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IMPURITY PROFILING METHOD DEVELOPMENT AND VALIDATION FOR METFORMIN HCL AND VILDAGLIPTIN FROM COMBINATION TABLET DOSAGE FORM BY RP-HPLC COUPLED WITH UV/PDA DETECTOR

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ABSTRACT: A precise, accurate, robust method was developed for quantitative estimation of related impurities of Metformin HCL and Vildagliptin from combination tablet dosage form. The mixture of ammonium dihydrogen orthophosphate and octane sulfonic acid sodium salt buffer at pH 4 was used as mobile phase A and mixture of methanol and buffer in the ratio of95:5 used as a mobile phase B. The mobile phase was pumped at 0.8 ml/minutes flow rate through BDS Hypersil C8, 250 x 4.6 mm, 5µ, HPLC column operated at 35°C. All the solutions were injected at 10µl injection volume, and the chromatograms were monitored at 210 nm based on optimum response of impurities and analytes. The % recovery of Metformin HCL, was found 90.4, 92.3, 99.4 and 99.2%, while for Vildagliptin was 90.5, 94.5, 99.6 and 98.3 % from LOQ, 50,100 and 150 % levels respectively. The method was found linear from LOQ to 150% with the correlation coefficients (r2) were 0.998 for Metformin HCL and Vildagliptin. No interference observed from diluent and placebo at the retention time of all known impurities and principle analytes in specificity study and no significantly affected chromatographic pattern for standard, system suitability criteria and sample chromatograms during robustness study, hence the method is specific and robust. The method can be used in quality control laboratories for analysis of related substances from these combination drug products in pharmaceutical industries.

INTRODUCTION: The Metformin HCL and Vildagliptin combination tablet is commonly prescribed medication worldwide to control the sugar level in the blood.

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The purity, safety and efficacy of the medication is depending on the impurity level in the drug and drug products hence for batter efficacy the medication should have impurities well within the limits as per ICH guideline. It is most important to have an accurate, precise and robust method that can detect and able to accurately quantitate all the known and unknown degradants from drug and drug products.

Metformin Hydrochloride (MTF): MTF **Fig. 1** is a biguanide derivative used to lower the blood

glucose concentration in patients with non-insulin dependent diabetes mellitus [1]. Chemically it is 1, 1-dimethylbiguanide hydrochloride easily soluble in water, marginally soluble in ethanol and almost insoluble in acetone and methylene chloride [2].

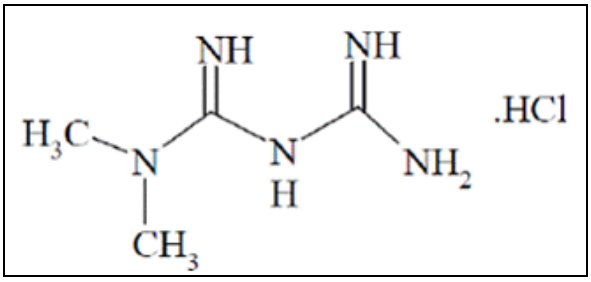

FIG. 1: STRUCTURE METFORMIN HCL (MTF)

Vildagliptin: The IUPAC name of Vildagliptin is (S)-1-[2-(3-Hydroxyadamantan-1-ylamino) acetyl] pyrrolidine-2- carbonitrileas shown in **Fig. 2**. It is anti-hyperglycemic agent having white to off-white solid powder that is soluble in water and Dimethyl Sulfoxide [3].

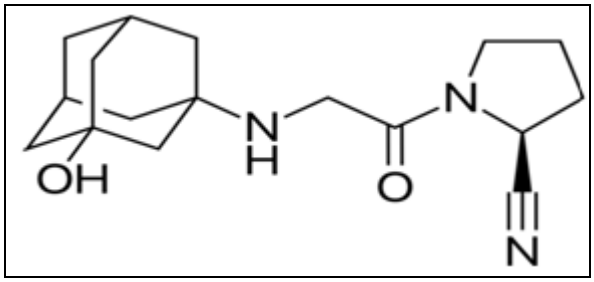

FIG. 2: STRUCTURE VILDAGLIPTIN (VIL)

After extensive literature review we come to know that, several assay methods for estimation of metformin HCL and Vildagliptin were reported by various technique like HPLC [4, 9], HPTLC [10, 11], HPLC-MS, and by UFLC [12]. Individual assay determination of Vildagliptin as well as combination with other drug also reported by RP-HPLC [13-17], Spectrophotometry and spectroflurometric [18], GC [19], electrophoresis [20]. Ramzia L. El Bagry and *et. al* reported the Vildagliptin determination in presence of its synthetic mixture in presence of Metformin HCL and Pioglitazone [21]. In this reported method, does not included the Vildagliptin known impurities as well as impurities of MTF like Melamine, Cyanoguanidine, MTF related compound B, MTF related compound C. Caroline Paola Uber and *et. al* developed the method by HPLC–MS for determination of Vildagliptin and Metformin HCL with its impurities, but no Vildagliptin impurities were reported and eluted by this method [22]. E. Al-Qudah, and *et. al*, did the Forced degradation studies of Vildagliptin API in the presence of excipients, by using HPLC-UV, but known impurities not reported and eluted [23]. Best of our knowledge to the date no methods are reported for the simultaneous quantitation of known and unknown impurity profiling for Metformin HCL and Vildagliptin form their combination tablet dosage forms. Present study focused on simultaneous quantitation of known and unknown degradants of MTF and VIL from their combination tablet dosage form. The developed method was validated as per ICH guideline [24, 25] and can be used in quality control laboratories for routine analysis of commercial batches.

MATERIALS AND METHOD: MTF (99.6% purity) and VIL (99.7% purity) was in-house qualified working standards used for the complete development and validation study. The AR grade octane sulfonic acid sodium salt (Merck), ammonium dihydrogen orthophosphate (RANKEM), orthophosphoric acid (RANKEM), and HPLC grade acetonitrile, methanol (RANKEM) were used for mobile phase preparation. The complete development and validation was carried out on Shimadzu LC 2010 CHT, HPLC with Lab solution software and UV detector. Wavelength selection, selectivity study and method precision study was performed on Water's HPLC with Empower 3 software connected with 2695 PDA detector. The in- house manufactured sample batches and placebo were used for the development and validation studies. The developed method was applied for the analysis of some marketed samples like TORGLIP M 50/1000, and TORGLIP 50/500.

Method Optimization: Method development trials were initiated by using monobasic phosphate buffer with ion pair reagents like hexane sulfonic acid , octane sulphonic acid sodium salt at different pH used as mobile phase A and acetonitrileasa mobile phase B, but the noisy base line and unwanted peaks was observed. Inertsil ODS 250 x 4.6 mm, 5 µ; BDS Hypersil, C18, 250 x 4.6 mm, 5µ; Baker bond C18, 250 x 4.6 mm, 5µ HPLC columns were tried to achieve the desire resolution between impurities. Finally 40 mm of ammonium dihydrogen orthophosphate buffer with 1.0

g/loctane sulfonic acid at pH 4 was selected as a buffer for mobile phase A. The mixture of buffer and methanol in the ratio of 88:12 was selected as mobile phase A and mixture of methanol and buffer in the ratio of 95:5 was used as a mobile phase B, which significantly improved retention of MTF and resolution between the VIL impurities on Hypersil BDS C8, 250 x 4.6 mm, 5µ HPLC column operated at 35°C. The gradient was optimized for better separation and chromatograms were monitored at 210 nm based on the optimum response of impurities and principle the analytes.

Chromatographic Condition: Accurately weighed 4.6 g ammonium dihydrogen orthophosphate, and 1g octane sulfonic acid sodium salt was transferred in 1 L HPLC grade water. The mixture was mixed well to dissolved, then sonicated and filtered through 0.45µ filter. The pH was adjusted to 4.0±0.1 with dilute orthophosphoric acid and used as buffer. The well mixed and degassed mixture of buffer and methanol in the ratio of 88:12 %v/v was used as mobile phase A. Degassed mixture of methanol and buffer in the ratio of 95:5 was used as mobile phase B. The mobile phase was pumped at 0.8ml/ minutes through.

BDS Hypersil C8, 250x4.6 mm, 5 µ columns which was operated at 35°C. The 10µl injection volume was load in to the column and the generated chromatograms were interpreted at 210 nm. The gradient as shown in **Table 1** was optimized for better separation.

Diluent: The mixture of water: methanol in the ratio of 80:20% v/v used as diluent.

TABLE 1: GRADIENT PROGRAM

Time in Minutes	% Mobile phase A	% Mobile phase B
0.1	100	0
15.0	100	0
50.0	80	20
65.0	80	20
65.1	100	0
80.0	100	0

Standard Preparation:

Preparation of MTF Standard Stock Solution: Accurately weighed 5 mg of MIF working standard was transferred into a 100 ml volumetric flask; about 70 ml of diluent was added and sonicated with intermittent shaking under controlled room temperature. The volume was made up to the mark with diluent.

Preparation of VIL Standard Stock Solution: Accurately weighed 10 mg of VIL working standard was transferred into a 100 ml volumetric flask; about 70 ml of diluent was added and sonicated with intermittent shaking under controlled room temperature. The volume was made up to the mark with diluent.

Preparation of Mix Standard Solution: (5ppm of MTF and 10 PPM of VIL): Mix standard was prepared by adding 5 ml of MTF standard stock solution and 5 ml of VIL standard stock solution in a 50 ml volumetric flask and adjusting the volume with diluent.

Preparation of MTF Impurity Stock Solution: Accurately weighed about 2.5 mg each, Melamine impurity, Cyanoguanidine, MTF related compound B, MTF Related compound C was transferred into a separate 10 ml volumetric flasks, dissolved by sonication and diluted up to the mark with diluent.

Preparation of VIL Impurity Stock Solution: Accurately weighed about 2.5mg each of VIL impurity A, B, C, and D, was transferred into a separate 10 ml volumetric flasks, dissolved by sonication and diluted up to the mark with diluent.

Preparation of System Suitability Solution: In a 50 ml volumetric flask, pipette 1 ml each of Cyanoguanidine, Melamine, MTF related compound B, and MTF related compound C, 5 ml MTF standard stock solution and 2 ml each of VIL Impurity A, B, C, D and 5 ml of VIL standard stock solutions and diluted up to the mark with diluent. The volume was made up the mark with diluent, mixed well and used as a system suitability solution.

Sample Solution Preparation: Tablet powder equivalent to 1 tablet was transferred into a 100 mL volumetric flask. About 70 mL of diluent was added, mixed well and sonicated for 20 minutes under controlled room temperature with intermittent shaking. The volume was made up to the mark with diluentand centrifuged the solution for 10 minutes at 5000 rpm. Then filtered through

0.45µ filter by discarding 5 ml of filtrate and injected.

System Suitability Criteria: The relative retention times for all the known impurities were calculated and listed in **Table 2**. The similarity factor between standard -1 and standard -2 should be within 95%-105%. The theoretical plates should not be less than 2000 and tailing factor not more than 2 for both the analytes in standard 1 and 2.

Impurity Calculations: The known impurities were calculated against respective drug standard area from standard solution 1. The unknown impurities were confirmed by injecting individual MTF and VIL API with placebo during FD study. Hence the rest of all unknown impurities were calculated against the MTF standard area from standard solution 1 as most of the generated unknown impurities were from MTF.

Analytical Method Validation: Analytical method validation for the developed method was carried out as per ICH guideline in terms of Accuracy, Precision, selectivity, Linearity, LOD/ LOQ and range.

Accuracy: Recovery of impurities in presence of active, placebo plays a vital role to assess the level and extent of recovery and extraction efficiency of the analytical method. Accuracy in terms of recovery for all the known impurities of MTF and VIL as well as principle analytes was done by spiking in the placebo from LOQ, 50%, 100% and 150% of specification level. All the preparations were injected in triplicate injections in to the HPLC system and recovery was calculated at each level.

Precision: In the precision study, the standard -1and Standard-2 were injected and calculated the similarity factor for both the analytes. The limit of similarity factor was set 95 to 105%.

Method Precision and Intermediate Precision: Six samples were separately prepared, injected and %impurities were calculated. The relative standard deviation of % individual known impurities, single maximum impurity and total impurities were calculated from the six sample preparations. The intermediate precision was conducted as like method precision but by different the analyst, different date, HPLC system, and column lots. Same study was repeated by spiking the known impurities at 100% level in the sample preparations.

Selectivity: Selectivity of the developed method was checked by specificity and by forced degradation study.

Specificity: Specificity of the method was checked by selectivity and force degradation study. In the selectivity all the individual solutions of each impurities and principle analytes were injected and confirmed the any interference from placebo, diluent at the retention time of each analytes.

Force Degradation Study: The stress study was applied for the developed method to check the stability indicating nature of the method. The sample, placebo and diluent were treated in acid, base, peroxide, condition, and thermal conditions. For acidic degradation 0.05NHCl at room temperature 6 Hrs and for base degradation study

0.05 N NaOH at room temperature for 15 minutes, were optimized. The solutions were neutralized; volume was adjusted and injected to check the degradation. The sample, placebo was kept at 60°C for 7 days in thermal degradation, after 7 days the samples were removed, prepared as per method of analysis and injected. Oxidation stress study was optimized by adding10% of total volume 30 % H_2O_2 and the solutions were kept at room temperature for 12Hrs. The mass balance was checked at every condition by using assay method and it was controlled between 95 to 105% of initial value.

LOD and LOQ: LOQ value was considered where the single to noise ratio (S/N) is more than 10 and for LOD it was consider 3. Limit of detection and limit of quantitation was determine by injecting minimum four levels below 50% level of working concentration. Each level was injected triplicate and LOD/LOQ was calculated by $10\sigma/S$ and $3\sigma/S$ method as per ICH guideline.

Linearity and Range: Linearity study prove that the method is capable to accurate measure the response of impurities and analytes which is directly proportional to it concentration. The study was conducted at various levels from LOQ to 150%. All the impurities solution and standard

solution were spiked from LOQ to 150% and volume was makeup with diluent. Each level was injected in triplicate in the sequence and the correlation coefficient of each individual impurities as well as principle analytes was calculated and controlled by the limit r^2=0.998.

The range of the method was established from lower to upper limits in which the response is directly proportional to analytes concentration.

Robustness: Ability of the developed method to remains unaffected by deliberate changes in the critical method parameters. Robust of the method was proved by varying the method parameters like change in column temperature ±5°C, wave length ±3nm, flow rate ± 0.1 ml, organic composition in mobile phase ±5% etc. The system suitability parameters were checked at each condition.

Solution Stability: Solution stability of the prepared samples, impurity standard solution, working standard solutions was monitored at room temperature. The solutions were injected at different time intervals like initial, after 12, 24, 48 Hrs. and check the system suitability criteria and any significant variation in peak response under the curve.

RESULTS AND DISCUSSION:

Selectivity: All the known impurities MTF and VIL along with principle analytes were well separate from each other's and no interference was observed from placebo and diluent at the retention time of each impurities and principle analytes.

The representative chromatograms of diluent as blank, placebo, system suitability, and sample were shown in **Fig. 3** to **Fig. 6** respectively. The retention time and relative retention time were calculated for the each known impurity against its respective API as shown in **Table 2**.

The force degradation study shows that the no significant degradation hence the product is stable at thermal condition. In the basic condition 11.9% degradation was found while 19.5 % degradation observed in peroxide condition and 12.8% in degradation observed in acidic condition.

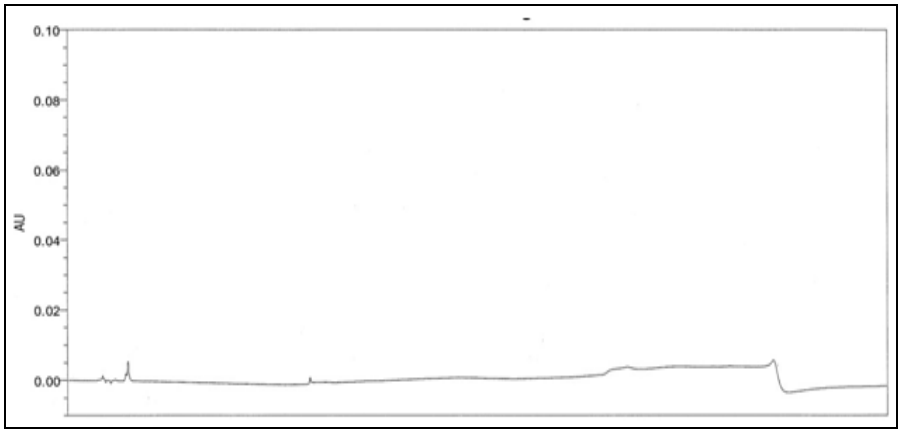

FIG. 3: REPRESENTATIVE CHROMATOGRAM OF DILUENT AS A BLANK

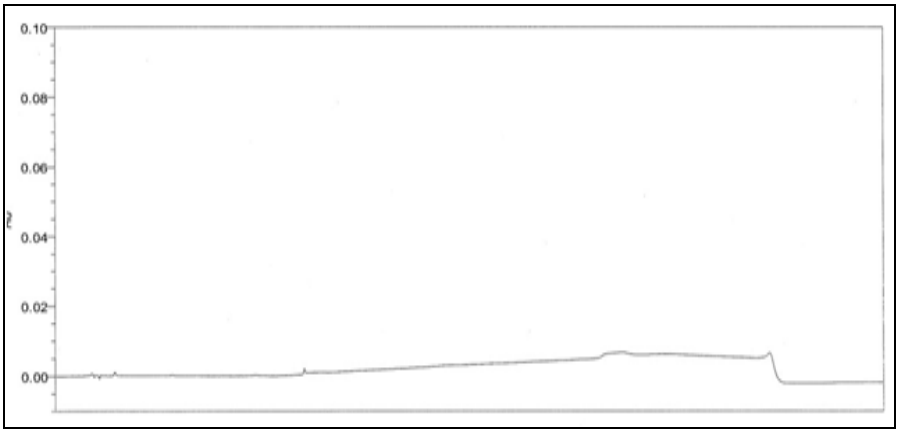

FIG. 4: REPRESENTATIVE CHROMATOGRAM OF PLACEBO

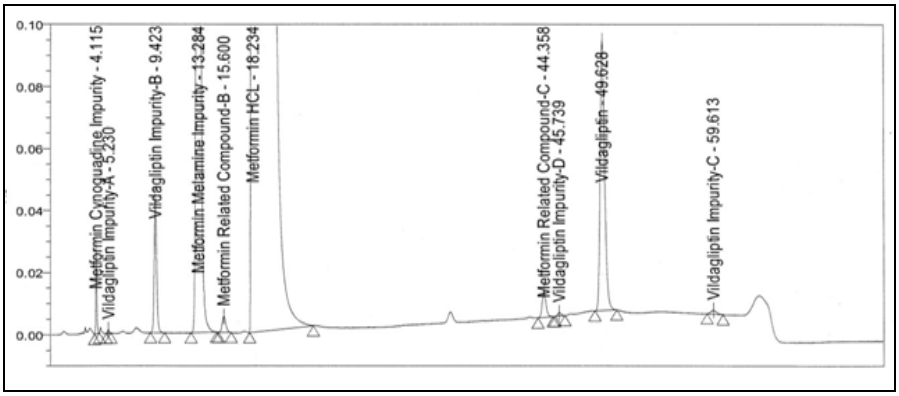

FIG. 5: REPRESENTATIVE CHROMATOGRAM OF SYSTEM SUITABILITY

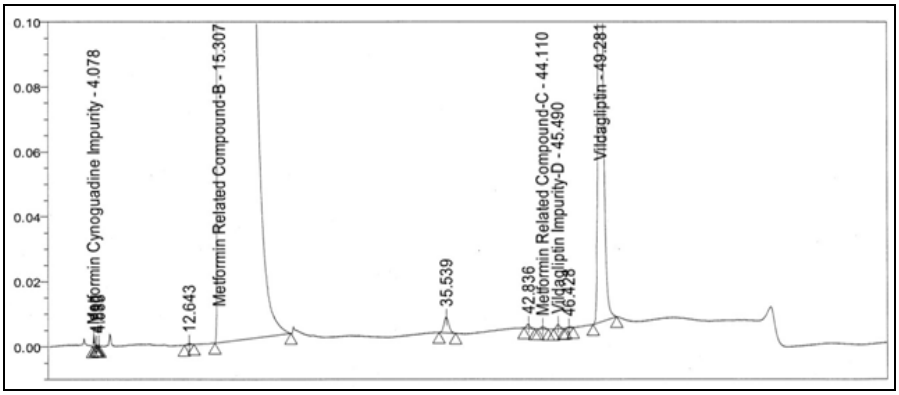

FIG. 6: REPRESENTATIVE CHROMATOGRAM OF SAMPLE

TABLE 2: RETENTION TIME AND RELATIVE RETENTION TIME OF IMPURITIES

Sr. no.	Name of Impurities	Retention time in minutes	RRT against respective API
1	Cyanoguanidine	4.1	About 0.22, w.r.t. MTF.
2	Melamine	13.2	About 0.78, w.r.t. MTF.
3	MTF Related Comp B	15.6	About 0.85, w.r.t. MTF.
4	Metformin HCL	18.2	1.0
5	MTF Related Comp. C	44.3	About 2.4, w.r.t. MTF.
6	Vil. Imp. A	5.2	About 0.105 w.r.t. VIL.
7	Vil. Imp. B	9.4	About 0.189 w.r.t. VIL
8	Vil. Imp. D	45.7	About 0.92 w.r.t. VIL
9	Vildagliptin	49.6	1.0.
10	Vil. Imp. C	59.6	About 1.20 w.r.t. VIL.

Precision: In precision study the % of each known impurity, highest unknown impurity and total impurities were calculated form the six sample preparations in method precision and six sample preparation in intermediate precision.

The % RSD of total impurities in method precision is 6.5% while in intermediate precision was found 4.8% which was well within the limit (NMT15.0%). All the known impurities were observed below detection limits while the % RSD of known impurities in spike study were found below 15% hence the developed method is precise.

Linearity: The method found linear to measure the accurate response of analytes over the range of LOQ to 150% of impurity limit. The correlation coefficient (r^2) for MTF and VIL is more than 0.998 as shown in **Fig. 7** and **8** the linearity plots for both the analytes.

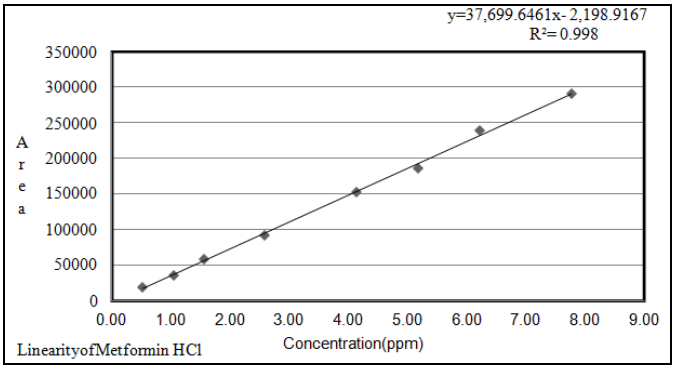

FIG. 7: LINEARITY OF METFORMIN HCL

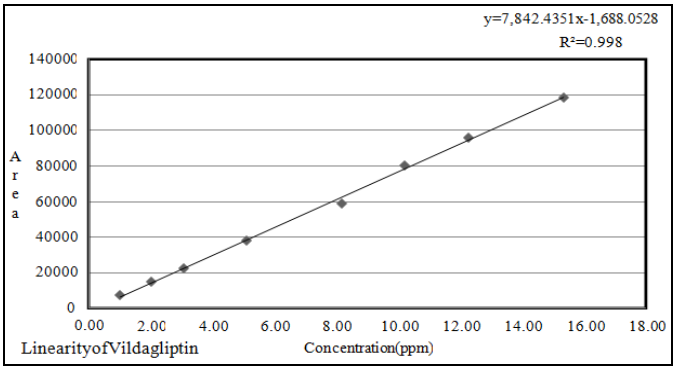

FIG. 8: LINEARITY OF VILDAGLIPTIN

Accuracy: Recovery of MTF and VIL in presence of placebo from LOQ to 150% was found between 85 to 115%. Hence, the developed method has ability to recover all the impurities as well as analytes in selected diluent and volume and accurately quantitate from LOQ to 150% as shown in **Table 3.**

TABLE 3: RECOVERY STUDY

Impurity name	LOQ Level	50 % Level	100% Level	150% Level
MTF	90.4	92.3	99.4	99.2
Melamine	104.1	106.3	105	106
Cyanoguanidine	104.8	106.5	100.8	103.2
MTF Related Comp B	89.2	92.1	92.1	91.1
MTF Related Comp C	94.5	88.3	91.4	85.5
VIL. Impurity A	89.6	92.3	91.5	90.6
VIL. impurity B	100.7	102	102.1	100.3
VIL. Impurity C	88.8	94	101.8	1006
VIL. Impurity D	95.7	104.7	103.6	103.2
VIL	90.5	94.5	99.6	98.3

LOD and LOD Determination: The LOD and LOQ values for MTF and VIL were calculated as per ICH guideline. The observed LOD and LOQ values of MTF is 0.33µg/ml and 1.03µg/ml where as for VIL is 0.19µg/ml and 0.57µg/ml.

Robustness and Solution Stability: During the robustness study, it was observed that no significant variation observed in system suitability criteria, like resolution, retention time and RRT. Hence the developed method is robust. In solution stability at room temperature the solutions were found stable up to 3 days at room temperature.

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