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Research Article

DEVELOPMENT OF RPHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN, METFORMIN AND LINAGLIPTIN

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ABSTRACT

A novel RP-HPLC method was developed and validated for the simultaneous estimation of Metformin, Linagliptin, and Empagliflozin in pharmaceutical dosage forms. This method is designed to handle a high throughput of samples efficiently, offering robustness, accuracy, and precision without requiring prior separation steps. The choice of methanol and potassium dihydrogen orthophosphate as the mobile phase allowed for effective solubility and separation of the analytes. The method's run time was optimized to 5 minutes, facilitating rapid analysis. Validation parameters, including system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, LOD, and LOQ, were thoroughly assessed. System suitability parameters were within acceptable limits, confirming the system's reliability. Linearity was observed within the 10-100 µg/mL concentration range, with percentage recoveries for Metformin, Linagliptin, and Empagliflozin ranging from 98.22% to 99.25%. No interference from excipients or the mobile phase was detected, ensuring specificity. The method demonstrated robustness and ruggedness through consistent results despite variations in flow rate, mobile phase composition, and different analysts conducting the analysis. This validated RP-HPLC method is rapid, accurate, and efficient, making it highly suitable for routine analysis and quality control in pharmaceutical laboratories.

Keywords: RP-HPLC, Metformin, Linagliptin, Empagliflozin, Simultaneous Estimation, Validation.

INTRODUCTION

Quality control in manufacturing industries, monitoring clinical and environmental samples, assaying geological specimens, and supporting both fundamental and applied research are the principal applications of chromatography [1]. Chromatography, first described by Russian scientist Mikhail Tswett in 1906 [2, 3] , is a method where components of a mixture are separated on an adsorbent column in a flowing system. Over the years, the stationary phase has evolved into various forms, including paper, thin layers of solids on glass plates, immobilized liquids, gels, and solid particles packed in columns [4]. Depending on whether the mobile phase is a liquid or a gas, chromatography can be subdivided into Liquid Chromatography (LC) and Gas Chromatography (GC). High Performance Liquid Chromatography (HPLC) is now one of the most powerful tools in analytical chemistry [5]. It has the ability to separate, identify, and quantify compounds present in any sample that can be dissolved in a liquid, even at trace concentrations as low as parts per trillion (ppt). HPLC has applications across various fields, including

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pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals. Normal phase HPLC (NP-HPLC) was the first kind of HPLC chemistry used, which separates analytes based on polarity. The retention time of the analyte decreases with more polar solvents in the mobile phase, whereas more hydrophobic solvents increase retention times [6]. Polar solvents can deactivate the column by occupying the stationary phase surface. Reversed phase HPLC (RP-HPLC) uses a non-polar stationary phase and an aqueous, moderately polar mobile phase. The retention time increases with the addition of polar solvents and decreases with more hydrophobic solvents [7]. The pharmaceutical industry frequently employs RP-HPLC for drug qualification before release. Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM) [8]. It decreases blood glucose levels by reducing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. Metformin is not associated with weight gain and may induce weight loss, making it the drug of choice for obese NIDDM patients. However, it can cause side effects such as dyspepsia, nausea, and diarrhea. Linagliptin is a highly selective DPP-4 inhibitor [9]. It works in patients with type 2 diabetes by slowing the inactivation of incretin hormones, thereby increasing their concentration and prolonging their action. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released by the intestine throughout the day and increase in response to a meal. These hormones are rapidly inactivated by the enzyme DPP-4 [10]. Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), found almost exclusively in the proximal tubules of the nephronic components in the kidneys. SGLT-2 accounts for about 90 percent of glucose reabsorption into the blood. By blocking SGLT-2, Empagliflozin reduces blood glucose by blocking glucose reabsorption in the kidneys, thereby excreting glucose via the urine. The FDA has approved Trijardy XR, a fixed-dose combination of empagliflozin, linagliptin, and metformin, for the oral treatment of type 2 diabetes in adults [11]. A review of the literature reveals fewer analytical methods reported for the simultaneous estimation of Metformin, Linagliptin, and Empagliflozin by RP-HPLC. Existing methods such as spectrophotometry, HPLC, and HPTLC are reported for these compounds individually or in combination with other dosage forms. Hence, there is a need for a new stability-indicating analytical method for the simultaneous estimation of Metformin, Linagliptin, and Empagliflozin in pharmaceutical dosage forms. This study aims to develop a new, simple, fast, accurate, efficient, and reproducible RP-HPLC method for the simultaneous analysis of Metformin, Linagliptin, and Empagliflozin. The developed method will be validated according to ICH guidelines.

METHODOLOGY

Wavelength selection

A solution of 10 µg/ml of Metformin, Linagliptin and Empagliflozin were prepared in milliQ water. The resulting solutions were scanned individually on HPLC PDA detector from 190 to 400 nm and also in UV-Visible spectrophotometer. The optimal response for three of them was obtained at 245 nm. Hence the complete method was processed at the wavelength of 240 nm.

Preparation of standard solution:

10 mg of Metformin,5 mg of Linagliptin and 25 mg of Empagliflozin were accurately weighed and transferred into a 100 ml clean dry volumetric flask, about 70 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 100 µg/ml,50µg/ml,250 µg/ml.(Stock solution) Further 1 ml was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent to give a concentration of 10µg/ml, 5µg/ml and 25 µg/ml respectively.

Preparation of sample solution

10 tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of Metformin,5 mg of Linagliptin and 25 mg of Empagliflozin was transferred into a 100 ml clean dry volumetric flask, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 100 µg/ml,50 µg/ml,250 µg/ml ,allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as par with standard solution. The solution was filtered through 0.45 mcm filter before injecting into HPLC system.

Preparation of Placebo

The amount of powdered inactive ingredient supposed to be present in 10 tablets were accurately weighed and transferred in to 10 ml volumetric flask, 7 ml of diluent was added and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 0.1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was

filtered through 0.45 mcm filter before injecting into HPLC system.

Preparation of mobile phase :(for Optimized Conditions)

Take 2.5 gm of potassium dihydrogen ortho phosphate into 1000ml volumetric flask dissolved in hplc grated water and adjust ph upto 3 with ortho phosphoric acid. From the above prepared buffer take 350 ml (35%) and 650ml of Methanol HPLC (65%) were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Validation

Various parameters such as System suitability, Linearity, Precision, Accuracy, Specificity, Robustness, Ruggedness, Limit of detection, Limit of quantification, Force degradation were done as per ICH guidelines [12].

RESULTS

Selection of wavelength

A solution of 10 µg/ml of Metformin, Linagliptin and Empagliflozin were prepared in milliQ water. The resulting solutions were scanned individually on HPLC PDA detector from 190 to 400 nm and also in UV-Visible spectrophotometer. The optimal response for three of them was obtained at 245 nm. Hence the complete method was processed at the wavelength of 245 nm.

Validation

SYSTEM SUITABILITY

Tailing factor Obtained from the standard injection is 1. Theoretical Plates Obtained from the standard injection is 2496

Tailing factor Obtained from the standard injection is 1.51. Theoretical Plates Obtained from the standard injection is 2281 Tailing factor Obtained from the standard injection is 1.25. Theoretical Plates Obtained from the standard injection is 2594.

Linearity

Accuracy

The % Recovery for each level should be between 98.0 to 102.0%.

Table 1: Chromatogram values for System suitability of Metformin

Injection	Rt	Peak Area	USP Plate count	USP Tailing
1	**1.631**	**1250763**	**2489**	**1.52**
2	**1.632**	**1247865**	**2484**	**1.52**
3	**1.633**	**1255849**	**2495**	**1.63**
Mean		**1251360**		
SD		**3750.674**		
%RSD		**0.20728**		

Table 2: Chromatogram values for System suitability of Linagliptin

Injection	Rt	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.561	740627	2381	1.51	3.04
2	2.562	731161	2245	1.45	3.09
3	2.563	740306	2262	1.45	3.05
Mean		837362.7			
SD		5374.93			
%RSD		0.473408			

Table 3: Chromatogram values for System suitability of Empagliflozin

Injection	Rt	Peak Area	USP Plate count	USPTailing	USP Resolution
1	3.211	832577	2507	1.24	12.96
2	3.212	834463	2595	1.24	13.25
3	3.213	820415	2566	1.23	13.17
Mean		829167.6			
SD		6598.938			
%RSD		0.617823			

Table 4: Calibration parameters for Metformin, Linagliptin and Empagliflozin

Parameter	Metformin	Linagliptin	Empagliflozin
Slope	19813	14314	78352
Intercept	65496	49165	48075

Correlation coefficient	0 .9993	0.99917	0.99903

Table 5: Chromatogram Values For Accuracy of Metformin

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	**5**	4.9	98%	**100%**
		5	**5.1**	**102%**	
		5	**5**	**100%**	
2	100 %	**10**	9.88	**98.8%**	**99.12%**
		10	**9.91**	**99.1%**	
		10	**9.95**	**99.5%**	
3	150 %	**15**	14.89	**99.2%**	**99.68%**
		15	14.86	**99%**	
		15	14.82	**99.79%**	

Table 6: Chromatogram Values For Accuracy of Linagliptin

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	**10**	9.8	98%	**100%**
		10	**10.2**	**102%**	
		10	**10**	**100%**	
2	100 %	**20**	19.8	**99%**	**100%**
		20	**20.2**	**101%**	
		20	**20**	**100%**	
3	150 %	**30**	29.6	**98.6%**	**99.35%**
		30	**30**	**100%**	
		30	**29.8**	**99.33%**	

Table 8: Sample Chromatogram values for Repeatability of Metformin

Injection No	Peak Area	R_t
1	1248257	1.631
2	1247578	1.632
3	1245272	1.633
4	1245264	1.634
5	1248573	1.635
Avg	1246487	
SD	2865.61	
% RSD	0.23783	

Table 9: Sample Chromatogram values for Repeatability of Linagliptin

Injection No	Peak Area	R_t
1	935136	2.561
2	929455	2.562
3	930458	2.563
4	934387	2.564
5	924058	2.565
Avg	927858.7	
SD	5875.15	
% RSD	0.5231	

Table 10: Sample Chromatogram values for Repeatability of Empagliflozin

Injection No	Peak Area	R_t
1	954857	3.212
2	937616	3.213
3	950692	3.214
4	940253	3.215
5	927055	3.216
Avg	935424.3	
SD	6301.561	
% RSD	0.562	

Figure 1: Overlay spectrum for Metformin, Linagliptin and Empagliflozin.

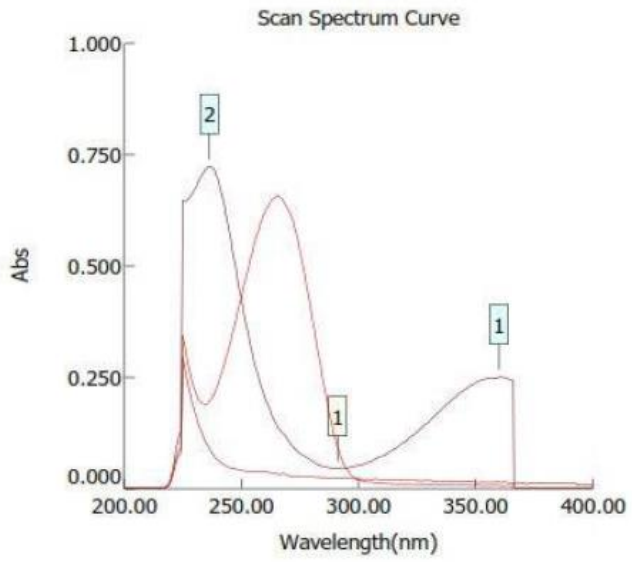


Figure 2: Calibration curve of Metformin

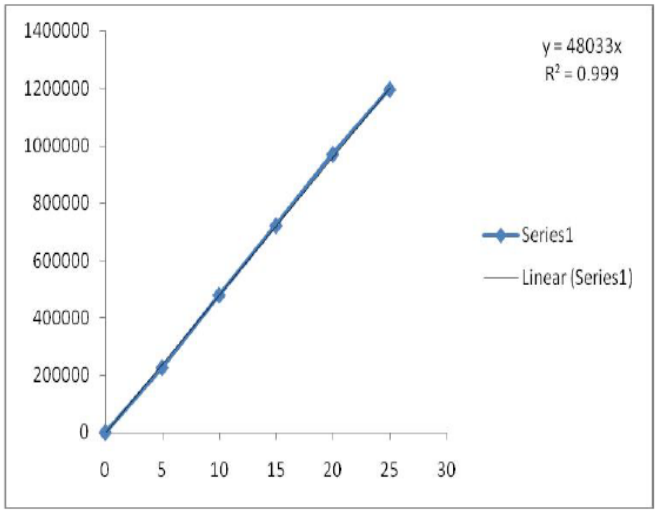


Figure 3: Calibration curve of Linagliptin

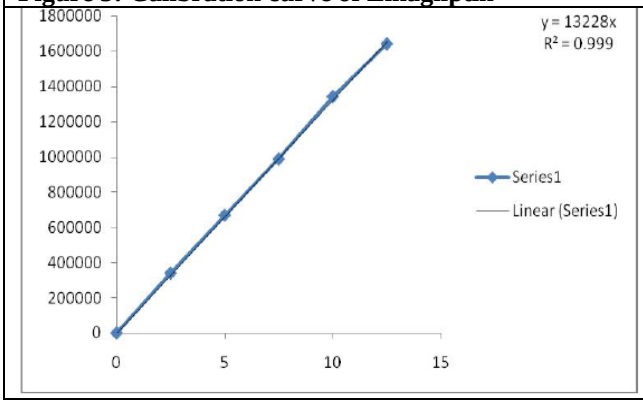


Figure 4: Calibration curve of Empagliflozin

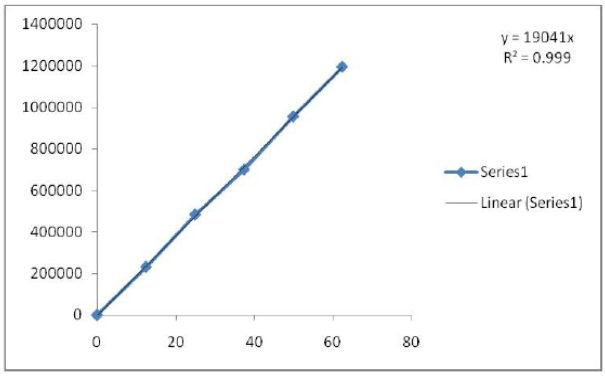


Figure 5: Standard Chromatogram for Identification of Metformin, Linagliptin and Empagliflozin

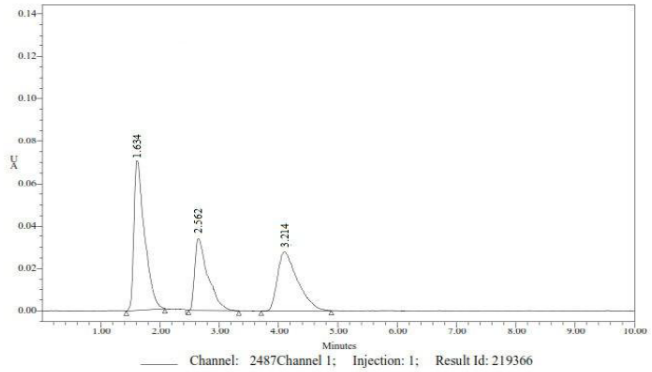


Figure 6: Sample Chromatogram for Identification of Metformin, Linagliptin and Empagliflozin

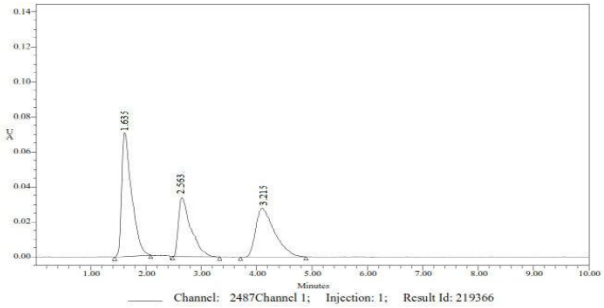


Figure 7: Chromatogram for flow rate of 0.8 ml/min

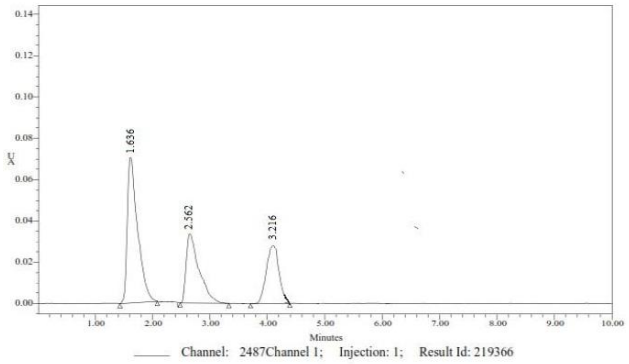


Figure 8: Chromatogram for flow rate of 1.0 ml/min

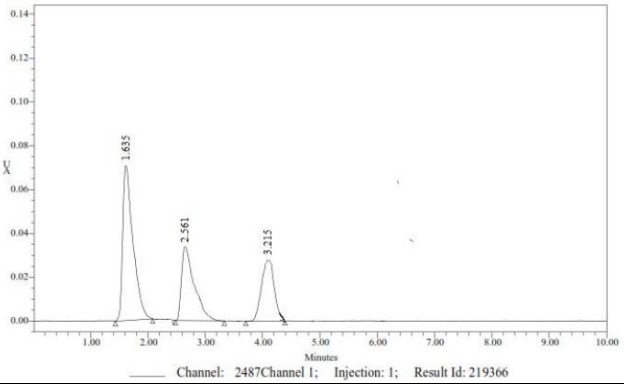


Figure 9: Chromatogram for flow rate of 1.2 ml/min

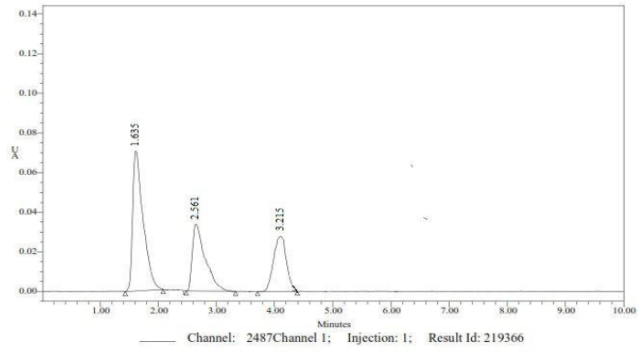


Figure 10: Chromatogram for Metformin, Linagliptin and Empagliflozin (LOD)

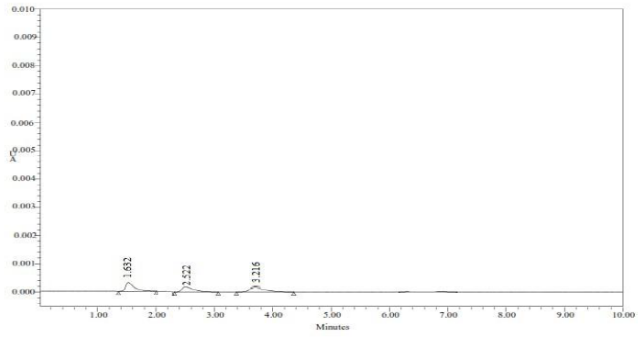
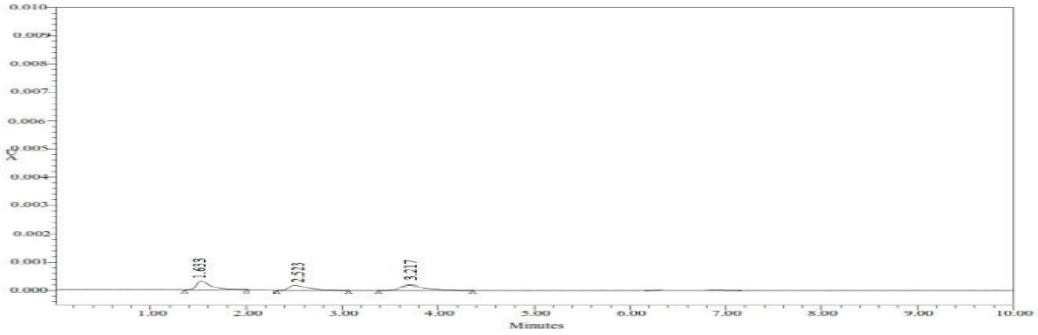


Figure 11: Chromatogram for Metformin, Linagliptin and Empagliflozin (LOQ)



DISCUSSION

The established HPLC method was designed to analyze a large number of samples in a short period, exhibiting excellent robustness, accuracy, and precision without requiring any prior separation steps. This efficiency is particularly advantageous for high-throughput environments. The method's capability to generate large amounts of quality data underscores its power and convenience as an analytical tool. In terms of solubility, Metformin and Linagliptin were freely soluble in methanol, while Empagliflozin showed sparing solubility in water. The choice of methanol and potassium dihydrogen orthophosphate as the mobile phase components facilitated the efficient separation and analysis of these compounds. The run time of the HPLC procedure was optimized to 5 minutes, allowing for rapid analysis. The method underwent thorough validation for various parameters, including system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, LOD, and LOQ. The system suitability parameters fell within acceptable limits, confirming the system's appropriateness for performing the assay. Linearity was demonstrated over a concentration range of 10-100 µg/mL, indicating that the method could accurately quantify different concentrations of the analytes. The percentage recovery for Metformin, Linagliptin, and Empagliflozin ranged from 98.22% to 99.25%, reflecting the method's high accuracy and ability to recover known amounts of these compounds reliably. Specificity was confirmed as there was no interference from excipients or the mobile phase, ensuring that the method could accurately identify and quantify the target analytes without extraneous influences. Robustness and ruggedness were demonstrated through insignificant variations in the results despite deliberate changes in flow rate, mobile phase composition, and different analysts performing the analysis. This indicates that the method is stable and reliable under varied conditions, making it suitable for routine use in diverse laboratory settings. Overall, the developed HPLC method is highly effective for the simultaneous estimation of Metformin, Linagliptin, and Empagliflozin. Its rapid run time, combined with high accuracy, precision, and robustness, makes it an excellent choice for quality control and routine analysis in pharmaceutical laboratories.

CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Metformin, Linagliptin, and Empagliflozin is robust, accurate, and precise. With a rapid run time of 5 minutes, this method efficiently handles high sample throughput, making it ideal for routine quality control and analysis in pharmaceutical settings. Its validated parameters ensure reliable performance under varied conditions, confirming its suitability for high-throughput environments without prior separation steps. This method enhances analytical efficiency, supporting the stringent demands of pharmaceutical quality control and research applications.

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