Stability-Indicating RP-HPLC Method for Simultaneous Determination of Metformin Hydrochloride and Vildagliptin in Tablet and Biological Samples

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The present work aimed to develop and validate a simple, rapid, sensitive, accurate, and precise method for simultaneous determination of metformin hydrochloride and vildagliptin in tablet and biological samples. Isocratic elution of both the analytes was performed at 35 °C by injecting 20 µL into Thermo Hypersil ODS C18 column (5 µm, 4.6 mm × 250 mm), while the flow rate was set to 0.8 mL/min. The mobile phase comprised of methanol, acetonitrile, and phosphate buffer (5:30:65, v/v, pH 3.5), and wavelength was selected at 212 nm. The overall run time per sample was 7.0 min with a retention time of 3.36 and 5.41 min for metformin hydrochloride and vildagliptin, respectively. The calibration curve was linear from 10–140 µg/mL for metformin and 1–14 µg/mL for vildagliptin with a coefficient of determination (R^2) ≤ 0.9919, while repeatability and reproducibility (expressed as relative standard deviation) were lower than 1.13 and 0.97%, respectively. Force degradation studies indicated a complete separation of the analytes in the presence of their degradation products providing a high degree of method specificity. The proposed reversed-phase high-performance liquid chromatography (RP-HPLC) method was demonstrated to be simple and rapid for the determination of metformin hydrochloride and vildagliptin in commercially available tablet and biological samples providing recoveries ranged between 100.13–100.29%.

Keywords: diabetes mellitus, metformin, vildagliptin, HPLC

1. Introduction

Metformin (MTF) is a biguanide class drug which is orally administered for the management of type 2 diabetes. In patients with type 2 diabetes, MTF improves glucose tolerance, as well as lowers the postprandial and basal plasma glucose. MTF are prescribed for patients that are obese or over weight with normal kidney function [1]. It improves insulin sensitivity by decreasing glucose intestinal absorption and hepatic production, and it is the most important therapy which is used in combination with other orally administered hypoglycemics [2]. Vildagliptin (VLD) is used for the treatment of type 2 diabetes. It is an orally administered anti-hyperglycemic agent of the new class of drugs called dipeptidyl peptidase-4 (DPP-4) inhibitors [3–5]. The synergic effect of MTF and VLD offers advantages over other anti-hyperglycemic drug with almost no risk of hypoglycemia. Therefore combination of these drugs in a single tablet is mostly used to achieve best glucose control in blood and for better compliance to therapy [6]. To the best of our knowledge, it is revealed that several reversed-phase high-performance liquid chromatography (RP-HPLC) methods are available for the determination of MTF and VLD (Figure 1) individually or in combination with other drugs in fixed dose pharmaceutical formulations, while a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method is presented for both the drugs in combination [7], but no stability-indicating assay method has been reported [8–10]. The

present research works to develop a rapid, sensitive, and stability-indicating liquid chromatographic (high-performance liquid chromatography [HPLC]) method for the simultaneous determination of MTF and VLD in tablet and biological samples (human plasma), and the proposed HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines [11]. Stability-indicating nature of the method was assessed by performing force degradation studies.

2. Experimental

2.1. Chemicals and Reagents. MTF and VLD reference standards were provided by Novamed Pharmaceuticals (Pvt.) Ltd. (Pakistan). Acetonitrile (ACN), potassium dihydrogen phosphate (KH₂PO₄), phosphoric acid (H₃PO₄, 85%), methanol (MeOH), and sodium hydroxide were supplied by Honeywell (USA). All the chemicals and reagents were of analytical grade, while GenPure water system (Thermo Scientific, USA) was used to obtain ultrapure water (18 MΩ. cm). Drug formulations in tablet form containing MTF and

Figure 1. Molecular structures of metformin hydrochloride (a) and vildagliptin (b)

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VLD were collected from local market shelves (Viglip-M[®], Atco Laboratories, Pakistan, Galvus Met[®], Novartis, Pakistan) in the capital city Lahore, Pakistan.

2.2. Chromatography. For HPLC analysis, PG LC200, a HPLC system with a LC210 pump, a PC220 UV/Vis detector, an LC250 column oven, a LC240 vacuum degasser, and LC Win 1.0 software equipped with a Thermo Hypersil ODS C18 column (5 μ m, 4.6 mm $\times$ 250 mm) was used. The mobile phase was comprised of MeOH, ACN, and phosphate buffer pumped in a ratio of 5:30:65 (v/v) at pH 3.5 adjusted by dilute NaOH (pH meter, Orion 5 Star, Thermo Scientific, UK). The detection was carried out at 30 $^{\circ}$ C by injecting 20 μ L, while the flow rate and wavelength were set at 0.8 mL/min and 212 nm, respectively. All the solutions including the standards, samples, and the mobile phase were filtered by a 0.45- μ m nylon filter (Sartorius, Germany) before injection into the LC system. Peak integration and quantification were performed by using LC Win 1.0 software.

2.3. Stock and Working Standard Solutions. Stock standard solutions of MTF and VLD (1000 μ g/mL and 100 μ g/mL, respectively) were prepared in water and placed in an ultrasonic bath and are sonicated for 15 min. Subsequently, working solutions were prepared by serial dilution of the individual stock standard solutions with the mobile phase to get final concentration 50 μ g/mL of MTF and 5 μ g/mL of VLD respectively.

2.4. Analysis of Pharmaceutical Dosage Form. The stated composition of tablets under investigation is MTF (1000 mg), and VLD (50 mg) was analyzed by the proposed HPLC methods. Twenty tablets were grinded, and aliquots equivalent to one tablet was diluted with ethanol in a 50-mL flask, sonicated for 15 min for complete dissolution, and filtered. Finally, appropriate concentration was diluted with the mobile phase, and the final concentration of analytes became 50 μ g/mL of MTF and 5 μ g/mL of VLD.

2.5. Analysis of Biological Samples. Drug-free human plasma samples were obtained from healthy volunteers. One milliliter of MTF and VLD standard containing each of 5000 μ g/mL, 5.4 mL methanol, and 3.6 mL of plasma were mixed in a centrifuge tube resulted in the final concentration 500 μ g/mL of each drug. The aliquots were centrifuged at 3000 rpm for 20 min after sonicating in an ultrasonic bath for 5 min. A linear range of concentration within 10–140 μ g/mL for MTF and 1–14 μ g/mL for VLD was made after diluting with mobile phase.

2.6. System Suitability. The working solution 50 μ g/mL of MTF and 5 μ g/mL of VLD was injected six times on HPLC, on 3 separate days, and conformity of chromatographic parameters was done as explained in the United States Pharmacopeia (USP) including retention time (<2% RSD), peak area (<2% RSD), tailing factor (<2), selectivity factor (>1), resolution (>2), and theoretical plates (>1000) [12,13].

2.7. Validation Studies. Validation studies were performed according to the ICH guidelines [11] in terms of specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). For LC-based methods (HPLC), the linear dynamic range was selected within 10–120 μ g/mL for MTF and 0.5–10 μ g/mL for VLD. A linear calibration curve in the form of $y = ax + b$ was obtained by plotting the peak area of the drugs MTF and VLD in triplicate, y against the nominal concentration x of 7 concentrations (10, 20.0, 40.0, 80.0, 100.0, 120.0, and 140.0 μ g/mL for MTF and 1.0, 2.0, 4.0, 8.0, 10.0, 12.0, and 14.0 μ g/mL for VLD), whereas a is the slope of the calibration curve, and b is the intercept. Linear regression equation was demonstrated, and the necessary parameters were tabulated. The accuracy of each method was determined in triplicate by spiking a known

amount of pure drug to pre-analyzed tablets at 50, 100, and 150% of analyte in the dosing formulation. The resulting final concentrations of 75.0, 100.0, and 125.0 μ g/mL obtained for MTF and 7.5, 1.0, and 12.5 μ g/mL for VLD. The precision studies were determined at different concentrations (75.0, 100.0, and 125.0 μ g/mL for MTF and 7.5, 10.0, and 12.5 μ g/mL for VLD), represented by mean recovery and %RSD. The intra-day precision (repeatability) was evaluated by replicates of 5 on 1 day, whereas the inter-day precision (reproducibility) was determined over 3 consecutive days. The resultant parameters of the linear regression including the standard deviation (SD) of the response based upon the slope and intercept determined the LOD and LOQ. The LOD and LOQ were defined as $3.3\sigma/S$ and $10\sigma/S$, respectively [14–19], where σ is the standard deviation, and S is the slope of regression line.

2.8. Force Degradation Studies. Force degradation studies for stability-indicating assay by the proposed HPLC method were performed under acidic, basic, oxidative, photolytic, and thermal conditions at final concentration of 50 μ g/mL of MTF and 5 μ g/mL of VLD from commercial product under investigation. Stock solution (50 μ g/mL) was treated with 0.1 M HCl and 0.1 M NaOH for acidic and basic hydrolysis for 1 h. 3% H₂O₂ was employed for oxidative stress (1 h), while photolytic and thermal stresses were performed after exposing the solid form for 6 h under UV (254 nm) and keep in an oven at 100 $^{\circ}$ C, respectively. After the described time, the stressed compound was dissolved in methanol, and then stocks were diluted using the mobile phase to get the final concentration of 50 μ g/mL of MTF and 5 μ g/mL of VLD.

3. Results and Discussion

3.1. Optimization of Chromatographic Conditions. HPLC method was developed for determination of MTF and VLD in tablet dosage form and human plasma, to achieve sufficient resolution of target compound in the presence of interfering substances including excipients, and matrix effect is vital in the development of a liquid chromatographic (LC) method. In the first step, a UV absorption spectrum was taken in the range of 200–400 nm resulting in a maximum absorbance at 212 nm for both analytes. Various mobile phase compositions and ratios were employed for sensitive and selective determination of MTF and VLD by LC method. Initially methanol and acetonitrile were utilized in different ratios with a phosphate buffer (5:15:85, 5:20:75, and 5:30:65, v/v) at pH 6.0, 5.0, 4.0, 3.5, and 3.0. These mobile phases were run on different columns like Thermo Hypersil ODS C18, Venusil XBP C18, and Purospher[®] RP-18 (Table 1).

The chromatograms have broad peaks and long retention time observed by using low ACN and higher buffer concentration keeping the MeOH concentration constant. The analysis time was reduced when ACN concentration increase and buffer concentration decrease, and peaks were sharp and more symmetrical. In the end, the mobile phase consisting of MeOH, ACN, and phosphate buffer in a ratio of 5:30:65 (v/v) with the pH adjusted to 3.5 was chosen for the best separation,

Table 1. Results of tested stationary phase^a

Column	Analyte	R_s	T	k'	N
Thermo Hypersil ODS C18 (250 mm $\times$ 4.6 mm, 5 μ m)	MTF	–	1.17	2.54	5546
	VLD	8.02	1.06	4.69	7234
Venusil XBP C18 (250 mm $\times$ 4.6 mm, 5 μ m)	MTF	–	1.21	1.86	3203
	VLD	6.22	1.34	3.53	4597
Purospher [®] RP-18 C18 (250 mm $\times$ 4.6 mm, 5 μ m)	MTF	–	2.27	1.67	3576
	VLD	4.93	1.96	3.08	3278

^aWorking conditions: mobile phase 5:30:65 (MeOH–CAN–phosphate buffer, pH = 3.5 at 212 nm), flow rate 0.8 mL/min, temperature 35 $^{\circ}$ C.

Figure 2. Typical HPLC chromatograms of (a) standard, (b) sample, (c) blank plasma, and (d) spiked plasma

as well as a column temperature of 35 °C, and the flow rate was kept at 0.8 mL/min. Sharp and symmetric peak shapes were obtained by using Thermo Hypersil ODS C18 columns for HPLC analysis, at a detection wavelength of 212 nm. The retention times for MTF and VLD were 3.36 and 5.41 min, respectively, by HPLC as shown in Figure 2.

3.2. Validation Studies. The system suitability is conformity of chromatographic parameters that ensures the performance of the analytical system. All critical parameters, as defined above, met the acceptance criteria on all days. The system suitability parameters that were monitored for MTF and VLD are presented in Table 2.

For chromatographic methods over a dynamic range of 10–140 µg/mL and 1.0–14 µg/mL, 7 concentrations (10.0, 20.0, 40.0, 80.0, 100.0, 120, and 140 µg/mL for MTF and 1.0, 2.0, 4.0, 8.0, 10.0, 12.0, and 14.0 µg/mL for VLD) were employed to construct a calibration graph by the peak area of the drug to the concentration. The calibration curves were linear for MTF and VLD with a coefficient of determination $R^2 \geq 0.9903$ (Table 3).

The accuracy of both the techniques under investigation was determined by evaluating the recovery studies after spiking the known amount of standard drugs in commercial products. LOD and LOQ determined by the proposed LC methods were 2.18 and 6.55 µg/mL of MTF while 0.13 and 0.38 µg/mL for VLD, respectively, as shown in Table 3.

Table 2. System suitability data of the reference solution of MTF and VLD

USP criteria	Specification	MTF			VLD		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Retention time (t_R in min)	%RSD < 2	0.43	0.35	0.42	0.61	0.67	0.71
Tailing factor (T)	< 2.0	1.17	1.19	1.16	1.06	1.09	1.06
Resolution (R_s)	> 2.0	-	-	-	8.06	8.04	8.02
Capacity factor (k')	> 1.0	2.54	2.36	2.43	4.69	4.61	4.63
Theoretical plates (N)/meter	> 1000	5546	5503	5531	7234	7225	7245
Area	%RSD < 2	0.87	0.74	0.88	0.81	0.79	0.67

Table 3. Regression data of MTF and VLD standard and biological samples by HPLC

Parameters	Standards		Plasma	
	MTF	VLD	MTF	VLD
Linearity range (µg/mL)	10–140	1–14	10–140	1–14
Slope	34,011	12,934	34,031	12,951
Intercept	445,810	8642	445,823	8662
Standard error of slope	2.6×10^3	571	2.6×10^3	586
Standard error of intercept	2.2×10^4	494	2.2×10^4	491
Coefficient of determination (R^2)	0.9917	0.9903	0.9913	0.9902
LOD (µg/mL)	2.18	0.13	2.19	0.14
LOQ (µg/mL)	6.55	0.38	6.57	0.41

The recovery results were obtained between the ranges 99.83–100.22% and 100.71–102.30% (Table 4) for MTF and VLD by the proposed HPLC method, respectively, which justified the suitability of the techniques for their intended applications.

For precision studies, the results of repeatability and reproducibility are presented in Table 5 by injecting 3 different concentrations (50, 100, and 150% level of analyte under investigation) of standard solutions of MTF and VLD ($n = 5$) on same day and 3 consecutive days, respectively. Relative standard deviation (RSD) values for repeatability and reproducibility were obtained less than 1.32 and 1.53, respectively, for MTF while below than 0.83 for repeatability and 1.35 for reproducibility assays of VLD.

Table 4. Accuracy studies of MTF and VLD by HPLC

Analyte	Concentration after spiking ^a (µg/mL)	Concentration found ^b (µg/mL) ± SEM; RSD	(%) Recovery [BIAS]
MTF	75.0	74.87 ± 0.49; 1.27	99.83 [0.17]
	100.0	100.22 ± 0.38; 0.72	100.22 [0.22]
	125.0	124.84 ± 0.17; 0.22	99.87 [0.13]
VLD	7.5	7.67 ± 0.32; 1.43	102.30 [2.3]
	10.0	10.13 ± 0.42; 1.12	101.30 [1.3]
	12.5	12.84 ± 0.21; 1.32	100.71 [0.71]

^aActual concentration each of MTF = 50 µg/mL and VLD = 5 µg/mL.

^bAll measurements were made in replicate of five; SEM: Standard error mean; RSD: Relative standard deviation.

Table 5. Precision studies of MTF and VLD by HPLC

Analyte	Repeatability (<i>n</i> = 5)		Reproducibility (<i>n</i> = 5)		
	Concentration (μg/mL)	Concentration found (μg/mL) ± SEM; RSD	Concentration found (μg/mL) ± SEM; RSD		
			Day 1	Day 2	Day 3
MTF	75.0	74.34 ± 0.22; 1.11	75.34 ± 0.22; 0.65	75.11 ± 0.32; 1.13	75.21 ± 0.32; 1.12
	100.0	99.23 ± 0.43; 1.04	100.21 ± 0.33; 0.43	99.83 ± 0.21; 1.12	100.11 ± 0.21; 1.53
	125.0	125.16 ± 0.42; 1.32	124.32 ± 0.41; 0.63	125.11 ± 0.43; 1.32	125.21 ± 1.23; 1.23
VLD	7.5	7.41 ± 0.32; 0.83	7.45 ± 1.21; 0.76	7.32 ± 0.76; 1.35	7.56 ± 0.76; 1.16
	10.0	10.43 ± 0.43; 0.76	10.11 ± 0.45; 0.66	9.89 ± 1.15; 0.33	10.18 ± 1.05; 0.81
	12.5	12.33 ± 1.12; 0.42	12.63 ± 0.34; 1.32	12.52 ± 0.43; 0.86	12.48 ± 1.13; 0.92

SEM: standard error mean; RSD: relative standard deviation.

Table 6. Force degradation results of MTF and VLD by HPLC

Nature of stress	Amount remaining, mean ± SEM (%) ^a		Extent of degradation	
	MTF	VLD	MTF	VLD
0.1 M HCl (1 h)	99.43 ± 0.21	99.76 ± 0.43	None	None
0.1 M NaOH (1 h)	86.32 ± 0.23	91.38 ± 0.21	Substantial	Substantial
3% H ₂ O ₂ (1 h)	88.64 ± 0.29	89.61 ± 0.29	Substantial	Substantial
6 h under 100 °C	99.86 ± 0.11	99.12 ± 0.12	None	None
6 h under UV-254	98.32 ± 0.11	99.41 ± 0.11	None	None

^aAll measurements were made in triplicate.

3.3. Force Degradation Studies. Force degradation studies under stress conditions (Table 6) were performed for presenting the stability-indicating capacity of the proposed HPLC method.

The chromatograms (Figure 3) after force degradation are presented, while the amount remaining and the extent of degradation are tabulated after each treatment. No degradations were observed for MTF and VLD under acidic, thermal, and photolytic stress, and the extent of degradation was declared as none.

Figure 3. Typical HPLC chromatograms of the samples of MTF and VLD under stress conditions: (a) acidic, (b) basic, (c) oxidative, (d) thermal, (e) photolytic, and (f) sample without stress

Table 7. Assay results of MTF and VLD by HPLC in commercial tablet formulation

Product	Label claim (mg)	Concentration found ^a mg $\pm$ SEM; RSD	Recovery (%)
Viglip-M [®]	MTF = 1000, VLD = 50	MTF = 997 $\pm$ 0.34; 1.11, VLD = 50.11 $\pm$ 1.12; 1.06	99.70, 100.22
Galvus Met [®]	MTF = 1000, VLD = 50	MTF = 1003 $\pm$ 1.21; 1.32, VLD = 50.43 $\pm$ 0.92; 1.12	100.30, 100.86
Spiked Plasma (μ g/mL)	MTF = 50, VLD = 5	MTF = 46.8 $\pm$ 0.91; 0.82, VLD = 4.59 $\pm$ 1.15; 1.31	93.68, 91.80

^aResults are expressed as average of 10 measurements.

In contrast, substantial degradation was observed under basic and oxidative stress conditions, and the remaining concentration of MTF and VLD were found to be 86.32 and 91.38%, respectively. Two impurity peaks at unique retention times $t_R = 0.25$ min and $t_R = 7.45$ min (DP-1 and DP-2, Figure 3b) were observed after basic degradation, while 3 impurity peak at $t_R = 0.25$ min, $t_R = 4.11$, and $t_R = 7.72$ min (DP-1, DP-2 and DP-3, Figure 3c) were observed under oxidative stress. As a result of these experiments, the proposed HPLC method was found to be specific to the analyzed active pharmaceutical ingredients.

3.4. Analysis of Commercial Tablet Formulation. The applicability of the proposed HPLC method was evaluated by examining the commercial tablets (Viglip-M[®], Galvus Met[®]) with a reported MTF and VLD dosage of 1000 mg and 50 mg, respectively. It was concluded that the proposed HPLC method was sufficiently accurate and precise (Table 7) with recovery and RSD values found to be ranged 99.70–100.86% and 1.06–1.32%, respectively.

3.5. Analysis of Biological Samples. For plasma medium, a good linear relationship between detector signal and spiked concentrations of MTF and VLD was found, as shown in Table 3. The value of the coefficient of determination is ≥ 0.9902 , while detection and quantification limits were calculated using the related equations, which are given in the ‘Validation Studies’ section. The spiked plasma concentration was obtained with excellent recovery (91.8–93.68%) as shown in Table 7. The chromatograms for blank and spiked plasma sample are presented in Figure 2.

4. Conclusion

A sensitive and selective HPLC method was validated for determination of MTF and VLD in pharmaceutical formulations and biological samples. The developed isocratic chromatographic method enabled unified quantification of MTF and VLD with convincingly good precision and accuracy in pharmaceutical dosage forms and plasma. This stability-indicating HPLC method was fully validated according to the ICH guidelines. The proposed method was optimized and

presented its suitability for quality control laboratories where time and economy are essentially required.

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References

1. Modi, D. K.; Parejiya, P. B.; Patel, B. H. *J. Chem.* **2013**, *2013*, 4.
2. Correia, S.; Carvalho, C.; Santos, M. S.; Seica, R.; Oliveira, C. R.; Moreira, P. I. *Mini Rev. Med. Chem.* **2008**, *8*, 1343.
3. Beers, M. H.; Fletcher, A. J.; Jones, T.; Porter, R.; Berkwitz, M.; Kaplan, J. *The Merck manual of medical information*, Pocket Books, 2003.
4. Karagiannis, T.; Paschos, P.; Paletas, K.; Matthews, D. R.; Tsapas, A. *BMJ* **2012**, *344*, e1369.
5. Stoimenis, D.; Karagiannis, T.; Katsoula, A., et al. *Expert Opin. Pharmacother.* **2017**, *18*, 843.
6. Banerji, M.; Purkayastha, D.; Francis, B. *Diabetes Res. Clin. Pract.* **2010**, *90*, 182.
7. Pontarolo, R.; Gimenez, A. C.; de Francisco, T. M. G.; Ribeiro, R. P.; Pontes, F. L. D.; Gasparetto, J. C. *J. Chromatogr. B.* **2014**, *965*, 133.
8. Mohammad, M. A.-A.; Elkady, E. F.; Fouad, M. A. *Eur. J. Chem.* **2012**, *3*, 152.
9. Santhosha, B.; Ravindranath, A.; Sundari, C. *Int. Res. J. Pharm. Appl. Sci.* **2012**, *2*, 22.
10. Martin, J.; Buchberger, W.; Santos, J. L.; Alonso, E.; Aparicio, I. *J. Chromatogr. B.* **2012**, *895*, 94.
11. Group, I. E. W. *Validation of analytical procedures: text and methodology Q2 (R1). Paper presented at: Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*, **2005**.
12. Mohammad, A.; Yen, C. H.; Schneider, M., et al. *Anal. Methods.* **2018**, *10*, 1842.
13. Pharmacopeia, U. *The Official Compendia of Standards (USP-40-2017). Paper presented at: United States Pharmacopoeial Convention*, Rockville, MD, **2017**.
14. Ali, A.; Ahmed, M.; Mahmud, T.; Qadir, M.; Nadeem, K.; Saleem, A. *Indian J. Pharm. Sci.* **2015**, *77*, 515.
15. Qadir, M. A.; Ahmed, M.; Hussain, W. A.; Tahir, M. S. *Indian J. Pharm. Sci.* **2015**, *77*, 434.
16. Ahmed, M.; Qadir, M. A.; Shahzad, S.; Waseem, R.; Tahir, M. S. *Int. J. Chem. Pharm. Sci.* **2013**, *2*, 536.
17. Ahmed, M.; Shahzadi, S. K.; Waseem, R.; Shahzad, S.; Ahmad, W. *Inter. J. Chem. Sci. Res.* **2013**, *3*, 1.
18. Jehangir, M.; Ahmed, M.; Imtiaz Shafiq, M.; Samad, A. *J. Anal. Methods Chem.* **2017**, 2017.
19. Tahir, M. S.; Ahmed, M.; Shafiq, M. I., et al. *Lat. Amer. J. Pharm.* **2017**, *36*, 2136.