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Development and Validation of HPTLC and RP-HPLC Methods for Simultaneous Estimation of Atorvastatin, Glimepiride, and Metformin Hydrochloride in Bulk and Combined Dosage Form

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ABSTRACT
Combined formulations of atorvastatin, glimepiride, and metformin hydrochloride are widely used for type 2 diabetes with dyslipidemia. Reliable analytical methods for simultaneous estimation are needed for quality control. To develop and validate simple, accurate, economical, and reproducible HPTLC and RP-HPLC methods for simultaneous determination of these three drugs in bulk and combined tablet dosage form. HPTLC was performed on precoated silica gel $60F_{254}$ aluminum plates with mobile phase water:methanol:ammonium sulphate (3.5:3.5:12.6 v/v/v) and densitometric detection at 245 nm. RP-HPLC used a Phenomenex C_{18} column (250×4.6 mm, 5 µm) with mobile phase 20 mM potassium dihydrogen phosphate:acetonitrile (65:35 v/v), flow rate 1.0 mL/min, UV detection at 245 nm. Both methods were validated according to ICH Q2(R1) guidelines.
HPTLC gave Rf values of 0.33±0.01 (metformin), 0.50±0.01 (atorvastatin), and 0.65±0.01 (glimepiride). Linearity ranges: 50-350 ng/spot, 1-7 ng/spot, and 0.1-0.7 ng/spot respectively. RP-HPLC gave retention times of 3.92±0.70 min (metformin), 8.51±0.51 min (atorvastatin), and 12.18±0.62 min (glimepiride). Linearity ranges: 50-250 µg/mL, 1-5 µg/mL, and 0.1-0.5 µg/mL respectively. Recovery was 98-102% with RSD<2%. Both methods were successfully applied to tablet formulation (label claim: 10 mg atorvastatin, 1 mg glimepiride, 500 mg metformin). The developed methods are simple, precise, accurate, and suitable for routine quality control analysis of the three drugs in combined dosage forms.

Keywords: Atorvastatin; Glimepiride; Metformin hydrochloride; HPTLC; RP-HPLC; Method validation; ICH guidelines

1. INTRODUCTION
Cardiovascular diseases and type 2 diabetes mellitus frequently coexist, requiring combination therapy to manage both conditions effectively. Atorvastatin, a competitive HMG-CoA reductase inhibitor, is widely used for hyperlipidemia and cardiovascular risk reduction. Glimepiride (third-generation sulfonylurea) and metformin hydrochloride (biguanide) are first-line oral antidiabetic agents. Fixed-dose combinations containing all three drugs improve patient compliance by reducing pill burden.
Several analytical methods have been reported for individual or binary combinations of these drugs. Dhaneshwar et al. (2010) described an HPTLC method for simultaneous estimation of metformin, atorvastatin, and glimepiride using water:methanol:ammonium sulphate (1:1:4 v/v/v) with detection at 237 nm. Devi Ramesh and Habibuddin (2011) developed an RP-HPLC method using acetonitrile:phosphate buffer (60:40, pH 3.0) at 235 nm. However, alternative validated methods with different mobile phase compositions and optimized chromatographic conditions are still valuable for quality control laboratories.
The present study aimed to develop and validate simple, economical HPTLC and RP-HPLC methods for simultaneous estimation of atorvastatin, glimepiride, and metformin hydrochloride in bulk and combined tablet dosage form, following ICH Q2(R1) guidelines.

2. MATERIALS AND METHODS
2.1 Chemicals and Reagents
Reference standards of atorvastatin, glimepiride, and metformin hydrochloride were obtained from [source name, city, country]. HPLC-grade methanol and acetonitrile, analytical-grade ammonium sulphate, potassium dihydrogen phosphate, and orthophosphoric acid were procured from S.D. Fine Chemicals Ltd., Mumbai, India. Commercial tablet formulation (label claim: atorvastatin 10 mg, glimepiride 1 mg, metformin hydrochloride 500 mg per tablet) was purchased from a local pharmacy.

2.2 Instrumentation

HPTLC: Camag HPTLC system equipped with a TLC scanner, WinCATS software, and Linomat 5 sample applicator. Stationary phase: precoated silica gel $60F_{254}$ aluminum sheets (20×10 cm, 0.2 mm thickness).
RP-HPLC: Shimadzu SPD-20A Prominence UV/Vis detector with Class AT10 VP system. Column: Phenomenex C_{18} (250×4.6 mm i.d., 5 µm particle size).

2.3 Preparation of Standard Solutions

HPTLC: Stock solutions of atorvastatin (10 µg/mL), glimepiride (1 µg/mL), and metformin hydrochloride (500 µg/mL) were prepared in methanol. Working standard solutions for linearity were prepared by appropriate dilution.
RP-HPLC: Stock solutions of atorvastatin (1 mg/mL), glimepiride (0.1 mg/mL), and metformin hydrochloride (5 mg/mL) were prepared in mobile phase. Working standards were prepared in concentration ranges: atorvastatin 1-5 µg/mL, glimepiride 0.1-0.5 µg/mL, metformin 50-250 µg/mL.

2.4 HPTLC Method Development and Chromatographic Conditions

After evaluating various mobile phase compositions (Table S1, Supplementary material), optimal separation was achieved with water:methanol:ammonium sulphate (3.5:3.5:12.6 v/v/v) . The mobile phase was freshly prepared, sonicated, and saturated for 20 min in a twin-trough chamber. Developing distance: 80 mm. Densitometric scanning was performed at 245 nm using a deuterium lamp in reflectance/absorbance mode. Band width: 6 mm; slit dimension: 5×0.45 mm; scanning speed: 20 mm/s. The Rf values were 0.33±0.01 (metformin), 0.50±0.01 (atorvastatin), and 0.65±0.01 (glimepiride).

2.5 RP-HPLC Method Development and Chromatographic Conditions

The mobile phase consisted of 20 mM potassium dihydrogen phosphate buffer:acetonitrile (65:35 v/v) , pH adjusted to 3.5 with orthophosphoric acid. Isocratic elution at a flow rate of 1.0 mL/min. Injection volume: 20 µL; detection wavelength: 245 nm; column temperature: ambient. Run time: 15 min. Retention times were 3.918±0.70 min (metformin), 8.51±0.51 min (atorvastatin), and 12.18±0.62 min (glimepiride). System suitability parameters are presented in Table 1.

Table 1. System suitability parameters for RP-HPLC method.

Parameter	Atorvastatin	Glimepiride	Metformin HCl
Theoretical plates (N)	2687	7056	1098
Resolution (Rs)	–	1.06	1.08
Tailing factor	1.26	1.33	1.25

2.6 Validation

Both methods were validated according to ICH Q2(R1) guidelines for linearity, accuracy, precision, specificity, LOD, LOQ, and robustness.

a. **Linearity:** Six concentration levels analysed in triplicate. Calibration curves constructed by plotting peak area versus concentration.
b. **Precision:** Intra-day (same day, three concentrations, three replicates) and inter-day (three consecutive days) precision. Repeatability of sample application and measurement was assessed.
c. **Accuracy:** Recovery studies by standard addition method at 80%, 100%, and 120% levels.
d. **LOD and LOQ:** Calculated as LOD = 3.3×(SD/slope), LOQ = 10×(SD/slope).
e. **Specificity:** Peak purity assessed by comparing spectra at peak start, apex, and end.

2.7 Assay of Formulation

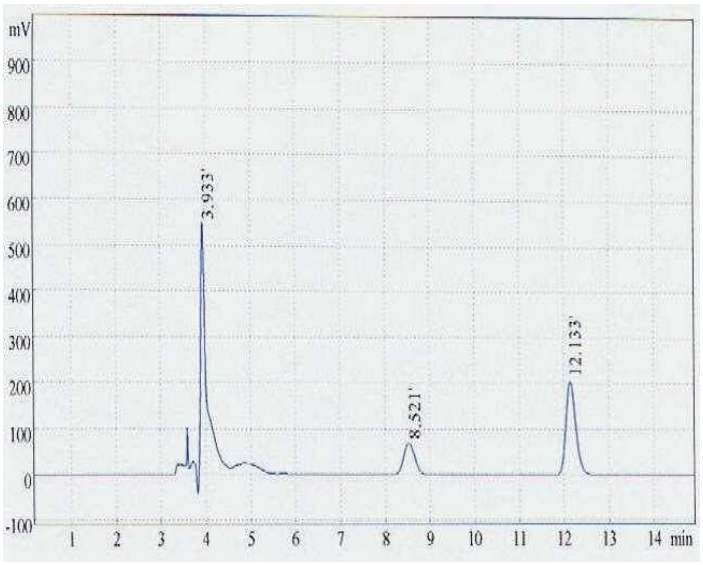
Twenty tablets were weighed and powdered. Powder equivalent to 1 mg atorvastatin, 0.1 mg glimepiride, and 50 mg metformin was extracted with methanol (30 min sonication), volume made to 100 mL, centrifuged at 3000 rpm for 5 min, and filtered. For HPTLC, 0.4 µL of this solution was spotted; for RP-HPLC, the solution was diluted appropriately and injected.

3. RESULTS AND DISCUSSION

3.1 Method Development

HPTLC: Among several mobile phases tried (Table S1), the system water:methanol:ammonium sulphate (3.5:3.5:12.6) gave compact, well-resolved spots with Rf values well separated from each other and from the solvent front. Detection at 245 nm provided optimal response for all three drugs.
RP-HPLC: The mobile phase 20 mM phosphate buffer:acetonitrile (65:35) produced symmetrical Gaussian peaks with resolution >2. Flow rate 1.0 mL/min gave acceptable retention times (around 4, 8.5, and 12 min)

allowing baseline separation within 15 min. System suitability parameters (Table 1) were within acceptable limits (N>2000, tailing<2).



3.2 Validation Results

3.2.1 Linearity

Calibration curves were linear over the tested ranges with correlation coefficients >0.99 (**Table 2**). Representative calibration graphs are shown in **Figures 1-3** (HPTLC) and **Figures 4-6** (RP-HPLC).

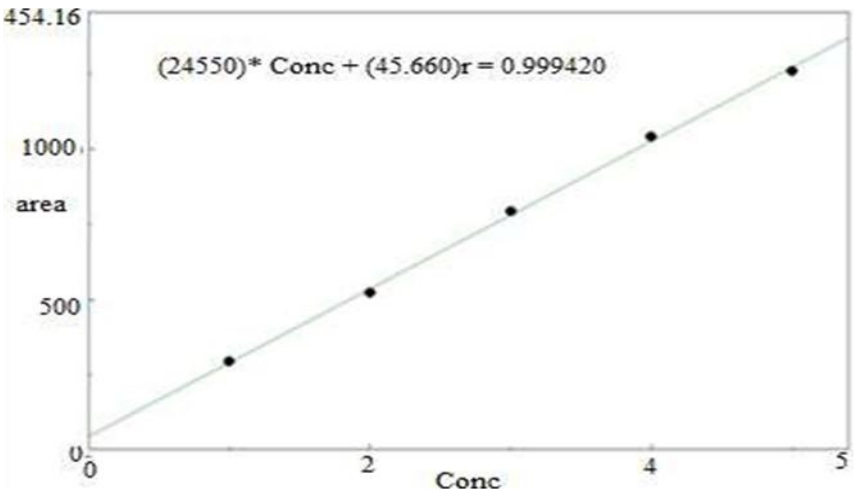


Fig.1. Calibration Graph of Atorvastatin (1-5ng/spot)

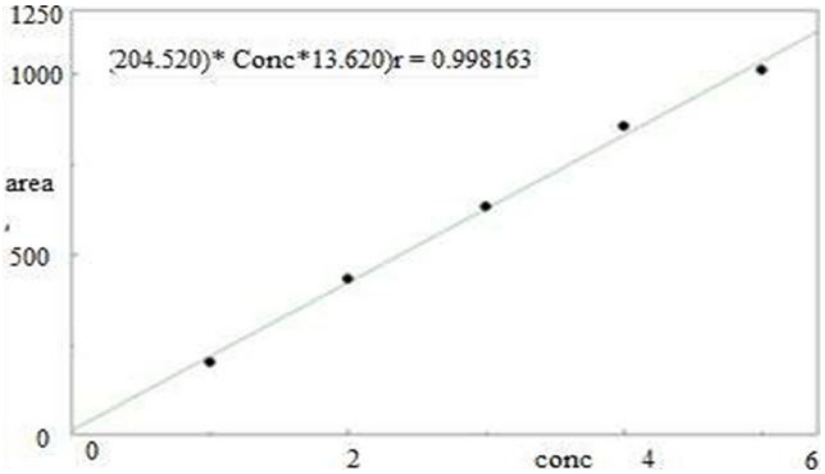


Fig.2: Calibration Graph of Glimepiride(0.1-0.5ng/spot)

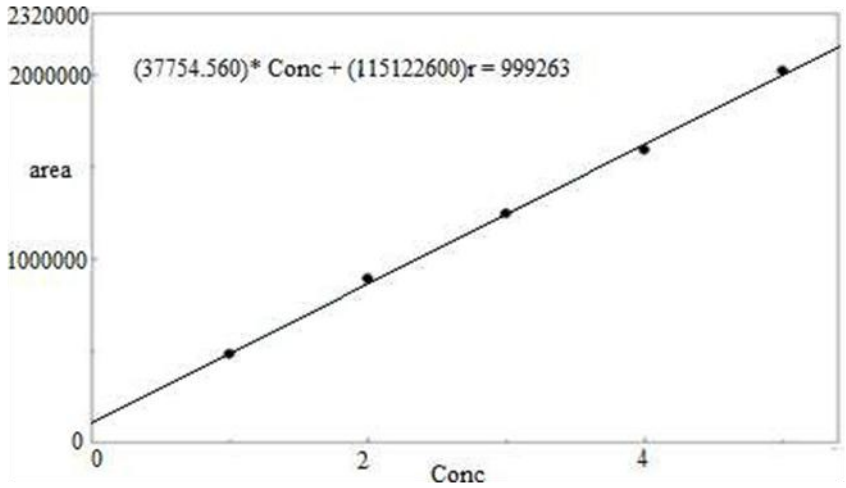


Fig.3 Calibration Graph of Atorvastatin (1–5µg/ml)

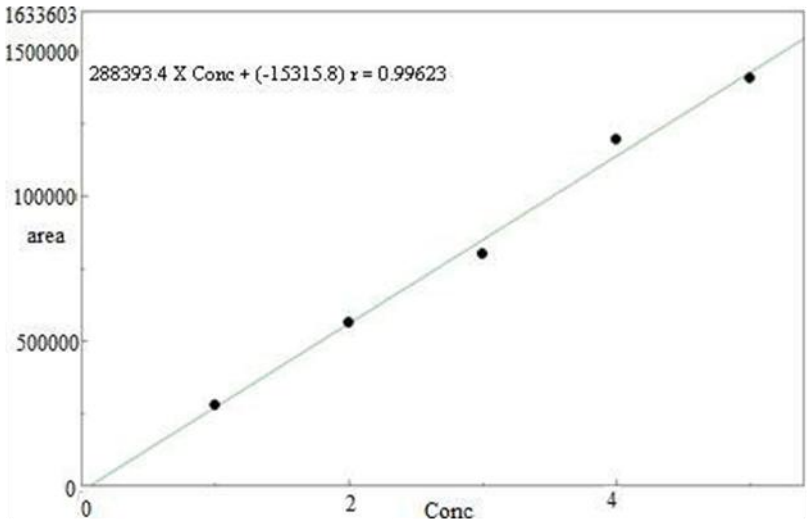


Fig.4.: Calibration Graph of Glimepiride (0.1-0.5µg/ml

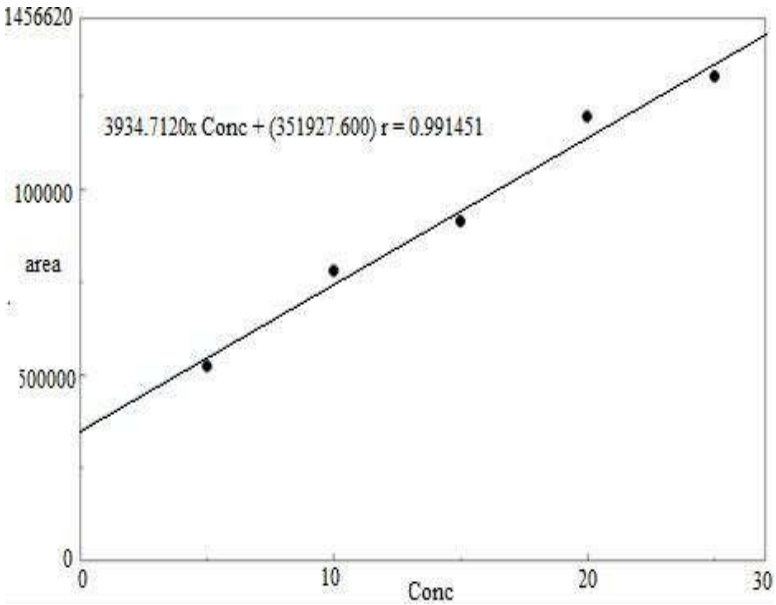


Fig.5.: Calibration Graph of Metformin Hydrochloride(50–250µg/ml)

Table 2. Linear regression data for HPTLC and RP-HPLC methods.

Method	Drug	Linearity range	Slope	Intercept	r^2
HPTLC	Atorvastatin	1-7 ng/spot	24.55	45.66	0.999
	Glimepiride	0.1-0.7 ng/spot	204.52	13.62	0.998
	Metformin HCl	50-350 ng/spot	0.8054	26.41	0.999
RP-HPLC	Atorvastatin	1-5 µg/mL	37754.56	115122.60	0.999
	Glimepiride	0.1-0.5 µg/mL	288393.40	-15315.80	0.996
	Metformin HCl	50-250 µg/mL	3934.71	351927.60	0.991

3.2.2 Precision

Low %RSD values (<2%) for intra-day and inter-day precision confirmed good precision of both methods (**Table 3**). Repeatability of sample application and measurement also gave %RSD <2%.

Table 3. Precision results (%RSD, n=6).

Method	Drug	Intra-day (%RSD)	Inter-day (%RSD)
HPTLC	Atorvastatin	0.57-0.86	0.43-0.56
	Glimepiride	0.07-0.60	0.88-1.35
	Metformin HCl	0.38-1.45	1.08-1.45
RP-HPLC	Atorvastatin	0.35-0.82	0.35-0.82
	Glimepiride	0.46-1.93	0.46-1.04
	Metformin HCl	1.08-1.28	0.81-1.40

3.2.3 Accuracy (Recovery Studies)

Recovery values ranged from 97.6% to 101.9% with %RSD <2%, indicating no interference from excipients (**Table 4**).

Table 4. Accuracy results for HPTLC method (n=3).

Drug	80% recovery (% ± RSD)	100% recovery (% ± RSD)	120% recovery (% ± RSD)
Atorvastatin	99.56 ± 0.74	101.23 ± 0.41	98.92 ± 0.63
Glimepiride	98.97 ± 0.46	100.89 ± 0.52	101.93 ± 0.32
Metformin HCl	101.23 ± 0.98	101.45 ± 1.09	99.12 ± 0.89

3.2.4 LOD and LOQ

The low LOD and LOQ values (**Table 5**) demonstrate adequate sensitivity for pharmaceutical analysis.

Table 5. LOD and LOQ values.

Method	Drug	LOD	LOQ
HPTLC	Atorvastatin	0.2 ng/spot	1.0 ng/spot
	Glimepiride	0.02 ng/spot	0.1 ng/spot
	Metformin HCl	5 ng/spot	50 ng/spot
RP-HPLC	Atorvastatin	0.01 µg/mL	0.3 µg/mL
	Glimepiride	0.001 µg/mL	0.03 µg/mL
	Metformin HCl	0.1 µg/mL	1.0 µg/mL

3.2.5 Specificity

Peak purity correlation coefficients (r(s,m) and r(m,e)) were >0.999 for all three drugs in both methods, confirming that no impurities or degradation products co-eluted with the analyte peaks.

3.3 Assay of Formulation

Both methods were successfully applied to the commercial tablet formulation. The results (**Table 6**) were in good agreement with the label claims, and the low %RSD values indicate good reproducibility.

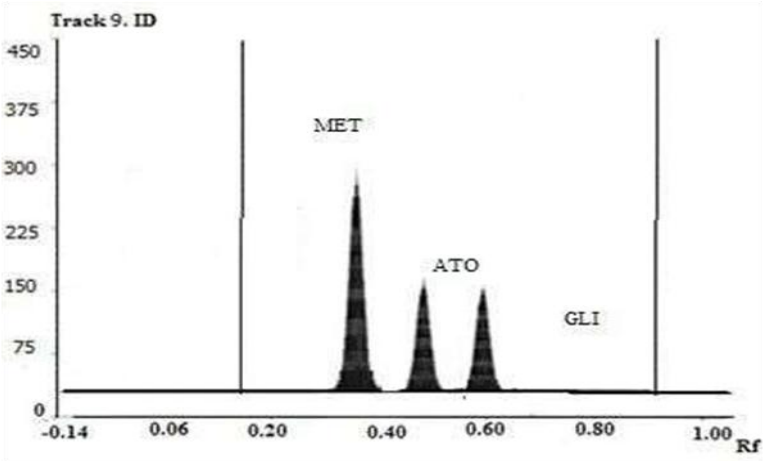


Fig.7 Atorvastatin (4ng/spot), Glimepiride (0.4ng/spot) and Metformin Hydrochloride (200ng/spot)

Table 6. Assay results of commercial tablet formulation (n=6).

Method	Drug	Label claim (mg/tablet)	Estimated amount (mg)	% Label claim	%RSD
HPTLC	Atorvastatin	10	9.84	98.40	0.93
	Glimepiride	1	0.98	98.00	1.12
	Metformin HCl	500	499.23	99.84	0.89
RP-HPLC	Atorvastatin	10	9.72	97.20	0.67
	Glimepiride	1	0.98	98.00	0.35
	Metformin HCl	500	498.12	99.62	0.87

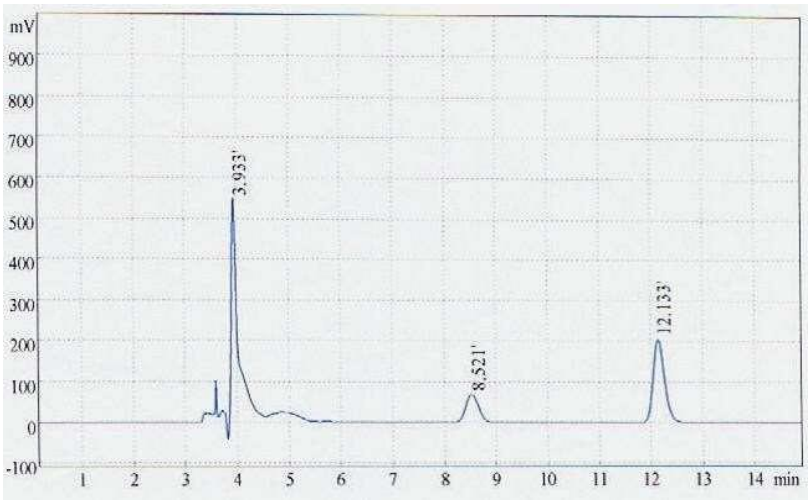


Fig.9.: Chromatogram of Formulation Atorvastatin (3g/ml), Glimepiride (0.3g/ml) and Metformin Hydrochloride (150g/ml)

3.4 Comparison with Published Methods

The developed methods compare favourably with previously reported methods (**Table 7**). The HPTLC method uses a slightly different mobile phase ratio, and the RP-HPLC method offers good resolution with a simple isocratic system.

Table 7. Comparison with published methods.

Parameter	Present HPTLC	Dhaneshwar et al. (2010)	Present RP-HPLC	Devi Ramesh et al. (2011)
Mobile phase	Water:MeOH:Ammonium sulphate (3.5:3.5:12.6)	Water:MeOH:Ammonium sulphate (1:1:4)	Buffer:ACN (65:35)	Buffer:ACN (40:60)
Detection (nm)	245	237	245	235
Run time	80 mm development	80 mm development	15 min	~10 min
Rf or RT	0.33, 0.50, 0.65	0.37, 0.59, 0.75	3.92, 8.51, 12.18 min	2.57, 7.06, 9.39 min

4. CONCLUSION

Two simple, precise, accurate, and economical methods (HPTLC and RP-HPLC) were developed and fully validated for the simultaneous estimation of atorvastatin, glimepiride, and metformin hydrochloride in bulk and combined tablet dosage form. Both methods met ICH acceptance criteria for linearity, precision, accuracy, specificity, LOD, and LOQ. Statistical analysis confirmed that the methods are reliable and free from excipient interference. These methods can be readily applied for routine quality control analysis in pharmaceutical laboratories.

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