RESEARCH ARTICLE

INTERNATIONAL RESEARCH JOURNAL OF MULTIDISCIPLINARY TECHNOVATION

Asian Research Association

DOI: 10.54392/irjmt24323

Stability indicating for Quality Control Assessment of three antidiabetic molecules using HPLC technique: Stability Assessment of three antidiabetic molecules

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DOI: https://doi.org/10.54392/irjmt24323

Received: 02-01-2024; Revised: 27-02-2024; Accepted: 29-04-2024; Published: 23-05-2024

Abstract: Diabetes mellitus responds better to co-formulated tablet dosages of dapagliflozin (DFZN), metformin (MFMN), and vildagliptin (VDGN). For the purpose of studying stability and quantifying DFZN, VDGN, and MFMN in bulk forms and in dosage forms, an efficient and fast HPLC method of analysis is currently developed. The mobile phase featured 80:20 (*v/v*) 0.2 M, pH 3.0, ammonium acetate buffer and acetonitrile mixed together and Luna's HPLC C-18 column, named Phenyl hexyl, was utilised for DFZN, VDGN, and MFMN separation and its quantification. The PDA type detector operating at 235 nm wavelength was deployed. The "International Conference on Harmonisation" recommendations were strictly adhered throughout the validation process. A strong linear association between response and quantity in the range of 2.5–15 µg/ml (DFZN), 25–150 µg/ml (VDGN), and 125–750 µg/ml (MFMN) is supported by the regression information for the DFZN, VDGN, and MFMN calibration plots. The precision, selectivity, accuracy, sensitivity, ruggedness and robustness were satisfactory for the method. The tablet sample of DFZN, VDGN, and MFMN was subjected to acid, water, base, sodium bisulfite, light, dry heat and peroxide degradations. Significant differences in retention times were observed between the well-resolved peaks of the degradants and the primary peaks (DFZN, VDGN, and MFMN). Thus, the assay might be characterised as stability indicating. The contents of DFZN, VDGN, and MFMN in dosage forms were assessed precisely and accurately by currently developed HPLC technique. This technique may be applied to ensure the quality of formulation doses for DFZN, VDGN, and MFMN contents.

Keywords: Diabetes, Anti-Diabetic Molecules, Stability Indicating Assay, HPLC, Stress Degradation

1. Introduction

A chronic metabolic disease titled diabetes mellitus is marked by continuing hyperglycemia [1]. It has been reported that 415 million persons aged 20 to 79 had diabetes mellitus during 2015. This information comes from the "International Diabetes Federation" [2, 3]. Since this number is predicted to increase to an additional 200 million by 2040, diabetes is proving to pose a burden on worldwide public healthcare [2, 3]. In those suffering from diabetes mellitus, chronic hyperglycaemia can exacerbate other metabolic abnormalities and harm multiple organ systems, including retinopathy, nephropathy, and neuropathy. It may additionally result in macrovascular complications, which may elevate the chances of cardiovascular disorders by two to four times [4, 5].

In diabetic individuals, the increases in blood glucose quantities can usually be multifactorial [6, 7]. As such, attempting to restore normal blood glucose quantities by influencing the activity of a single hypoglycemic mechanism is challenging. As a result, various single medication combinations are now utilized as an appealing strategy for the medical management of diabetes [8]. Getting blood glucose quantities into the acceptable range without experiencing uncomfortable side effects is the main focus of every antidiabetic therapy, and this could be achieved by combining medications with multiple strategies of action. The need to develop appropriate and effective analytical techniques for the simultaneous assessment of the co-administered pharmaceutical molecules increases due to this treatment trend.

The co-formulated tablet doses of vildagliptin (VDGN), metformin (MFMN), and dapagliflozin (DFZN) have been displayed to be better effective in decreasing blood glucose quantities compared with individual drug dosages [9]. Respective structures and chemical names of DFZN, VDGN, and MFMN are displayed in Figure. 1 [10-12].

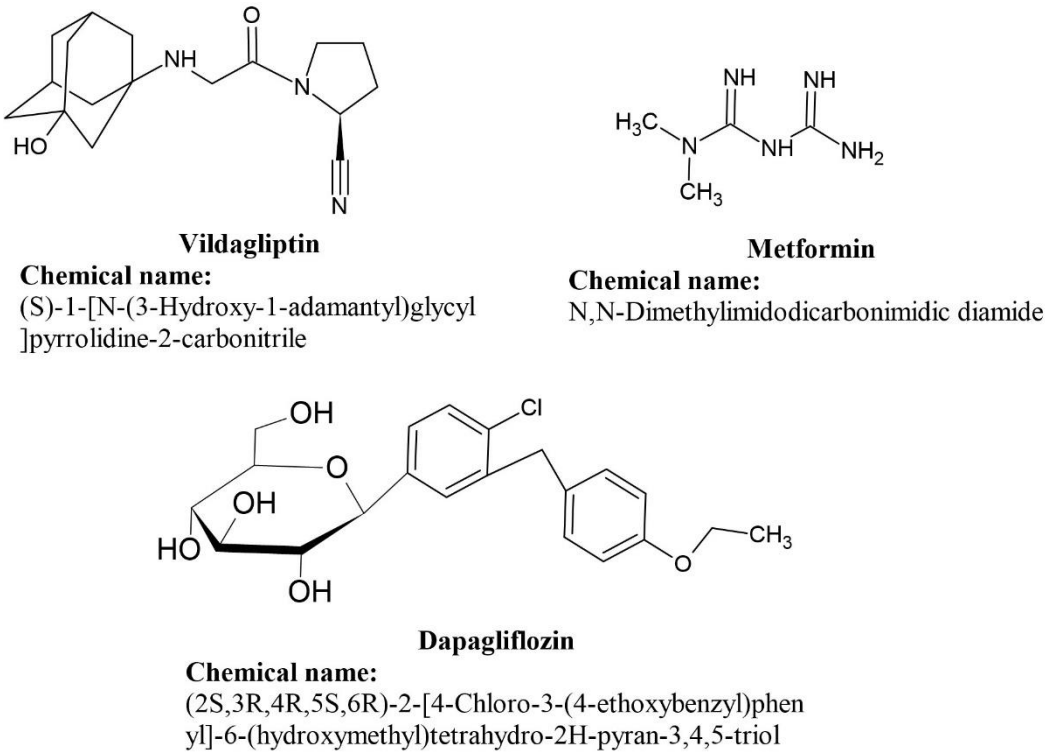


Figure 1. Structures and chemical names of drugs of investigation

The hypoglycemic effects of these three medications (DFZN, VDGN, and MFMN) are produced by distinct mechanisms. With the DFZN, blood glucose quantities are lowered through increasing urine glucose excretion [13]. By raising the body's incretin levels, which induce the pancreas to build up more insulin, the VDGN aids with blood glucose regulation [14]. In cases of hyperglycemia, the VDGN further instructs the liver to cease synthesising glucose. Food-derived glucose absorption and hepatic glucose synthesis are both reduced by the MFMN [15, 16]. Additionally, the MFMN strengthens the body's responsiveness to insulin, a naturally occurring chemical that manages blood glucose quantities.

As a result, it's critical to possess a reliable analytical methodology that could be implemented to analyze various medications together. Also, the analysis need to hold applicable to pharmaceutical dose formulations. Additionally, the analysis must be reliable when respective degradation products are present [17-20]. Separations using HPLC offer a number of benefits, including quick analytical times. Furthermore, it only needs less injection quantities (in microliters quantity). Additionally, minimal solvent is used, and no previous extraction processes need to be done [21-23]. To far, no study has yet been published on the combined analysis of DFZN, VDGN, and MFMN using any kind of strategy.

Thus, the key objective of this research is to establish an efficient RP-HPLC technique that uses simple mobile phase components to determine DFZN, VDGN, and MFMN simultaneously despite the presence of respective degradation products and in their tablet forms, resulting in a safe analytical approach. It should therefore be implemented for regular quality control examination of DFZN, VDGN, and MFMN. It can additionally be exploited to indicate stability in DFZN, VDGN, and MFMN analyses.

2. Materials and Methods

2.1. Instruments

The "Waters" HPLC system was the device put to use for DFZN, VDGN, and MFMN analysis. The "Waters" PDA system was the device that was executed to detect DFZN, VDGN, and MFMN. Selected reactions were conducted in solution while the stress degradation analysis by using an accurate water bath fitted with an internal temperature controller. An investigation of thermal stability on DFZN, VDGN, and MFMN was done in a thermostatic enclosed hot air oven. Analytical balances and sonicators were among the other instruments employed. For data processing in the current study project, “Waters” Empower software 2® was utilized.

2.2. Chemicals Requirements

Ammonium acetate (analytical category), phosphoric acid (analytical category), acetonitrile (HPLC category), distilled water (HPLC category) were used. All of the above came from India's “Merck Life Science Private Limited”. Water was collected using the Milli Q

apparatus. Vylda DM® tablets ("Emcure Pharmaceuticals Ltd", India) with DFZN (10 mg), VDGN (100 mg), and MFMN (500 mg) labelled content were used. The samples of DFZN, VDGN, and MFMN were collected from "Biocon Limited" India.

2.3 Chromatographic conditions

The mobile phase featured 80:20 (*v/v*) 0.2 M, pH 3.0, ammonium acetate buffer and acetonitrile mixed together and filtered using an 0.45 µ membrane sieve. Luna's HPLC C-18 column, named Phenyl hexyl, was utilised; its physical dimensions were 4.6 mm × 250 mm and 5 µ. With a 10 µl injection volume, the mobile phase transfer rate was preset at 1.0 ml/min and the column's temperature stayed constant with ambient. The PDA type detector operating at 235 nm wavelength was deployed to measure the analyte (DFZN, VDGN, and MFMN) concentrations.

2.4. Solutions

A 100 ml volumetric flask was loaded with precisely weighed quantities of DFZN (10 mg), VDGN (100 mg), and MFMN (500 mg). The materials were dissolved and subsequently diluted to the appropriate level using methanol. This is stock DFZN (100 µg/ml), VDGN (1000 µg/ml), and MFMN (5000 µg/ml) solution. Another 100 ml volumetric flask was loaded with 10 ml of this is stock DFZN, VDGN, and MFMN solution, which was then subsequently diluted with methanol to the appropriate level. This constitutes working DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) solution.

2.5. Forced Degradation Studies

Stress experiments were conducted pursuant to ICH-recommended circumstances to assess the stability indicating feature of the newly proposed HPLC technique [24]. The tablet Vylda DM® sample (DFZN - 100 µg/ml, VDGN - 1000 µg/ml and MFMN - 5000 µg/ml) was tested against the following kinds of stress conditions in an attempt to cause intentional degradation: acid (1.0 N HCl at 60 °C for reaction time of 15 min; 30 min; 45 min; 60 min), base (1.0 N NaOH at 60 °C for reaction time of 15 min; 30 min; 45 min; 60 min), oxidation (10% H_2O_2 at 60 °C for reaction time of 15 min; 30 min; 45 min; 60 min), water (distilled water at 60 °C for reaction time of 15 min; 30 min; 45 min; 60 min) dry heat (105 °C, for exposure period of 2 hr; 4 hr; 6 hr; 8 hr), reduction (sodium busulfite at 60 °C for reaction time of 15 min; 30 min; 45 min; 60 min) and UV light (320 nm to 400 nm range, for exposure period of 1 hr; 2 hr; 3 hr; 4 hr). It was investigated if the suggested strategy could evaluate the analytes (DFZN, VDGN, and MFMN) responses while its degradation products were present. Additionally, this will be applied for testing the consistency of DFZN, VDGN, and MFMN when administered with deliberate degradations.

2.6. Assay of DFZN, VDGN, and MFMN marketed formulation

After precisely weighing twenty Vylda DM® pills to determine their mean weight, they were crushed in a pestle and mortar around ten minutes to turn them into a fine powder. A precisely measured amount of tablet powder, about equal to 10 mg of DFZN, 100 mg of VDGN, and 500 mg of MFMN, was put into a 100 ml volumetric flask, into which 30 ml of mobile phase was included. The flask's contents were thereafter sonicated for approximately 15 minutes, and mobile phase was then included to bring the volume equal to the desired level. This is stock Vylda DM® solution (DFZN - 100 µg/ml, VDGN - 1000 µg/ml and MFMN - 5000 µg/ml). A membrane sieve with a thickness of 0.45 µ was used to filter the stock Vylda DM® solution. Using mobile phase again, an aliquot holding 10 ml of stock Vylda DM® solution was diluted to 100 ml. To acquire the results, the resultant solution (10 µl) was put on to HPLC column, chromatograms were established and an assessment of DFZN, VDGN, and MFMN in Vylda DM® tablets using PDA was carried out.

2.7. Placebo solution

A placebo constituted of a homogenous powder comprising 40 mg each of stearate (a lubricant), lactose (a binder), hydroxyl cellulose (a binder), magnesium sodium alginate (a lubricant), starch (a binder), ferric oxide yellow (a colorant), and talc (a lubricant). A precise 20 mg placebo homogenous powder was utilised, and the extract was made according to the tablet extract preparation.

3. Results and Discussion

3.1. Optimization Outcomes

Before the methodology validation, HPLC assay parameters for DFZN, VDGN, and MFMN were optimised ensuring a smoother elution procedure with improved separation performance. Two separate columns were put into use to investigate DFZN, VDGN, and MFMN peaks separation. One is Luna's HPLC C-18 column, named Phenyl hexyl; its physical dimensions were 4.6 mm × 250 mm and 5 µ. Another one is Scimadzu's HPLC C-18 column, named Symmetry; its physical dimensions were 4.6 mm × 250 mm and 5 µ. Luna's HPLC C-18 column adequately separated all peaks (Figure. 2), consequently it was picked for investigation.

The mobile phase combination was assessed by varying the solvents ratio.

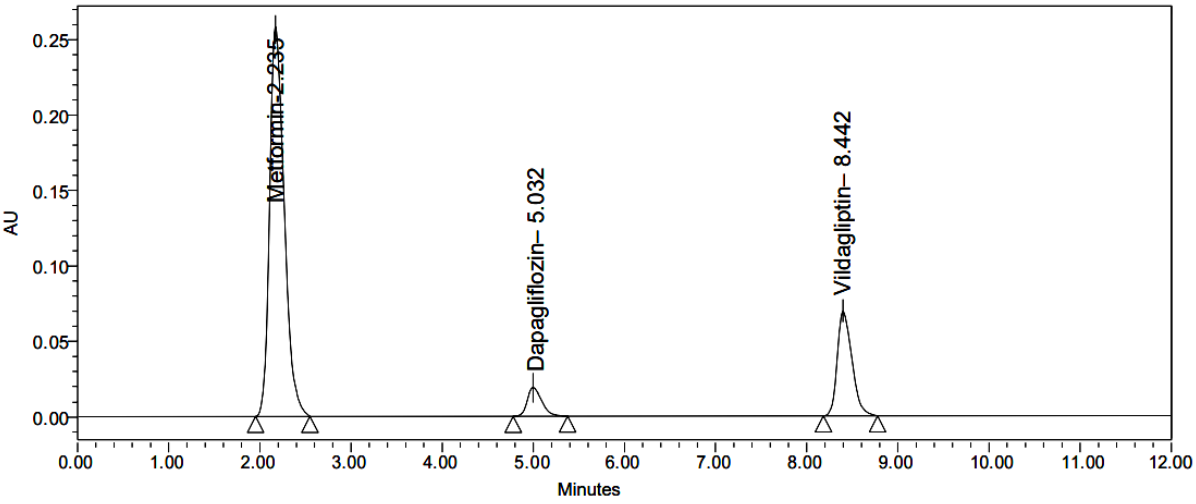


Figure 2. DFZN, VDGN, and MFMN chromatogram with optimized HPLC: DFZN, VDGN, and MFMN - assay parameters

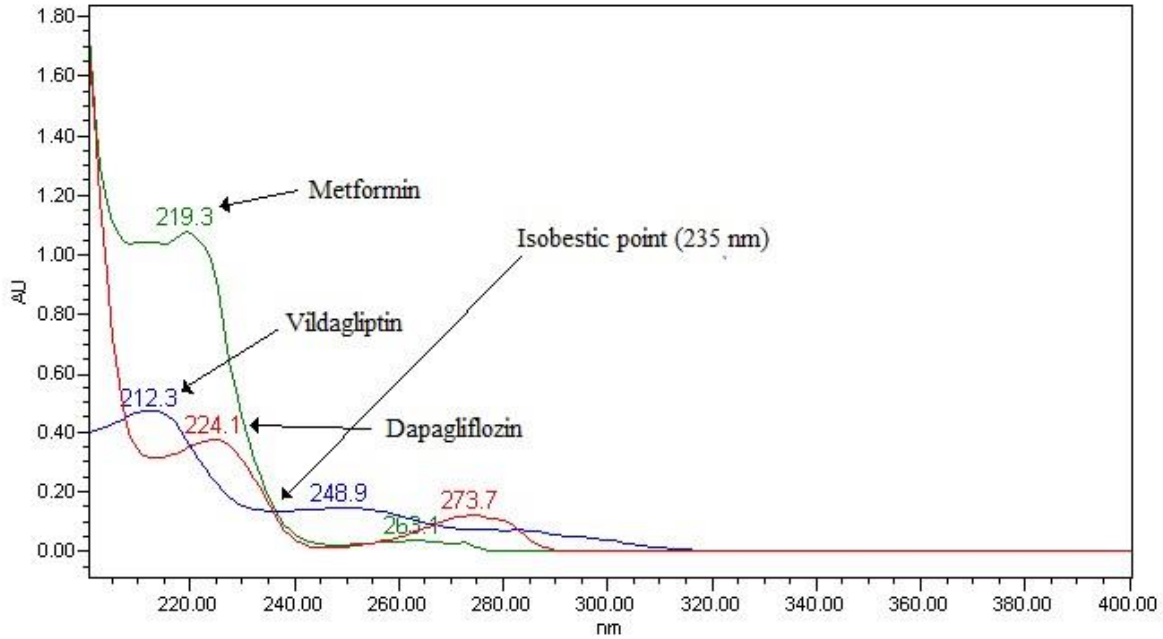


Figure 3. DFZN, VDGN, and MFMN ultra violet (UV) spectra

The solvent mixtures investigated were: acetonitrile with 0.1% (pH 3.0) trifluoroacetic acid and acetonitrile with 0.2 M (pH 3.0) ammonium acetate buffer. The mobile phase featured 80:20 (*v/v*) 0.2 M, pH 3.0, ammonium acetate buffer and acetonitrile mixed together resulted in good sensitivity and resolution (Figure. 2). It became apparent that using ambient temperature and flow rate of 1.0 ml a min produced finest peak separations (Figure. 2) as well as good reproducibility. A scan of DFZN, VDGN, and MFMN solutions was performed over a wavelength spanning of 200 to 400 nm. The isobestic point of the DFZN, VDGN, and MFMN was spotted at 235 nm (Figure. 3), consequently it was picked for DFZN, VDGN, and MFMN investigation.

3.2. Method validation

The validation elements were tested in tandem with the ICH's suggested directives for completing the method validation procedures [25].

3.3. Linearity

By taking measurements at six concentration levels in a span of 2.5-15 mg/ml (DFZN), 25-150 mg/ml (VDGN) and 125-750 mg/ml (MFMN), the linear connection concerning peak areas and drug concentrations were assessed over an array of concentrations represented in mg/ml. For DFZN, VDGN, and MFMN, the correlation coefficient values of 0.99996, 0.99995 and 0.99989, respectively demonstrated a linear pattern that was deemed acceptable.

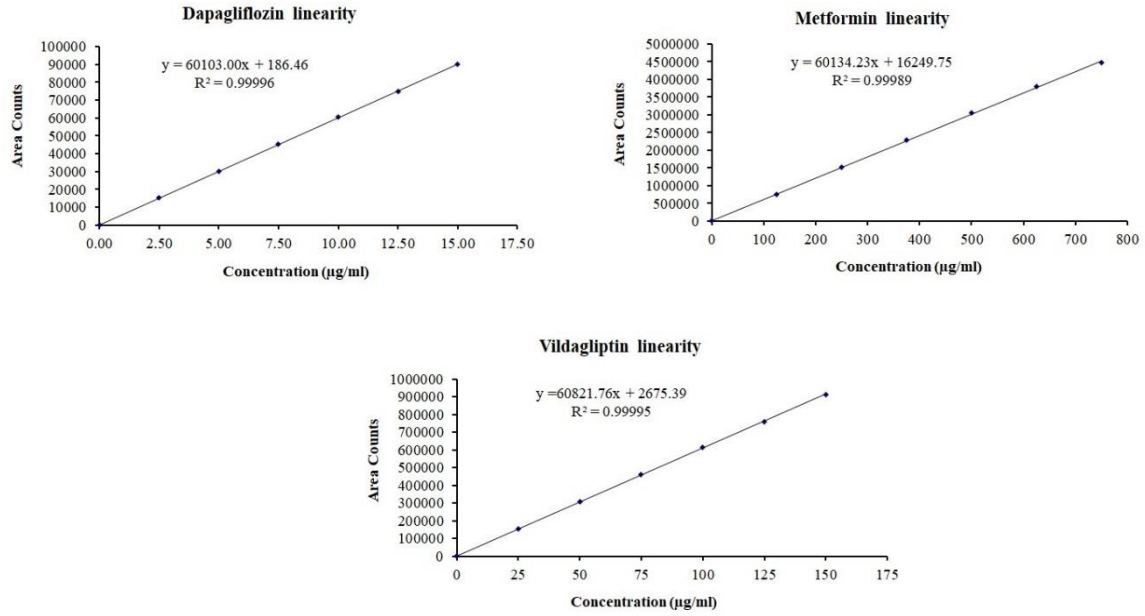


Figure 4. Regression equation and linearity curves

The assay's most accurate and consistent range is 2.5-15 mg/ml (DFZN), 25-150 mg/ml (VDGN) and 125-750 mg/ml (MFMN). For DFZN, VDGN, and MFMN, respectively, Figure. 4 shows their linearity and also regression equations.

3.4. Robustness

Essential investigative parameters, such as the column chromatographic temperature and the proportion of organic modifier solvent, were changed so as to show robustness. This resulted in a change in the flow rate to 1.1 ml/min and 0.9 ml/min from 1.0 ml/min and a change in the volume percent ratio of acetonitrile to 15% vol. and 25% vol. from 20% vol. As a means to evaluate robustness, amounts of DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) in diluent solution were used. The effects were expressed in assay percentile of DFZN, VDGN, and MFMN in modified procedures. By modifying the volume percent ratio of acetonitrile, the percent assays were 100.434 and 100.329% for DFZN, 100.381 and 99.837% for VDGN, and 100.252 and 100.933% for MFMN. During change in flow rate, the percent assays were 100.704 and 100.534% for DFZN, 100.198 and 100.469% for VDGN, and 100.970 and 100.030% for MFMN (Table 1). By demonstrating that observed values remain consistent even as chromatographic parameters like column chromatographic temperature and the proportion of organic modifier solvent are changed, robustness can be verified.

3.5. Accuracy

By repeatedly (n = 3 per level) evaluating the tablet Vylda DM® sample spiked with different three measures of DFZN, VDGN, and MFMN, the recovery of DFZN, VDGN, and MFMN in a simulated tablet Vylda DM® sample shows the accuracy of DFZN, VDGN, and MFMN extraction from tablet as well as sample processing. Percentage recovery is a means for reporting accuracy characteristics. The percent mean (n = 3 per level) recovery was 100.633% for DFZN (Table 2), 99.733% for VDGN (Table 3), and 100.367% for MFMN (Table 4). Good recoveries in a sample of spiked tablets demonstrated unequivocally that other excipient components of tablets have no influence on the extraction as well as evaluation of DFZN, VDGN, and MFMN.

3.6. Precision

The precision of the chromatographic evaluation of DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) in diluent solution (n = 6) and in tablet Vylda DM® sample (n = 6) was assessed. The RSD% of measurements (n = 6) made in a single day is used to express precision. The novel method's precision for DFZN, VDGN, and MFMN was found to be inside of the acceptable range of less than 2.0%, with an RSD percentage ranging from 0.4400% to 0.8234 % (Table 5) for system precision and 0.3295% to 0.7342% (Table 5) for method precision.

3.7. Ruggedness

The ruggedness of the chromatographic evaluation of DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) in diluent solution (n = 6) was assessed. By analysing the DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) in diluent solution six times over the course of two days in two separate labs (n = 6 per day, two days), ruggedness was evaluated.

Table 1. DFZN, VDGN, and MFMN robustness reports

Condition varied	DFGN assayed (%)	VDGN assayed (%)	MFMN assayed (%)	Condition varied	DFGN assayed (%)	VDGN assayed (%)	MFMN assayed (%)
Flow (-), 0.9 ml/min	100.918	99.975	101.891	Flow (+), 1.1 ml/min	100.905	100.742	99.791
	100.438	100.471	99.875		100.253	100.137	101.000
	100.756	100.148	101.143		100.445	100.528	99.299
Average[a]	100.704	100.198	100.970	**Average[a]**	100.534	100.469	100.030
SD[b]	0.2442	0.2518	1.0191	**SD[b]**	0.3351	0.3068	0.8753
RSD[c]	0.2425	0.2513	1.0093	**RSD[c]**	0.3333	0.3054	0.8750
Condition varied	**DFGN assayed (%)**	**VDGN assayed (%)**	**MFMN assayed (%)**	**Condition varied**	**DFGN assayed (%)**	**VDGN assayed (%)**	**MFMN assayed (%)**
Acetonitrile ratio (-), 15% vol.	100.294	100.835	100.465	Acetonitrile ratio (+), 25% vol.	100.658	99.936	101.559
	100.757	100.232	99.708		100.518	99.452	100.210
	100.434	100.077	100.582		100.329	100.124	101.029
Average[a]	100.495	100.381	100.252	**Average[a]**	100.502	99.837	100.933
SD[b]	0.2375	0.4005	0.4744	**SD[b]**	0.1651	0.3467	0.6796
RSD[c]	0.2363	0.3990	0.4732	**RSD[c]**	0.1643	0.3473	0.6733

a -Average of three values; b - standard deviation of three values; c - relative standard deviation

Table 2. DGFN accuracy reports

Level (%)	Added DFZN (µg/ml)	DFGN area	Found DFZN (µg/ml)	Recovered DFZN (%)
50% fortified	5.00	30556	5.054	101.086
	5.00	30348	5.020	100.398
	5.00	30715	5.081	101.612
100% fortified	10.00	60612	10.026	100.260
	10.00	60478	10.004	100.038
	10.00	60848	10.065	100.650
150% fortified	15.00	91254	15.095	100.630
	15.00	92361	15.278	101.851
	15.00	90191	14.919	99.458
Average[a]				100.633
SD[b]				0.3512
RSD[c]				0.3490

a -Average of nine values; b - standard deviation of nine values; c - relative standard deviation

Table 3. VDGN accuracy reports

Level (%)	Added VDGN (µg/ml)	VDGN area	Found VDGN (µg/ml)	Recovered VDGN (%)
50% fortified	50.00	307481	49.84	99.680
	50.00	309944	50.24	100.480
	50.00	306958	49.76	99.520
100% fortified	100.00	617422	100.09	100.090
	100.00	619587	100.44	100.440
	100.00	613510	99.45	99.450
150% fortified	150.00	916958	148.64	99.093
	150.00	918748	148.93	99.287
	150.00	919891	149.12	99.413
Average[a]				99.733
SD[b]				0.3786
RSD[c]				0.3796

a -Average of nine values; b - standard deviation of nine values; c - relative standard deviation

Table 4. MFMN Accuracy Reports

Level (%)	Added MFMN (µg/ml)	MFMN area	Found MFMN (µg/ml)	Recovered MFMN (%)
50% fortified	250.00	1520463	249.46	99.784
	250.00	1549108	254.16	101.664
	250.00	1535847	251.98	100.792
100% fortified	500.00	3055155	501.25	100.250
	500.00	3039943	498.75	99.750
	500.00	3061958	502.37	100.474
150% fortified	750.00	4583594	752.02	100.269
	750.00	4568586	749.55	99.940
	750.00	4591540	753.32	100.443
Average[a]				100.367
SD[b]				0.2887
RSD[c]				0.2876

a -Average of nine values; b - standard deviation of nine values; c - relative standard deviation

Table 5. DFZN, VDGN, and MFMN precision (system and method) reports

Drug →	**DFZN (10 µg/ml) precision**		**VDGN (100 µg/ml) precision**		**MFMN (500 µg/ml) precision**	
Precision →	**Method (%assay)**	**System (peak area)**	**Method (%assay)**	**System (peak area)**	**Method (%assay)**	**System (peak area)**
Test 1	100.106	60585	99.477	618544	99.923	3052647
Test 2	99.467	60184	100.155	612022	101.428	3013511

Test 3	99.601	60614	99.711	617484	100.781	3034154
Test 4	100.351	60255	100.361	619467	100.493	3054666
Test 5	99.841	60848	100.018	614658	99.276	3088744
Test 6	99.724	60246	100.487	619118	100.248	3041511
Average[a]	99.848	60455	100.035	616882	100.358	3047539
SD[b]	0.3290	265.9892	0.3851	2945.2451	0.7368	25092.3798
RSD[c]	0.3295	0.4400	0.3850	0.4774	0.7342	0.8234

a -Average of six values; b - standard deviation for six values; c - relative standard deviation

Table 6. DFZN, VDGN, and MFMN Ruggedness Experiment Reports

Test	**DFZN assay (%) at 10 µg/ml level**		**VDGN assay (%) at 100 µg/ml level**		**MFMN assay (%) at 500 µg/ml level**	
	Analyst 1 Lab 1	**Analyst 2 Lab 2**	**Analyst 1 Lab 1**	**Analyst 2 Lab 2**	**Analyst 1 Lab 1**	**Analyst 2 Lab 2**
1	100.106	100.145	99.477	99.456	99.923	100.199
2	99.467	99.699	100.155	99.851	101.428	99.171
3	99.601	100.425	99.711	100.216	100.781	100.031
4	100.351	99.502	100.361	99.656	100.493	100.856
5	99.841	100.293	100.018	99.385	99.276	99.054
6	99.724	100.645	100.487	100.280	100.248	99.500
Average[a]	99.983		99.921		100.080	
SD[b]	0.3952		0.3830		0.7393	
RSD[c]	0.3953		0.3833		0.7387	

a -Average of 12 values; b - standard deviation for 12 values; c - relative standard deviation for 12 values 0.3833% to 0.7387%

The RSD% of all measures (n = 12) serves as an indication of ruggedness. The novel method's ruggedness for DFZN, VDGN, and MFMN analysis was found to be inside of the acceptable range of less than 2.0%, with an RSD percentage ranging from 0.3833% to 0.7387% (Table 6).

3.8. Selectivity

We assessed the selectivity for chromatographic evaluation of DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) using the interference experiment parameter. It became apparent that the mobile phase nor excipients (placebo) were not the cause of any noteworthy or credible disturbances (as depicted in Figure. 5). The method's selectivity for the assessment of DFZN, VDGN, and MFMN is evident.

3.9. System suitability

The plate count, resolution, and tailing factor were evaluated using the same injection concentrations of DFZN (10 µg/ml), VDGN (100 µg/ml), and MFMN (500 µg/ml) in six repetitions, and the system suitableness was assessed. Following an analysis of six analytical replicates, it appears that there have been no statistically noteworthy variations in the responses of MFMN (Table 7), DFZN (Table 8), and VDGN (Table 9). The RSD (%) for system suitableness was assessed to be less than 2 (0.0798% to 1.9578% for DFZN; 0.0798% to 1.9578% for VDGN; 0.0443% to 1.9698% for MFMN), confirming that the device has a high level of precision.

3.10. Forced degradation study

Studies on stress degradation offer insights on the stability of pharmaceuticals (DFZN, VDGN, and MFMN) and their formulation products.

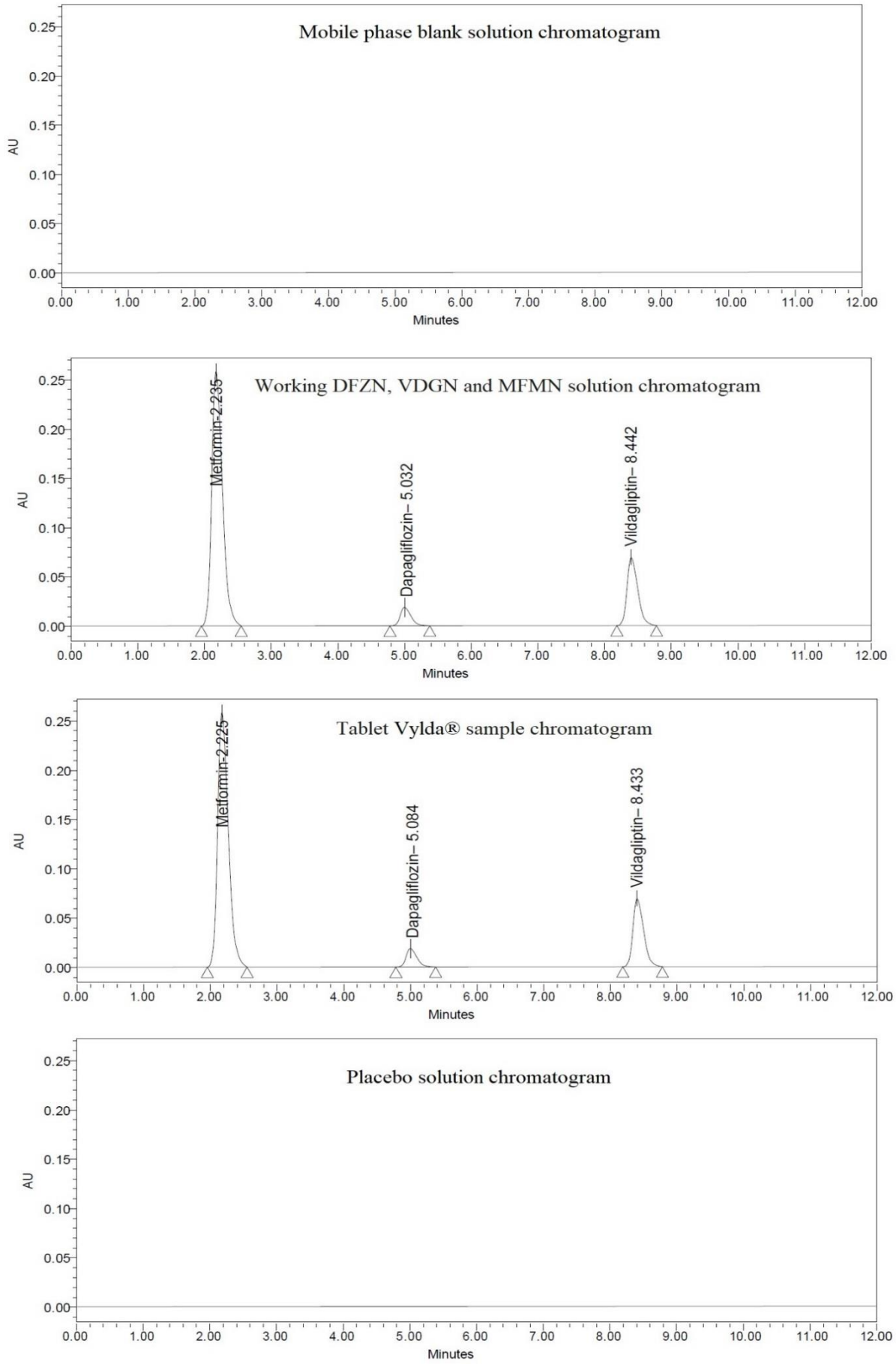


Figure 5. Chromatogram evidence for method's selectivity

Table 7. MFMN system suitability reports

Test	Tailing	Plate count	Retention time	Area
1	1.12	13524	2.235	3052647
2	1.14	13164	2.233	3013511
3	1.16	13365	2.231	3034154
4	1.13	13558	2.229	3054666
5	1.12	13854	2.227	3088744
6	1.16	13255	2.225	3041511
Average[a]	1.138	13453	2.230	3047539
SD[b]	0.0183	247.9255	0.0037	25092.3798
RSD[c]	1.6119	1.8429	0.1678	0.8234

a -Average of six values; b - standard deviation for six values; c - relative standard deviation

Table 8. DFZN System Suitability Reports

Test	Tailing	Plate count	Retention time	Area	Resolution
1	1.11	5695	5.032	60585	8.63
2	1.13	5693	5.035	60184	8.66
3	1.15	5690	5.037	60614	8.68
4	1.17	5688	5.039	60255	8.7
5	1.15	5686	5.041	60848	8.72
6	1.12	5683	5.043	60246	8.74
Average[a]	1.138	5689	5.038	60455	8.688
SD[b]	0.0223	4.4460	0.0040	265.9892	0.0402
RSD[c]	1.9578	0.0781	0.0798	0.4400	0.4628

a -Average of six values; b - standard deviation for six values; c - relative standard deviation

Table 9. VDGN system suitability reports

Test	Tailing	Plate count	Retention time	Area	Resolution
1	1.07	8647	8.442	618544	12.37
2	1.09	8649	8.444	612022	12.39
3	1.11	8651	8.446	617484	12.41
4	1.1	8649	8.448	619467	12.43
5	1.13	8647	8.45	614658	12.45
6	1.08	8645	8.452	619118	12.47
Average[a]	1.097	8648	8.447	616882	12.420
SD[b]	0.0216	2.0976	0.0037	2945.2451	0.0374
RSD[c]	1.9698	0.0243	0.0443	0.4774	0.3013

a -Average of six values; b - standard deviation for six values; c - relative standard deviation

It provides details on how stable the pharmaceuticals (DFZN, VDGN, and MFMN) are in situations of acidity, basicity, light, thermal, hydrolysis, reduction, and oxidation. It has a direct impact on the choice of formulation development, packaging, preservation, shelf life, drug stability, and drug product stability.

Forced degradation analysis serves as insights on the stabilities of DFZN, VDGN, and MFMN in acid (1.0 N HCl at 60 °C, reaction time 15 min; 30 min; 45 min; 60 min), base (1.0 N NaOH at 60 °C, reaction time 15 min; 30 min; 45 min; 60 min), oxidation (10% H_2O_2 at 60 °C, reaction time 15 min; 30 min; 45 min; 60 min), water (distilled water at 60 °C, reaction time 15 min; 30 min; 45 min; 60 min), dry heat (105 °C, exposure time 2 hr, 4 hr, 6 hr, 8 hr), reduction (sodium bisulfite at 60 °C, 15 min; 30 min; 45 min; 60 min) and UV light (320 nm to 400 nm range; exposure time 1hr, 2 hr, 3 hr, 4 hr) conditions. Tables 10 -12 summarises the forced degradation analysis findings. The degradation of DFZN, VDGN, and MFMN increased as time of exposure increases (Tables10-12). From results (Tables 10-12), DGFN and VDGN appear to be far more susceptible to oxidation (10% H_2O_2 at 60 °C), whereas MFMN proved to be more sensitive towards dry heat (105 °C) conditions. DGFN and MFMN appear to be far more stable to reduction (sodium bisulfite at 60 °C) condition, whereas VDGN proved to be more stable towards dry heat (105 °C) conditions.

Forced degradation was also used to measure method's specificity for the assessment of DFZN, VDGN, and MFMN. There was no interference of any additional peaks corresponding to the degradants generated across any of the stressed sample chromatograms (Figure. 6, Chromatograms A-G).

Table 10. Stabilities of DFZN in forced degradation analysis

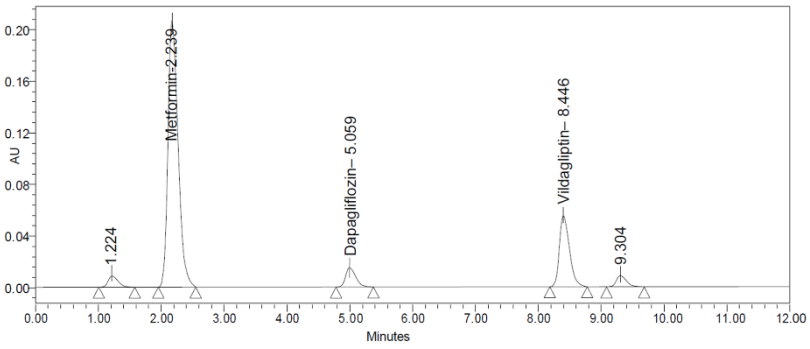
Accelerated condition	**S (%)**	**D (%)**	**S (%)**	**D (%)**	**S (%)**	**D (%)**	**S (%)**	**D (%)**
	At 15 min		At 30 min		At 45 min		At 60 min	
Acid	96.808	3.192	95.950	4.050	95.418	4.582	94.990	5.010
Peroxide	86.970	13.03	86.289	13.711	85.125	14.875	83.112	16.888
Reduction	98.402	1.598	98.228	1.772	97.669	2.331	97.669	2.331
Hydrolysis	95.681	4.319	95.056	4.944	94.453	5.547	93.765	6.235
Alkali	94.464	5.536	93.993	6.007	93.097	6.903	92.606	7.394
	At 2 hr		At 4 hr		At 6 hr		At 8 hr	
Thermal	98.904	1.096	97.973	2.027	97.439	2.561	96.041	3.959
	At 1 hr		At 2hr		At 3 hr		At 4 hr	
Photolytic	93.993	6.007	92.091	7.909	91.252	8.748	88.743	11.257

S – Stability in percentage; D – Degradation in percentage

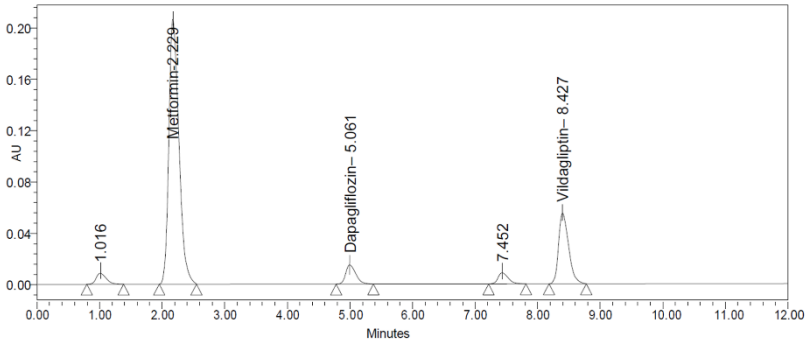
Table 11. Stabilities of VDGN in forced degradation analysis

Accelerated condition	**S (%)**	**D (%)**	**S (%)**	**D (%)**	**S (%)**	**D (%)**	**S (%)**	**D (%)**
	At 15 min		At 30 min		At 45 min		At 60 min	
Acid	88.201	11.799	87.649	12.351	85.587	14.413	83.147	16.853
Peroxide	85.45	14.574	84.722	15.278	82.863	17.137	80.456	19.544
Reduction	89.647	10.353	11.127	2.345	13.591	2.314	14.755	2.337
Hydrolysis	96.885	3.115	96.483	3.517	96.202	3.798	95.882	4.118
Alkali	91.568	8.432	91.032	8.968	88.744	11.256	87.365	12.635
	At 2 hr		At 4 hr		At 6 hr		At 8 hr	
Thermal	99.744	0.256	99.477	0.523	99.045	0.955	97.653	2.347
	At 1 hr		At 2hr		At 3 hr		At 4 hr	
Photolytic	90.128	9.872	87.533	12.467	85.633	14.367	83.690	16.310

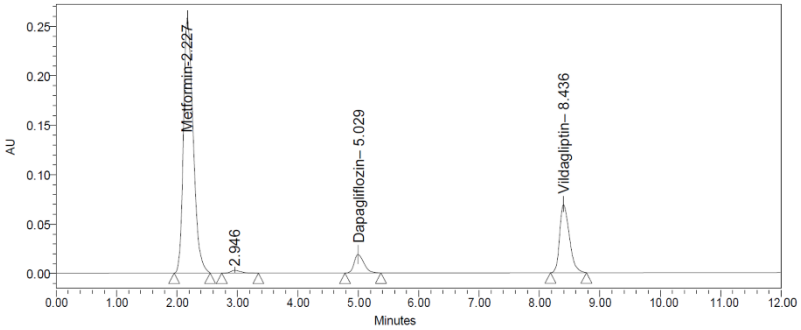
S – Stability in percentage; D – Degradation in percentage



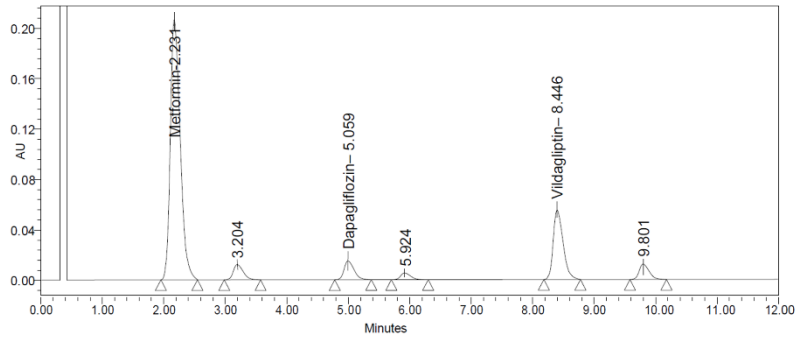
Chromatogram A: Acid (1.0 N HCl at 60 °C, 15 min reaction time) condition



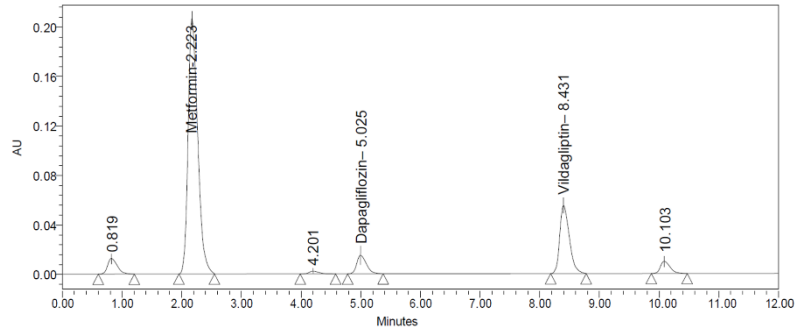
Chromatogram B: Alkali (1.0 N NaOH at 60 °C, 15 min reaction time) condition



Chromatogram C: Hydrolysis (Distilled water at 60 °C, 15 min reaction time) condition



Chromatogram D: Oxidation (10% peroxide at 60 °C, 15 min reaction time) condition



Chromatogram E: UV light (3 hr exposure time) condition

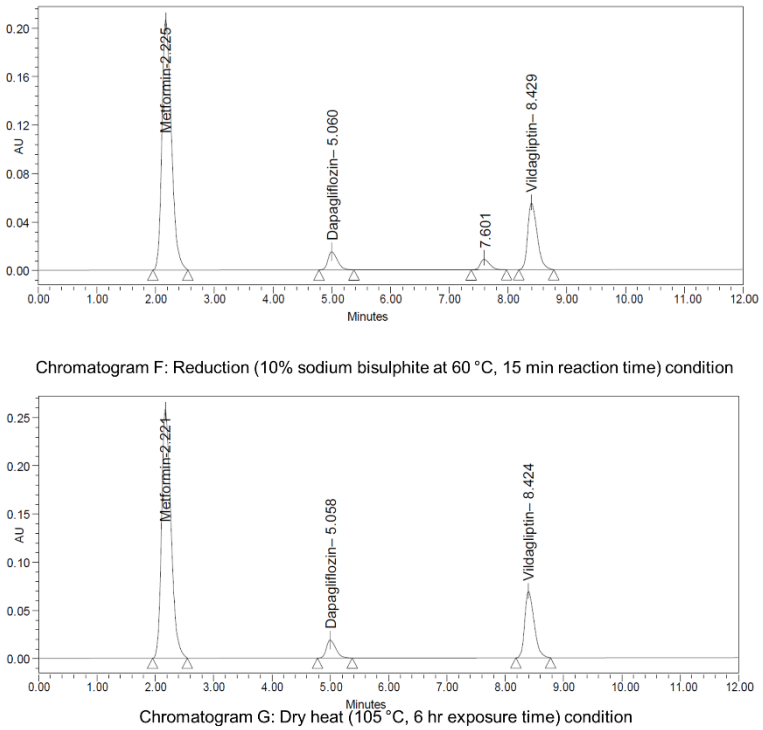


Figure 6. Chromatogram evidence for method's specificity

Table 12. Stabilities of MFMN in forced degradation analysis

Accelerated condition	S (%)	D (%)	S (%)	D (%)	S (%)	D (%)	S (%)	D (%)
	At 15 min		At 30 min		At 45 min		At 60 min	
Acid	86.872	13.128	85.977	14.023	83.161	16.839	81.558	18.442
Peroxide	84.257	15.743	83.879	16.121	82.631	17.369	79.683	20.317
Reduction	97.269	2.731	97.028	2.972	96.375	3.625	95.535	4.465
Hydrolysis	90.370	9.63	89.81	10.19	85.726	14.274	83.857	16.143
Alkali	89.247	10.753	88.951	11.049	86.019	13.981	84.743	15.257
	At 2 hr		At 4 hr		At 6 hr		At 8 hr	
Thermal	96.804	3.196	96.187	3.813	95.295	4.705	94.530	5.470
	At 1 hr		At 2hr		At 3 hr		At 4 hr	
Photolytic	86.142	13.858	83.035	16.965	81.541	18.459	79.975	20.025

S – Stability in percentage; D – Degradation in percentage

Table 13. Purities of DFZN, VDGN, and MFMN peaks in forced degradation analysis

Accelerated condition	DFZN purity angle	DFZN purity threshold	VDGN purity angle	VDGN purity threshold	MFMN purity angle	MFMN purity threshold
Thermal	0.248	5.741	2.311	9.584	1.506	8.138
Acid	0.257	5.707	2.364	9.588	1.522	8.121
Peroxide	0.286	5.728	2.347	9.531	1.511	8.143
Reduction	0.222	5.789	2.395	9.546	1.547	8.175
Photolytic	0.273	5.777	2.361	9.528	1.557	8.151
Hydrolysis	0.207	5.705	2.342	9.575	1.534	8.102
Alkali	0.234	5.725	2.303	9.554	1.564	8.115

Empower software was used to conduct a peak purity study in the 200–400 nm region. The spectral purity of the MFMN, VDGN, and DFZN peaks in all stress Vylda DM® tablet samples were verified by analysis. In every tablet samples, the peak purity values (purity threshold > purity angle, Table 13) of DFZN, VDGN, and MFMN showed that no other peaks were co-eluting with those three peaks. The new method's capacity to indicate stability was confirmed as there was no observable co-elution of substances from degradation with DFZN, VDGN, and MFMN within any of the stressed Vylda DM® tablet samples.

3.11. Assay of Vylda DM® tablets

Formulated Vylda DM® tablets containing 500 mg of MFMN, 100 mg of VDGN, and 10 mg of DFZN were selected for their evaluation investigations. The assay results for Vylda DM® tablets were figured out to be 100.216% for DGFN, 100.057% for VDGN and 99.890% for MFMN, respectively. The applicability of the developed HPLC approach was signified by the findings presented in Table 14 and Figure. 7.

3.12. Comparison study

Simultaneous quantification of MFMN, Saxagliptin, and DFZN was reported by Hadir *et al.* (using capillary electrophoresis) [26], Abdelrahman et al., (using HPTLC) [27], Dhanya *et al.* (using UV spectroscopy) [28], Priya *et al.* (using UV Spectroscopy) [29], Krishna et al., (using HPLC) [30]. The combination of MFMN, saxagliptin, and DFZN in tablet formulations can be quantified using any of these techniques [26-30]. The simultaneous measurement of MFMN and DFZN using an HPLC approach has been described by Nandre *et al.* [31] Reddy & Mathur [32], and Nachiket *et al.* [33] However, Nasser et al., proposed both HPTLC and HPLC methods for MFMN and DFZN simultaneous quantification [34].

Similarly, Jani *et al.* reported using UV spectroscopy approach for the same combination (MFMN and DFZN) [35]. For the simultaneous quantification of MFMN and DFZN in tablet formulations and synthetic combinations, all of these techniques are suitable [31-35]. The DFZN, VDGN, and MFMN are not simultaneously quantified in any of the publications that are presented [26-35]. For the first time, in this study, we apply the RP-HPLC approach for assessing DFZN, VDGN, and MFMN simultaneously. The developed method can be exploited for quality control examination of DFZN, VDGN, and MFMN and also to indicate stability in DFZN, VDGN, and MFMN analyses

Table 14. Evaluation of DFZN, VDGN and MFMN in Formulated Vylda DM® tablets

DFZN Content (mg)	DFZN Found (mg)	DFZN Assay (%)	VDGN Content (mg)	VDGN Found (mg)	VDGN Assay (%)	MFMN Content (mg)	MFMN Found (mg)	MFMN Assay (%)
10	10.041	100.415	100	99.862	99.862	500	501.181	100.236
10	10.002	100.017	100	100.252	100.252	500	497.723	99.545
	Average[a]	100.216		**Average[a]**	100.057		**Average[a]**	99.890
	SD[b]	0.2817		**SD[b]**	0.2760		**SD[b]**	0.4891
	RSD[c]	0.2811		**RSD[c]**	0.2758		**RSD[c]**	0.4897

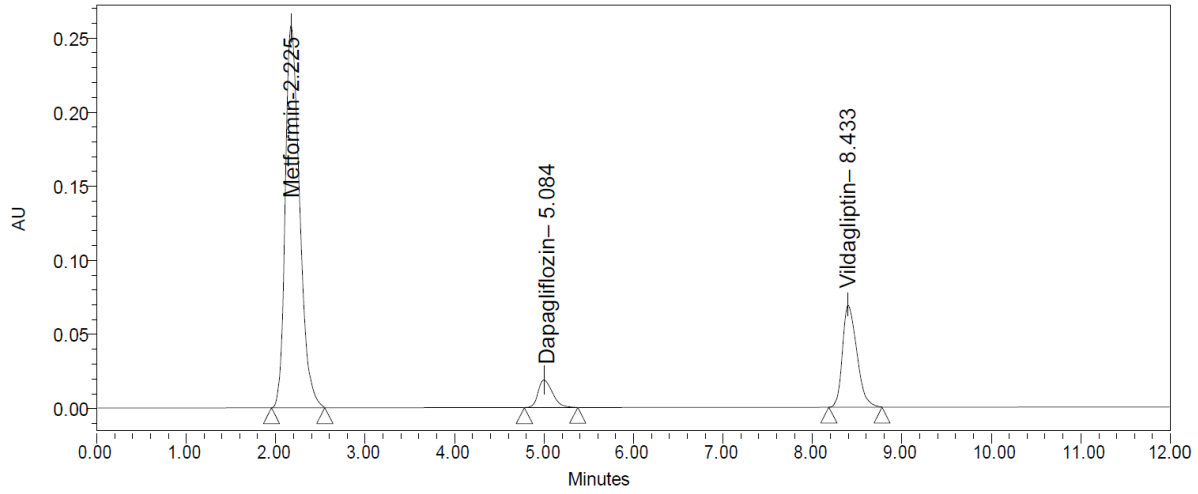


Figure 7. DFZN, VDGN and MFMN Chromatogram of formulated Vylda DM® tablets

4. Conclusion

The HPLC approach for DGFN, VDGN and MFMN assay seemed advantageous for both qualitative and quantitative examination. The HPLC approach seemed like a reliable, ideal technique for determining DGFN, VDGN, and MFMN simultaneously. The current strategy is also quite accurate, simple, and precise enough. All of these factors therefore point to the possibility of using the proposed and validated approach as a basis for quality control of DGFN, VDGN, and MFMN in the dosage forms as well as bulk forms. Degradation products that were obtained under the influence various stress circumstances illustrated good separation from DGFN, VDGN, and MFMN, showing that the approach represents stability-indicating.

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M. Haritha Kumari - Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, K. Bala Murali Krishna- Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, S. Jaganmohana Rao- Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, and R. Ramesh Raju- Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing. All the authors read and approved the final copy of the manuscript.

Funding

There was no funding provided by any source for this study.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Data Availability

Data and resources connected to the investigation work will be available upon request.

Has this article screened for similarity?

Yes