Analytical Quality by Design (AQbD) for Developing a Validated High-Performance Thin Layer Densitometry Method for Estimating Mangiferin in Human Plasma

Rajneet Kaur Khurana, Atul Jain, OP Katare, Bhupinder Singh*

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India 160014

Emails: khurana.neeti@gmail.com, bsbhoop@yahoo.com

ABSTRACT

Of late, Analytical Quality by Design (AQbD) has been gaining increased acceptance in the industrial, academic and regulatory circles. Considered as a science and risk-based approach, AQbD provides rational understanding of the critical method parameters (CMPs) affecting the critical analytical attributes (CAAs) of an analytical method. The present work aims at systematic development of a simple, rapid and highly sensitive bioanalytical high-performance thin-layer densitometry method for the analysis of mangiferin. Initially, the quality target method profile (QTMP) was defined and critical analytical attributes (CAAs) were earmarked. Preliminary studies were conducted for selecting the suitable mobile phase mixture, followed by primary and secondary screening studies employing D-optimal design and Plackett-Burman design, respectively for selecting the ideal mobile phase composition and prioritizing the critically influential method parameters on the CAAs. The CAAs chosen included retardation factor (Rf), peak height, capacity factor, theoretical plates and separation number. Response Surface Methodology (RSM) was conducted as per the face centered cubic design (FCCD) for optimizing volume loaded and plate dimension as the critical method parameters (CMPs) selected initially from the screening studies. The mobile phase containing mixture of ethyl acetate: acetic acid: formic acid: water in 7:1:1:1, v/v/v/v ratio was finally selected as the optimized combination owing to apt chromatographic separation for mangiferin at 262 nm with Rf 0.68±0.02, with all other parameters being within the acceptance limits. Method validation studies revealed high linearity for mangiferin in the concentration range of 50–800 ng/band with r^2 =0.998 ± 0.005. The developed method showed high accuracy, precision, ruggedness, robustness, specificity, sensitivity, selectivity, recovery, detection limit (12.1 ng/band) and quantification limit (36.6 ng/band). The bioanalytical method for analysis of mangiferin in plasma revealed the presence of well-resolved peaks and high recovery of mangiferin.

INTRODUCTION

Mangiferin, a polyphenolic C-glucosylxanthone, primarily exists as a principal phytoconstituent in the leaves and stem bark of Mangifera indica (family, Anacardiaceae). Chemically, 2-C-β-d-gluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone. Presence of the phenolic xanthone moiety owes to the powerful antioxidant activity for scavenging free radicals and protection against ROS-induced oxidative stress. The important therapeutic applications of mangiferin include as antidiabetic, antiobesitic potential, antiosteoclastogenic, antiasthmatic, antidiarrhoeal, immunomodulator, analgesic, antiallergic, antibacterial, antimicrobial, antiviral and anticancer agent.

RATIONALE:

- Analytical methods are highly vital at every stage of the product development starting from characterization of drug substance to its estimation in dosage form, biological samples and stability studies. Development of analytical methods require complete understanding of the method variables to attain the superior method performance
- Method validation helps in ensuring the accuracy, precision, robustness, specificity and sensitivity of an analytical method for the specified analyte.
- The objectives of the present studies were to obviate the tedious, expensive and prolonged sample preparation, use of intricate mobile phase compositions and sensitive analytical method employing AQbD for Mangiferin

DEFINING THE QTMP, CAAs & CMPs

QTMP
Summary of critical quality attributes to develop an efficient HPTLC method with enhanced densitometric separation

CAAs
Retardation factor (Rf), peak height, capacity factor, theoretical plates and separation number

CMPs
Mobile phase ratio, saturation time, volume loaded, developmental phase, scanning speed, slit dimension, distance between tracks, migration distance, application position, stationary phase, plate dimension

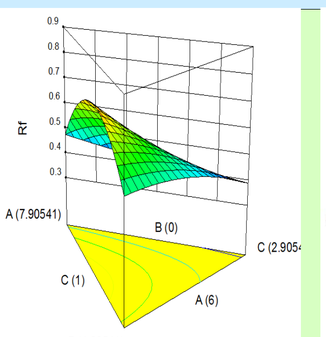
AQbD involves primary and secondary factor screening employing D-optimal and Plackett-Burman design. Further, optimization was performed with face centered cubic design

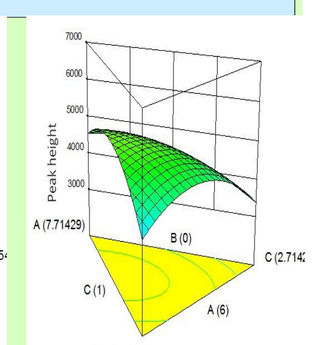
PRELIMINARY SCREENING

Developmental Phase	Ratio (v/v)	Rf	Inference drawn
Ethyl acetate: Methanol	4:6	0.12	Poor resolution of spots with high solvent front
Ethyl acetate: Methanol (with four drops of glacial acetic acid)	4:6	0.15	Solvent front decreased with no significant improvement in the resolution
Toluene: Acetone: Glacial acetic acid	6:3:1	0.14	Poor resolution of the spots
Methanol: Ethyl acetate: Glacial acetic acid	5:4:1	0.46	Better resolution of the spots with presence of tailing
Ethyl acetate: Methanol: Toluene	6:3:1	0.15	No apt resolution of the spots
Ethyl acetate: Methanol: Chloroform	6:3:1	0.17	No apt resolution of the spots
Hexane: Methanol: Ethyl acetate: Glacial acetic acid	2:2:5:1	0.21	Low spot resolution with tailing
Ethyl acetate: Glacial acetic acid: Formic acid: Water	5:2:2:1	0.56	Efficient spot resolution with improved Rf

PRIMARY PARAMETER SELECTION: D-OPTIMAL MIXTURE DESIGN

Experimental Run	Ethyl acetate	Acetic acid	Formic acid	Water
1	6	1	1	2
2	6	1	2	1
3	6	2	1	1
4	7	1	1	1
5	8	0	1	1
6	6.5	0.5	1	2
7	6	2	1	1
8	7	0	2	1
9	6.25	1.25	1.25	1.25
10	6	0	3	1
11	7	0	1	2
12	7	0	2	1
13	7.5	0	1	1.5
14	6.5	0	1.5	2
15	6	1	2	1
16	6	0.5	1.5	2
17	7	1	1	1
18	6	0	3	1
19	6.25	0.25	2.25	1.25
20	6	0	2	2



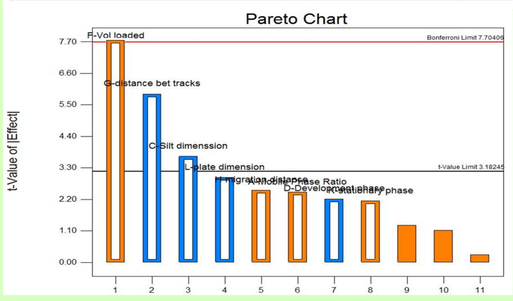


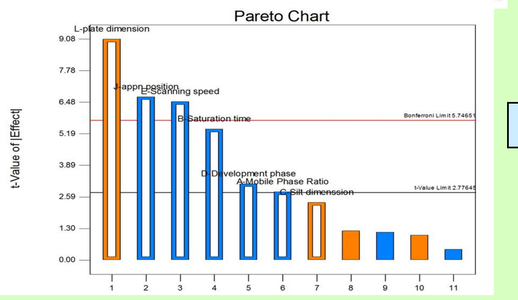
A= Ethyl acetate	B=Formic acid	C= Acetic acid	D=Water	Rf	Peak height	Desirability
7.00	1.00	1.00	1.00	0.654	5384.78	1
7.5	0.5	1	1	0.690	5367.46	1
6.5	1	1.5	1	0.671	5491.10	1

SECONDORY PARAMETER SELECTION: PLACKETT-BURMAN DESIGN

Mobile Phase Ratio	Saturation time	Volume loaded	Developmental Phase	Scanning speed	Slit dimension	Distance between tracks	Migration distance	Application position	Stationary phase	Plate dimension
1	1	1	-1	-1	-1	1	-1	1	1	-1
-1	1	1	1	-1	-1	-1	1	-1	1	1
-1	1	-1	1	1	-1	1	1	1	-1	-1
1	1	-1	1	1	1	-1	-1	-1	1	-1
1	1	-1	-1	-1	1	-1	1	1	-1	1
-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
-1	1	1	-1	1	1	1	-1	-1	-1	1
-1	-1	-1	1	-1	1	1	-1	1	1	1
1	-1	-1	-1	1	-1	1	1	-1	1	1
1	1	-1	-1	-1	1	-1	1	1	-1	1
1	1	1	-1	-1	-1	1	-1	1	1	-1

Name of the factors	Levels	
	Low (-1)	High (+1)
Mobile Phase Ratio	6:1.5:1.5:1	8:0.5:0.5:1
Saturation time (min)	2	4
Volume loaded (µL)	2	6
Developmental Phase (mL)	10	15
Scanning speed (mm/sec)	10	15
Slit dimension (mm)	5	6
Distance between tracks (mm)	5	10
Migration distance (mm)	70	80
Application position (mm)	5	10
Stationary phase	Aluminum plate 60	Aluminum plate 60F254
Plate dimension (cm)	10×10	20×10





AQbD OPTIMIZATION STUDIES AS PER FACE CENTERED CUBIC DESIGN (FCCD)

Linear regression data for calibration curve of mangiferin in human plasma (n=3)

Parameters	Values
Linearity range (ng/spot)	50–800
Regressed equation	Y=3.046x + 1066
Correlation coefficient	0.996± 0.0012
Slope± SD	3.046 ± 0.557
LOD	12.06 ng/ml
LOQ	36.55 ng/ml

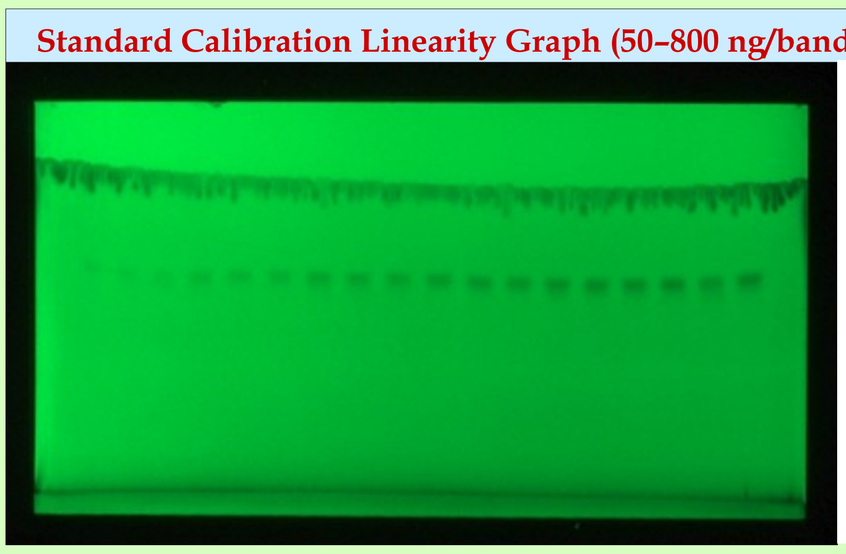
Design Matrix: Actual and Coded Levels of CMAs

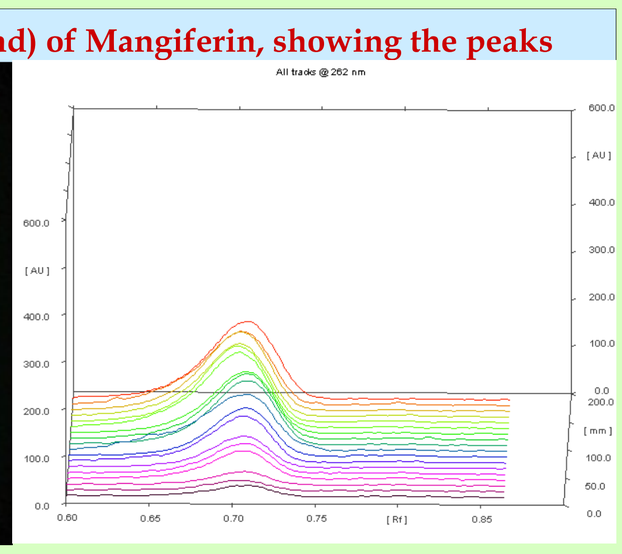
Trial No.	Coded factor levels	
	Factor 1	Factor 2
1.	1	-1
2.	1	1
3.	0	0
4.	0	0
5.	0	0
6.	-1	1
7.	0	-1
8.	-1	-1
9.	1	0
10.	0	0
11.	0	1
12.	-1	0
13.	0	0

Translation of coded levels in actual units

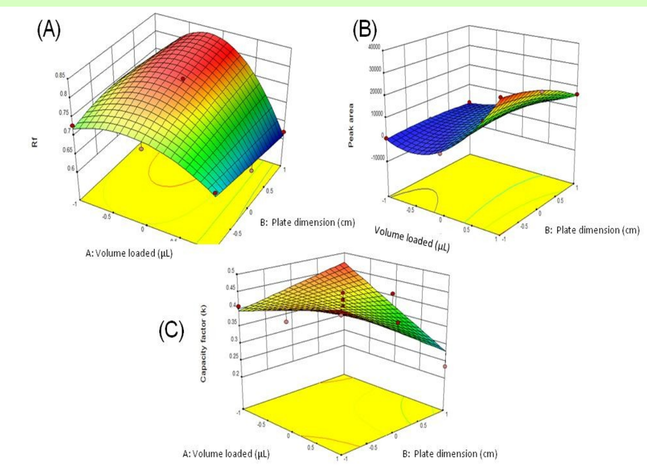
Coded level	-1	0	1
Volume loaded (µL)	2	4	6
Plate dimension (cm)	10×10	15×10	20×10

Standard Calibration Linearity Graph (50–800 ng/band) of Mangiferin, showing the peaks

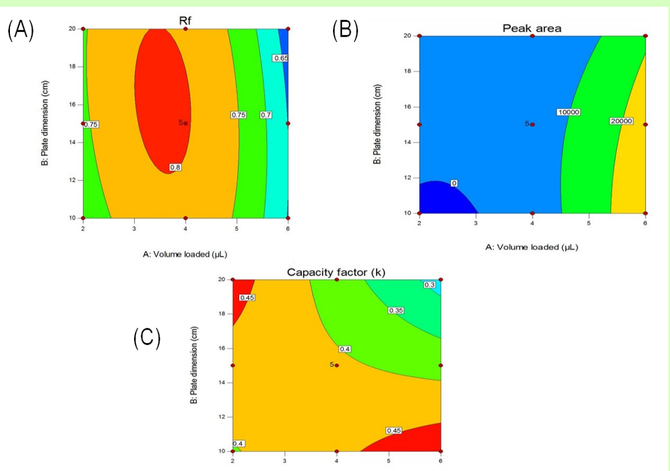




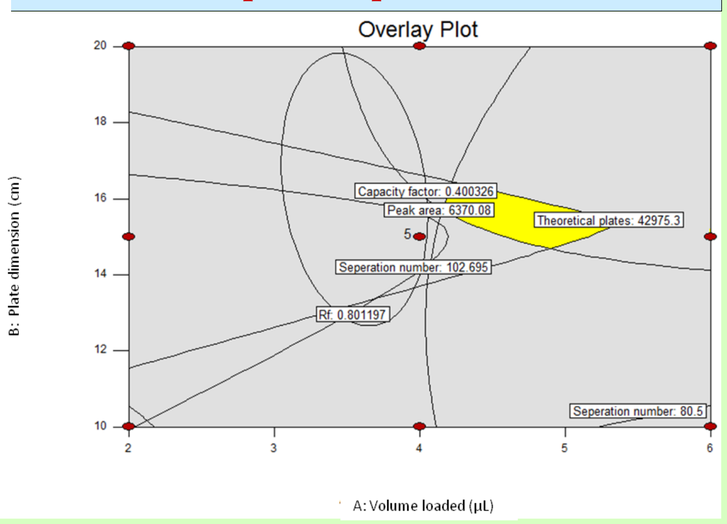
3D-Response Surface Analysis



Contour Charts



Graphical Optimization



Accuracy Data for Mangiferin

Standard concentration	Levels (%)	Concentration (ng/spot)	Amount recovered (ng/spot) ± S.D.	Recovery (%)	RSD (%)
Mangiferin (100 ng/spot)	LQC: 50	150	146.13 ± 2.52	96.75	1.72
	MQC: 100	200	194.69 ± 3.51	97.34	1.80
	HQC: 150	250	248.69 ± 3.51	99.47	1.41

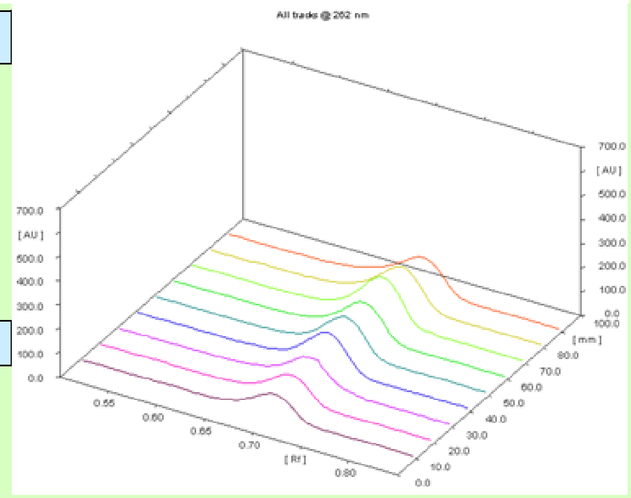
Intra- and Inter-Day Precision Data of Bioanalytical HPTLC Method of Mangiferin

Intra-day precision (within day)			
Standard concentration (ng/spot)	Amount recovered (ng/spot) ± S.D.	Recovery (%)	RSD (%)
LQC: 100	95.06 ± 1.10	95.06	1.15
MQC: 150	146.92 ± 1.53	97.94	1.04
HQC: 200	195.10 ± 1.00	97.55	1.02

Inter-day precision (between day)			
Standard concentration (ng/spot)	Amount recovered (ng/spot) ± S.D.	Recovery (%)	RSD (%)
LQC: 100	92.06 ± 1.10	92.06	1.19
MQC: 150	144.92 ± 1.53	96.13	1.59
HQC: 200	193.10 ± 1.00	96.55	1.03

CONCLUSIONS

- A simple, rapid, sensitive and economical bioanalytical method has been successfully developed employing AQbD approach for quantification of mangiferin in plasma.
- The final developmental phase composition was selected as Ethyl acetate: Formic acid: Acetic acid: Water (7:1:1:1). Method validation studies corroborated excellent linearity, accuracy, precision, and system suitability of the developed HPTLC method.



REFERENCES

- Singh *et al.* Crit Rev Ther Drug Carrier Syst, 2005. 22(1): p. 27-105.
- Khurana *et al.* Curr Pharm Anal,.2015; 11: 3 (10)