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Research Article

Application of TLC-Spectrodensitometric and Chemometric Methods for Determination of Momenta® Cream: A Comparative Study Applied on Ternary Mixture

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Abstract

A comparative study for the validation and advancement of two analytical approaches applied for the simultaneous determination of Mometasone Furoate (MF), Miconazole Nitrate (MIC) and Gentamicin (GM) formulated in Momenta® cream. The first approach was TLC-spectrodensitometric method, which was advanced by separating the three components on TLC aluminum plates coated with silica gel 60 F_{254} using chloroform: methanol: formic acid (4:0.3:0.15, v/v/v) as a mobile phase, then scanned at 254nm using Camage TLC scanner 3 operated in reflectance-absorbance mode. The second approach was the chemometric method using two models: Partial Least Squares (PLS) and Principle Component Regression Model (PCR). The proposed approaches were validated according to ICH guidelines and were applied for the determination of the ternary mixtures in their analytical mixtures and pharmaceutical preparation.

Keywords: Mometasone furoate; Miconazole nitrate; Gentamicin; Momenta® cream; TLC-spectrodensitometry; PLS; PCR

Introduction

Mometasone Furoate (MF) is a glucocorticoid or corticosteroid used topically to reduce inflammation of the skin or in the airways; inflammatory skin disorders (such as eczema and psoriasis) (topical form), allergic rhinitis (such as hay fever) (topical form), and asthma (inhalation form). A review of the literature showed that methods reported for the determination of (MF) alone or in combinations were spectrophotometry [1-3], HPLC [4-15], HPTLC [7,16-18], electrochemical method [19], and LC [20]. Miconazole Nitrate (MIC) an imidazole antifungal agent that is used topically and by intravenous infusion, miconazole nitrate is the nitrate salt form of miconazole, an antifungal synthetic derivative of imidazole and used in the treatment of candidal skin infections. A review of the literature showed that methods reported for the determination of (MIC) alone or in combinations were spectrophotometry [21-28], HPLC [29-35], HPTLC [31,36-38], gas chromatography [39,40], and electrochemical methods [41]. Gentamicin (GM) is a mixture of isomeric aminoglycoside antibiotics (gentamicin C_1, gentamicin C_{1A}, and gentamicin C_2) produced by *Micromonospora purpurea* or *M. echinospora*. They are broad-spectrum antibiotics, but may cause ear and kidney damage. They act to inhibit protein synthesis [42]. GM was determined alone or in combinations by variety of methods as electrochemical methods [43-47], spectrophotometry [48-51], HPLC [52-59], and LC [60-67]. There is no reported method for the determination of the three drugs together either in their ternary mixture or in the presence of their degradation products.

Therefore, the objective of this work was to develop a validated and simple TLC-spectrodensitometric and chemometric methods for the determination of MF, MIC and GM in bulk powders, laboratory prepared mixtures and pharmaceutical dosage form. The chemical structures of the cited drugs were displayed in Figure 1.

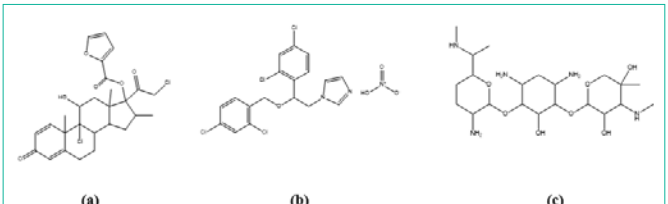

Figure 1: The chemical structures of (a) Mometasone Furoate (MF); (b) Miconazole Nitrate (MIC) and (c) Gentamicin (GM).

Experimental

Apparatus and software

- **The TLC-spectrodensitometric system:** CAMAG TLC scanner 3 S/N 130319 operated with winCATS software, Linomat 5 autosampler (CAMAG, Muttenz, Switzerland), CAMAG microsyringe (100µL). TLC aluminum sheets (20x20 cm) pre-coated with silica gel 60 F_{254} (Merck KgaA, Darmstad, Germany) were used. Calculations were performed using the Excel program.
- Ultraviolet/Visible spectrophotometer (Spectronic Genesys® with WINPEC® application software) with1 cm quartz cell, Spectronic, (USA).
- **The centrifugation system:** Laboratory Centrifuge, Sigma 2-16KL, Sigma 2-16KHL, with order number 10350, 10353.
- Sonicated water bath BranSonic 220, (Zurich, Switzerland).
- All calculations and statistics were carried out on computer using MATLAB® program version 7.9.

Chemicals and reagents

Pure samples: Standard (MF), (MIC) and (GM) were kindly

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donated by SIGMA Pharma Co., Quesna, Egypt. Their purity was found to be 100.08±0.39 [2], 100.17±0.13 [36], and 100.02±0.30 [48] according to reported methods, respectively.

Market sample: Momenta® cream (Jamjoom pharmaceuticals Co., Ltd., Jeddah, Saudi Arabia), labeled to contain 1mg (MF), 20mg (MIC), and 1mg (GENTA) per one gm cream (batch No. TH0101) were purchased from the Egyptian local market.

Solvents: Methanol and chloroform (Analar grade) and formic acid solution was supplied from (Adwic, El Nasr pharmaceutical Chemicals Co., Egypt).

Standard solutions

Stock solutions: Solutions were prepared in methanol of concentrations: 1mgmL^{-1} MF, 2mgmL^{-1} MIC mL^{-1} and in methanol and water of concentration 4mgmL^{-1} GM.

Working solutions: Working solutions were freshly prepared by further dilution of suitable volumes from each stock solutions with methanol to get solutions of final concentration for TLC-spectrodensitometric method, 0.5mgmL^{-1} MF, 1mgmL^{-1} MIC, and 2mgmL^{-1} GM; for chemometric method, 100µgmL^{-1} MF, 200µgmL^{-1} MIC, and 2000µgmL^{-1} GM.

Procedure

For TLC-densitometric method:

Chromatographic conditions: TLC aluminum sheets 20 x 20 cm pre-coated with 0.25mm silica gel 60 F_{254} were used. The samples were applied as bands (bandwidth: 6mm, bands were spaced 1cm apart from each other and 1.5cm from the bottom edge of the plate). The developing system used was chloroform: methanol: formic acid (4:0.3:0.15, v/v/v) as a mobile phase of total volume approximately 10 milliliters. Linear ascending development was done in a chromatographic tank previously saturated with the developing system for 15min. at room temperature (25±2 °C) to a distance of approximately 8cm from the lower edge (approximately 10min). The developed plates were dried in air for approximately 5min. and scanned at 254nm. The detection was done using Camage TLC scanner 3 operated in the reflectance-absorbance mode. The slit dimension was kept at 3mm x 0.45mm and the scanning speed was 20mm/s. All measurements were performed by winCATS software.

Application to pharmaceutical preparation: A four-gram portion of cream was transferred to a 50mL volumetric flask, taking care to avoid sticking cream to the walls of the volumetric flask. A 30mL portion of methanol and 10mL portion of water was added to the flask, and the cream was allowed to melt by warming at 60°C in a water bath with constant shaking. The solution was allowed to cool to room temperature. The volume was made up to the mark with methanol and mixed. The solution was centrifuged at 10000rpm for 10min, and a clear supernatant solution was obtained. A portion of the supernatant was diluted with methanol to obtain a final concentration 40µgmL^{-1} of MF, 800µgmL^{-1} of MIC, and 40µgmL^{-1} of GM.

Linearity and construction of calibration curves: Aliquot volumes (1-12 µg band^{-1}) of MF, (20-45 µg band^{-1}) of MIC and (1-6 µg band^{-1}) of GM were separately transferred from their working solutions into 10mL volumetric flasks and diluted to volume with methanol. Aliquot of 10µL of each solution was applied to the TLC plate using a 100µL syringe. The chromatographic conditions were applied and the chromatograms were recorded. The calibration curves were constructed by plotting the recorded peak area *versus* the corresponding drug concentrations, from which the regression equations were calculated. The calibration curves were made from the average of three experiments.

For chemometric method:

Construction of calibration Set: Multilevel partial factorial design [68] was used for the construction of the calibration and validation sets. A five-level, five-factor calibration design was used. Thirteen mixtures were used for building the calibration model. The laboratory-prepared mixtures of MF, MIC and GM were prepared within their corresponding concentration ranges. The absorption spectra of the prepared mixtures were recorded in the range of 200-400 nm and transferred to Matlab® for subsequent data manipulation.

Application to validation set: Into a series of 10mL volumetric flask, accurate aliquots of each component were transferred from their working solutions to prepare twelve mixtures containing different ratios of the cited drugs. The spectra of the prepared solutions from 200 to 400 nm were recorded and transferred to Matlab®. The concentration of each component was calculated using the constructed model.

Application to pharmaceutical preparation: As described before, then the solution was centrifuged at 10000rpm for 10min, and a clear supernatant solution was obtained. Further dilution was done to obtain a final concentration 40µgmL^{-1} of MF, 800µgmL^{-1} of MIC, and 40µgmL^{-1} of GM.

The concentration of each component was calculated using the constructed PCR and PLS models. When carrying out the standard addition technique, different known concentrations of the pure standard of each drug were added to the pharmaceutical dosage form before proceeding in the previously mentioned procedure.

Results and Discussion

This work was aimed to develop, and validate simple, accurate, selective, and precise analytical approaches which were TLC-spectrodensitometric and chemometric methods, for the simultaneous assessment of the ternary mixture of MF, MIC, and GM in their pure form and pharmaceutical dosage form.

TLC-densitometry

This approach offers a simple manner for quantification directly on TLC plate by calculating the optical density of the separated bands. The amounts of compounds are determined by comparing to a standard curve from reference materials chromatographed simultaneously under the same circumstances.

Optimization of the method: To optimize the approach conditions, it was necessary to test the effect of different variables. In order to separate the three drugs from each other's, several ratios of different developing systems were investigated. Certainly, it was established that the best separation of the cited drugs was achieved by applying the developing system using chloroform: methanol: formic acid (4:0.3:0.15, v/v/v). R_f for MF, MIC, and GENTA were 0.30 ± 0.01, 0.52±0.02, and 0.03±0.01, respectively. Different scanning

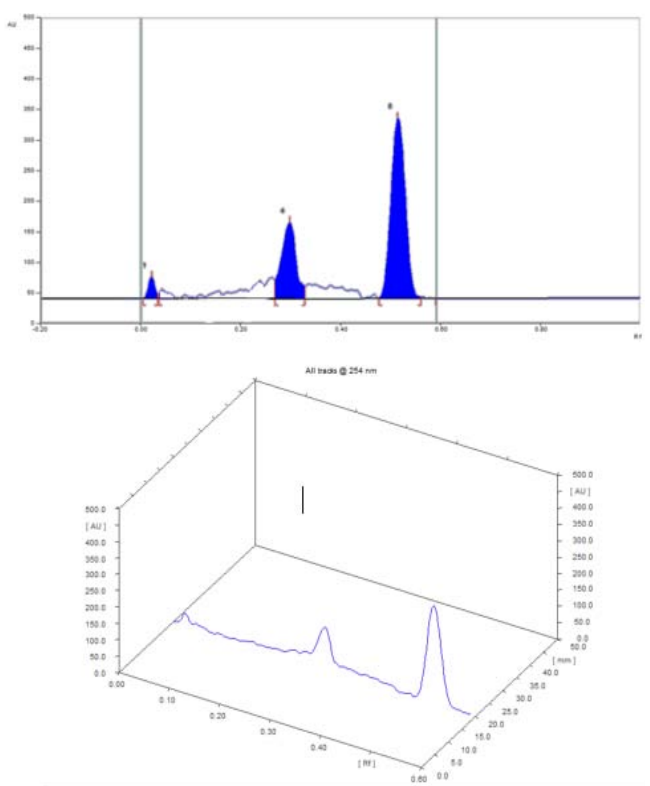

Figure 2: (A) 2D-TLC chromatogram; (B) 3D-TLC chromatogram of (a) 0.4µg band^{-1} of GM, (b) 24µg band^{-1} of MF, and (c) 4µg band^{-1} of MIC, using chloroform: methanol: formic acid (4:0.3:0.15, v/v/v) as the developing system.

wavelengths were tried; on using 245 nm where the separated peaks were sharp and symmetrical with minimum noise, as shown in Figure 2.

Method validation: Method validation was performed according to the International Conference on Harmonization (ICH) guidelines [69] regarding linearity, range, precision, and accuracy, limit of detection and limit of quantitation.

Range and linearity: The linearity of the suggested method was assessed by preparing different calibration curves. Analysis was carried out on a series of standard drug solutions, the calibration curves were constructed between AUC and corresponding concentrations of bands. Linear regression analysis was applied and

Table 1: Assay parameters and method validation sheet obtained by applying the proposed TLC-spectrodensitometric method for determination of MF, MIC, and GM in ternary mixture.

Parameter	MF	MIC	GM
Concentration range (µg band^{-1})	1 - 12	20 - 45	1 - 6
Slope	4.7103	1.9829	5.0829
Standard deviation of the slope (SD_b)	0.0664	0.0201	0.0327
Intercept	157.7	290.39	69.76
Standard deviation of the intercept (SD_a)	0.4764	0.6743	0.1274
Standard deviation of the residuals (SDy/x)	0.5968	0.4198	0.1368
Number of determinations	6	6	6
Accuracy	100.34 ± 1.48	100.00 ± 0.34	100.29 ± 0.97
Correlation coefficient (r)	0.9996	0.9998	0.9999
Determination coefficient (r^2)	0.9992	0.9996	0.9998
Limit of detection, LOD (µg band^{-1})	0.3338	1.1222	0.0827
Limit of quantitation, LOQ (µg band^{-1})	1.0114	3.4006	0.2506

analytical parameters were calculated. The linear ranges were found to be 1-12 µg band^{-1}, 20-45 µg band^{-1}, and 1-6 µg band^{-1} for MF, MIC, and GM, respectively. The method at a good linearity and indicated by the values of correlation coefficient. The linear concentration ranges and other statistical parameters for the proposed method were listed in Table 1.

Limits of detection and quantitation: The Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the proposed method were calculated, for drugs using a ratio of 3.3 and 10 standard deviations of the blank and the slope of the calibration line, Table 1. The limits of detection were calculated as SD×3.3/slope. Whereas, Limits of quantitation were calculated as SD×10/slope.

Accuracy: For the study of the accuracy of the suggested method, repeated analysis (three times) of different concentrations of MF, MIC, and GM within the linearity range were performed. The accuracy asserted as percentage recoveries and Standard Deviations (SD) (Table 1). By applying standard addition technique to the pharmaceutical formulation, the conflict of excipients was studied. The acceptable accuracy demonstrated that the excipients in the pharmaceutical formulation did not interfere in the analysis of these compounds in the pharmaceutical formulation (Table 2).

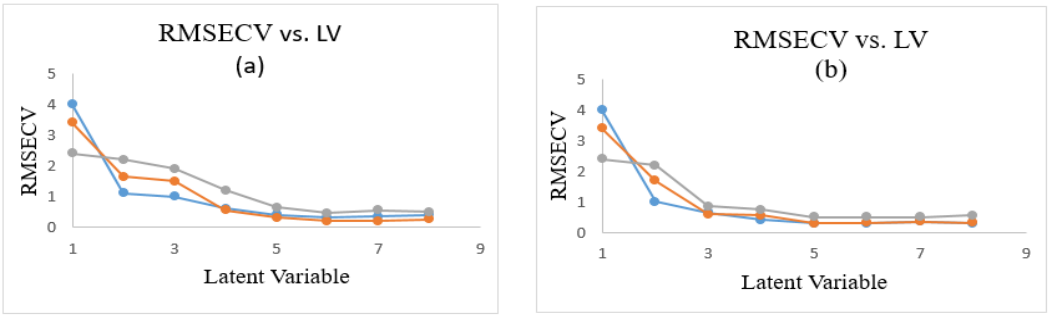

Figure 3: RMSECV of the calibration set of MF, MIC, and GM as a function of latent variables of (a) PCR and (b) PLS models.

Table 2: Application of standard addition technique to the analysis of Momenta® cream by applying the proposed methods.

	TLC–densitometry			PLS		
	Taken in µg band^{-1}	Found in µg band^{-1}	Recovery%*	Taken in µg band^{-1}	Found in µg band^{-1}	Recovery%*
MF	5	5.02	100.35	20	20.03	100.15
MIC	25	24.88	99.5	40	40	100
GM	4	4	100.11	30	30.01	100.03

*Average of three experiments.

Table 3: Application of Intra-day and Inter-day technique to the analysis of MF, MIC, and GM in Momenta® cream by the proposed TLC–spectrodensitometric method.

	Drug	Conc. Level (µg mL^{-1})	*% Recovery ± SD	%RSD
Inter-day	MF	5	99.67 ± 0.59	0.59
		7	99.85 ± 0.60	0.6
		9	100.67 ± 0.18	0.18
	MIC	25	99.74 ± 0.54	0.54
		30	100.58 ± 0.22	0.22
		35	99.55 ± 0.30	0.3
	GM	3	99.32 ± 1.19	1.2
		4	100.13 ± 0.41	0.41
		5	99.87 ± 0.59	0.59
Intra-day	MF	5	99.69 ± 0.55	0.55
		7	100.32 ± 0.31	0.31
		9	99.93 ± 0.39	0.39
	MIC	25	99.55 ± 0.68	0.68
		30	100.18 ± 0.26	0.26
		35	99.71 ± 0.54	0.54
	GM	3	100.45 ± 0.40	0.4
		4	99.53 ± 0.49	0.49
		5	100.08 ± 0.31	0.31

*Average of three experiments.

Table 4: Determination of MF, MIC, and GM in laboratory prepared mixtures by the proposed TLC-spectrodensitometric method.

	Mix ratio	MF	MIC	GM
1	1:10:02	99.75 ± 0.57	100.54 ± 1.20	99.90 ± 0.98
2	1:04:01	99.75 ± 0.69	99.97 ± 0.98	100.09 ± 1.05
3	2:05:01	99.00 ± 1.05	99.95 ± 0.95	99.59 ± 0.79
4	1:20:1*	99.51 ± 0.77	100.59 ± 0.95	99.90 ± 1.05
5	3:05:01	100.54 ± 0.77	99.89 ± 0.89	99.00 ± 0.99

*Ratio in Momenta® cream.

Precision: The inter-day and intra-day precision of the proposed method were determined by the analysis of three distinct concentrations of each component, within the linearity range, by three replicate analyses of three pure samples of both drugs on a single day and three ensuing days, for the inter-day and intra-day precisions, respectively. The results expressed as mean percentage recoveries and RSD (Table 3).

Selectivity: Selectivity was confirmed by analyzing different mixtures containing drugs in different ratios within the linearity range. Satisfactory results were shown in Table 4. The conflict of excipients in the pharmaceutical formulations were studied by applying standard addition method to the pharmaceutical formulation Table 2; which did not interfere in the analysis of these compounds in the pharmaceutical formulation.

System suitability: System suitability was checked by calculating different parameters (Table 5). The obtained values were in the acceptable ranges when compared to the reference values [70].

Table 5: System suitability parameters of the proposed TLC-spectrodensitometric method of MF, MIC and GM in Momenta® cream.

Parameter	MF	MIC	GM	Reference value
R_f value	0.30 ± 0.01	0.52 ± 0.02	0.03 ± 0.01	
T (tailing factor)	0.91	0.9	0.87	T≤ 1.15 - 0.95 & T = 1 for symmetric peak
R_s (experimental resolution)	2.66	3.42	1.72	R_s >1.5

Table 6: Concentration of MF, MIC, and GM in the calibration set using PCR and PLS models.

Experimental No.	Concentration (µg mL^{-1})		
	MF	MIC	GM
1	6	12	6
2	10	10	4
3	4	14	10
4	6	14	4
5	4	12	4
6	8	11	10
7	10	13	8
8	6	13	10
9	10	12	10
10	2	14	8
11	8	10	2
12	6	10	8
13	8	12	8
14	4	13	2
15	4	10	6

Table 7: Summary of results obtained by applying the diagnostic tools for model validation of the PCR and PLS models with MF, MIC, and GM.

Validation parameters	PCR			PLS		
	MF	MIC	GM	MF	MIC	GM
Slope	0.9997	1.0541	0.9701	0.9351	0.9054	1.0451
Intercept	0.2534	0.0981	0.1535	0.1234	0.2157	0.7311
Correlation coefficient (r)	0.9999	0.9996	0.9997	0.9995	0.9999	0.9999
RMSEP	0.259	0.095	0.141	0.053	0.155	0.094

Table 8: Statistical comparison between the results obtained by the proposed TLC-spectrodensitometric method and the reported methods for the determination of MF, MIC and GM in pure powder form.

Parameter	MF			MIC			GM		
	Reported Method	TLC	PLS	Reported Method	TLC	PLS	Reported Method	TLC	PLS
Mean	100.1	100.34	-	100.17	100	-	100.02	100.29	-
Standard Deviation (SD)	1.039	1.48	-	0.13	0.34	-	0.3	0.97	-
N	7	0	-	3	6	-	3	6	-
Variance	1.08	2.19	-	0.0169	0.1156	-	0.09	0.94	-
Student *t*		0.33 (2.228)	-		1.08 (2.306)	-		0.62 (2.306)	-
F		2.03 (4.39)	-		6.84 (19.3)	-		10.44 (19.3)	-

Chemometric method

Among the different regression methods existing for multivariate calibration, the factor analysis based on Principal Component Regression Model (PCR) and Partial Least Squares (PLS) regression have received considerable attention in the chemometrics literature [71]. PCR predates PLS. In cases where only partial knowledge of components is present, PCR and PLS can work well. PCR assumes that error is only in the instrumental response and concentration matrix is error-free, while PLS assumes that error is equally distributed between concentration matrix and instrumental response (spectral) matrix. Thus, PLS produces more robust model as it removes noise from both absorbance and concentration data [72].

The calibration set was constructed using the absorption spectra set of 15 mixtures, as listed in Table 6. The initial models were found to give bad results, so the regions below 205 and above 300nm were rejected. Cross-validation methods leaving out one sample at a time was employed. The Root Mean Squares Error of Cross-Validation (RMSECV) was calculated which is used as a diagnostic test for examining the errors in the predicted concentrations. It indicated both precision and accuracy of predictions. The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with additional factor. Four factors were found to be optimum for the mixture, as shown in Figure 3 for PCR and PLS.

Model validation: To assess the prediction ability of the suggested models, an external validation set of 12 mixtures was used as listed in Table 6. The predicted concentrations were compared with the true concentrations of each component in each sample. The Root Mean Squared Errors of Prediction (RMSEP) and the regression equations for the predicted versus actual concentration are listed in Table 7 as diagnostic tools for model validation. The results indicated the higher predictive ability of the PLS model than that of PCR model to analyze the laboratory-prepared mixtures (validation set) within the accepted range, as shown in Table 7, where PCR was unable to interpret this complex model, as it might require a larger number of samples for accurate calibration. The proposed model was also applied for the determination of Momenta® cream, and the validity of the proposed procedures was further assessed by applying the standard addition technique showing no excipients interference. The results obtained are shown in Table 2.

Statistical analysis

Table 8 showed statistical comparison of the results obtained by the proposed methods and reported methods for MF [7], MIC [36], and GM [48] for TLC-spectrodensitometric method, and official method {-} for chemometric method. The calculated *t* and *F* values were less than the theoretical ones indicating that there was no significant difference between the proposed and the official methods with respect to accuracy and precision.

Conclusion

This work presented a comparative study on two analytical techniques based on UV spectrophotometry which were TLC-densitometric method and chemometric-assisted spectrophotometric method (PLS) and (PCR). Both techniques were successfully applied for the simultaneous estimation of the ternary mixture of MF, MIC, and GM in their pure form and topical pharmaceutical formulation. The TLC-densitometric method has the advantage over HPLC methods as it minimizes the usage of reagents which supports the eco-friendly behavior of green chemistry, it minimizes the time required for analysis, and it utilizes the merit of applying several sample bands on TLC plate, which may be more advantageous for regulatory quality control laboratories. In addition, the method is inexpensive and does not require certain types of stationary phases, but still, the method fulfills the same validation parameters and efficiency when compared to reported HPLC method. Meanwhile, the chemometric method has the advantage of being simpler as it does not require special reagents or chemicals, and it is considered to be time- and cost-saving, but it requires a special software (Matlab). It was found that PLS preceded PCR in the analysis of such complex mixtures. As a final conclusion, the results obtained by the two proposed methods were reliable, accurate, and precise. Hence, both methods can be employed for routine quality control analysis as alternative methods to different HPLC techniques in quality control laboratories lacking the required facilities for those expensive techniques.

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