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Design of Experiments for Wet Granulation of Valsartan and Pravastatin Fixed-Dose Combination Tablet

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Design of experiment provides a quality risk management to the manufacturing process of a product. This study was performed to select the water amount and identify the critical process parameters for wet granulation. Design of experiment, one of the quality by design approaches, was used in the study. The manufacturing process of valsartan and paravastatin fixed-dose combination tablets involves wet granulation, drying, sieving, blending and tableting. For wet granulation, a 3-factorial (granulating time, agitator speed and the amount of granulating water), 2-level (granule density and dissolution), 1-center (n = 3) point was used in the design of experiment batches and analyzed using Design Expert Software. Previously, this formulation was found to show good assay and content uniformity. Thus, only physical properties, bulk density and dissolution were evaluated for the design of experiment study. The amount of granulating water was identified as an important factor affecting the mean dissolution ($p < 0.05$) in contrast to the granulating time and agitator speed. Present results indicated that granulating time (6 to 10 min), amount of granulating water (40 to 60 g) and agitator speed (150 to 250 rpm) were optimal for wet granulation of valsartan and pravastatin fixed-dose combination tablets.

Keywords: Valsartan, Pravastatin, Quality by design, Design of experiment, Wet granulation.

INTRODUCTION

Valsartan is an angiotensin II type 1 receptor antagonist; it is rapidly absorbed, reaching the maximum plasma level 3 h after oral administration [1]. Valsartan has low bioavailability of 25 % and pH-dependent solubility as its solubility decreases in low pH conditions such as the acidic environment of the upper gastrointestinal tract [2]. Pravastatin sodium is a selective HMG-CoA reductase inhibitor, a class of lipid-lowering agents that reduce cholesterol biosynthesis. Pravastatin sodium is most soluble and stable at neutral pH, but it degrades due to lactonization and oxidation at pH below 4 [3]. Although valsartan and pravastatin sodium differ in their mechanisms of action, they have beneficial effects as a combination therapy to reduce LDL cholesterol and blood pressure [4].

In the present study, wet granulation was used in the formulation development of valsartan and pravastatin fixed-dose combination (FDC) tablets. Wet granulation has several advantages such as increased content uniformity in low-dose drugs and improved flowability and compactibility of granules in high-dose drugs [5]. The objective of this development study was to establish a robust and stable wet granulation method for the formulation of valsartan and pravastatin fixed-dose combination tablets through quality by design approach. Quality decision in traditional drug development does not take into consideration science and risk evaluation. In other words, this approach has limitations because statistical and process controls are applied at the manufacturing stage through quality assurance inspection [6]. However, quality by design allows complete understanding of the process and monitoring of all critical steps to reduce product failure [6]. Valsartan and pravastatin fixed-dose combination tablet is studied as an example to reduce variation and build robustness in the wet granulation process through scientific understanding using the design of experiment approach of the quality by design. Composition, critical process parameter, quality target product profile, control methods, proven acceptable ranges (PARs) and design space of the target formulation were established for application of the design of experiment method [6]. For application of the design of experiment method, it is unrealistic to analyze all of the parameters which impact the drug critical quality attribute (CQA). Thus, it is accepted to use prior knowledge to determine the most important parameters such as operational parameters (speed, time, *etc.*) and possible product outcomes (dissolution, friability, *etc.*) [7]. In this study, a full -factorial design with three factors, two levels per factor

and one center (n = 3) was used. This enables us to study a sample size of N = 2k [8]. In this way, 3 factors can be tested within 11 runs, so the number of trials may be reduced down to the absolute minimum. Generally, wet granulation involves agglomeration of various particles through mixing and liquid addition. A typical formulation consists of the active pharmaceutical ingredient, diluents (fillers), polymeric binder, granulating liquid such as ethanol or water, lubricant and disintegrating agent [9]. The aim of the study was to analyze the impact of different process parameters on the drug product quality attributes in case of wet granulation of valsartan and pravastatin fixed-dose combination tablets.

EXPERIMENTAL

Valsartan was purchased from MSN Laboratories Private Limited (Hyderabad, India). Pravastatin was obtained from Hisun Pharmaceutical Co. Ltd. (Zhejiang, China). Microcrystalline cellulose (Vivapur types 101, JRS), magnesium oxide (Heavy, Tomita), low-substituted hydroxypropyl cellulose (LH22, Shinetsu), croscarmellose sodium (Acdisol, FMC biopolymer), magnesium stearate (Hyqual, Mallinckrodt) were also purchased. All other chemicals and reagents were of the pharmaceutical grades commercially available. The reference drugs used were Diovan® (valsartan) and Mevalotin® (pravastatin).

Preparation of valsartan and pravastatin fixed-dose combination tablets: Immediate-release fixed-dose combination tablets of valsartan and pravastatin were prepared by wet granulation technique. Valsartan (160 mg), pravastatin (40 mg), microcrystalline cellulose (45 mg), magnesium oxide (5 mg) and low-substituted hydroxypropyl cellulose 40 (mg) per tablet were first mixed in the high-speed mixer (Nara Machinery Co. Ltd, NMG-1L, Tokyo, Japan). Water was used for wet granulation of the mixture and then wet granules were screened out through a 1.7 mm screen. The main purpose of the screening process is to crush the agglomerates. Wet granules were transferred to a fluid bed drier to obtain dried granules having loss on drying (LOD) from 1.3 to 1.7 % w/w and sieved by a comil (Quadro 197S, Quadro Engineering, Canada) with a 990 μm screen at 1200 rpm. Finally, croscarmellose sodium (45 mg) and magnesium stearate (5 mg) were blended with the sieved granules in a double cone blender (HS-DCM-10, Hansung F&C co., Korea) at 15 rpm for 10 min. Each batch was compressed into tablets with a target weight of 340 mg. To compare the tablet dissolution of different batches, the main compression force was adjusted to achieve a range of 5.0 to 7.0 kP hardness using Piccola Nova tablet press (BD4+4, Buenos Aires, Argentina). Based on the initial risk assessment of the overall manufacturing process presented in Table-1, the risk of the mixing and wet granulation step to impact the dissolution was identified as high. Tables 1 and 2 summarize the risk assessment of the mixing and wet granulation process variables (granulating time, the amount of granulating water and agitator speed) on tablet dissolution. For this study, a 2^3 full factorial design of experiment was used and three center points were included to evaluate if any curvature effects exist. As shown in Table-3, eleven batches of tablets were prepared for the design of experiment study where each batch amounted to 0.85 kg in size (Design Expert Software, Version 9.0.5.1, Stat-Ease Inc., Minneapolis, MN, USA). The responses (granule density and dissolution) of each factor were assessed by experiments. Generally, granulating time, agitator speed and the amount of granulating water can affect numerous quality attributes including the density of granules and tablet dissolution [10].

Loss on drying (LOD): Loss on drying was analyzed by halogen moisture analyzer (HG63, Mettler Toledo GmbH, Greifensee, Switzerland) using 5 g of wet granules at 105 °C for 15 min.

Dissolution study: Tablet dissolution rate was assessed using the USP dissolution apparatus 2 procedure (the paddle method) where eight individual tablets of each batch were

TABLE-1
INITIAL RISK ASSESSMENT OF THE MANUFACTURING PROCESS FOR VALSARTAN AND PRAVASTATIN FIXED-DOSE COMBINATION TABLETS

Process step	Drug product critical quality attributes			
	Assay	Content uniformity	Dissolution	Degradation products
Mixing and wet granulation	Low	Low	High	Low
Drying	Low	Low	Medium	Medium
Granulate screening	Low	Low	High	Low
Final blending	Low	Medium	Low	Low
Tablet compression	Medium	High	High	Medium

TABLE-2
DESIGN OF THE 2^3 FULL FACTORIAL DESIGN OF EXPERIMENT TO STUDY MIXING AND WET GRANULATION PROCESS VARIABLES

	Factors: Process parameter	Range and levels		
		-1	0	+1
A	Granulating time (min)	6	8	10
B	Agitator speed (rpm)	150	200	250
C	Amount of granulating water (g)	40	60	80
	Responses	Goal	Acceptable ranges	
Y_1	Granule density (g/mL)	Define acceptable range	To be defined based on other responses	
Y_2	Mean Dissolution similarity of valsartan and pravastatin (f_2)	Maximize	≥ 55 (f2)	

TABLE-3
EXPERIMENTAL RESULTS OF THE 2^3 FULL FACTORIAL DESIGN OF EXPERIMENT TO STUDY MIXING AND WET GRANULATION PROCESS VARIABLES

Batch No.	A	B	C	Y_1	Y_2
1	10	150	80	0.38	54.01
2	6	250	40	0.42	58.99
3	6	150	80	0.38	54.25
4	10	250	40	0.44	56.22
5	10	150	40	0.43	57.55
6	8	200	60	0.42	63.07
7	10	250	80	0.38	52.58
8	6	150	40	0.43	57.98
9	6	250	80	0.38	53.33
10	8	200	60	0.42	64.78
11	8	200	60	0.42	65.51

A = Granulating time (min); B = Agitator speed (rpm); C = Amount of granulating water (g); Y_1 = Granule density (g/mL); Y_2 = Mean Dissolution similarity of valsartan and pravastatin (f_2)

tested at 37 ± 0.5 °C and 50 rpm, using water as a dissolution medium. 5 mL aliquots were withdrawn at 5, 10, 15, 30, 45 and 60 min using a filtering rod (0.45 μm). The samples were assayed by HPLC (Agilent Technologies, 1200 series, USA) column (Phenomenex Synergi Polar RP, 150 mm × 4.6 mm, 4 μm) and detected at wavelength 230 nm using a UV detector (Agilent Technologies, 1200 series, Photo-Diode Array UV/ visible detector, USA).

Bulk density: Bulk density was analyzed by weighing 50 g sample of each batch and placing it in a 100 mL graduated cylinder. Bulk density was calculated using the formula: sample weight (g)/sample volume (mL).

RESULTS AND DISCUSSION

Preliminary study evaluation: The manufacturing process of valsartan and pravastatin fixed-dose combination tablets consists of wet granulation, drying, sieving, blending and tableting. In a preliminary study valsartan and pravastatin fixed-dose combination tablets were formulated by wet granulation, direct compression and dry granulation processes to select proper process with good flowability and compaction. It was found that wet granulation was the most suitable method, ensuring good blend uniformity, dose uniformity, good tablet compaction, assay, *etc*. Hence, the factors influencing the wet granulation process were chosen for evaluation in the design of experiment study in order to determine their acceptable ranges.

All manufacturing process parameters can affect the finished product quality attributes. Accordingly, all input materials and apparatus determine the quality of the intermediate and final process steps [11]. Viscosity of the granulating solution is an important factor affecting the wet granulation process [12]. In general, an increase in the binder solution concentration could result in the production of larger and harder granules [13]. However, the use of the binder was omitted in this study because the mixture of pravastatin in water itself has sufficiently high viscosity, resulting in equal dissolution of the test drug compared to that of the reference drug. Although the granulator fill level has not been evaluated, it may also impact the granule density and dissolution based on experience. In this study, the suitability of wet granulation in the manufacturing process of valsartan and pravastatin fixed-dose combination tablets was confirmed by eleven lab-scale studies with 0.85 kg. The critical steps including wet granulation and mixing steps will be evaluated in the design of experiment study because they can impact the product critical quality attributes including the assay, dissolution and tableting.

Evaluation of wet granulation: Risk assessment of the wet granulation process was carried out to select suitable ranges of granulating time, agitator speed and the amount of granulating water (Table-2). For each batch, granule density and dissolution testing was conducted according to the formulation variables. The experimental results for the impact of three factors (granulating time, agitator speed and amount of granulating water) on two responses (granule density and mean dissolution) are presented in Table-3. The three factors can affect numerous quality attributes such as granule density and dissolution. The excessively dense or loose granule may affect the dissolution [14]. The bulk density with angle of repose, tapped density, Carr's compressibility index, or Hausner ratio are better predictors for tableting efficiency [15]. The associated risk of all process variables in the design of experiment study was identified and ranked from low to high based on feasibility studies and previous experience [16]. All the design of experiment studies were performed under controlled moisture and temperature to eliminate the possibility of lactonization and oxidation of pravastatin as other statins [17-19]. The dissolution profiles of the fixed-dose combination tablets were analyzed using a similarity factor ($f_2 \geq 55$) compared to that of the reference drug. As shown in the half-normal plot (Fig. 1), the amount of water whose effect shows 5.63 is a highly significant factor affecting the similarity factor (f_2) in wet granulation. However, granule density varied inconsistently from 0.38 to 0.44 g/mL at different levels of three factors without specific effects. The effect of granulating time and agitator speed is less valuable than that of the amount of granulating water. The effect of the amount of granulating water is also shown in Fig. 2, which determines the importance of the variables based on Bonferroni and standard t. It means that all factors above the t-value limit can significantly affect the process [20]. As shown in Table-3, the dissolution of batches (1, 3, 7 and 9) using 80 g granulating water demonstrated unacceptable results ranged from 52.58 to 54.25 as compared with the value of acceptance criteria ($f_2 \geq 55$). Regardless of various granulating time (6-10 min) and agitator speed (150-250 rpm), high amount of granulating water (80 g) is found to be a significant factor impacted on tablet dissolution. Under the circumstances, the fixed tablet hardness (5-7 kP) was applied because equal dissolution rate of the test drug was obtained compared to those of original drugs. Generally, the limited surface and porosity affect the creation of agglomerates in wet granulation. Nkuzinna *et al.* [21] suggested that fast agitation in wet granulation increase both the energy and frequency of particles collisions. Increased temperature due to high speed may also impact the viscosity of the binder solution and the plasticity of the particles. In other words, the amount of granulating water and agitator

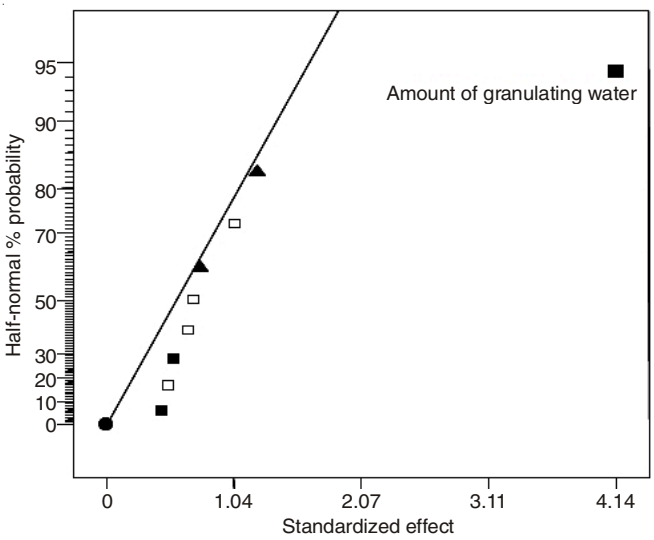

Fig. 1. Half-normal plot of the process variable effect on dissolution. ■ Positive effect, □ Negative effect, △ Error estimates

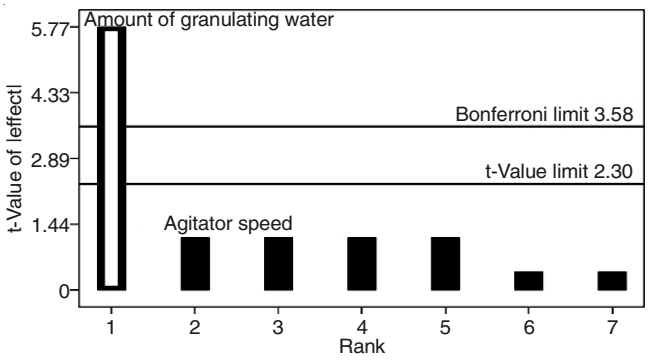

Fig. 2. Pareto chart for standardized effect

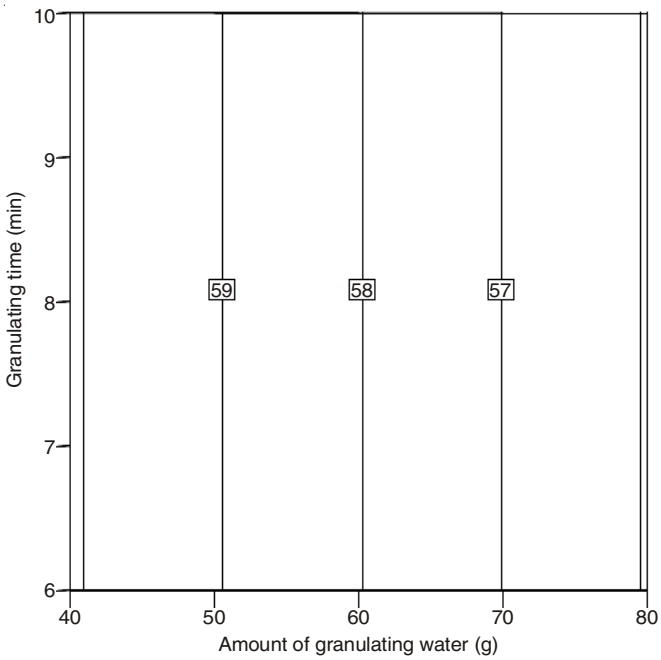

Fig. 3. Main effect of the amount of granulating water on dissolution of final product

speed used during wet granulation are highly important factors. In our experiment, the amount of granulating water is found to be crucial to dissolution of fixed-dose combination tablets (Fig. 2). When the amount of granulating water was increased, the similarity factor of dissolution tends to decrease slightly (Fig. 3). Thus, it is confirmed that dissolution depends mainly on the amount of granulating water and a high level of the amount of granulating water in wet granulation could result in unacceptable dissolution in fixed-dose combination tablets. The analysis of variance (ANOVA) results included three center points to extract the key points of the design space as presented in Table-4. Design of experiment results including three center points of wet granulation indicated that the curvature was not significant. According to Fig. 1 and Table-4, the most important factor affecting tablet dissolution in the wet granulation process was the amount of granulating water. The selected model (Table-4) indicated that the effect of the amount of granulating water ($p < 0.0004$) on the dissolution rate of tablets is significant at $p < 0.05$. It also showed that lack of fit is not significant ($p = 0.6515$), which indicates that this model can be adjusted based on our desire [22]. To determine the design space where the difference between the predicted and adjusted regression cofficients (R^2) is generally less than 0.2 and their ratio is greater than 4, a full factorial model was used [21]. Our results showed that the difference and ratio of the predicted and adjusted coefficients of regression (R^2) are 0.19 and 4.79, respectively. The values of the amount of granulating water were changed descendingly, which showed unacceptable dissolution ($f_2 \leq 55$) at a high level (80 g) of the amount of granulating water.

Design space and updated risk assessment: The design space is shown in Fig. 4. The region of black colour indicates the most successful operating ranges for wet granulation. In general, a point within the design space indicates no change and fulfills the desired quality for the product. Fig. 4 also displays a 95 % confidence interval (CI) of the mean values of granule density and dissolution for wet granulation. The initial risk assessment in the wet granulation and mixing step was identified and reduced by formulation development through the design of experiment study. Accordingly, the risk assessment of valsartan and pravastatin fixed-dose combination tablets was well defined in the range where there is no effect on granule density or dissolution as agitator speed (150 to

TABLE-4
ANOVA RESULTS OF THE SELECTED FACTORIAL MODEL

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value (Probe > F)
Model	28.13	1	28.13	33.33	0.0004
The amount of granulating water	28.13	1	28.13	33.33	0.0004
Residual	6.75	8	0.84	–	–
Lack of fit	4.75	6	0.79	0.79	0.6515
Pure error	2.00	2	1.00	–	–
Cor total	206.73	10	–	–	–

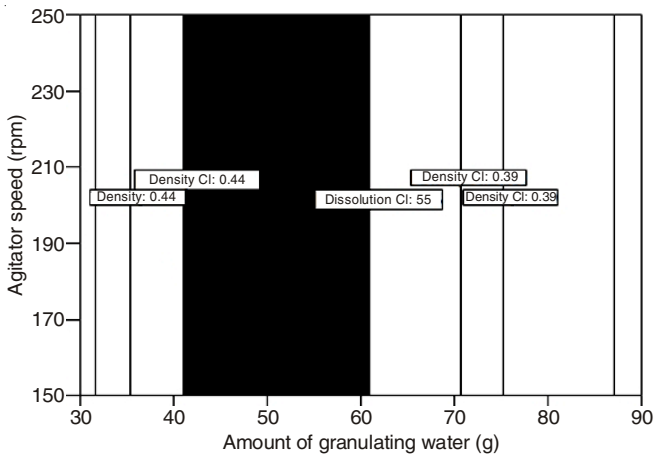

Fig. 4. Design space for wet granulation of valsartan and pravastatin fixed-dose combination tablet

250 rpm), granulating time (6 to 10 min) and the amount of granulating water (40 to 60 g).

Conclusion

All process variables can potentially affect the drug dissolution including assay and content uniformity, *etc*. Associated risks should be evaluated by rank such as low and high. The high risks (agitator speed, granulating time and the amount of granulating water) were evaluated using the design of experiment study to determine their acceptance criteria for wet granulation. The water amount in wet granulation was selected as the most important factor in the formulation development study. We concluded that the amount of granulating water (40 to 60 g) has a negative or null effect on tablet dissolution on the lab-scale. Based on the results of the design of experiment study on the lab-scale, the factors and process variables for wet granulation of the scale-up size should finally be set and optimized according to the acceptance criteria.

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REFERENCES

1. A.N. Zaid, S. Natur, A. Qaddomi, M. Abualhasan, R. Al-Ramahi, N. Shraim, S. Khammash and N. Jaradat, *Pak. J. Pharm. Sci.*, **27**, 755 (2014).
2. Q.-R. Cao, Y. Liu, W.-J. Xu, B.-J. Lee, M. Yang and J.-H. Cui, *Int. J. Pharm.*, **434**, 325 (2012).
3. T. Hirano, F. Komuro, S. Furukawa, S. Nagano and T. Takahashi, *Metabolism*, **39**, 605 (1990).
4. K.K. Koh, S.H. Han, P.C. Oh, E.K. Shin and M.J. Quon, *Atherosclerosis*, **209**, 307 (2010).
5. V.K. Rai, N. Pathak, R. Bhaskar, B.C. Nandi, S. Dey and L.K. Tyagi, *Int. J. Pharm. Sci. Drug Res.*, **1**, 51 (2009).
6. International Conference on Harmonisation (ICH) Guideline, Q8 Pharmaceutical Development (2008).
7. Product Quality Research Institute (PQRI), Process Robustness-A PQRI White Paper, *Pharm. Engine*. **26**, 1 (2006).
8. D.C. Montgomery, Design and Analysis of Experiments, J. Wiley & Sons Inc., Hoboken New Jersey, edn 6 (2005).
9. B.J. Ennis, in ed.: D.M. Parikh, Theory of Granulation: An Engineering Perspective, Handbook of Pharmaceutical Granulation Technology, Taylor and Francis group, New York, pp. 140-144 (2006).
10. A.J. Rogers, A. Hashemi and M.G. Ierapetritou, *Processes*, **1**, 67 (2013).
11. S. Bajaj, D. Singla and N. Sakhuja, *J. Appl. Pharm. Sci.*, **2**, 129 (2012).
12. M. Benali, V. Gerbaud and M. Hemati, *Powder Technol*., **190**, 160 (2009).
13. G. Levy, J.M. Antkowiak, J.A. Procknal and D.C. White, *J. Pharm. Sci.*, **52**, 1047 (1963).
14. N.C. Ngwuluka, B.A. Idiakhoa, E.I. Nep, I. Ogaji and I.S. Okafor, *Res. Pharm. Biotechnol.*, **2**, 25 (2010).
15. N. Tavakoli, N.G. Dehkordi, R. Teimouri and H. Hamishehkar, *Jundishapur J. Nat. Pharm. Prod.*, **3**, 33 (2008).
16. L.X. Yu, *Pharmaceut. Res.*, **25**, 781 (2008).
17. M. Litviæ, K. Smic, V. Vinkovic and M. Filipan-Litvic, *J. Photochem. Photobiol. Chem.*, **252**, 84 (2013).
18. A. Kocijana, R. Graheka and L. Zupancic-Kraljb, *Acta Chim. Slov.*, **53**, 464 (2006).
19. S. Brain-Isasi, C. Requena and A. Alvarez-Lueje, *J. Chil. Chem. Soc.*, **53**, 1684 (2008).
20. A. Mishra, S. Kumar and S. Kumar, *J. Sci. Ind. Res.*, **67**, 1098 (2008).
21. O.C. Nkuzinna, M.C. Menkiti, O.D. Onukwuli, G.O. Mbah, B.I. Okolo, M.C. Egbujor and R.M. Government, *Nat. Resour.*, **5**, 299 (2014).
22. E.O. Akala, S. Adesina and O. Ogunwuyi, *Int. J. Environ. Res. Public Health,* **13**, 47 (2016).