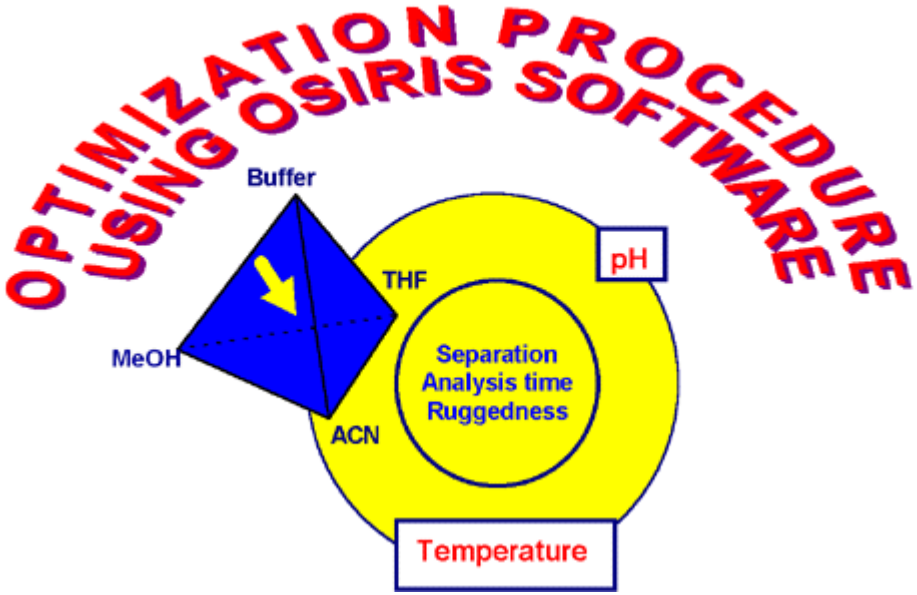
USE OF AN OPTIMIZATION SOFTWARE FOR THE GOOD PREDICTIONS OF RUGGED ANALYSIS CONDITIONS IN RP-HPLC

S. Goga, S. Heinisch, J.L. Rocca

Laboratoire des Sciences Analytiques, UMR 5619 CNRS,

Université Claude Bernard, 43 Bd du 11 Novembre 1918,

69622 Villeurbanne Cedex, France

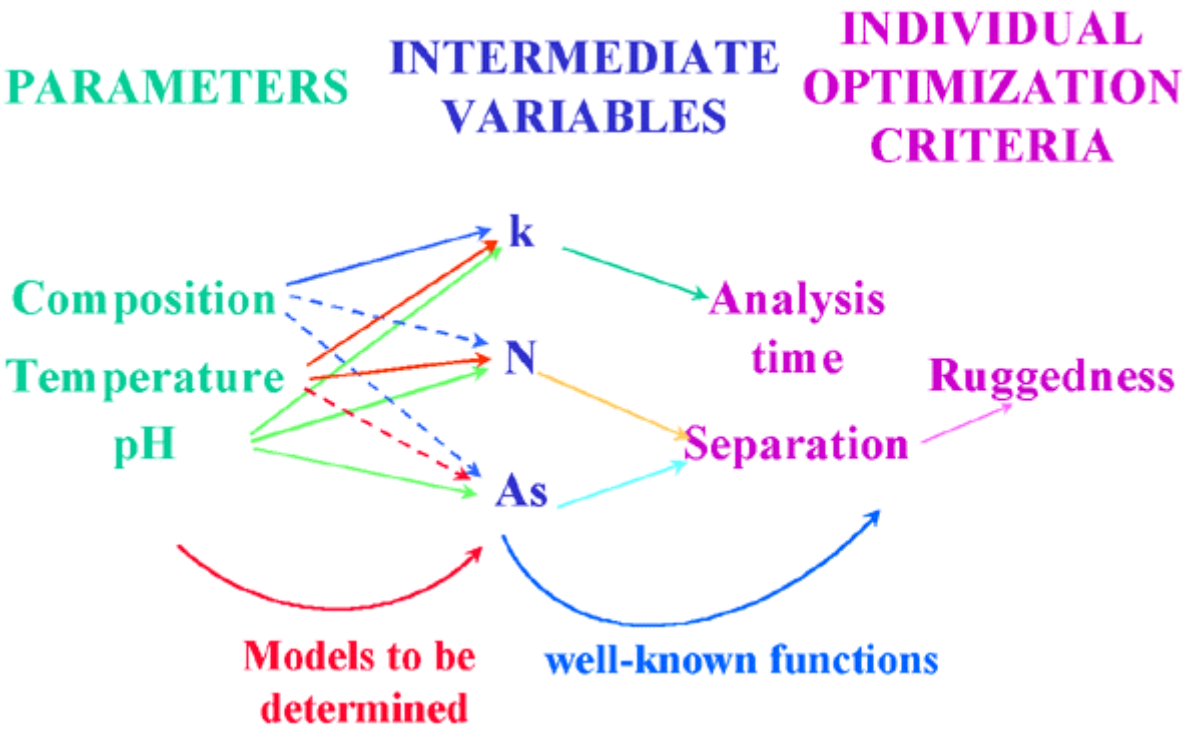


Optimization consists in predicting the **best analysis conditions** by using a **minimum of preliminary experiments**. The OSIRIS optimization procedure requires 3 stages :

1. Determination of the relevant **parameters P_i** which have a significant effect on the separation.

2. Modelization of the **intermediate variables** (retention factor k, theoretical plates number N, Asymmetry As) as a function of these parameters. The prediction accuracy depends on the quality of the chosen models and is of prime importance for the optimization results.

3. Evaluation of the chromatogram quality with a **Response Function** that has to take into account different **optimization criteria** : analysis time, separation quality (Resolution Rs), separation ruggedness for each parameters ($Ru(P_i)$).

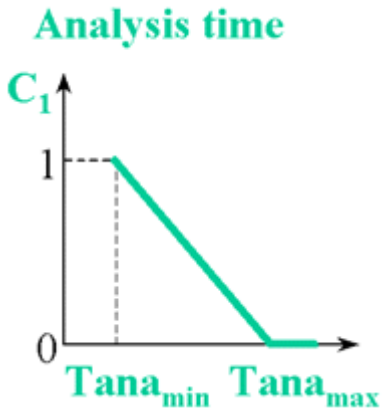


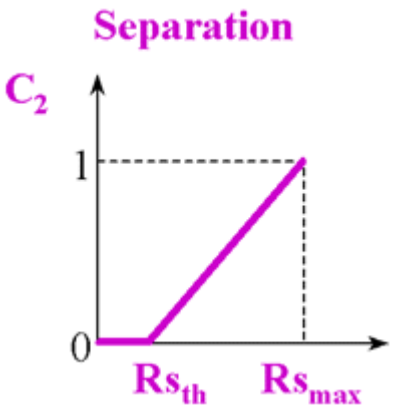
Each individual criteria comply with the following requirements :

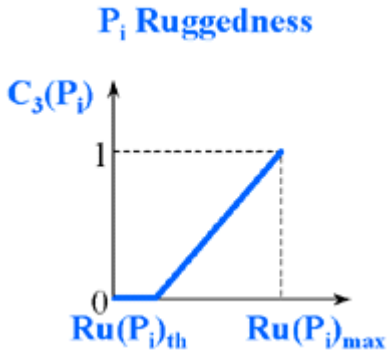
Analysis time (Tana)	as short as possible (**$Tana_{min}$** being the minimum analysis time observed within the response area) lower than a threshold value (**$Tana_{th}$**)
Separation (Rs_{min}) *Resolution of the worst separated peak pair*	as high as possible (**Rs_{max}** being the maximum observed resolution) upper than a threshold value (**Rs_{th}**)

P_i Ruggeness ($Ru(P_i)$) *variation that can be affected to P_i while keeping a resolution value upper than the threshold value Rs_{th}*	as high as possible (**$Ru(P_i)_{max}$** being the maximum observed ruggedness for the parameter P_i) upper than a threshold value (**$Ru(P_i)_{th}$**)

A response function according to the **Derringer Desirability's function** [1,2] was developped in order to take into account these various **individual criteria simultaneously**. This function is calculated after a **transformation** of each optimization **individual criterion in a dimensionless desirability scale**.







$$\text{Response Function for n parameters} = \left(C_1 \times C_2 \times \prod_{i=1}^{n} C_3(P_i) \right)^{\frac{1}{n+2}}$$

[1] *S.N. Deming,* J. of Chromatogr., **550** (1991) 15-25

[2] *B. Bourguignon, D.L. Massart,* J. of Chromatogr., **586** (1991) 11-20

EXAMPLE 1

Simultaneous optimization of temperature and acetonitrile composition for a 16 PAHs separation

Column Cosmosil 250x4,6 mm, Flow rate 1mL.min^{-1}.

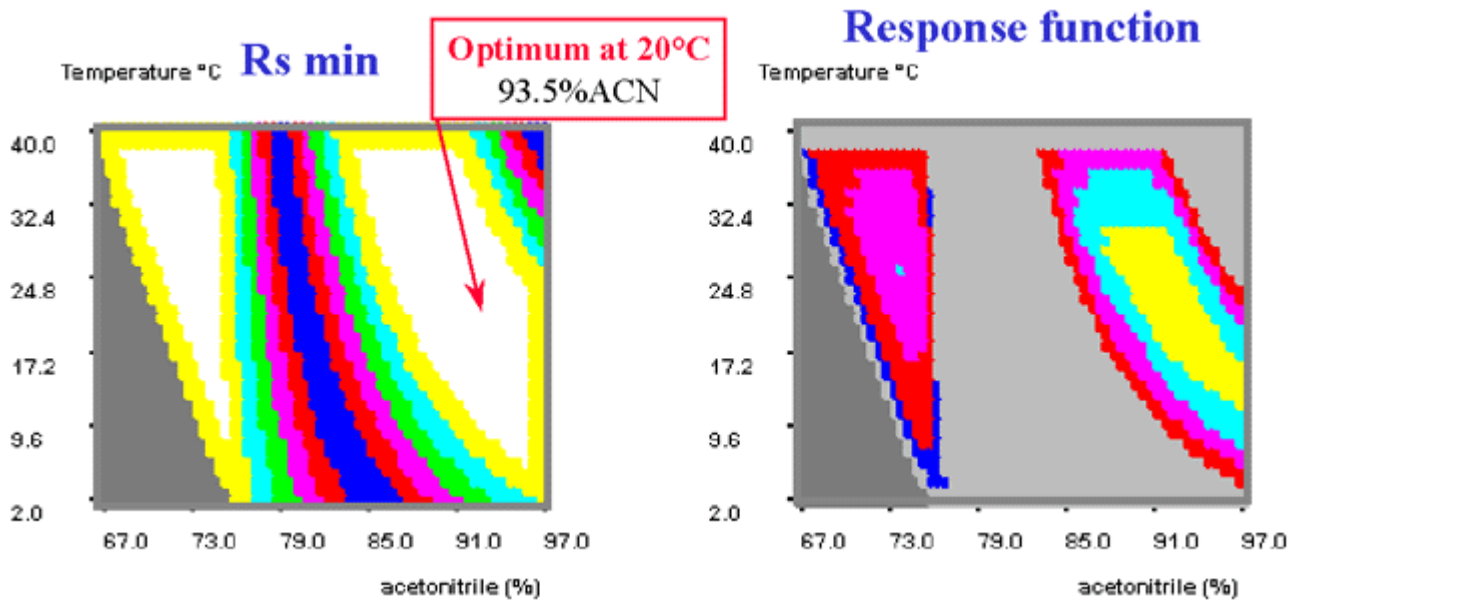
Objectives	Rs_{th}=1 for the 6 most pollutant PAHs $Tana_{max}$=50 min $Ru(Température)_{th}$=2℃
Application	Separation at room temperature

4 preliminary experiments :

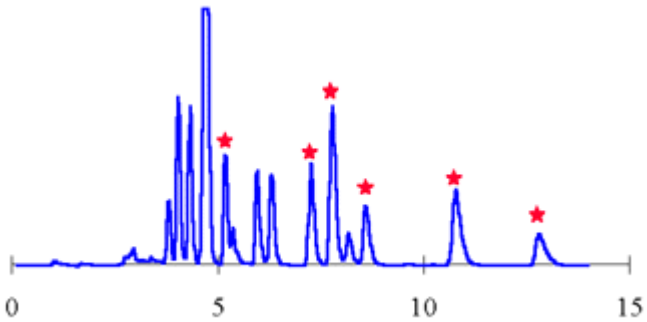
2 x 2 experiments performed at 2℃ and 40℃ :

gradient run from 50% to 100% ACN in 50 min

gradient run from 50% to 95% ACN in 15 min



	Rs_{min}
	out of range
	0.0 - 0.2
	0.2 - 0.4
	0.4 - 0.6
	0.6 - 0.8
	0.8 - 1.0
	> 1.0



	Response Function
	out of range
	0
	0 - 0.18
	0.18 - 0.36
	0.36 - 0.55
	0.55 - 0.73
	0.73 - 0.91

Reproducibility of the separation quality

at room temperature (18-22°C)

		Optimum
	Number of performed analysis	**5 (within 2 months)**
Rs_{min} value obtained	**Minimum value**	**1.08**
	Maximum value	**1.16**
	Average value	**1.12**

Conclusion : the separation is rugged at room temperature

EXAMPLE 2

Simultaneous optimisation of pH and acetonitrile composition

for the separation of 7 acidic and basic solutes (pKa in the range 3 to 5)

Column Capcell C18 150x4,6 mm, Temperature 30°C, mo bile phase Citrate buffer 0.05 M / ACN, Flow rate 1mL.min^{-1}

Objectives	Rs_{th}=2 $Tana_{max}$=20 min $Ru(composition)_{th}$=1% $Ru(pH)_{th}$=0.05 pH units

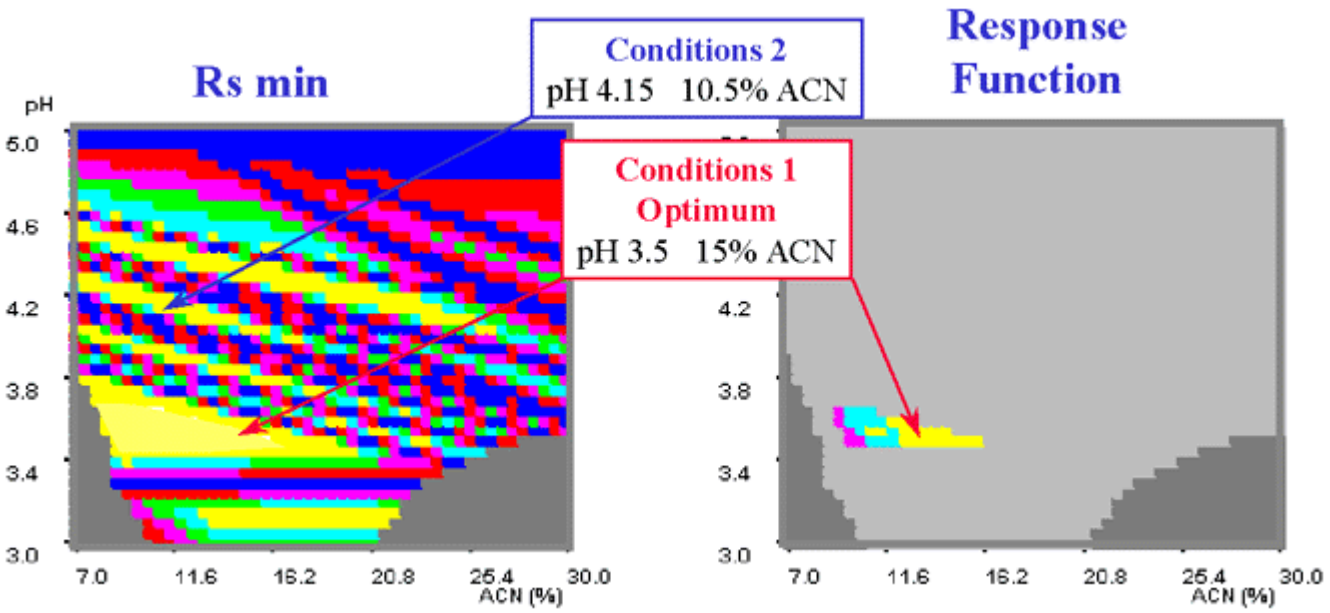
9 preliminary experiments :

3 x 3 experiments performed at pH 3, 4 and 5 :

gradient run from 5% to 50% ACN in 45 min

gradient run from 5% to 50% ACN in 15 min

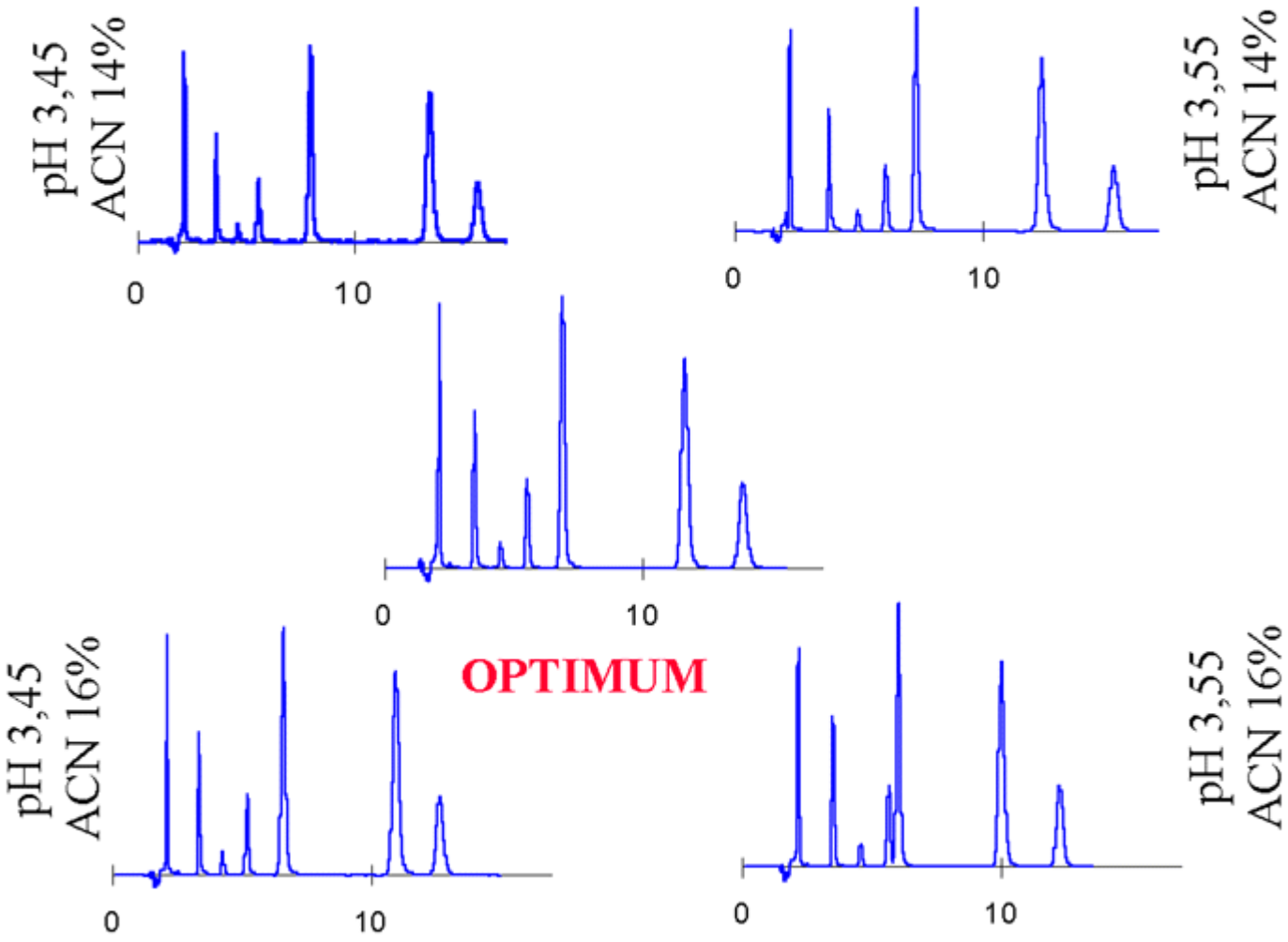
isocratic run at 40% ACN



	Rs_{min}
	out of range
	0.0 - 0.4
	0.4 - 0.8
	0.8 - 1.2
	1.2 - 1.6
	1.6 - 2.0
	> 2.0

	Response Function
	out of range
	0
	0 - 0.14
	0.14 - 0.28
	0.28 - 0.41
	0.41 - 0.55
	0.55 - 0.69

No effect on the separation quality with small variations in optimum conditions


pH 3,45
ACN 14%
0
10
pH 3,55
ACN 14%
0
10
0
10
OPTIMUM
pH 3,45
ACN 16%
0
10
pH 3,55
ACN 16%
0
10

Reproducibility of the separation:

comparison of the results obtained with optimum conditions 1 and conditions 2

		Conditions 1 (Optimum)	Conditions 2
	Number of performed experiments	15 (within 4 months)	11 (within 1 month)
Rs_{min} value obtained	Minimum value	2.3	0
	Maximum value	3.9	1.6
	Average value	2.9	0.4

Conclusion :

the separation quality is reproducible with optimum conditions. It is not reproducible with conditions 2.

CONCLUSION

The Osiris software allows to take into account the ruggedness that is of prime importance for method validation. No adding experiment is required in the proposed procedure.

The suggested ruggedness criterion takes into account a threshold value, assigned by the chromatographer.

The proposed response function can consider several individual criteria simultaneously and consequently the chromatogram quality may be well evaluated according to all the objectives of the chromatographer.