Namur – 20/01/2016

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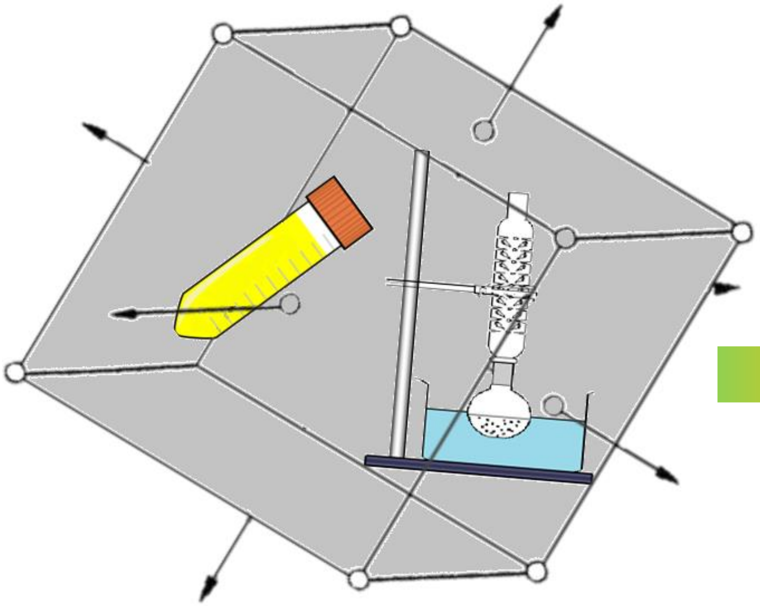
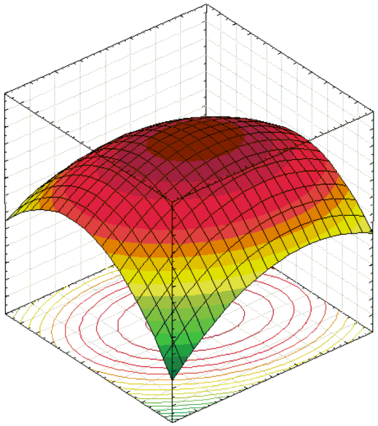
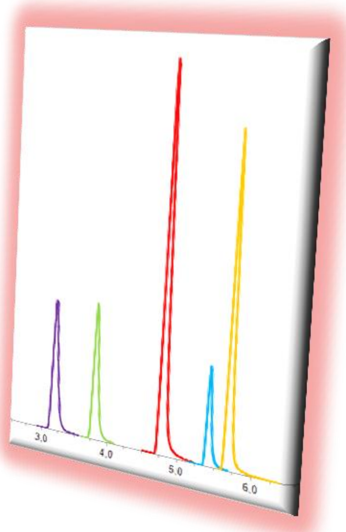
Use of response surface methodology to optimize samples preparations in laboratory







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Context - lifecycle of an analytical method

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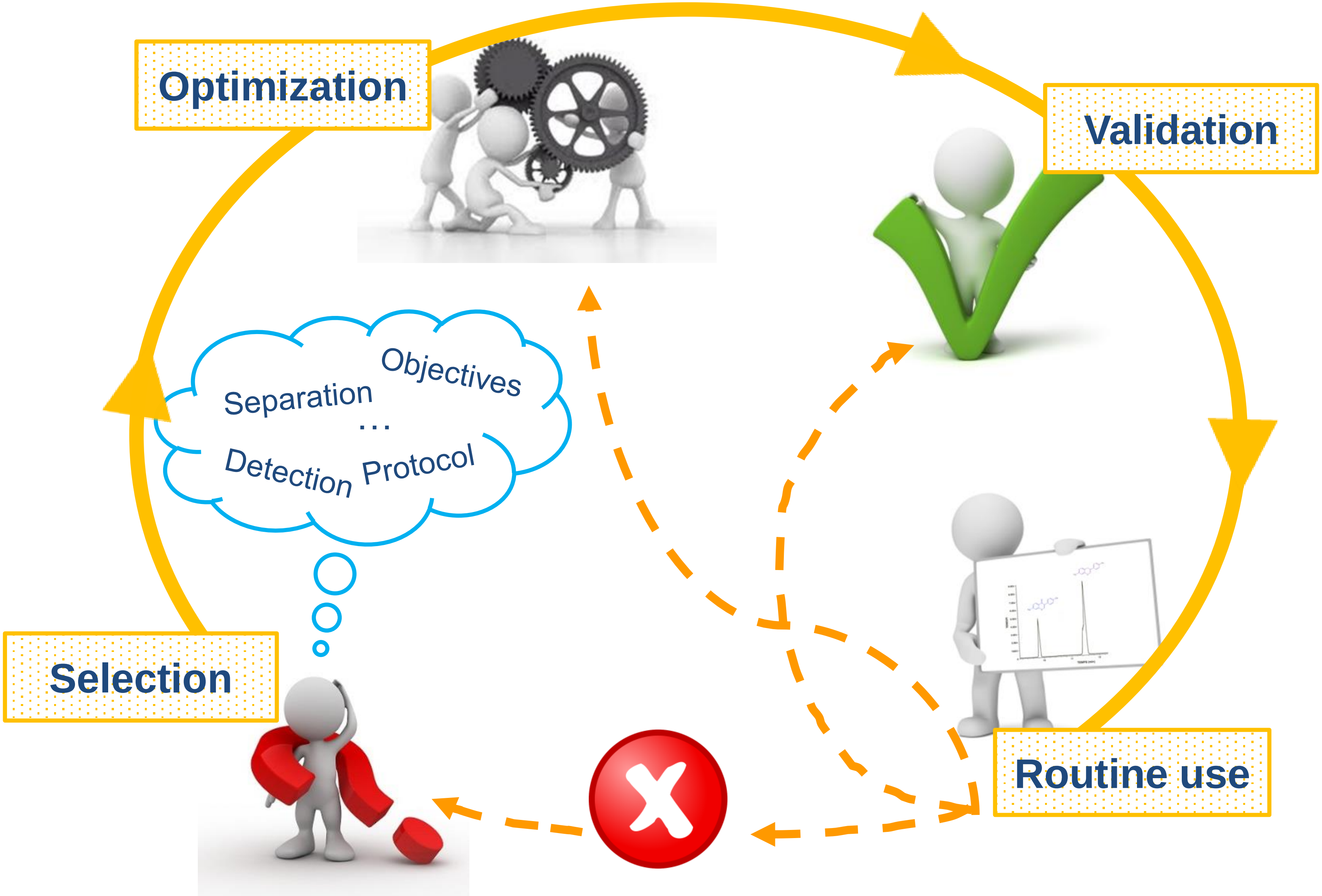
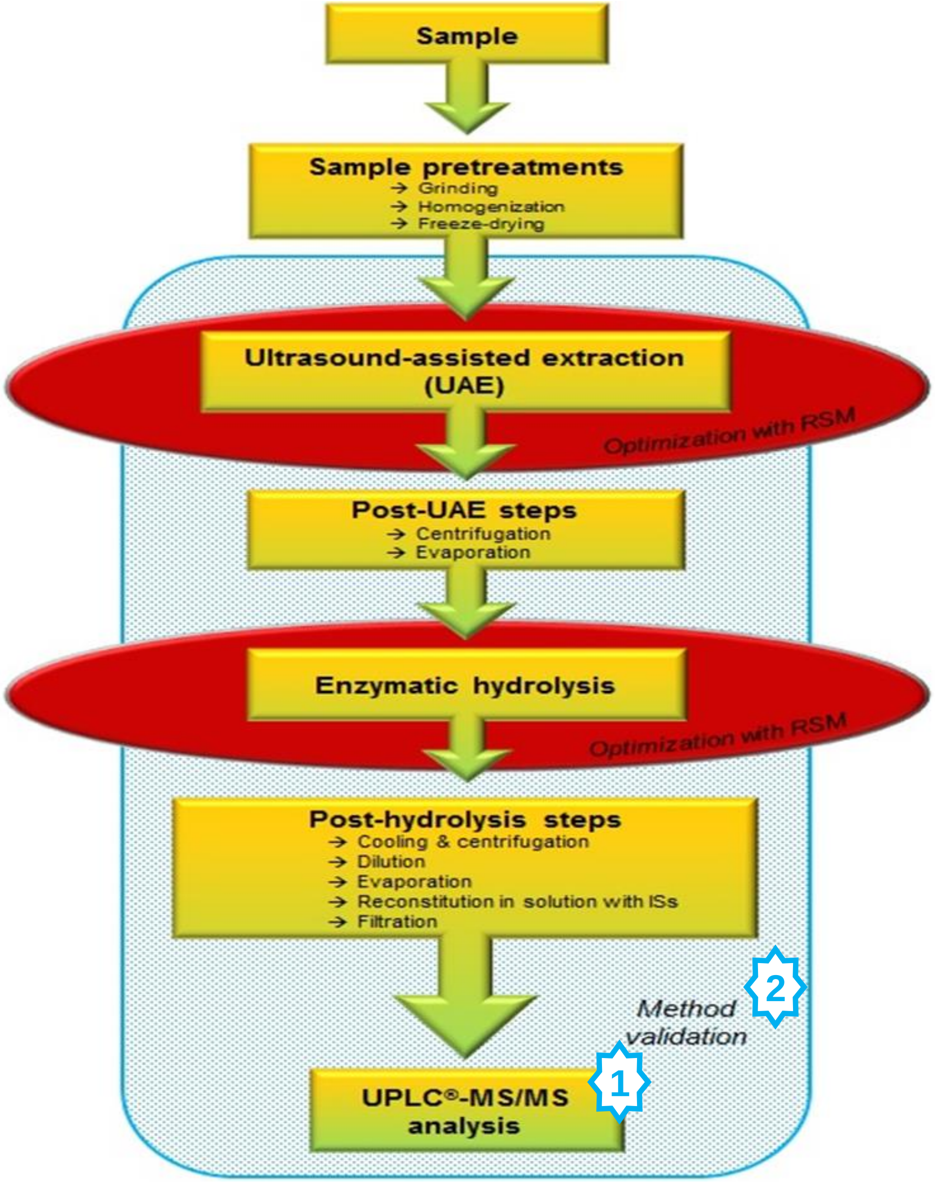


Context – lots of protocols

Methodology



Methodology - Box-Behnken

Experimental Design

Objectives:

Researching factors of influence // Understanding the impact of factors and their possible interactions // **Finding optimal conditions** //

"Decreasing the number of assays --> decreasing development costs"

Number of factors

Number of experiments

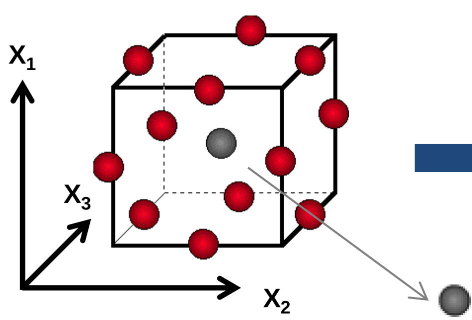
Number of central points

$$N = 2k(k-1) + C_0$$

Box-Behnken

≥ 3 factors (X_n)

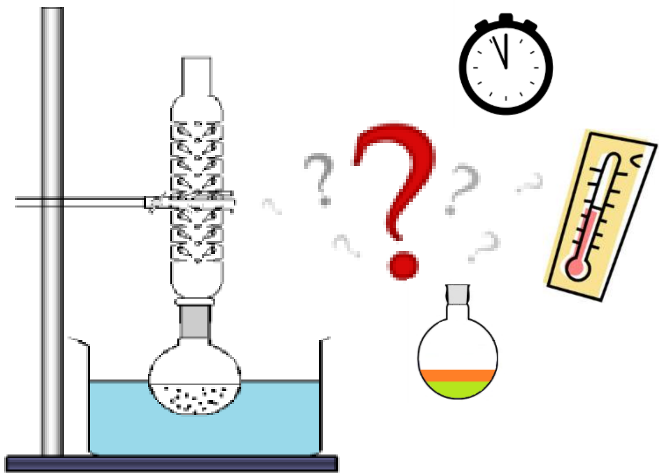
3 levels/factor

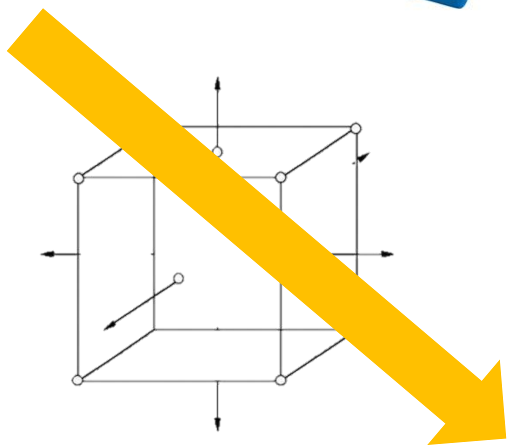


Assay	Temperature (°C)	Time (min.)	Solvent concentration (%)
1	0	0	0
2	0	0	0
3	1	0	1
4	0	1	1
5	-1	0	1
6	-1	1	0
7	1	1	0
8	0	0	0
9	-1	-1	0
10	1	-1	0
11	0	1	-1
12	0	0	0
13	0	-1	1
14	-1	0	-1
15	0	-1	-1
16	+1	0	-1
…	0	0	0

Application – UAE optimization with Box-Behnken design

Level	Temperature (°C)	Time (min.)	Solvent concentration (%)
-1	20	10	10
0	50	50	50
1	80	90	90

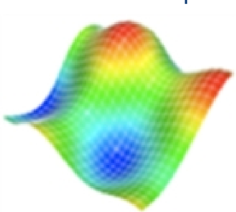


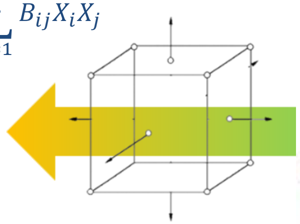


Assay	Temperature (°C)	Time (min.)	Solvent concentration (%)
1	50	50	50
2	50	50	50
3	80	50	90
4	50	90	90
5	20	50	90
6	20	90	50
7	80	90	50
8	50	50	50
9	20	10	50
10	80	10	50
11	50	90	10
12	50	50	50
13	50	10	90
14	20	50	10
15	50	10	10
16	80	50	10

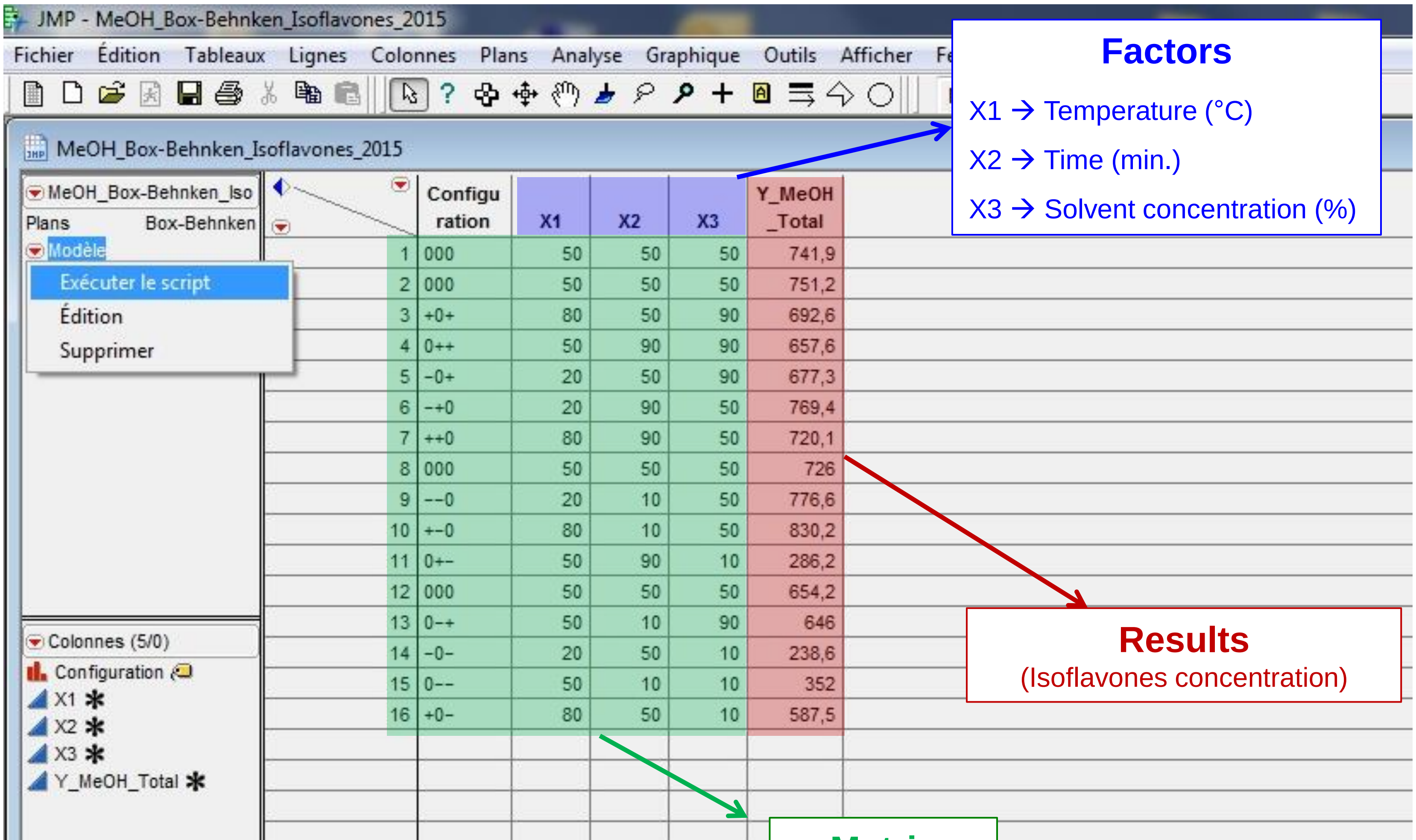
$$Y = \beta_0 + \sum_{i=1}^{3} B_i X_i + \sum_{i=1}^{3} B_{ii} X_i^2 + \sum_{i=1}^{3} B_{ij} X_i X_j$$

Second-order equation





Application - results processing



	Configuration	X1	X2	X3	Y_MeOH_Total
1	000	50	50	50	741,9
2	000	50	50	50	751,2
3	+0+	80	50	90	692,6
4	0++	50	90	90	657,6
5	-0+	20	50	90	677,3
6	-+0	20	90	50	769,4
7	++0	80	90	50	720,1
8	000	50	50	50	726
9	--0	20	10	50	776,6
10	+-0	80	10	50	830,2
11	0+-	50	90	10	286,2
12	000	50	50	50	654,2
13	0-+	50	10	90	646
14	-0-	20	50	10	238,6
15	0--	50	10	10	352
16	+0-	80	50	10	587,5

Factors

X1 → Temperature (°C)

X2 → Time (min.)

X3 → Solvent concentration (%)

Results
(Isoflavones concentration)

Matrix

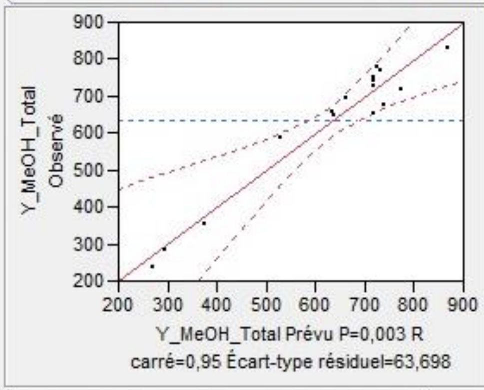
Application - results processing

Fichier Édition Tableaux Lignes Colonnes Plans Analyse Graphique Outils Afficher Fenêtre Aide

MeOH_Box-Behnken_Isoflavones_2015

Réponse Y_MeOH_Total

Graphique des valeurs observées en fonction des valeurs prévues



Résumé de l'ajustement

R carré	0,949675
R carré ajusté	0,874187
Écart-type résiduel	63,69784
Moyenne de la réponse	631,7125
Observations (ou sommes pondérées)	16

87,4% of the variations can be explained by the model

Analyse de la variance

Source	Degré(s) de liberté	Somme des carrés	Carré moyen	Rapport F
Modèle	9	459399,71	51044,4	12,5805
Résidus	6	24344,49	4057,4	Prob. > F
Total	15	483744,20		0,0030*

The model is significant
(p-value<0,05)

Manque d'ajustement

Source	Degré(s) de liberté	Somme des carrés	Carré moyen	Rapport F
				3,1919
Manque d'ajustement	3	18537,022	6179,01	Prob. > F
Erreur pure	3	5807,468	1935,82	0,1830
Erreur totale	6	24344,490		R carré max.
				0,9880

The lack-of-fit is not significant
(p-value>0,05)

Application - results processing

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Fichier Édition Tableaux Lignes Colonnes Plans Analyse Graphique Outils Afficher Fenêtre Aide

MeOH_Box-Behnken_Isoflavones_2015

Réponse Y_MeOH_Total

Tests des coefficients

Source	Nombre de coefficients	Degré(s) de liberté	Somme des carrés	Rapport F	Prob. > F
X1(20,80)	1	1	16974,03	4,1835	0,0868
X2(10,90)	1	1	3676,53	0,9061	0,3779
X3(10,90)	1	1	182770,58	45,0461	0,0005*
X1*X2	1	1	2647,10	0,6524	0,4501
X1*X3	1	1	27822,24	6,8571	0,0397*
X2*X3	1	1	1497,69	0,3691	0,5658
X1*X1	1	1	14232,49	3,5078	0,1102
X2*X2	1	1	60,84	0,0150	0,9065
X3*X3	1	1	209718,20	51,6876	0,0004*

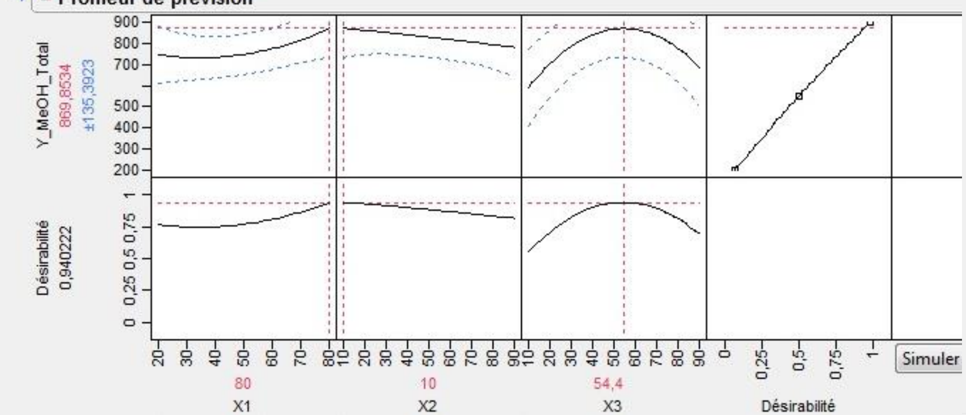
Estimations des coefficients triés

Terme	Estimation	Écart-type	Rapport t	Prob.>|t|
X3*X3	-228,975	31,84892	-7,19	0,0004*
X3(10,90)	151,15	22,52059	6,71	0,0005*
X1*X3	-83,4	31,84892	-2,62	0,0397*
X1(20,80)	46,0625	22,52059	2,05	0,0868
X1*X1	59,65	31,84892	1,87	0,1102
X2(10,90)	-21,4375	22,52059	-0,95	0,3779
X1*X2	-25,725	31,84892	-0,81	0,4501
X2*X3	19,35	31,84892	0,61	0,5658
X2*X2	-3,9	31,84892	-0,12	0,9065

Détails des coefficients

Surface de réponse

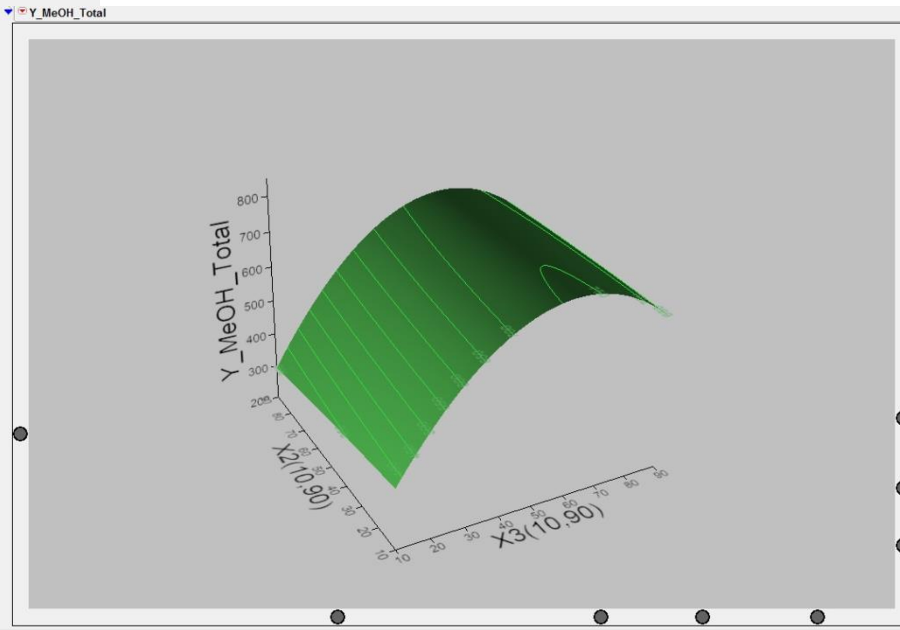
Profileur de prévision



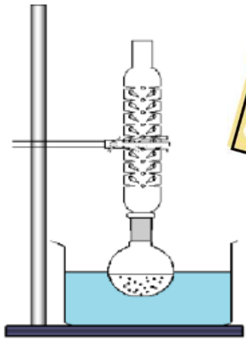
Second-order polynomial model

$$Y_{MeOH-Total} = 718.325 + 46.0625X_1 - 21.4375X_2 + 151.15X_3 - 25.725X_1X_2 - 83.4X_1X_3 + 19.35X_2X_3 + 59.65X_1^2 - 3.9X_2^2 - 228.975X_3^2$$

Significant impact
(p-value<0,05)



Application – to find a compromise

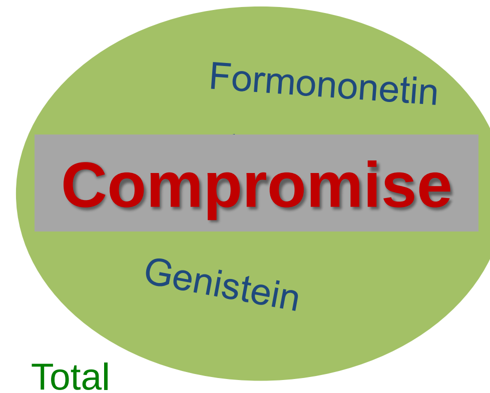



80°C

10 min.

54,4% MeOH

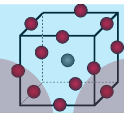
869,8 ± 135,4 µg.g^{-1} MS



Compound	Factors → / Conditions ↓	X_1 Temperature (°C)	X_2 Time (min., sec.)	X_3 Solvent concentration (%)	Y (µg.g^{-1} MS) Predicted [l]	Pred. Inter. [m]	Obtained (n=7) [n]
Total	*Optimal*	*80*	*10*	*54,4*	*869,8 ± 135,4*	*1036,6 – 703,1*	**892,3 ± 57,4**
	Selected	**80 [a]**	**10 [b]**	**55 [c]**	**869,7 ± 135,6**	**1036,6 – 703,0**	
Formononetin	*Optimal*	*80*	*19,54*	*50,7*	*476,3 ± 85,9*	*619,1 – 333,4*	**517,2 ± 50,1**
	Selected	**a**	**b**	**c**	**474,2 ± 98,6**	**620,4 – 328,2**	
Biochanin A	*Optimal*	*80*	*10*	*57,1*	*305,1 ± 45,6*	*374,5 – 235,8*	**291,6 ± 26,8**
	Selected	**a**	**b**	**c**	**304,9 ± 45,4**	**360,8 – 249,0**	
Genistein	*Optimal*	*80*	*90*	*68,4*	*66,0 ± 6,7*	*73,9 – 58,1*	**62,9 ± 6,2**
	Selected	**a**	**b**	**c**	**63,6 ± 6,4**	**71,4 – 55,7**	
Daidzein	*Optimal*	*80*	*29,44*	*60,7*	*19,2 ± 2,7*	*23,3 – 15,1*	**20,7 ± 2,3**
	Selected	**a**	**b**	**c**	**18,9 ± 3,5**	**23,2 – 14,6**	

[l] Predicted content ± half confidence interval (α=0.05), [m] Prediction interval of 95%, [n] Mean value ± standard deviation

Optimized and validated method



Sample pretreatments
(Grinding, homogenization & freeze-drying)

Ultrasound Assisted Extraction
(80 °C, 10 min & 55% MeOH)

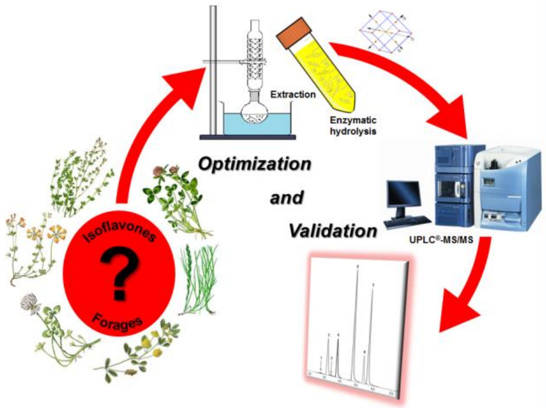
Enzymatic hydrolysis
(20 °C, 18 h & pH 6)

Post-hydrolysis steps
(Dilution, evaporation, solubilization of dry residues & filtration)

UPLC®-MS/MS analysis

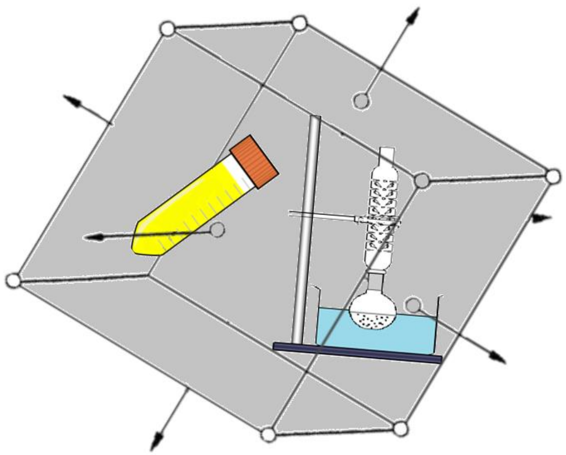
EMA VICH GL49 (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/04/WC500105053.pdf)

Conclusions



- This methodology allows to propose a <u>reliable and robust analytical method</u> for simultaneous quantification of isoflavones in forages. *("Quality")*

Experimental design



- *To obtain a maximum of information with a small number of assays → allowing to find the <u>optimal conditions for UAE and hydrolysis process</u> among the numerous and divergent conditions proposed in literature.*

Thank you for your attention

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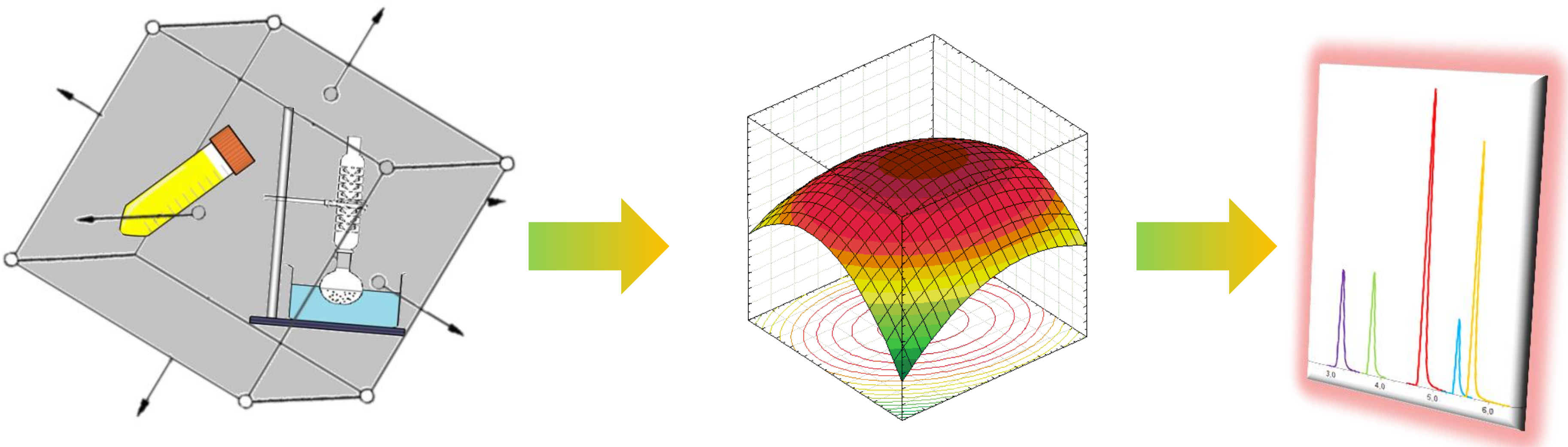

Acknowledgements:

- Public Service of Wallonia (PhytoHealth project, Moerman funds)
- Members of PhytoHealth project, C. Rasse (SMCS-IMMAQ, UCL) and C. Jasselette

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