

Application of Design of Experiment® AQbD Approach for Systematic Optimization of Reverse-Phase High Performance Liquid Chromatography Method to Analyse Simultaneously Two Drugs (Metformin HCl and Sitagliptin phosphate monohydrate) in Tablet Dosage form

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INTRODUCTION & AIM

Diabetes, characterized by hyperglycaemia, arises from defects in insulin secretion, insulin activity, or both. The World Health Organization (WHO) broadly classified Diabetes mellitus in 1980 as either insulin-dependent diabetes mellitus (Type 1) or non-insulin-dependent diabetes mellitus (Type 2). A fixed-dose combination of metformin hydrochloride (MF) and sitagliptin phosphate monohydrate (SITA) has proven effective in achieving glycaemic control in Type 2 diabetic (DM) patients. The Food and Drug Administration has approved two such fixed-dose combinations. Currently, both immediate and extended-release tablets of MF and SITA are available worldwide. Chemically, MF is an N, N-diethyl imido dicarbonimidic diamide hydrochloride and belongs to the biguanide class of anti-diabetic drugs. Its mechanism of action includes enhancing peripheral insulin sensitivity by upregulating glucose in skeletal cells and inhibiting both intestinal glucose uptake and hepatic gluconeogenesis from non-sugar precursors. Sitagliptin phosphate monohydrate (SITA) is associated with the class dipeptidyl peptidase-4. Its chemical name is trifluorophenyl) (trifluoromethyl)-[(3R)-3-amino-1-oxo-4-(2,4,5butyl]-5,6,7,8-tetrahydro-3--1,2,4-triazolo[4,3-a] pyrazine phosphate. It functions by extending the anti-diabetic effects of incretin hormones, such as glucagon-like peptide and glucose-dependent insulinotropic polypeptide, by inhibiting their degradation. Although various analytical techniques for the simultaneous estimation of MF and SITA are documented in the literature, they are often time-consuming for quantitative analysis or costly due to high organic solvent consumption. Therefore, there is a current need for a simple, fast, ecological, and cost-effective method. This study aimed to develop a robust, quick, and inexpensive technique for the simultaneous estimation of MF and SITA using reverse-phase HPLC, validated according to the International Council for Harmonization (ICH Q2R2) guidelines for Technical Requirements for Pharmaceuticals for Human Use. The study focused on achieving linearity, system suitability, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and specificity for the quantitative evaluation of drugs in marketed dosage forms. The developed method was assessed for greenness, and this method was developed by using AQbD principles.

METHOD

Chemicals and Reagents: MF and SITA were kindly provided as gift samples from Viobility Research Private Limited, Dehradun, Uttarakhand, India.

Equipment and Software: The chromatographic separation was done on a JASCO 4000 series, composed of a quaternary gradient pump, integrated with a PDA (Photo Diode Array) detector, a manual injector, and ChromNAV 2.0 software, which is a data-acquiring software by JASCO, was used for response.

Mobile Phase: The trials were done using ethanol: 0.025 M ammonium acetate buffer at various pH levels in various flow rates, with different ratios of ethanol to buffer were operated at an ambient temperature (25 °C).

Preparation of Standard Solution: The standard working concentration of mixed MF (10 µg/mL) and SITA (10 µg/mL) was prepared in the mobile phase. This solution was subjected to liquid chromatographic analysis.

Chromatographic Conditions: The JASCO 4000 series, Quaternary gradient pump, integrated with PDA (Photo Diode Array) detector, was used for chromatographic separation. The HiQ sil C18HS (4.6 mm ID. x 250 mm L 5µm 100 Å) with an injection volume of 20 µL was used. The mobile phase consisted of Ethanol: 0.025 M ammonium acetate at various pH levels in several ratios with various flow rates. The HPLC system was operated at ambient temperature (25 °C).

Method Development: The optimized and predicted data from Design Expert software consisted of mobile Ethanol: 0.025 M ammonium acetate buffer (55:45 % v/v), pumped at pH 4.5 (pH adjusted with 1 M orthophosphoric acid) and a flow rate of (1.157 mL/min), giving the desirability function around 1. The UV detector was set at 256 nm. The retention time obtained for MF 2.623 and SITA 3.337 minutes

Experimental Design: A Box Behnken Design was used for method development. The amount (% v/v) of the organic phase (X1), pH of the mobile phase (Buffer pH) (X2), and flow rate (X3) were selected as independent variables. The independent variables were varied at three levels (-1, 0, +1). Different ranges of three parameters (X1) 45-55 % organic phase,(X2) pH 3.5-4.5, and(X3) flow rate of 0.8-1.2 mL/min were taken, respectively, as shown in Table 1. The factors and ranges selected were established in previous chromatographic separation studies. The dependent variables (or responses) were resolution, tailing factor, and number of theoretical plates. The study design of 17 experimental runs was generated and analyzed by Design-Expert software version 13.

Table 1: Independent factors and factor levels

Factors	Factors level		
	-1	0	+1
Amount of organic phase (X1)	45	50	55
pH (X2)	3.5	4	4.5
Flow rate (X3)	0.8	1	1.2

RESULTS & DISCUSSION

Table 2: Design summary

Factor	Name	Units	Type	SubType	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	% of Organic Solvent	v/v	Numeric	Continuous	45.00	55.00	-1 ↔ 45.00	+1 ↔ 55.00	50.00	3.54
B	Buffer pH		Numeric	Continuous	3.50	4.50	-1 ↔ 3.50	+1 ↔ 4.50	4.00	0.3536
C	Flow Rate	Minutes	Numeric	Continuous	0.8000	1.20	-1 ↔ 0.80	+1 ↔ 1.20	1.0000	0.1414

Response	Name	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	Resolution b/w MF and SITA	17.00	1.704	4.503	3.13	0.8401	2.64
R2	Tailing Factor of MF	17.00	1.232	1.845	1.50	0.1662	1.50
R3	Tailing Factor of SITA	17.00	1.209	1.669	1.52	0.1393	1.38
R4	NTP of MF	17.00	2323	2562	2385.00	73.13	1.10
R5	NTP of SITA	17.00	2423	2468	2450.47	14.37	1.02
R6	Area of MF	17.00	50960	98458	76735.59	12863.29	1.93
R7	Area of SITA	17.00	33449	39831	37731.53	1914.69	1.19

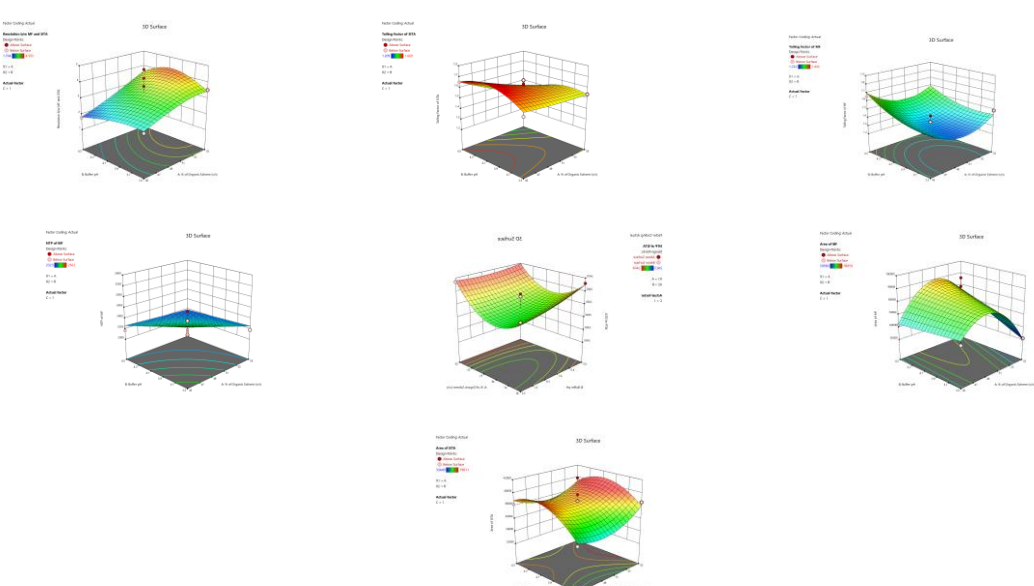


Fig 1. 3D plot for independent variables v/s dependent variables

Method Validation: The developed method was linear over the concentration range of 5-15 µg/mL, with a correlation coefficient (r²) of 0.9858 and 0.9928 for MF and SITA, respectively. This showed that all the responses were within the specified acceptance limit, indicating a high degree of closeness of predicted data with the observed ones. For the accuracy studies at 50, 75, 100, 125, and 150 % levels. The summary of method validation parameters is shown in the table below

Parameter	Limit	Result	
		MF	SITA
Linearity and Range	(r ²)	0.9858	0.9928
Inter-Day Precision	CV < 2	0.81-1.40	0.63-1.72
% Recovery	98-102 %	98.12-102.3	98.13-102.4
Robustness	C.V. < 2	0.17-1.76	0.87-1.79
Intraday Precision	C.V. < 2	0.83-1.86	0.93-1.63

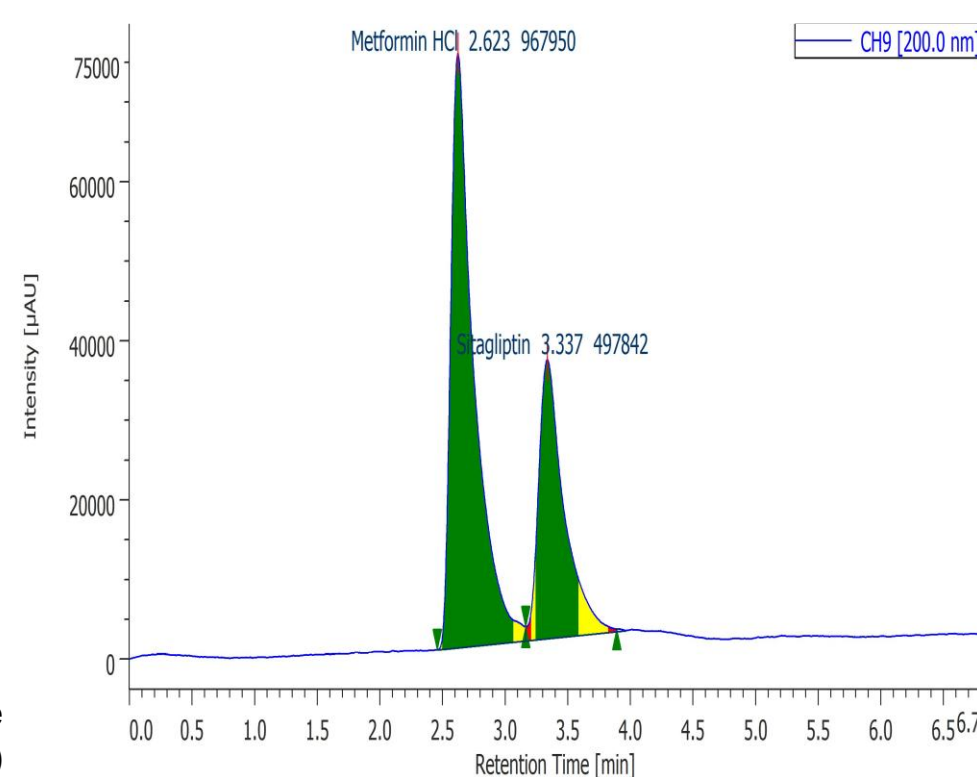


Fig 2. Optimized Chromatograms for MF and SITA

Greenness Score of Method:

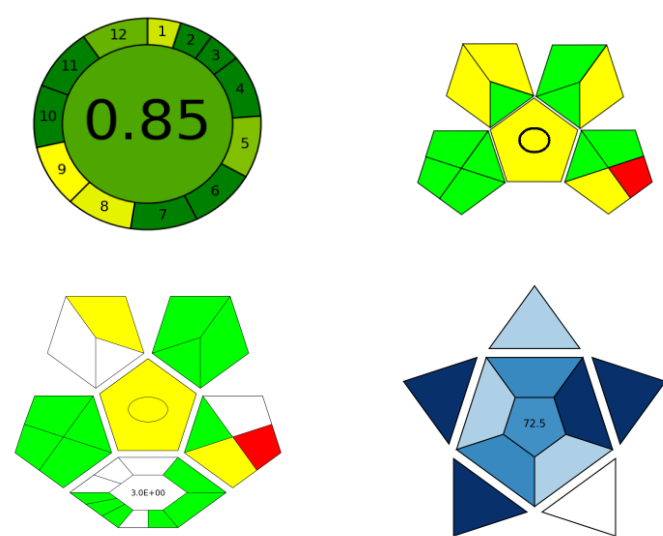


Fig 3. Greenness Assessment by AGREE, GAPI, Complex GAPI, BAGI, AES, AMVI, AMGS

CONCLUSION

A green RP-HPLC method was successfully optimized by using Design Expert software version 13, using AQbD approach study was done Box Behnken Design response surface design was done by 3 factors, i.e., % of organic phase, pH of buffer, and flow rate, and 4 responses, i.e., resolution between MF and SITA, tailing factor, NTP, area for MF and SITA, respectively. The developed method was validated by ICH guidelines (Q2R2) for linearity, % recovery, accuracy, precision, robustness, and assessed the greenness of the method by AGREE (score 0.85), GAPI, Complex GAPI, BAGI (score 72.5), AES (score 96), AMVI (score 75), AMGS (score 552.46) for the simultaneous estimation of MF and SITA. This RP-HPLC method will be useful for routine analysis for quality control in the Pharmaceutical industry.

FUTURE WORK / REFERENCES

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