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Development and validation of analytical method for vildagliptin encapsulated poly-ε-caprolactone microparticles

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Abstract

Statistical experimental designs (DoE) are used as an advancement over conventional 'One Variable at Time' (OVAT) experimental approach. It provides more accurate results in fewer runs compared to conventional methods. In the present work a simple, rapid, precise and highly accurate RP-HPLC method was developed and validated for vildagliptin (VLG) in VLG-poly-ε-caprolactone (PCL) microparticles by using Box-Behnken design (BBD). Microparticles (MPs) were prepared by W/O/W solvent evaporation method. The prepared MPs were characterized by employing scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR).The RP-HPLC separation was carried out on HyperClone ^{TM} C18 column (250 mm x 4.6 mm I.D., 5µm) with mobile phase consisting of mixture of Phosphate buffer (0.01 M, pH 7): acetonitrile (75:25,v/v) and UV detection set at 203 nm with steady flow rate of 1.0 ml/min. The measurement was linearly correlated with concentration in the 10 - 100 µg/ml range ($r > 0.9999$). The proposed method was applied for the analysis of VLG in developed MPs. The results showed that the method is suitable to determine VLG in microparticle formulation without any interference from the formulation variables.

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Keywords: Vildagliptin microparticles; response surface methodology; RP - HPLC; method validation.

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1. Introduction

VLG is dipeptidyl peptidase-4 (DPP-4) inhibitor class drug which constitutes a new agent for treatment of type-2 diabetes mellitus (T2DM) and employed successfully as second line antihyperglycemic therapy [1,2].VLG is reported to have rapid metabolism and short elimination half-life of 1.6-2.5 h [3]. The drug is not official in the pharmacopoeia. Despite the current use of VLG to treat hyperglycemia, its multiple administration are needed to attain optimal therapeutic concentration which has the potential to cause severe side effects [4]. Therefore, it is of great importance the development of new approaches with higher specificity and effectiveness to deliver as anti-hyperglycemic agent to T2DM. Certainly, microparticulate drug delivery system containing VLG might be a powerful tool to overcome the shortcomings associated with conventional drug delivery. Microparticles formulation with sustained drug release property might result in enhanced anti-hyperglycemic activity. Some work are reported to encapsulate VLG by different carrier system [3,5,6]. PCL, a hydrophobic, biodegradable and biocompatible polymer due to its in vitro stability is ideally suitable for controlled release of encapsulated drug for long term [7,8]. Several methods were established for the determination of VLG in a variety of matrices such as rat plasma [9], dog plasma [10] and in human plasma by using LC-ESI-MS/MS and HPLC [11,12]. A few analytical methods are reported for estimation of VLG in alone and in combination [13-16]. However, these methods are suitable for pharmaceutical products without complex matrices, and not for formulations based on polymers and surfactants, as likely microparticles. The objective of the present work was to prepare and characterize the VLG-PCL microparticles and to develop simple, precise, highly accurate, and economic RP-HPLC method for the estimation of VLG in microparticles. Box-Benkhen (BBD) experimental design was used to assess robustness of the method.

2. Materials and methods

2.1 Chemicals and reagents

Active pharmaceutical ingredient (API) VLG was kindly gifted by S. P. Pharmaceuticals (Jalgaon, India). Poly-e-caprolactone (Mn ≈ 70,000-90,000 g/mol) and Polyvinyl alcohol (PVA; Mn ≈ 1,25,000) were purchased from Sigma-Aldrich (USA) and Thomas Baker (Mumbai, India) respectively. Dichloromethane (DCM) was procured from Merck Specialties Private Limited (Mumbai, India). All other chemicals and reagents used were of HPLC or analytical grade and used as provided. HPLC grade water was used to prepare different solutions.

2.2 Preparation of VLG-PCL microparticles

VLG-PCL microparticles were prepared by water-in-oil-in-water (W/O/W) double emulsion solvent evaporation technique [16]. Briefly, the drug (500 mg) predissolved in 2 ml distilled water as internal aqueous phase (W) was emulsified in 15 ml solution of dichloromethane (DCM) containing PCL polymer (2 gm) as oil phase (O) on magnetic stirring at 600 rpm for 10-15 min. which was resulted in primary W/O emulsion. The drug: polymer ratio was selected based on preliminary trials with different ratios and formulation with maximum encapsulation efficiency was selected for this study. The primary (W/O) emulsion was then injected using a glass syringe (21.5 G needle) into a 100 ml aqueous PVA (surfactant) solution as external aqueous phase (W) to produce W/O/W double emulsion at a speed of 800 rpm at room temperature using four blade stirrer (Remi electrotechnik Limited, India). After complete addition of W/O primary emulsion to the external aqueous phase W, n-hexane was added for hardening of microparticles. Stirring was continued for 1.5 h for the purpose of complete evaporation of dichloromethane. The resultant microparticles were collected by vacuum filtration and rinsed twice with n-hexane to remove non encapsulated drug. Filtered microparticles were allowed to air drying for 24 h and utilized for further evaluation.

2.2 Instrumentation and chromatographic conditions

The HPLC system (Younglin, ACME 9000 Korea was used in this work consisted of isocratic pump (SP-930D), UV-detector (YLUV-730D) , Rheodyne injector with loop of 20µl capacity and column oven. The signal outputs were recorded and integrated using Autochro 3000 software. The liquid chromatographic system was operated isocratically, at ambient temperature, using HyperClone ^{TM} C18 column (250 mm x 4.6 mm I.D., 5µm) with mobile phase consisting of mixture of phosphate buffer (0.01 M, pH 7) : acetonitrile (75:25,v/v) and UV detection set at 203 nm at steady flow rate of 1.0 ml/min. The sample injection volume was 20µl.Buffer solution for mobile phase was freshly prepared. Mobile phase was filtered through 0.45µm Nylon filter under vacuum and degassed by sonication for 15 min before use.

2.3 Experimental design

During the assay method development and validation of VLG multiple initial trials were conducted to improve the drug separation. The robustness of an analytical method refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. To assess the robustness of the method BBD was specifically selected, since it requires fewer runs than a central composite design in cases of three or four variables [17,18]. A 3-factor, 3-level BBD constructed 17 experimental runs by using Design-Expert® Software. The independent variables selected were the acetonitrile conc. in mobile phase (A, %v/v), flow rate (B, ml/min) and column temperature (C, ºC). Retention time (RT) (Y_1, min) and theoretical plate count (TP) (Y_2, N) were selected as dependent factors. Dependent and independent variables along with different levels are presented in Table 1. The significance of the design was determined by the comparisons of statistical parameters, on the basis of higher values of R^2. Two-dimensional (2D) and three-dimensional (3D) response plots from the resulting equations were constructed.

2.4 Standard and sample preparation

The standard stock solution of VLG was prepared by dissolving an accurately weighed working standard in mobile phase to obtain a solution with final concentration of 1 mg/ml. Further, for linearity assessment serial dilutions were done to get calibration standards of 10 - 100 µg/ml and injected in triplicate.

For the estimation of VLG content from VLG-PCL microparticles, an appropriate amount of dried microparticles were completely dissolved in minimum amount of dichloromethane, followed by addition of water to precipitate the polymer. After that solution was centrifuged at 4000 rpm for 15 min and filters the supernatant through 0.22µ PVDF micro filter.

Table 1. Box-Behnken experimental design for robustness testing and observed responses for selected variables

Coded values				Actual values	
			A	B	C
-1 (Low level)			20	0.8	23
0 (Middle level)			25	1	25
+1 (High level)			30	1.2	27
Run	Independent variable			Dependent variable	
	A	B	C	Y_1	Y_2
1	-1	0	-1	3.64	2013
2	0	-1	-1	3.42	2614
3	0	-1	+1	3.55	2536
4	0	0	0	3.28	2468
5	-1	+1	0	3.58	1645
6	0	0	0	3.25	2487
7	0	+1	-1	3.41	2356
8	+1	0	-1	3.1	1964
9	+1	-1	0	3.18	1694
10	+1	+1	0	3.38	1697
11	0	0	0	3.21	2455
12	+1	0	+1	3.37	1568
13	0	0	0	3.18	2570
14	0	+1	+1	3.48	2698
15	0	0	0	3.21	2541
16	-1	-1	0	3.67	1725
17	-1	0	+1	3.32	1958

A is Acetonitrile conc. in mobile phase (%v/v), B is flow rate (ml/min) and C is column temperature (ºC). Y_1, Y_2 are dependent variables Retention time (RT, min) and theoretical plate count (TP, N) respectively.

2.4 Characterization of microparticles

2.4.1 Fourier transform infrared spectrophotometer (FTIR) study

FTIR spectra of VLG, PCL and VLG loaded microparticles were determined by KBr-pellet method using FTIR (Perkin-Elmer, FTIR Spectrum-Two, Waltham, USA). The pellets were prepared in KBr disk by taking sample: KBr quantities in ratio of 1:10. These pellets were scanned in the range from 4000 to 400 cm^{-1}.

2.4.2 Field emission scanning electron microscopy (FE-SEM)

The surface morphology (shape and surface characteristics) of the microspheres were examined by field emission scanning electron microscope (Hitachi, Model-S4800). To view the image, sample was mounted on a metal stub using double-sided adhesive tape and coated with gold for 80 seconds, then placed into the specimen chamber. The SEM was operated at a distance of 8.2-8.5 mm and accelerating voltage of 15.0 kV.

2.4.3 Encapsulation efficiency (% EE) and drug loading (% DL)

The amounts of VLG in biodegradable microparticles were determined by reverse-phase (RP) HPLC system. The chromatographic conditions were followed as per section 2.2

The drug encapsulation efficiency (% EE) and drug loading (%) of the blend microspheres were calculated according to previous formula [7].

2.5 Validation study

The method for the determination of VLG was validated as per the ICH guidelines for validation of analytical procedures for different validation parameters. It was validated to include the essential demands of International Conference on Harmonization (ICH) guidelines [19,20-23].Robustness test is verified during the validation procedure and as per USP/ICH in this test influence of small deliberate changes in method parameter, such as operational, environmental and peak analysis are evaluated.

2.5.1 System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were determined by six replicate injections of the system suitability solution (20µg/ml).

2.5.2 Accuracy

The accuracy of the HPLC method was assessed by recovery test. Accuracy was evaluated by performing the assay of samples and calculated the peak area responses of different samples by recovery method. Sample with known concentration (10µg/ml) was prepared according to section 2.4 and spiked with 5, 10 and 15 µg/ml µg of working standard. The mean percent recovery was significantly determine the accuracy of the method.

Table 2. Recovery results for VLG using proposed HPLC method.

Amount of VLG found in MP[a]	Amount of spiked WS of VLG (µg)	Estimated amount (µg/ml)[a]	Recovery (%)	RSD (%)
9.68 ± 0.69	5	15.04±0.03	102.45	0.75
	10	19.87±0.26	100.96	0.31
	15	25.36±0.09	102.75	0.64
Mean of recovery			**102.05**	

MP- Microparticle formulation, [a] Mean of 3 runs ± standard deviation

2.5.3 Precision

The repeatability of the method was determined by evaluation of standard solution at three concentration level. Intra-day precision was determined by repeating each level five times within the same day.

Table 3. Intra-day precision and inter-day precision of VLG

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	% of amount found (n=5)[a]	RSD (%)	% of amount found (n=10)[b]	RSD (%)
20	101.51±1.052	0.64	103.1±1.024	1.14
40	100.84±0.234	0.27	101.69±0.87	0.58
60	99.87±0.985	0.81	101.32±0.438	1.34

[a] Mean of 5 replicates in a day± standard deviation,

[b] Mean of 5 replicates per day for 2 days± standard deviation

Intermediate precision (iner-day) was performed by analyzing the samples similarly in two days. Relative standard deviation was used to determine the precision.

3. Results and discussion

3.1 Method optimization

During the method development [11,13-15] number of experimental trials are performed to optimize the chromatographic conditions. Based on the previous trials attempted some responses like peak separation, resolution, relative retention time and peak symmetry was not up to the mark and hence a rapid, accurate and precise method for VLG detection from polymeric matrix was desired. A chromatographic separation was achieved with HyperClone ^{TM} C18 column (250 mm x 4.6 mm I.D., 5µm) with mobile phase consisting of mixture of phosphate buffer (0.01 M, pH 7): acetonitrile (75:25, v/v) and UV detection set at 203 nm at steady flow rate of 1.0 ml/min.

3.2 Validation study

System suitability was obtained by checking various parameters and observed values were in conformity with ICH limit. Linearity was performed at five different concentrations for VLG. The proposed method shows outstanding linearity over a range of 20, 30, 40, 50, 60 µg/ml with correlation coefficient ($r^2 = 0.999$).

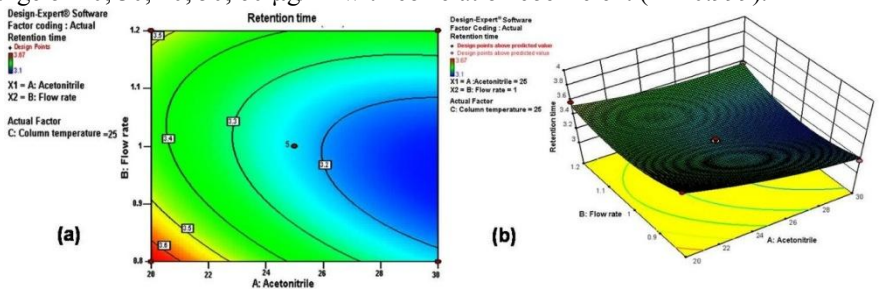

Fig.1. 2D contour plot (a) and 3D response surface plot (b) for retention time.

Recovery study was performed by spiking VLG sample of microparticle formulation with three different level standard solutions (Table 2) and the mean recovery value was found 102.05 with acceptable percent relative standard deviation (% RSD < 1). Mean of retention time by the proposed method was 3.23 min. It indicates accuracy of the method. Intra-day and Intermediate precision (iner-day) were performed by analyzing the samples in replicates for three level of concentration and similarly replicates were analyzed for two days. Table 3 summarises the results obtained which showed % RSD was less than 2%. However % RSD values were maximum for intermediate precision as compared to intra-day precision, but found within acceptable limit this might be due to stability of VLG in solvent which has kept for 48 h.

3.3 BBD for Robustness evaluation

Table 1 shows A Box–Behnken experimental design with 3 independent variables (A, B, C) at 3 different levels (-1, 0, +1) was used to study the effects on the dependent variables (Y_1,Y_2) and the RT and TP of all HPLC runs. The polynomial quadratic equation for RT (Y_1) and TP (Y_2) are given as

$$Y_1 = +3.23 - 0.15\,A + 3.750B + 0.019C + 0.073AB + 0.15AC - 0.015BC + 0.060A^2 + 0.17B^2 + 0.072C^2 \quad (1)$$

$$Y_2 = +2504.20 - 89.00A - 3.00B + 1.00C - 16.50AB - 37.00AC + 105.00BC - 659.35A^2 - 32.35B^2 + +79.15C^2 \quad (2)$$

A positive value in the regression equation for a response represents an effect that favours the optimization (synergistic effect), while a negative value indicates an inverse relationship (antagonistic effect) between the factor and the response [7,17-24]. From the Table 1 it is observed that RT and TP varies from 3.1-3.67 min and 1568-2698 N.

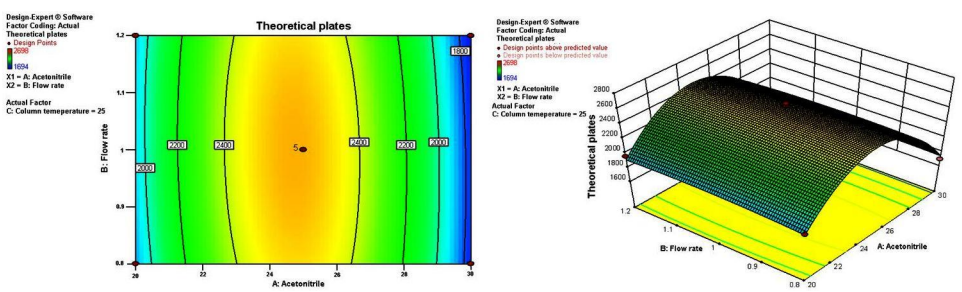

Fig.2. 2D contour plot (a) and 3D response surface plot (b) for theoretical plates

The results showed that RT affected by the independent variables like A, AB, AC B^2 and C^2 and these are

significant terms. TP was affected by A, BC, A^2. In both regression equations the main effects of A, B and C represents the average results of changing one variable at a time from its low level to high levelThe interaction terms AB, AC and BC show how the RT and TP changes when two variables are simultaneously changed. Table 4 showed summary statistics for Y_1 and Y_2 in bold faces for significant models respectively. From the ANOVA results, p values ($p<0.05$) for both response suggested significance of the model.

Table 4. Summary statistic for regression analysis for dependent variables Y_1 and Y_2

Response	Model	SD	R^2	Adjusted R^2	Predicted R^2	PRESS	Significance
	Linear	0.15	0.3712	0.2261	-0.1044	0.53	-
Y_1	2FI	0.14	0.5997	0.3595	-0.1757	0.56	-
[RT]	Quadratic	**0.058**	**0.9508**	**0.8876**	**0.3986**	0.29	Suggested
	Cubic	0.039	0.9872	0.9487	-	-	-
	Linear	388.34	0.0313	-0.8250	-0.8250	3.694	-
Y_2	2FI	437.01	0.0564	-3.0059	-3.0059	8.108	-
[TP]	Quadratic	**87.27**	**0.9737**	**0.6478**	**0.6478**	7.128	Suggested
	Cubic	49.27	0.9952	0.9808	-	-	-

The effects of factor A and B with their interaction on the RT and TP at C = 0 level are visualized by constructing 2D contour and 3D response surface plots as shown in Fig. 2 and Fig.3.It is observed that factor A has negative effect and factor C has positive effect on both responses. Unlikely, factor B has positive effect on RT and negative effect on TP and hence it shows that the relationship between factors and response is not always linear, when one or more than one factor is altered simultaneously then a factor can result in different grade of responses. The statistical results for RT and TP indicated that the analytical method was robust since variations in the experimental conditions did not affect on the quantitative analysis (% assay) of VLG.

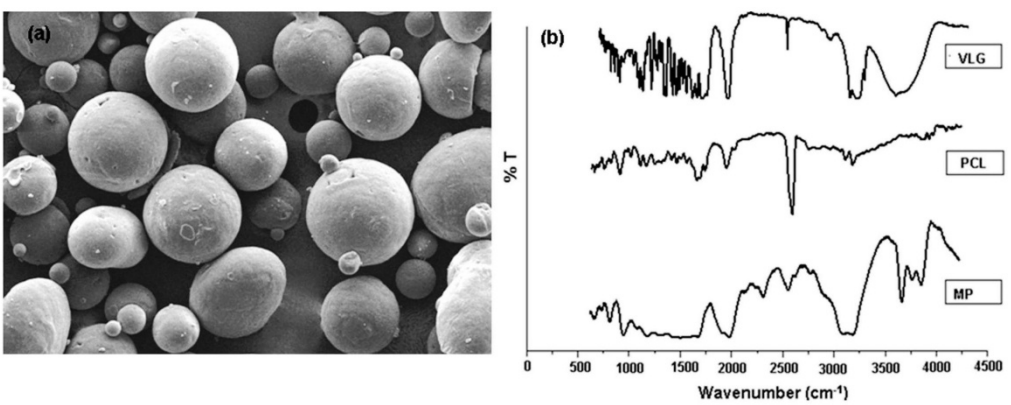

Fig.3. SEM Photomicrographs (a) of microparticles and FTIR spectra (b) for VLG, PCL, MPs (microparticles)

3.4 Microparticles characterization

The FTIR spectra of vildagliptin (Fig.4, b), the principle peaks were found at 3011−3370 cm^{-1} and 3294 cm^{-1} attributed to OH and N−H stretching vibrations. The prominent peak at 2230 cm^{-1},1659 cm^{-1} and 1245 cm^{-1} were assigned to nitrile, amide C=O and C−N stretching vibrations The FTIR spectra of VLG-PCL microparticles showed overlapping of the characteristic peaks at 3444 cm^{-1}, 2963 cm^{-1}, 2332 cm^{-1}, 1693 cm^{-1}, 1216 cm^{-1} suggesting absence of any interaction between drug and polymer during the microparticle preparation. Fig. 4(a) showed surface morphology of VLG-PCL microparticles and found spherical in shape with smooth surface and distributed uniformly.

Encapsulation efficiency of the VLG-PCL microparticles was determined by proposed method and found 82.42 ±0.37%. Depending upon the molecular weight of the PCL and viscosity of the internal phase it had successfully entrapped the hydrophilic VLG. DL was found 24.6%. Double emulsion solvent evaporation method was found suitable preparation technique for VLG microparticles. *In vitro* release study was performed for the prepared microparticles to assess their ability to sustain the action of VLG to minimize the dosing frequency and to achieve better patient compliance. The *in vitro* release was performed for 10h and found incomplete dissolution (68.12%) of the drug indicating that PCL microparticles could be used as effective new approach for long term delivery of VLG.

4. Conclusion

VLG-PCL microparticles were prepared by solvent evaporation method and found successfully encapsulated by PCL polymer. The method represented rapid, precise, accurate and highly robust analytical technique to analyse VLG in VLG-PCL microparticles. This method could be helpful for routine quality control analysis of VLG for such type of dosage form.

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