DEVELOPMENT AND VALIDATION OF HPTLC TECHNIQUE FOR ASSESSMENT OF DAPAGLIFLOZIN AND METFORMIN HCl

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Abstract

For the detection of two anti-diabetic medications, Dapaglifloxin and Metformin hydrochloride, in formulations, a high-performance thin layer chromatography (HPTLC) approach was created and validated. Toluene, ethyl acetate, and formic acid (3: 6.5: 0.5 v/v/v) were used as the mobile phase in the study. For direct analysis of the chromatograms in the absorbance mode, a scanner tuned at 235 nm was employed. Method was approved in accordance with ICH recommendations. The calibration curves for dapaglifloxin and metformin hydrochloride were found to have correlation values of 0.9953 and 0.9983 in the concentration ranges of 50-300 and 2500-15000 ng band1, respectively. The percentage recovery was determined to be between 99.25% - 101.73% for dapagliflozin and between 99.31% -101.17% for metformin hydrochloride, respectively. The technique had the capacity to concurrently identify these medications from dosage forms without the excipients of the tablet interfering. As a result, it was claimed that the proposed chromatographic method was quick, easy, focused, sensitive, exact, accurate, robust, and reliable and could be successfully used for analysis at research organizations and quality control divisions in enterprises.

Keywords: High-performance thin-layer chromatography, Dapaglifloxin, Metformin hydrochloride, Method Development, Validation.

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Introduction

Metformin is a component of first-line therapy for type II diabetes [1]. High efficacy, safety, favourable cardiovascular and metabolic effects, and therapeutic benefit when combined with other anti-diabetic medications are among the key characteristics [2]. Dapagliflozin is a selective sodium glucose co-transporter subtype 2 (SGLT2) inhibitor with

anti-hyperglycemic activity. With certain limitations, both Type 1 and Type 2 diabetes are treated with dapagliflozin [3,4].

The method of thin layer chromatography has been improved by HPTLC. It is an effective method for separation in quantitative analysis. The procedures of thin layer chromatography can be altered in various ways to automate the various steps, increase the level of determination attained, and enable more precise quantitative estimations [5]. An efficient instrument for chromatographic data of compound mixtures of organic, inorganic, and natural compounds is HPTLC. It offers automated plate development, sample application, detection, and documentation [6,7]. In order to isolate and quantify the blend of Dapagliflozin and Metformin HCl in a single run, HPTLC technique with minimal runtime is being developed and validated in accordance with ICH rules [8,9].

Materials and Methods

Metformin HCl and Dapaglifloxin Active Pharmaceutical ingredients (API's) were attained as free samples from Spectrum Pharma Limited, Hyderabad. The local market provided the pharmaceutical dosage form that was employed in this trial, which was stated to comprise 10 mg of dapaglifloxin and 500 mg of metformin hydrochloride per tablet. All chemicals and solvents are of Analytical grade.

Selection of mobile phase and chromatographic conditions

The working standard solution of dapagliflozin (100 ng/band) and metformin HCl (5000 ng/band) was used for the chromatographic separation experiments. To get the desired system suitability parameters, initial tests were conducted using several solvents in varying amounts on standard TLC plates [10,11]. Following a few tests, the mobile phase of toluene, ethyl acetate, and formic acid (3: 6.5: 0.5 v/v/v) was selected because it provided good resolution and appropriate peak characteristics. In order to produce repeatable Rf values and a symmetrical peak shape for the drug peak, other chromatographic settings, such as chamber saturation time, duration, sample application volume, and detection wavelength, were optimized.

The samples were spotted using a 100 µL sample syringe from Hamilton (Bonaduz, Switzerland) and a Linomat 5 sample applicator on a precoated silica gel aluminium plate 60 F254 (10 10) with a thickness of 250 µm from E. MERCK (Darmstadt, Germany). The slit size was 5 mm X 0.45 mm, and the scanning speed was 20 mm/sec. The linear ascending

development was conducted in a twin trough glass box measuring 10 cm by 10 cm (CAMAG, Muttenz, Switzerland). 15 minutes was the perfect compartment saturation period for the mobile phase with run length of 9 cm. Using a hair dryer, TLC plates were dried in an air current [12]. All advancements were scanned densitometrically using a CAMAG scanner at 235 nm and WINCATS software version 1.4.2. The deuterium lamp used as the radiation source continuously emitted UV light between the wavelengths of 200 - 400 nm.

Preparation of Standard Stock Solution

By combining 10 mg of dapagliflozin with 10 ml of methanol, a standard stock solution with a concentration of 1000 µg/ml was created [13]. Metformin HCl standard stock solution was created by combining 100 mg of the drug with 10 ml of methanol to achieve a concentration of 10,000 µg/ml. Working standard solution was made from the corresponding standard stock solution and contained separately 25 µg/ml (25 ng/µl) of dapagliflozin and 1250 µg/ml (1250 ng/µl) of metformin HCl in methanol.

Preparation of sample solution (Tablet Formulation Analysis)

20 tablets were weighed, powdered each comprising 500 mg of metformin HCl and 10 mg of dapagliflozin and transferred to a 10 ml volumetric flask, diluted to a final volume with methanol [15]. The solution was filtered, and methanol was used to further dilute it until it had a final concentration of 1250 µg/ml of metformin HCl and 25 µg/ml of dapagliflozin. A TLC plate with a 4 µl capacity was applied and developed under ideal circumstances.

Selection of Analytical Wavelength

By appropriately diluting dapagliflozin and metformin hydrochloride with methanol, a standard stock solution was created. To determine the best absorbance for choosing the HPTLC wavelength, all solutions were scanned [16]. These two solutions were run through a UV-visible spectrophotometer between 200 and 400 nm. The right wavelength was chosen from the overlapped spectra of the solutions of dapagliflozin and metformin hydrochloride.

Method Validation

In order to ascertain the validation characteristics, the developed HPTLC method was authenticated in accordance with ICH recommendations Q2 (R1).

Specificity

Peak purity profiling studies confirmed the method's specificity.

Linearity

A solution comprising 25 µg/ml of dapagliflozin and 1250 µg/ml of metformin HCl was made from a standard stock solution (1000 µg/ml) of dapagliflozin and 10000 µg/ml of metformin HCl [17]. To get a linear range, several volumes were put to a TLC plate. There were six replicates used for each concentration. For Dapagliflozin and Metformin HCl, the linearity was assessed over the concentration ranges of 50–300 ng/band and 2500–15000 ng/band, respectively.

Precision

In the intra-day experiments, the % RSD was computed when three replicates of 3 distinct concentrations were studied in a single day [18]. For the interday variation investigations, the percentage RSD was computed after the analysis of 3 dissimilar concentrations over the course of three consecutive days.

Assay

The tablet formulation analysis was completed as defined in the earlier section. The process was carried out six times. For each API, a sample solution was administered, and the area was noted [19]. A linear equation was used to calculate concentration and purity %.

Accuracy

Recovery experiments were completed using 3 dissimilar doses of standard drug—50, 100, and 150% to test the method's accuracy. The basic concentrations of the sample selected were 5000 ng/band of metformin HCl and 100 ng/band of dapagliflozin [20]. To create the densitogram, these solutions were applied in triplicate to TLC plates. Using the linearity equations for dapagliflozin and metformin HCl, the concentrations of both drugs were determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The formulas used to compute the LOD & LOQ of the developed technique from the standard deviation of the y-intercepts and slope of the calibration curves of dapagliflozin and metformin HCl as follows:

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

Where,

σ = standard deviation response for the lowermost concentration

S = slope of the standardization plot.

Robustness

By doing the study under settings where wavelength, chamber saturation time, and time from application to development were changed and the effect on area was observed, the robustness of the method was assessed.

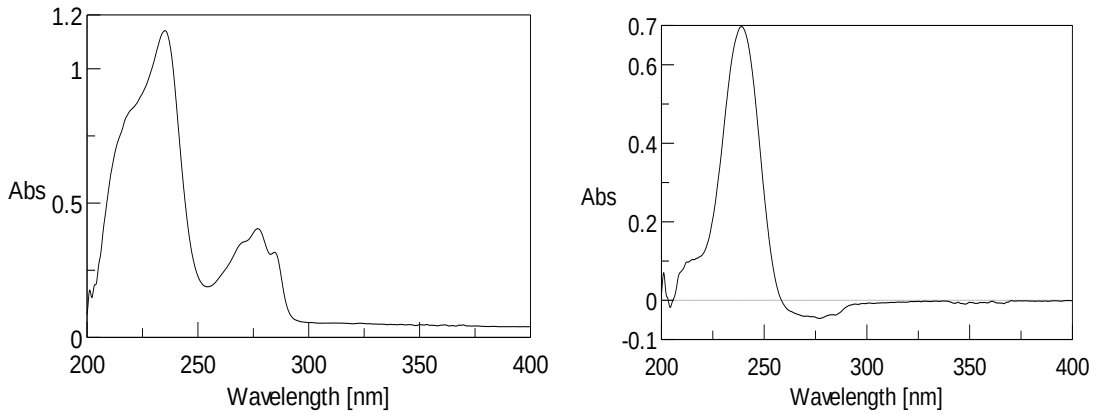
RESULTS AND DISCUSSION

Optimization

The mobile phase made up of toluene, ethyl acetate, and formic acid in the proportions of 3: 6.5: 0.5 v/v/v provided the greatest resolution with distinct peaks for the analytes dapagliflozin and metformin HCl, respectively. The lack of a peak in the densitogram of the empty mobile phase (Fig. 2) further demonstrated the purity of the standard peaks attained with the suggested mobile phase.

Table 1: Chromatographic parameters

Parameter	Settings for Analysis
Stationary phase	TLC aluminium plate precoated with silica gel 60 F_{254}
Mobile phase	Toluene: ethyl acetate: formic acid (3: 6.5: 0.5 v/v/v)
DetectionWavelength	235 nm
Saturation time	15 min
Band width	6 mm
Development time	15 min



A) B)

Fig.1: UV-VIS Spectra of A) Dapagliflozin (10 µg/ml) and B) Metformin HCl (10 µg/ml)

System suitability parameters of drug

Chromatogram of Methanol blank, Dapagliflozin, Metformin HCl and standard Blend are shown in figures 2-5, individually.

Table 2: System suitability parameters

API	RT (min) Mean ± % RSD	Concentration (µg/ml)	Area	Asymmetry
Dapagliflozin	0.45 ± 0.02	100	862.20	1.07
Metformin HCl	0.74 ± 0.02	5000	4040.20	1.12

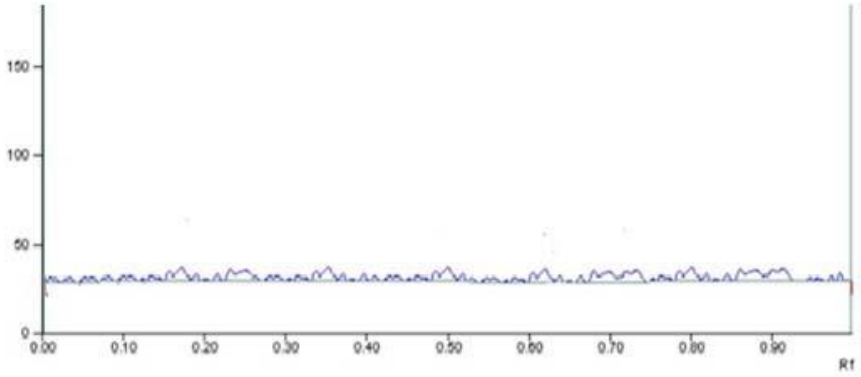


Fig. 2: Densitogram of Mobile Phase blank (Methanol)

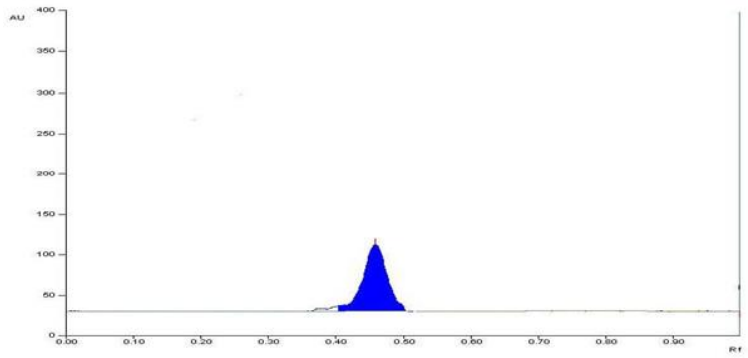


Fig 3: Densitogram of Dapagliflozin (100 ng/band).

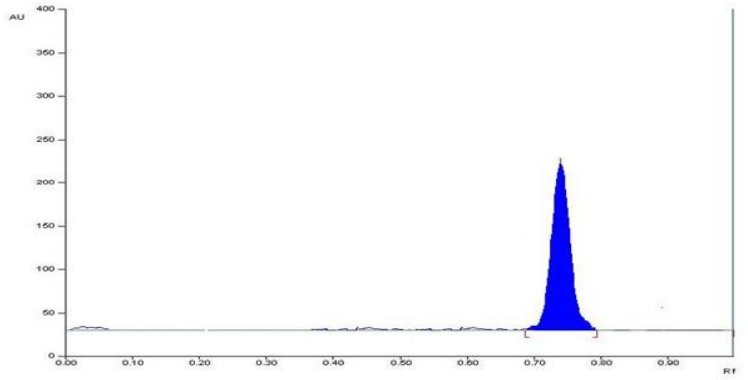


Fig 4: Densitogram of Metformin HCl (5000 ng/band).

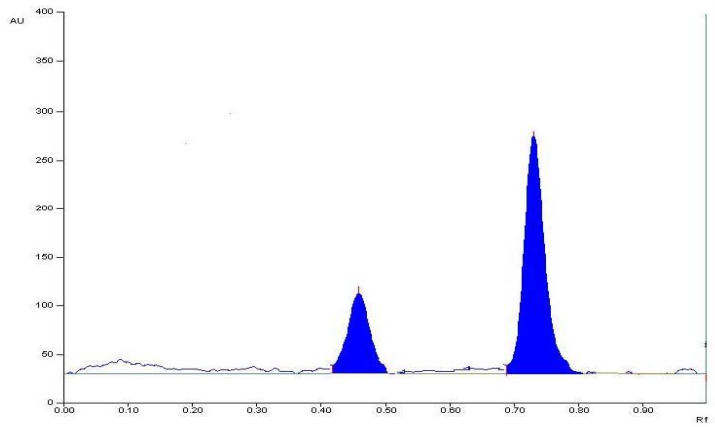


Fig 5: Densitogram of Standard mixture of Dapagliflozin (100 ng/band) and Metformin HCl (5000 ng/band)

Specificity

The peak purity values were determined to be > 0.997, suggesting that no other peak of a degradation product or impurity interfered.

Linearity

Metformin HCl and dapagliflozin's linearity responses were evaluated in the concentration ranges of 2500–15000 ng/band and 50–3000 ng/band, respectively.

Table 3: Linearity study of Dapagliflozin

Replicates	**Concentrations of Dapagliflozin (ng/ band)**					
	50	**100**	**150**	**200**	**250**	**300**
	Peak Area					
1	403.90	862.20	1525.50	2039.60	2683.00	3064.00
2	432.1	849.00	1598.00	2041.00	2643.00	3088.00
3	456.20	824.00	1542.00	2014.00	2622.00	3044.00
4	423.20	857.00	1534.00	2028.00	2641.00	3040.00
5	412.30	842.00	1511.00	2065.00	2662.00	3011.00
6	421.20	822.00	1562.00	2100.00	2642.00	3055.00
Mean	424.82	842.70	1545.42	2047.93	2648.83	3050.33
Std.dev.	18.16	16.75	30.86	30.56	20.99	25.77
%RSD	1.96	1.99	2.00	1.49	0.79	0.84

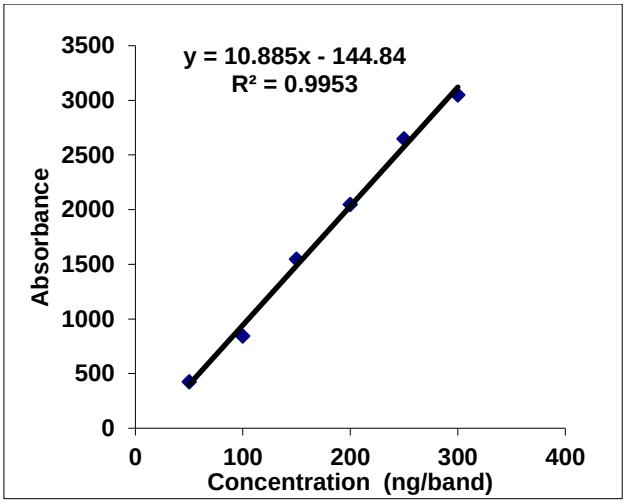


Fig. 6: Calibration curve for Dapagliflozin

The calibration plots' linear equations were y = 10.885x - 144.84 and y = 0.5936x + 1037.5, with the correlation coefficients (r^2) for dapagliflozin and metformin HCl, respectively, being 0.9953 and 0.9983.

Table 4: Linearity study of Metformin HCl

Replicates	**Concentrations (ng/band)**					
	2500	**5000**	**7500**	**10000**	**12500**	**15000**
	Peak Area					
1	2420.60	4040.20	5698.00	7076.00	8618.20	9814.90
2	2410.00	3970.00	5610.00	7015.00	8515.00	9805.00
3	2402.00	3912.00	5682.20	7043.10	8469.00	9807.00
4	2452.00	3862.00	5625.20	6993.10	8495.20	9805.00
5	2282.00	3822.00	5610.00	6894.20	8596.10	9851.00
6	2501.20	3886.00	5814.20	6932.20	8498.30	9812.20
Mean	2461.3	3915.37	5673.27	6992.27	8531.97	9815.85
Std.dev.	69.36	78.75	78.57	68.32	60.48	17.68
%RSD	2.02	2.01	1.38	0.98	0.71	0.18

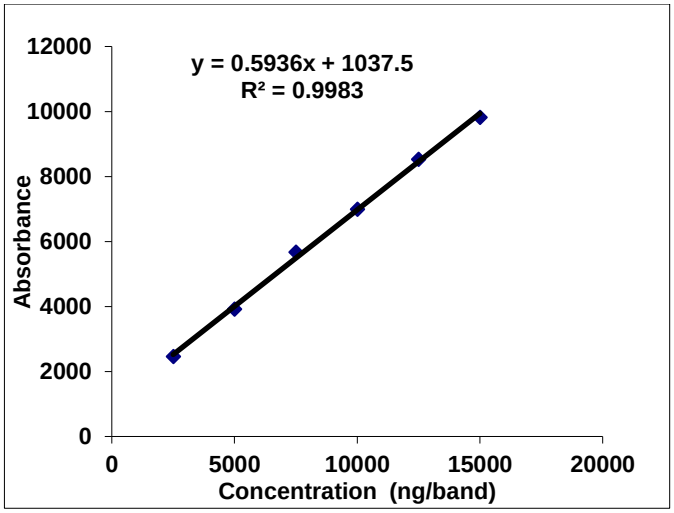


Fig. 7: Calibration curve for Metformin HCl

Precision

The method's accuracy was assessed using intra-day and inter-day fluctuations (%RSD). By testing calibration-range standard drug solutions three times on the same day, intraday precision was determined to be 0.27–0.72 for metformin HCl and 0.53-0.74 for dapagliflozin. By evaluating standard drug solutions within the calibration range on three distinct days over the course of a week, interday precision was evaluated. %RSD was found to be 0.47–0.64 for metformin HCl and 0.66-0.82 for dapagliflozin. This shows that the procedure is sufficiently exact.

Table 5: Intra-day precision study Dapagliflozin

Concentration (ng/band)	**Area**	**% Recovery**	**Avg % Recovery ± % RSD**
50	400	100.108	100.231± 0.741
	397	99.557	
	405	101.027	
100	941	99.756	99.970 ± 0.538
	950	100.582	
	939	99.572	

150	1500	100.740	99.965± 0.715
	1485	99.822	
	1477	99.332	

Table 6: Inter-day precision of Dapagliflozin

Concentration (ng/band)	**Area**	**% Recovery**	**Avg % Recovery ± % RSD**
50	400	100.108	99.925 ± 0.663
	395	99.190	
	402	100.476	
100	950	100.582	99.847 ± 0.695
	941	99.756	
	935	99.204	
150	1500	100.740	99.944 ± 0.829
	1488	100.006	
	1473	99.087	

Table 7: Intra-day precision study Metformin HCl

Concentration (ng/band)	**Area**	**% Recovery**	**Avg % Recovery ± % RSD**
2500	2510	99.158	99.630 ± 0.443
	2523	100.034	
	2518	99.697	
5000	3985	99.242	99.770 ± 0.721
	3992	99.478	
	4025	100.589	
7500	5501	100.191	99.892 ±0.275
	1488	100.006	
	1473	99.087	

Table 8: Inter-day precision of Metformin HCl

Concentration (ng/band)	**Area**	**% Recovery**	**Avg % Recovery ± % RSD**
2500	2514	99.428	100.123 ±0.646
	2526	100.236	
	2533	100.707	
5000	3995	99.579	100.174 ±0.624
	4011	100.118	
	4032	100.825	
7500	5540	101.066	100.535 ± 0.474
	5510	100.393	
	5499	100.146	

Assay

The acquired assay for two API's was determined to be between 99 and 100%, and the % RSD for both drugs was found to be within 2%.

Table 9: Assay of Tablet Formulation

S. no.	**Dapagliflozin**			**Metformin HCl**		
	Peak area	**Amount recovered (ng/band)**	**% Recovery**	**Peak area**	**Amount recovered (ng/band)**	**% Recovery**
1	945	100.123	100.123	3984	4960.438	99.209
2	940	99.664	99.664	4015	5012.626	100.253
3	950	100.582	100.582	4007	4999.158	99.983
4	952	100.766	100.766	4022	5024.411	100.488
5	941	99.756	99.756	4045	5063.131	101.263
6	935	99.204	99.204	3992	4973.906	99.478
Mean	943.83	100.016	100.016	4011	5005.612	100.112
% RSD	0.681	0.591	0.591	0.546	0.737	0.737

Accuracy

As per ICH recommendations, the recovery experiments were performed at 50%, 100%, and 150% of the test concentration. Dapagliflozin and metformin hydrochloride percentage recoveries at each of the three levels were found to be adequate (Table 10). The recovery rates for Dapagliflozin and metformin hydrochloride, respectively, ranged from 99.25% to 101.73% and 99.31% to 101.17%.

Table 10: Recovery readings of Dapagliflozin

Level	Conc. (ng/band)		Area	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.			
50 %	100	50	1489	100.067	100.700 ± 0.839
			1515	101.659	
			1494	100.373	
100 %	100	100	2070	101.738	100.529 ± 1.286
			2047	100.682	
			2014	99.166	
150 %	100	150	2580	100.132	99.617 ± 0.461
			2562	99.470	
			2556	99.250	

Table 11: Recovery values of Metformin HCl

Level	Conc. (ng/band)		Area	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.			
50 %	5000	2500	5490	99.944	99.375 ± 0.545
			5442	98.866	

			5462	99.315	
100 %	5000	5000	7013	100.598	100.227 ± 0.407
			6965	99.790	
			6995	100.295	
150 %	5000	7500	8550	101.178	100.527 ± 0.589
			8491	100.384	
			8464	100.020	

LOD and LOQ

Dapagliflozin's LOD and LOQ were 4.127 and 12.506 ng/band, respectively. LOD and LOQ were determined to be 191.832 and 581.308 ng/band, respectively, for metformin HCl. This shows that the method's sensitivity is sufficient.

Robustness

It was clear from the results in Table 12 that intentional adjustments had no effect on the system suitability parameters of Dapagliflozin and metformin HCl.

Table 12: Robustness

API	**Wavelength**			**Chamber Saturation Time (Min)**			**Time form application to development (min)**		
	234	**235**	**236**	**14**	**15**	**16**	**0**	**30**	**60**
Dapagliflozin	0.452	0.710	0.654	0.872	1.480	1.118	1.554	1.654	1.762
Metformin HCl	0.459	0.733	0.665	0.875	1.508	1.074	0.646	0.950	0.812

CONCLUSION

The new HPTLC method has a number of benefits, including speed, the usage of a straightforward solvent phase and sample preparation procedures. Therefore, this method can be used to analyze pharmaceutical tablet dosage forms and pure drug dose forms. Results of the validation parameters showed that the investigative technique is suitable for its projected use and complies with ICH Q2 (R1) requirements.

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