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Simultaneous Determination of Atorvastatin, Metformin and Glimepride in Pharmaceutical Dosage Form by a Stability Indicating RP HPLC Method

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ABSTRACT

A specific, accurate, precise and sensitive stability indicating RP HPLC method was developed and validated for the simultaneous determination of atorvastatin (ATR), metformin (MET) and glimepiride (GLM) in bulk drug and pharmaceutical dosage form in the presence of its degradation products. An isocratic RP HPLC method was developed with a Hibar C_{18} (250 X 4.6 mm i.d., 5µ) column and Acetonitrile: 10 mM Ammonium acetate (pH 3.0, adjusted with acetic acid) in the ratio of 40:60 % v/v as mobile phase. The flow rate was maintained at 1 mL/min and detection was carried out using UV detector (245 nm). The drugs were subjected to stress conditions of degradation in aqueous solutions including hydrolysis, oxidation and photolysis. Degradation was carried out for 24 hrs at room temperature. The developed method provided better separation of the drugs from the degradation products. The linearity of the proposed method was carried out in the range of 0.5 – 12 µg/mL, 25 – 250 µg/mL and 0.2 – 1.2 µg/mL for ATR, MET and GLM respectively. The limits of detection and quantification were found to be 10 ng/mL, 1 ng/mL, 1ng/mL and 30 ng/mL, 4 ng/mL, 5 ng/mL for ATR, MET and GLM respectively.

Keywords: Atorvastatin; Metformin; Glimepiride; RP-HPLC; Validation

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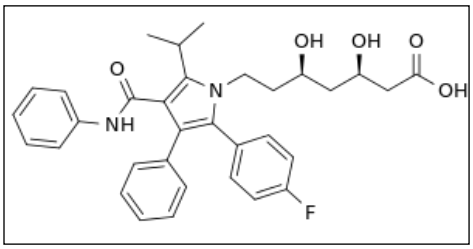
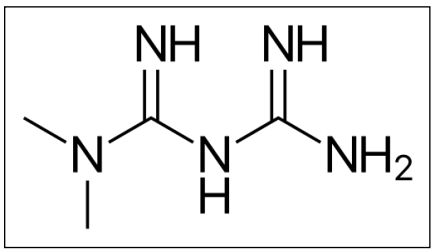
INTRODUCTION

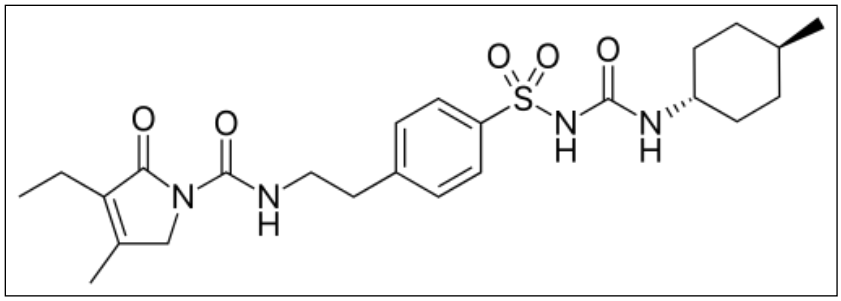
Atorvastatin is chemically (3R, 5R)-7-[2-(4-fluoro phenyl)-3-phenyl-4-(phenyl carbonyl)-5-(propan-2-yl)-1H-pyrol-1-yl]-3,5-dihydroxy heptatonic acid. It is a competitive inhibitor of hydroxyl methyl glutaryl-coenzyme A (HMG-CoA) reductase and is used for lowering the blood pressure. Metformin falls under the category of oral anti-diabetic drug and is chemically 1-carbamimidamido-N, N-di methyl di methyl methan imidamide. Glimepiride is the first III generation sulphonyl urea. It is chemically 3-ethyl-4-methyl-N-(4-[N-((1r, 4r)-4-methyl cyclo hexyl carbamoyl) sulfamoyl] phenethyl)-2-oxo-2, 5-dihydro-1H-pyrrole-1-carboxamide.



Atorvastatin



Metformin



Glimepiride

Figure 1: Structure of the selected drug candidates

A literature survey on various analytical methods for the simultaneous estimation of the three drugs revealed an LC MS/MS [1] method for the determination of ATM, MET and GLM in human plasma. Also a stability indicating RP HPC method was reported by Devi Ramesh and Mohammed Habibudin [2] wherein the stress studies were carried out for 1 hr at 60°C. This method does not discuss in detail the degradation of the selected drug candidates at room temperature. Further UPLC [3], RP HPLC [4], UV[5] and HPTLC [6, 7] methods for the estimation of ATR, MET and GLM in various pharmaceutical formulations were also reported. However there are various other analytical methods viz HPLC, UV, SFC and MS/MS, available for the estimation of ATR, MET and GLM individually and in combination with other drugs [8-28]. A literature search on the stability indicating analytical methods for the simultaneous estimation of ATR, MET and GLM revealed no methods for the degradation studies at room temperature. Hence the present work is designed to develop a simple, sensitive, rapid, precise and accurate analytical method for the simultaneous estimation of the selected drug candidates and to carry out the stability studies of the bulk drugs under various stress conditions. The developed method is validated as per International Conference on Harmonization (ICH) Q2 (R2) guidelines. [29, 30]

EXPERIMENTAL SECTION

Chemicals, reagents and solutions

All the reagents used were of analytical grade. HPLC grade Methanol, HPLC grade Acetonitrile, and Ammonium acetate were procured from S D Fine chem. Ltd., (India). Millipore water from milli-Q RO system was used. Borosilicate glass wares (Class A) were used for preparation of solutions.

Chromatography

Chromatographic separation and quantitative determination were performed using an LC system with LC-10AT-VP solvent delivery system, an SPD M-10A VP UV detector, an auto injector system with 100 µl loop volume and a Class VP data station (Shimadzu, Japan). As the stationary phase, Hibar C_{18} column, 5µ particle size, 250 X 4.6 mm (Merck, Darmstadt, Germany) was used. The mobile phase consisted of 10 mM Ammonium acetate (pH 3.0, Adjusted with Acetic Acid) and Acetonitrile in the ratio of 60: 40 % v/v. The flow rate of the mobile phase was maintained at 1.0 mL/min. The eluents were monitored at 245 nm using UV detector. The pH measurements were carried out using Systronics digital pH meter 335 equipped with a glass electrode. The pH meter was calibrated using standard buffer between pH 4.0 and 7.0.

Assay of the commercial formulation

The assay of the commercially available formulation was performed by using the developed RP HPLC method. 20 tablets were accurately weighed and crushed into fine powder. A tablet powder equivalent to 1 mg of ATR, 50 mg of MET and 0.2 mg of GLM were accurately weighed and transferred to a 100 mL volumetric flask. The drugs were extracted with sufficient quantity of mobile phase [Acetonitrile: 10mM Ammonium acetate (pH 3.0)] and sonicated for 15 mins at room temperature. The above solution was filtered; 1 mL of the above filtrate was taken in a 10 mL volumetric flask and made up to the mark with the mobile phase. This solution was then filtered through 0.45 µ syringe filter before analysis by the developed and validated RP HPLC method.

Stress degradation studies

The stress degradation studies of the ATR, MET and GLM were carried out under acid, alkaline, oxidative and photolytic stress conditions as per ICH Q1 guidelines. Under all stress conditions, the mixture of drugs were prepared at a concentration of 1 mg/mL; and after the studies the aliquots of the samples were diluted with the mobile phase to obtain 100 µg/mL and analysed under optimized chromatographic conditions. The samples from acid and base hydrolysis were neutralized suitably before analysis. Blank solutions were also prepared at the same time as that of the stock solutions.

Preparation of standard stock solutions

An accurately weighed quantity of 10 mg of ATR, MET and GLM were carefully transferred in to a 10 ml volumetric flask, and dissolved completely in a mixture of Water: Acetonitrile [50:50% v/v] to obtain 1mg/mL. The solutions for the stress studies were also prepared as above for acid hydrolysis, base hydrolysis and oxidation with HCl (0.1N), NaOH (0.1N) and hydrogen peroxide (3%) respectively. Photo degradation study was carried out for the standard stock solutions under UV lamp for 24 hours.

Acid hydrolysis

1mg/mL mixed stock solutions of ATR, MET and GLM were prepared in 0.1N HCl at room temperature. The solution was kept at room temperature for 24 hrs. Aliquots of the samples were withdrawn at different time intervals, further neutralized with 0.1N NaOH and diluted to 10 ml with mobile phase. Blank solutions were prepared at the same time of preparation of stock solutions. The samples were injected against blank under the optimized chromatographic conditions and the chromatograms were recorded.

Base hydrolysis

1mg/mL mixed stock solutions of ATR, MET and GLM were prepared in 0.1N NaOH at room temperature. The solution was kept at room temperature for 24 hrs. Aliquots of the samples were withdrawn at different time intervals, further neutralized with 0.1N HCl and diluted to 10 mL with mobile phase. Blank solutions were prepared at the same time of preparation of stock solutions. The samples were injected against blank under the optimized chromatographic conditions and the chromatograms were recorded.

Oxidative degradation

1mg/mL mixed stock solutions of ATR, MET and GLM were prepared in 3% hydrogen peroxide at room temperature. The solution was kept at room temperature for 24 hrs. Aliquots of the samples were withdrawn at different time intervals and diluted to 10 mL with mobile phase. The samples were injected against blank under the optimized chromatographic conditions and the chromatograms were recorded.

Photo degradation

Photo degradation studies were carried out by exposing the stock solutions to UV lamp (short wavelength) for 24 hours. After degradation, the samples were then suitably diluted with the mobile phase, injected under optimized chromatographic conditions and the chromatograms were recorded.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The preliminary experiments were carried out in reverse phase mode using a HPLC system. The drugs were subjected to separation by using water and methanol as organic modifier in the ratio of 68:32. ATR and GLM were un retained in the column. Thus to improve the retention of ATR and GLM buffers of various pH were tried. Phosphate buffers were avoided to make the method LC/MS compatible. Methanol was replaced with Acetonitrile in terms of resolution and peak shapes. Initially 10 mM Ammonium acetate buffer at pH 7.0 (60% v/v) was chosen. However ATR and GLM were un retained and MET showed tailing. The effects of different pH and mobile phase composition were also checked.

A solvent combination of 10mM Ammonium acetate (pH 3.0, adjusted with acetic acid): Acetonitrile (60:40% v/v) on a C_{18} column (5µ particle size, 250 X 4.6 mm) as stationary phase gave satisfactory separation of ATR, MET, GLM from their degradation products formed under various stress conditions. The prepared mobile phase solvents were filtered through a 0.45 µ filter using vacuum and degassed through a sonicator. The flow rate was maintained constant at 1.0 mL/min. The injection volume was 100 µL and the detection was carried out at 245 nm. Typical retention times of ATR, MET and GLM were about 6.5, 2.8 and 8.6 min respectively. (Figure 2)

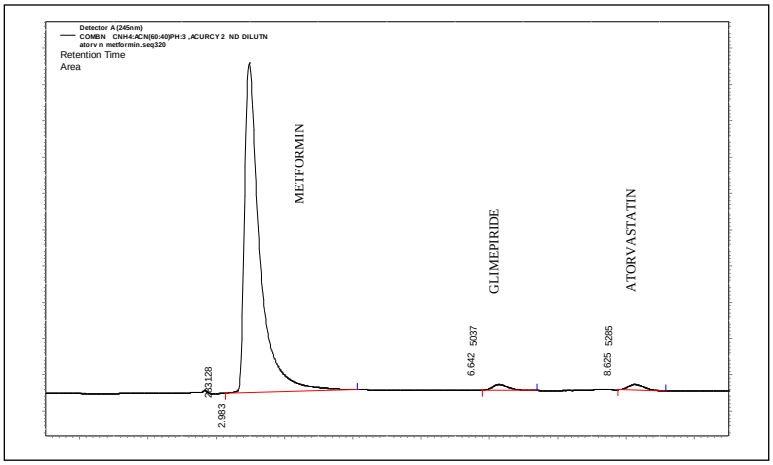


Figure 2: Typical Chromatogram of Standard drugs Atorvastatin, Metformin and Glimepiride

Validation of the stability indicating analytical method

The method was validated as per ICH (Q2) guidelines with respect to linearity, accuracy, precision, specificity, robustness, limit of detection and limit of quantification. [29, 30]

Specificity

Forced degradation studies were performed on the mixture of the three drugs to support the specificity of the stability indicating method. The standard solutions were stressed under conditions of hydrolysis (0.1N HCl, 0.1N NaOH, at room temperature), Photolysis (solution, exposed to UV lamp for 24 hrs) and oxidation (3 % H_2O_2 at room temperature). The degradants formed during the various degradation studies were adequately separated from ATM, MET and GLM indicating the specificity of the developed RP HPLC method.

Linearity

The linearity of the detector response to different concentrations of ATR, MET and GLM were studied in the range of 0.5 – 12 µg/mL, 25 – 250 µg/mL and 0.2 – 1.2 µg/mL for ATR, MET and GLM respectively. The samples were analyzed in triplicate. The calibration curves were described by the equation Y=mX+c; i.e. Y = 2303X – 4261, Y = 30725X – 5877 and Y = 60668X – 3356 for ATR, MET and GLM respectively. The correlation coefficients were 0.990, 0.994 and 0.990 for ATR, MET and GLM respectively indicating the linear response of the drugs. The calibration curves were constructed on six different days over a period of two weeks to determine the variabilities of slope and intercept. The interday variability of slope and intercept were not significant.

Accuracy

The accuracy of the optimized method was determined by absolute recovery experiments. Standard drugs in the range of 80, 100 and 120 % of the sample concentrations were added to the sample and each sample was analysed in triplicate. The results of the recovery studies were found to be within 98-100% for ATR, MET and GLM. The results for the assay of marketed formulation are presented in Table I. The results are tabulated in Table II.

Table 1: Assay

Drug	Labeled Amount (mg/Tablet)	Amount found (mg/Tablet ± SD)	Label claim (% ± SD)
Atorvastatin	10	9.85 ± 0.067	98.51 ± 0.667
Metformin	500	497.75 ± 1.099	99.55 ± 0.22
Glimepiride	2	1.95 ± 0.028	97.5 ± 1.42

Table 2: Accuracy

Drug	Amount Added (µg/mL)	Amount Recovered (µg/mL ± S.D); R.S.D % (n=6)	% Recovery
Atrovastatin	0.5	0.49 ± 0.01; 2.22	98
	2	1.98 ± 0.01; 0.87	99
	8	7.95 ± 0.05; 0.64	99.33
Metformin	25	24.85 ± 0.13; 0.52	99.4
	100	98.94 ± 0.91; 0.92	98.9
	200	198.28 ± 1.49; 0.75	99.1
Glimepiride	0.2	0.20 ± 0.005; 2.84	100
	0.6	0.58 ± 0.015; 2.60	96.66
	1.2	1.18 ± 0.02; 0.02	98.33

Precision

The data for intraday and interday precision studies were performed at three different concentrations in the linearity range [ATR: 0.5, 2.0 and 8.0 µg/mL; MET: 25,100 and 200 µg/mL; and GLM: 0.2, 0.6 and 1.2 µg/mL]. The relative standard deviation (RSD) values for intraday and interday precision were < 2 % indicating the precision of the method (Table III).

Table 3: Precision studies

Drug	Actual concentration (µg/mL)	Intra-day calculated concentration (µg/mL) ± S.D.; R.S.D % (n=6)	Inter-day calculated concentration(µg/mL) ± S.D.; R.S.D % (n=6)
Atorvastatin	0.5	0.46 ± 0.01; 2.17	0.45 ± 0.01; 2.52
	2	1.93 ± 0.05; 2.98	1.90 ± 0.05; 0.30
	8	7.91 ± 0.07; 0.94	7.89 ± 0.10; 1.28
Metformin	25	24.78 ± 0.22; 0.88	24.75 ± 0.22; 0.91
	100	98.81± 1.22; 1.12	98.61± 1.32; 1.33
	200	197.28 ± 2.39; 1.21	196.94 ± 2.64; 1.34
Glimepiride	0.2	0.194 ± 0.02; 1.34	0.192 ± 0.022; 1.35
	0.6	0.57± 0.01; 2.00	0.57± 0.02; 3.50
	1.2	1.18 ± 0.02; 1.69	1.17 ± 0.02; 2.26

Limit of Detection and Limit of Quantification

The LOD and LOQ parameters were determined based on signal to noise ratio. For LOD the S/N ratio of 3:1 and for LOQ determination a S/N ratio of 10: 1 were used. The LOD values for ATR, MET and GLM were found to be 10 ng/mL, 1 ng/mL and 1 ng/mL respectively. The LOQ values for ATR, MET and GLP were found to be 50 ng/mL, 4 ng/mL and 5 ng/mL respectively. (Table 4).

Table 4: System suitability parameters

Parameters	Atorvastatin	Metformin	Glimepiride
Linearity and Range	0.5-12µg/mL	25-250µg/mL	0.2-1.2µg/mL
Regression equation	Y=2303x-4261	Y=30725x-5877	Y=301.8x-5849
Correlation coefficient	0.99	0.994	0.99
Theoretical plates	9482	2458	7980
Asymmetric factor	1.5	1.5	1.8
Limit of Detection(LOD)	10 ng/mL	1 ng/mL	1 ng/mL
Limit of Quantification(LOQ)	50 ng/mL	4 ng/mL	5 ng/mL

Robustness test

The robustness of the develop method was evaluated by analyzing the samples after changing the following parameters: the composition of mobile phase, the mobile phase flow rate, pH of the buffer and concentration of the buffer. For each parameter change, its influence on the retention time, resolution and peak shape were evaluated. The results were tabulated in Table V. The method was robust for all the tested parameters.

Table 5: Robustness Studies

Parameters modified	Retention Time (min)		
	ATR	MET	GLM
Mobile Phase Ratio			
58:42:00	6.4	2.6	8.5
60:40:00	6.6	2.8	8.6
62:38:00	6.7	2.9	8.8
pH of the Aqueous Phase			
2.5	6.5	2.6	8.6
3	6.6	2.8	8.6
3.5	6.8	3	8.8
Flow Rate (ml/min)			
0.9	6.7	2.9	8.7
1	6.6	2.8	8.6
1.1	6.4	2.7	8.5
Strength of Aqueous Phase			
5mM	6.4	2.6	8.5
10mM	6.6	2.8	8.6
15mM	6.7	2.9	8.8

Stress degradation studies

Acid induced degradation:

Initial degradation trails were performed in 0.1 N HCl and it was observed that the drugs ATR, MET and GLM showed negligible degradation after 2 hrs at room temperature. Upon treatment with 1N HCl at room temperature for 24 hrs, the % degradation for ATR, MET and GLM were found to be 18.9, 15.4 and 56.2% respectively. One degradation product eluted at the retention time of 9.3 min. The results of the degradation at various time intervals and the chromatogram are shown in Table VI and Figure 3.

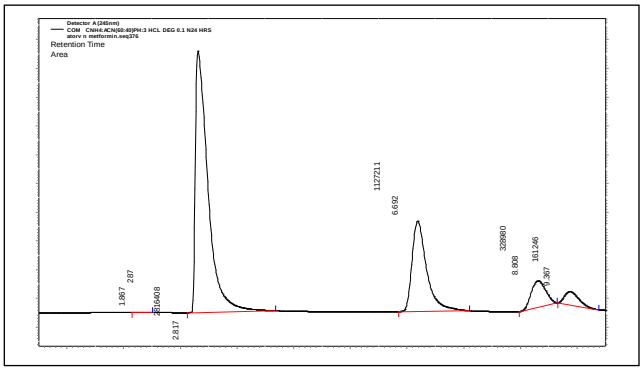


Figure 3: Typical Chromatogram of Acid Degradation Studies of ATR, MET and GLM after 24 Hrs at Room Temperature

Base induced degradation

In basic degradation studies, when the drugs were exposed to 0.1N NaOH for 2 hrs at room temperature, the drugs showed negligible degradation. Upon treatment with 0.1N NaOH at room temperature for 24 hrs, Atorvastatin was found stable, whereas MET and GLP showed 13.7 and 13.1% degradation respectively. The results of the degradation at various time intervals and the chromatogram are shown in Table VI and Figure 4.

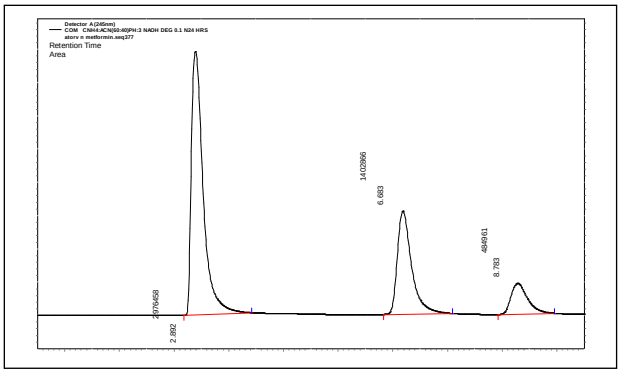


Figure 4: Typical Chromatogram of Base Degradation Studies of ATR, MET and GLM after 24 Hrs at Room Temperature

Oxidative degradation:

When the drug candidates were exposed to oxidative degradation with 3% hydrogen peroxide for 24 hrs at room temperature, considerable degradation was observed. ATR, MET and GLP showed 53.9, 59.8 and 62.3% degradation respectively. The results of the degradation at various time intervals and the chromatogram are shown in Table 4 and Figure 5.

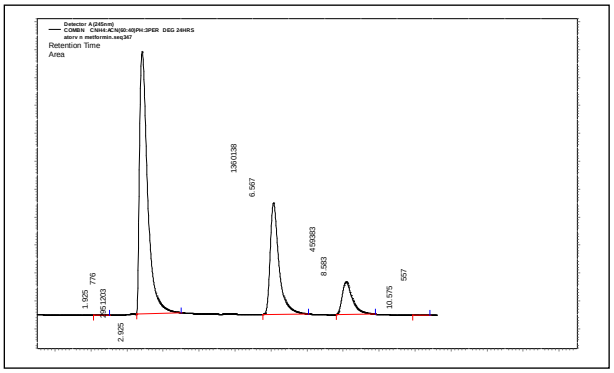


Figure 5: Typical Chromatogram of Oxidative Degradation Studies of ATR, MET and GLM after 24 Hrs at Room Temperature

Photolytic degradation:

When the drugs ATR, MET and GLM were exposed to white light (illumination of NLT 1.2 million Lux hours) for 24 hrs, the drugs were found to be stable showing negligible degradation. The results are tabulated in Table VI and the chromatograms are shown in Figure 6.

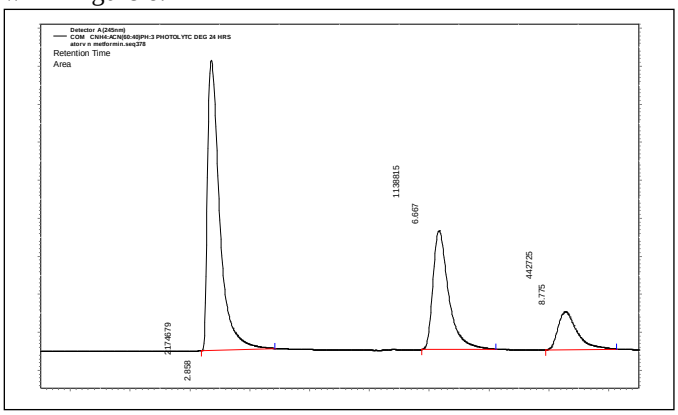


Figure 6: Typical Chromatogram of Photo Degradation Studies of ATR, MET and GLM after 24 Hrs at Room Temperature

Table 6: Stress degradation studies

Time (hrs)	% Degradation											
	0.1N HCl			0.1N NaOH			3% H_2O_2			Photo degradation		
	ATR	MET	GLM	ATR	MET	GLM	ATR	MET	GLM	ATR	MET	GLM
2	7.4	2.5	20.4	5	2.1	0.1	2.3	0.4	8.2	--	--	--
4	12.8	4.7	20.7	11	3.9	3.5	3.1	2.4	56.8	--	--	--
6	15	8	33.5	21.2	4.7	4.7	4.3	11.2	58.6	--	--	--
12	15	13	45.5	35.4	9.8	9.8	4.1	39.5	59.5	--	--	--
24	18.9	15.4	56.2	38.9	13.7	13.7	53.9	59.8	62.3	22.9	18.7	10.4

CONCLUSION

A simple, specific, accurate, reliable stability indicating RP HPLC method was developed for the simultaneous estimation of ATR, MET and GLM in presence of their degradation products and validated according to ICH guidelines. The method was found to be specific, accurate and robust for the routine assay of the combination drugs.

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