:1:0.2	7045.6	39.63	0.562	4.5:5.5:1:0.2	8539.83	73.7	0.863
4.6:5.6:1.1:0.3	8766.6	25.06	0.285	4.6:5.6:1.1:0.3	11,482.1	61.47	0.52

the mobile phase. For estimation of beclomethasone dipropionate and fusidic acid, a three-solvent system was chosen, i.e., first mobile phase(ethyl acetate:chloroform:diethyl ether 3.2:1.6:0.2 v/v/v) where the R_f value of beclomethasone dipropionate is not acceptable, in the second mobile phase ratio (methanol:ethyl acetate:toluene:chloroform 1:4.5:2:1.6 v/v/v/v) the peak separation is not appropriate, and in the third mobile phase (n-hexane:ethyl acetate:toluene:diethyl ether 4.5: 5.5: 1: 0.2 v/v/v/v) is final because of good resolution and a sharp peak. The second major problem is saturation time, on the same mobile phase (n-hexane:ethyl acetate:toluene:diethyl ether 4.5:5.5:1:0.2 v/v/v/v) with different saturation times of 45, 30, and 25 min. A superior resolution peak was shown at 30 min of saturation time.

Thus, a reliable analytical method for beclomethasone dipropionate and fusidic acid was developed and validated with a concentration range of 0.8–4.0 µg/band and 0.4–2.0 µg/band by using HPTLC-based analysis. Our study may be helpful to analytical laboratories, industry, and researchers. Hence, the developed methods provide good analytical techniques for quantifying and are used for routine analysis of bulk drug and formulation quality checks of beclomethasone dipropionate and fusidic acid.

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Conflicts of interest

There were no apparent conflicts of interest that the author disclosed

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Authors' contributions

In this study project, each author contributed equally and participated in equal numbers. P. Gehlot has worked on HPTLC and has conducted formulation analysis and literature reviews. The manuscript was prepared by A. Tiwari and I. Parmar. The manuscript has been revised by I. Parmar. The final manuscript was reviewed and approved by the authors.

REFERENCES

1. M. Blanco, D. Serrano, and J. L. Bernal, *Talanta*, **50**(3), 527 – 532 (1999); doi: 10.1016 / s0039-9140(99)00141-1
2. N. K. Patel and S. K. Koradia, *Asian J. Pharm. Anal*., **6**(2), 83 – 90 (2016); doi:10.5958 / 2231-5675.2016.00013.2
3. S. A. Gaballa, E. L. Garhy, and H. Moharram, *Pharm. Res.*, **37**, 1 – 23 (2020).
4. M. A. Jakasaniya, J. S Shah, and D. G Maheswari, *Pharmacophore*, **5**(2), 231 – 238 (2014)
5. S. Shaikh, M. S. Muneera, O. A. Thusleem, et al., *J. Chromatogr. Sci.*, **47**(2), 178 – 183 (2009); doi: org / 10.1093 / chromsci / 47.2.178
6. L. Evans, J. N. Hedger, D. Brayford, et al., *Phytochemistry*, **67**(19), 2110 – 2114 (2006); doi.org / 10.1016 / j.phytochem.2006.06.033
7. S. Vladimirov, Z. Fiser, D. Agbaba, et al., *J. Pharm. Biomed. Anal*., **13**(4), 675 – 678 (1995).
8. K. R. Dhudashia, A. V. Patel, and C. N. Patel, *Invent. J.*, **2**, 94 – 97 (2012).
9. V. K. Parmar, H. N. Patel, and B. K. Patel, *J. Chromatogr. Sci.*, **52**(10), 1255 – 1266 (2014); doi.org / 10.1093 / chromsci / bmt208
10. S. Görög, *Anal. Sci.*, **20**, 767 – 782 (2004); doi.org / 10.2116 / analsci.20.767
11. S. Shaikh, M. S. Muneera, O. A. Thusleem, et al., *J. Chromatogr. Sci.*, **47**(2),178 – 183 (2009); doi.org / 10.1093 / chromsci / 47.2.178
12. U. R. Mallu, K. H. Reddy, B. Varaprasad, et al., *Int. J. Pharma Bio Sci.*, **2** (2011).
13. N. Goswami, V. Gupta, and H. A. Jogia, *Scientia Pharmaceutica*, **81**(2), 505 – 518 (2013); doi.org / 10.3797 / scipharm.1301 – 21
14. B. V. Patel, M. B. Shankar, G. V. Chaudhari, et al., *Pharma Science Monitor*, **10**(2), 11 – 28 (2019).
15. S. V. Gandhi, A. D. Ogale, *Eurasian J. Anal. Chem.*, **12**, 395 – 404 (2017).
16. K. R. Dhudashia, A. V. Patel, and C. N. Patel, *Chronicles Young Scientists*, **4** (2013).
17. S. Shewale, V. Undale, A. Deshmukh, et al., *J. Pharm. Negat.*, **13**(1), 1272 – 1285 (2022); DOI: 10.47750 / pnr.2022.13.S01.154
18. N. S. Sinnarkar and M. S. Phoujdar, *World J. Pharm. Res.*, **27**, 186 – 190 (2017).
19. A. Samir, H. M. Lotfy, H. Salem, et al., *Mol. Biomol. Spectrosc*., **128**, 127 – 136 (2014); doi.org / 10.1016 / j.saa.2014.02.044
20. N. Patel and V. K. Parmar, *Pharm. Sci.*, **24**(2), 131 – 140 (2018).
21. T. H. Fereja, A. Hymete, and A. A. Bekhit, *AASCIT J. Chem.*, **2**(2), 32 – 42 (2015).
22. ICH Expert Working Group. Q2 (R1). Federal Register **60**, 11260 (1995).
23. K. Ferenczi-Fodor, B. Renger, and Z. Végh, *J. Planar Chromatogr. - Modern TLC*, **23**(3), 173 – 179 (2010); doi.org / 10.1556 / jpc.23.2010.3.1