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Experimental design approach for robustness testing of HPTLC methods

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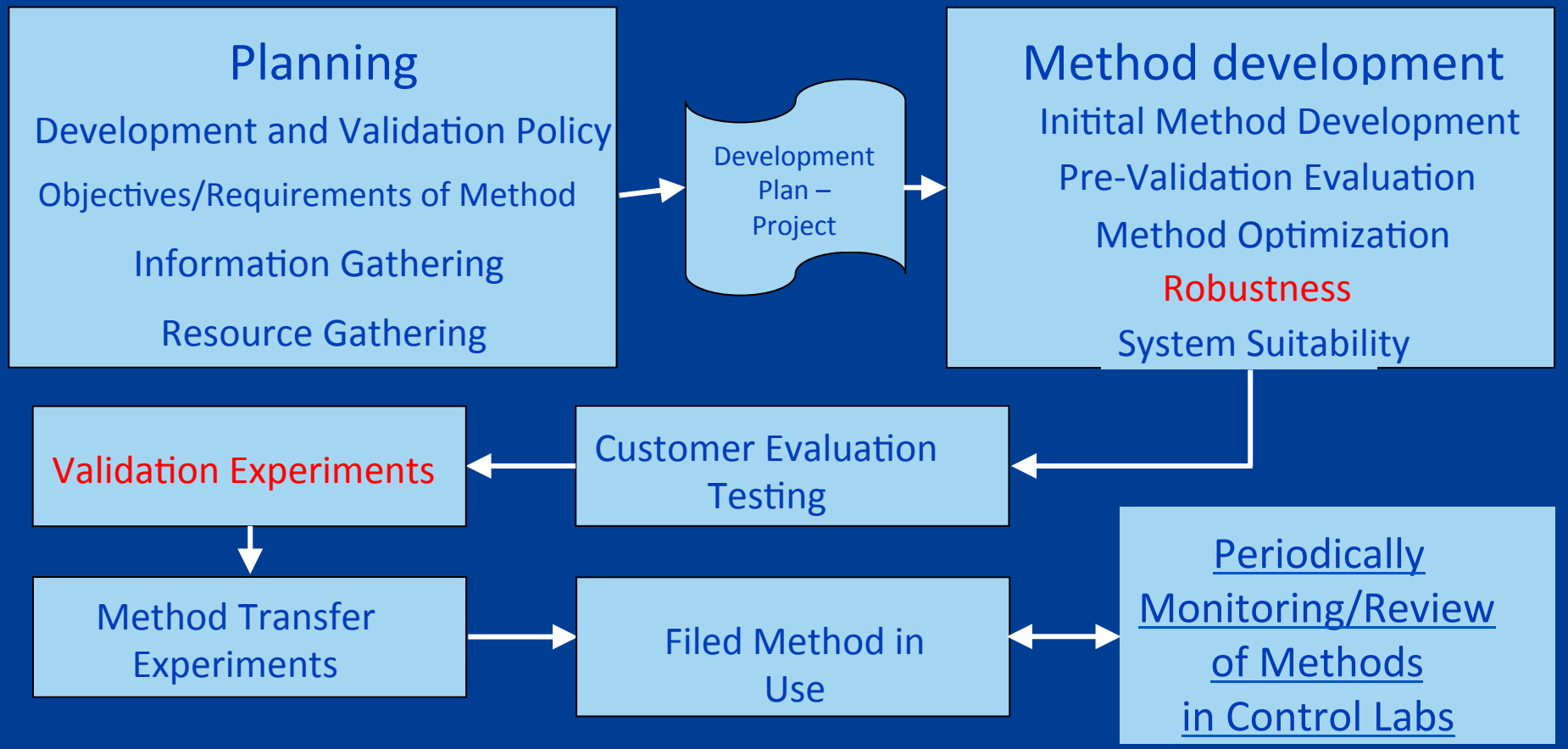
Analytical Method – Life Cycle



From: Analytical Chemistry in a GMP Environment. Edited by J.M. Miller and J.B. Crowther, ISBN 0-471-31431-5, Wiley & Sons Inc.

Analytical Method Validation

- Validation is the formal proof that method is suitable for its intended use.
- Validation Characteristics

	Identification	Impurities		Assay
		quantitative	limit	
Accuracy	-	+	-	+
Precision	-	+	-	+
Specificity	+	+	+	+
Detection Limit	-	-	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+
Robustness	+	+	+	+

- Robustness is one of the key elements in validation of separation methods

Robustness Testing

- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
- A robustness testing is an experimental set-up to evaluate the robustness of a method.
- The robustness test can be viewed as a part of method validation that is performed at the end of method development or at the beginning of the validation procedure.
- Indeed, ICH Q2(R1) guideline advocates that "The evaluation of robustness should be considered during the development phase".

ICH, Q2 (R1); Validation of analytical procedure: Text and Methodology, 2005, pp. 1-17

Traditional Approach for Robustness Testing

- COST / OVAT / OFAT / Shotgun Approach
- Changing One Single (or Separate) variable or factor at a Time

Pitfalls…….

- Unsystematic approach
- Sequential scheme
- Requires many experiments
- These approach gets stuck --- when there is presence of interactions—i.e., the influence of one or more variable(s) on others.

Optimizing Drug Delivery Systems Using Systematic "Design of Experiments." Part I: Fundamental Aspects. ***Bhupinder Singh, Rajiv Kumar, & Naveen Ahuja,*** *Critical Reviews™ in Th erapeutic Drug Carrier Systems, 22(1):27–105 (2004)*

Design of Experiment

- Design of experiments (DOE) is a well-proven characterization approach within product and process development and a key aspect of quality by design.
- DoE is the branch of applied statistics that deals with planning, conducting, analyzing and interpreting controlled tests to evaluate the factors that control the value of a parameter or group of parameters.
- *Advantages of DOE:*

✓ Development of a robust method.

✓ Understand, reduce and control sources of variability.

✓ Applicable throughout the life cycle of the method.

✓ Regulatory flexibility.

Steps in robustness testing by DoE approach

(a) identification of the factors to be tested,

(b) definition of the different levels for the factors,

(c) selection of the experimental design,

(d) definition of the experimental protocol (complete experimental set-up),

(e) definition of the responses to be determined,

(f) execution of the experiments and determination of the responses of the method,

(g) calculation of effects,

(h) statistical and/or graphical analysis of the effects, and

(i) drawing chemically relevant conclusions from the statistical analysis and, if necessary, taking measures to improve the performance of the method.

Guidance for Robustness/Ruggedness Tests in Method Validation. Y.V. Heyden, A. Nijhuis, J. Smeyers-Verbeke, B.G.M. Vandeginste and D.L. Massart

DoE : A New Paradigm in HPTLC

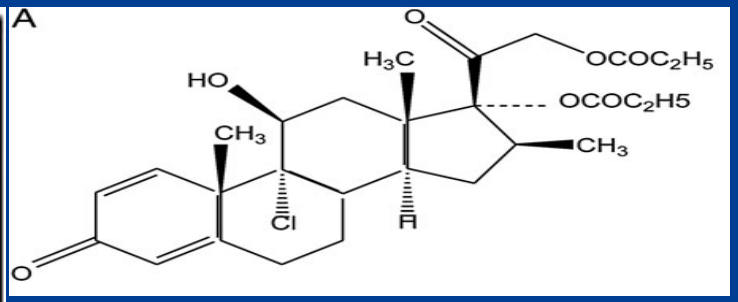
- A large number of reports are available with respect to robustness testing of HPLC method.
- However, very few published reports are available with respect to robustness testing of HPTLC method using Experimental Design approach.

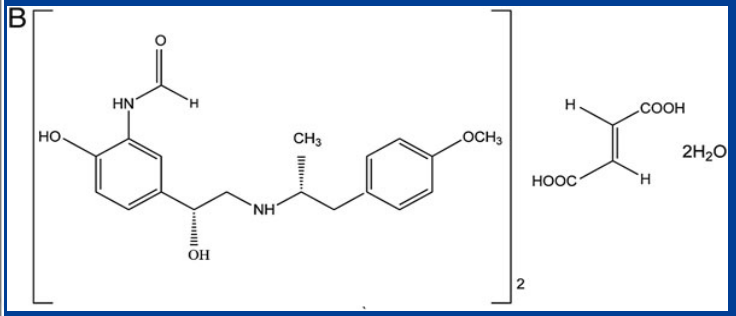
Experimental Design Approach for Robustness testing of…

1. HPTLC Method for Simultaneous Determination of Beclomethasone Dipropionate (BDP) and Formoterol Fumarate Dihydrate (FFD) in Rotacaps

2. HPTLC method for estimation of Diosgenin from *Balanites aegyptiaca* Extract using Spraying Reagent.

Case 1 - HPTLC method for simultaneous estimation of beclomethasone dipropionate (BDP) and formoterol fumarate dihydrate (FFD) from rotacaps





Label Claim: Each capsule contains
Beclomethasone dipropionate.....200 µg
Formoterol fumarate dihydrate..... 6 µg

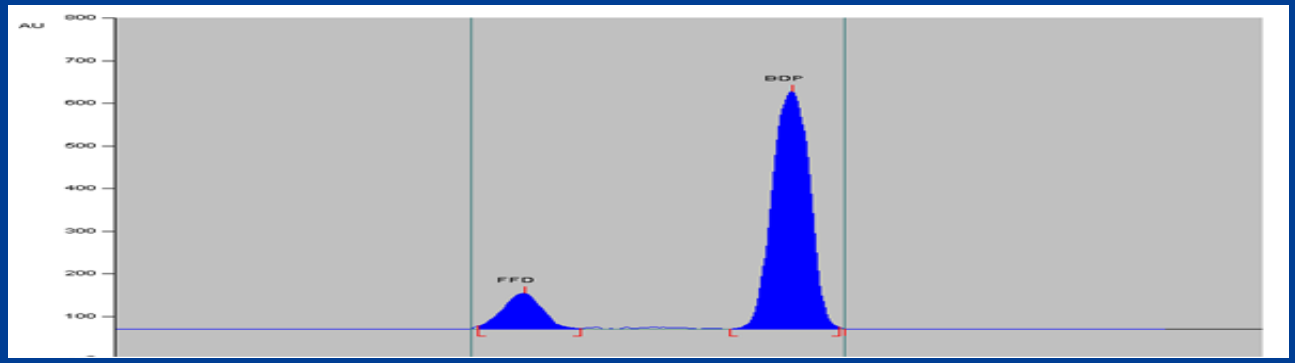
Parmar, V. K., Patel, H. N., & Patel, B. K. (2014). *Journal of chromatographic science*, bmt208

Nominal chromatographic conditions of HPTLC method for simultaneous determination of BDP and FFD

Stationary Phase	Precoated Silica gel G60 F254 aluminium Sheets 10×10 cm^2, layer Thickness 0.2 mm
Mobile Phase	Hexane: Ethyl acetate: Methanol: Formic acid 2.0:2.5:2.0:0.2 v/v/v/v
Pretreatment	TLC plates prewashed with methanol and activated in Oven at 110°C for 5mins
SPOTTING PARAMETER	
Band width	6 mm
Distance between two tracks	12mm
Spraying rate	150 nL/sec

Nominal chromatographic conditions of HPTLC method for simultaneous determination of BDP and FFD

DEVELOPMENT PARAMETERS	
Chamber saturation time	10 min
Migration distance	70 mm
Temperature	Room temperature
SCANNING PARAMETER	
Slit dimension	4.00 mm × 0.30 mm
Wavelength of detection	220 nm
Lamp	Deuterium
Measurement mode	Absorption/Reflection
Scanning speed	20 mm/sec



Factors and their Levels selected for Robustness Testing of HPTLC method for BDP and FFD (Case – 1)

	Factors	Levels		
		Low (-1)	Nominal (0)	High (1)
A	Change in volume of hexane in mobile phase composition (mL)	1.8	2.0	2.2
B	Change in volume of ethyl acetate in mobile phase composition (mL)	2.25	2.50	2.75
C	Change in volume of methanol in mobile phase composition (mL)	1.8	2.0	2.2
D	Change in saturation time (min)	9	10	11
E	Change in detection wavelength (nm)	219	220	221
F	Change in band width (mm)	4	6	8
G	Change in solvent run distance (cm)	6.5	7.0	7.5

Experimental Design

- **Plackett Burman Design**
- Full Factorial Design
- Fractional Factorial Design
- Asymmetric Factorial Design
- Central Composite Design
- Box- Behnken Design

- The **DOE ++ software** (Reliasoft Corporation, AZ, USA; ver 1.0.7) was used to set up the experimental designs.
- The **% Recoveries and Rf values** were observed **as responses** at each experiment designed.
- The experiment was repeated three times.
- The experiments were executed in random order.
- The significance of the factor effects was determined statistically, using error estimates in the calculation of critical effects, and graphically, by means of Pareto charts.

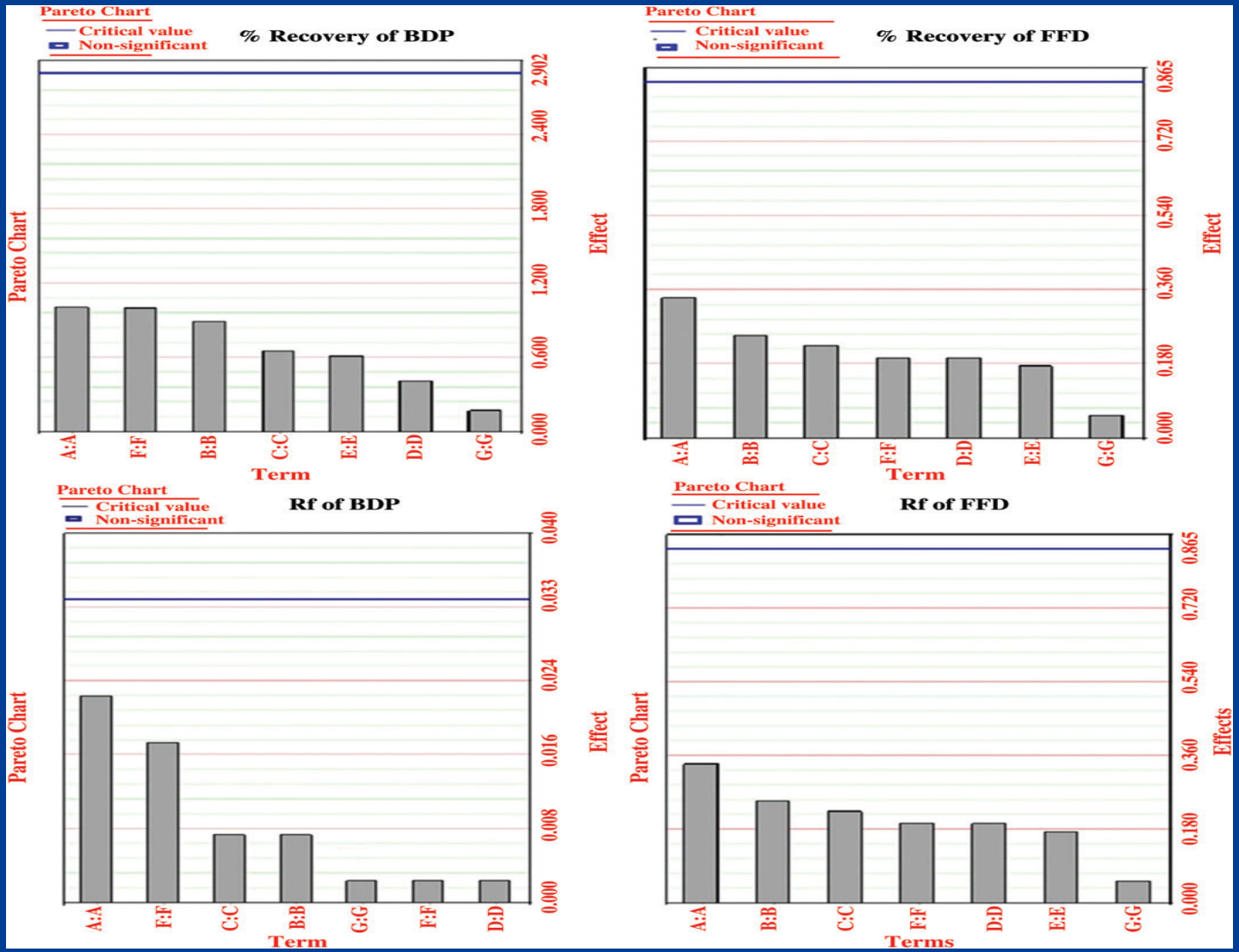
Eight experiment Plackett-Burman design to examine the seven factors (A-G) selected for robustness testing of HPTLC method

Experiments	Factors							Responses			
								% Recovery		Rf Values	
	A	B	C	D	E	F	G	BDP	FFD	BDP	FFD
1	-1	+1	+1	+1	-1	+1	-1	100.46	99.25	0.67	0.35
2	-1	-1	+1	+1	+1	-1	+1	99.77	99.54	0.67	0.36
3	-1	-1	-1	-1	-1	-1	-1	101.65	99.73	0.64	0.34
4	+1	-1	+1	-1	-1	+1	+1	98.79	100.10	0.64	0.33
5	+1	+1	-1	+1	-1	-1	+1	100.94	99.68	0.64	0.34
6	-1	+1	-1	-1	+1	+1	+1	100.74	99.91	0.68	0.33
7	+1	-1	-1	+1	+1	+1	-1	98.59	100.25	0.66	0.38
8	+1	+1	+1	-1	+1	-1	-1	100.25	99.77	0.65	0.36

Statistical Analysis

Responses	Effects of Factors							Critical Effect
	A	B	C	D	E	F	G	ME $_{(\alpha=0.05)}$
% Recovery (BDP)	-1.01	0.90	-0.66	-0.42	-0.62	-1.01	-0.18	1.729
% Recovery (FFD)	0.34	-0.25	-0.22	-0.20	0.18	0.20	0.06	0.525
Rf Values (BDP)	-0.018	0.007	0.002	0.008	0.018	0.013	0.003	0.028
Rf Values (FFD)	0.0075	-0.0075	0.0025	0.0175	0.0175	-0.0025	-0.0175	0.029

Representative Pareto charts to show the influence of variables studied in the response of BDP and FFD using PB experimental design for HPTLC method.



Case 2 - **HPTLC method for estimation of Diosgenin from *Balanites aegyptiaca* Extract using Spraying Reagent.**

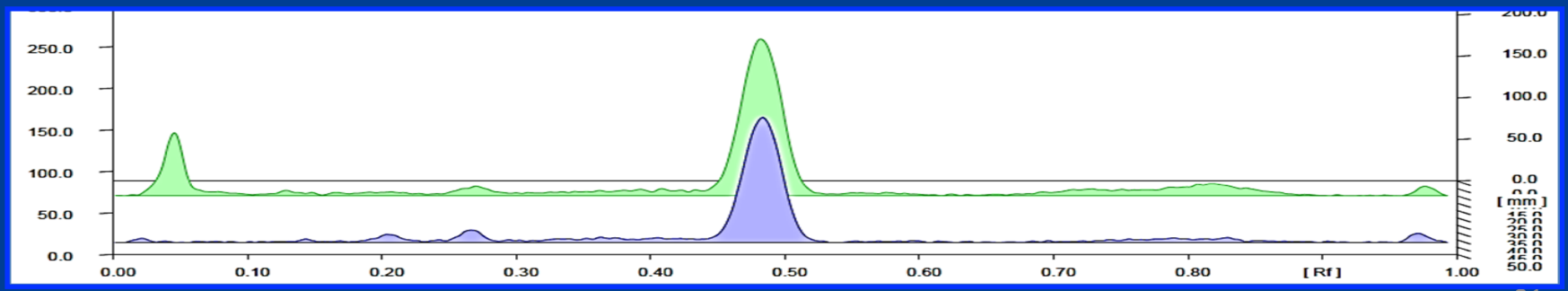
- *Balanites aegyptiaca (L) Del.,* also known as 'Desert date' in English, a member of the family Balanitaceae, is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia.
- Seed is used as expectorant, antibacterial, and antifungal. Fruit is used in whooping cough, also in leucoderma and other skin diseases.
- In Egyptian folk medicine, the fruits are used as an oral hypoglycemic and an antidiabetic;
- An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice.
- Widely used as traditional herbal medicine.
- It is major source of saponin of yamogenin and diosgenin (Balanitin-1 to 7).

Nominal chromatographic conditions of HPTLC method for estimation of diosgenin from *Balanites aegyptiaca* Extract

Stationary Phase	Precoated Silica gel G60 F254 aluminium Sheets 10×10 cm^2, layer Thickness 0.2 mm
Mobile Phase	Toluene : Ethyl acetate : Formic acid (7 : 2.8 : 0.2 v/v/v)
Pretreatment	TLC plates prewashed with methanol and activated in Oven 60 ± 3°C for 2.5 min
SPOTTING PARAMETER	
Band width	6 mm
Distance between two tracks	8 mm
Spraying rate	150 nL/sec
DEVELOPMENT PARAMETERS	
Chamber saturation time	20 min
Migration distance	80 mm
Temperature	Room temperature

Nominal chromatographic conditions of HPTLC method for estimation of diosgenin from *Balanites aegyptiaca* Extract

DERIVATIZATION PARARMETERS	
Spraying reagent	Anisaldehyde + 5 mL sulphuric acid + 10 mL glacial acetic acid diluted up to 100 mL with methanol
Oven temperature & time	60 ± 3°C for 18 min
SCANNING PARAMETER	
Slit dimension	4.00 mm × 0.30 mm
Wavelength of detection	426 nm
Lamp	Tungsten
Measurement mode	Absorption/Reflection
Scanning speed	20 mm/sec



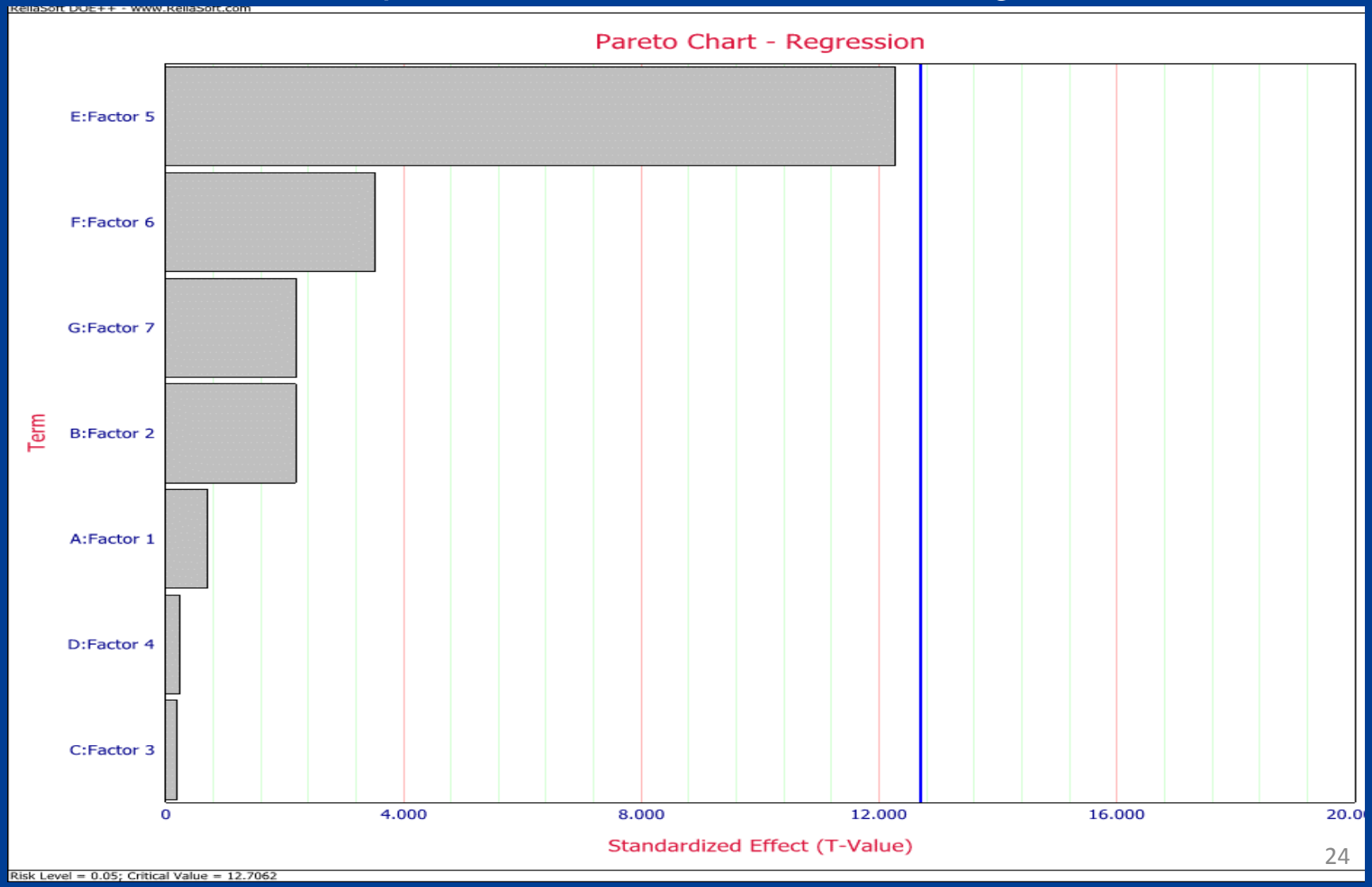
Factors and Their Levels for Robustness Testing of HPTLC method for diosgenin (Case – 2)

Factor Label	Factor Name	Level		
		Low (-)	Nominal (0)	High (+)
A	Toluene	6.3 mL	7 mL	7.7 mL
B	Ethyl Acetate	2.5 mL	2.8 mL	3.1 mL
C	Saturation Time	18 min	20 min	22 min
D	Solvent migration distance	7.5 cm	8 cm	8.5 cm
E	Ratio of application band to detection slit width	3/2 mm	6/4 mm	9/6 mm
F	Detection Wavelength	425 nm	426 nm	427 nm
G	Oven Temperature	55°C	60°C	65°C

Eight experiment Plackett-Burman design to examine the seven factors (A-G) selected for robustness testing of HPTLC method

	Factor							Response
	A	B	C	D	E	F	G	Peak Area (n=3)
Run 1	1	1	1	-1	1	-1	-1	3171.000
Run 2	-1	1	1	1	-1	1	-1	8454.667
Run 3	-1	-1	1	1	1	-1	1	4856.333
Run 4	1	-1	1	-1	-1	1	1	9539.333
Run 5	1	1	-1	1	-1	-1	1	7753.000
Run 6	1	-1	-1	1	1	1	-1	4989.333
Run 7	-1	1	-1	-1	1	1	1	5136.333
Run 8	-1	-1	-1	-1	-1	-1	-1	7894.667
Run 9	0	0	0	0	0	0	0	6948.000
Response	Effect of factors							Critical Effect ME $_{(\alpha=0.05)}$
Peak Area	2213.0	-1044.7	1581.2	-691.2	288.8	-1530.7	2419.5	3711.6581

Representative Pareto charts to show the influence of variables studied in the peak area measurement for diosgenin



Conclusions

- The robustness of the proposed methods was studied using DoEs and found to be robust at deliberate changes made in experimental conditions.
- Plackett-burman design can be used as an effective statistical tool for robustness testing of HPTLC methods.

Acknowledgement

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