ELSEVIER

Journal of Chromatography A, 697 (1995) 3–16

JOURNAL OF CHROMATOGRAPHY A

Criteria for developing rugged high-performance liquid chromatographic methods

Pascale F. Vanbel[a,*], Bernard L. Tilquin[a], Peter J. Schoenmakers[b]

[a]*Pharmaceutical School, Université Catholique de Louvain, UCL 7230, Av. E. Mounier 72, B-1200 Brussels, Belgium*

[b]*Analytical Department, Royal Dutch/Shell Laboratory (Shell Research), Badhuisweg 3, NL-1031 CM Amsterdam, Netherlands*

Abstract

An approach is described that allows the ultimate ruggedness of chromatographic methods to be rigorously included as an objective from the outset of systematic method development. Ruggedness criteria are defined as derivatives of other (typically resolution-based) criteria. Numerical estimates of ruggedness criteria can readily be obtained during selectivity optimization. It is necessary to consider ruggedness simultaneously with other objectives of the separation using multi-criteria decision making (MCDM) procedures. Three MCDM methods were considered in this work, viz., Pareto-optimality (PO) plot, Derringer's desirability function and the multiple-threshold approach (MTA). The characteristics of these three methods are discussed and the feasibility of developing rugged separations by systematically varying the pH and solvent composition is demonstrated.

1. Introduction

Owing to the widespread use of HPLC in routine analysis, it is very important that good HPLC methods are developed and that these are thoroughly validated. Many systematic methods exist for method development and optimization. The ruggedness or robustness of a method is typically evaluated independently at a much later stage as part of the method validation process. Full or fractional factorial experimental designs are used extensively for this purpose [1–5]. Developing rugged HPLC separations is of great practical importance. By considering ruggedness at an early stage of method development, both the amount of work required and the chance of failure during the method validation stage can be greatly reduced.

In this paper, we describe an original approach for evaluating the ruggedness of a predicted optimum (and of all experimental points in the parameter space) without the need to perform additional experiments. The defined ruggedness criteria (R_u) are based on numerical estimates of the derivatives of a specific criterion (e.g., minimum effective resolution) with respect to the optimized parameters (pH and solvent composition).

However, it is difficult to envisage ruggedness as a goal in itself in method development procedures. Obviously, highly rugged methods that are inadequate in terms of other criteria (e.g., resolution, analysis time) are unacceptable. Therefore, quality of separation (resolution) and robustness criteria represent a good example of a set of goals to be considered in a multi-criteria decision making (MCDM) process. Recently, several MCDM methods were reviewed [6,7].

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00933-3

Simultaneous optimization of both types of criteria (resolution and robustness) is achieved here by using three MCDM strategies. The first is based on the pareto-optimality concept, which was introduced into chromatography by Smilde et al. [8]. An experiment is called pareto-optimal if there is no other experiment that has a better result on one criterion without having a worse result on another. The second procedure uses Derringer's desirability function, which has been applied to the optimization of several chromatographic performance goals by Bourguignon and Massart [9]. It is based on the transformation of the measured properties to a dimensionless desirability scale for each criterion, so that values of several properties, obtained from different scales of measurement, may be combined. The desirability scale ranges between $d = 0$ (undesirable level of quality) and $d = 1$ (target value). The geometric mean of the desirability values for all criteria is then used to compare different experiments. The third method defines a threshold value for one criterion (generally resolution) and optimizes the other criterion for all situations in which the threshold for the first criterion is reached. A multiple-threshold approach (MTA) can also be used.

Although an MCDM process involving the pareto-optimality concept and a robustness coefficient has already been described in the field of pharmaceutical formulations [10–14], this concept is new in chromatography. By way of example, we shall apply our method for optimizing RP-HPLC separations by varying simultaneously the pH and solvent composition.

2. Theory

2.1. Resolution criteria

Optimization procedures require adequate response criteria to assess the quality of each chromatogram obtained during the process.

When pH is one of the optimization parameters, variations in efficiency and peak symmetry occur during the optimization procedure. The effective resolution defined by Schoenmakers et al. [15] is then the logical and recommended choice for characterizing the quality of the separation between two peaks [16]. It takes into account the individual widths of the two peaks, the asymmetry factors and peak heights. Considering the separation of a pair of peaks, two values of the resolution exist for each peak. The first, R_n, describes the extent to which a peak i is separated from the next peak (j), and the second value, R_p, reflects the extent to which peak i is separated from the previous peak. Generally, the lowest of these two values is kept.

R_n and R_p are calculated by using the following equations:

$$R_n = \frac{(t_j - t_i)(1 + A_{S,i})(1 + A_{S,j})\sqrt{N_i N_j}}{4A_{S,i}t_i(1 + A_{S,j})\sqrt{N_j} + 4t_j(1 + A_{S,i})\sqrt{N_j}\sqrt{1 + \frac{1}{2}\ln\left(\frac{h_j}{h_i}\right)}} \quad (1)$$

$$R_p = \frac{(t_j - t_i)(1 + A_{S,i})(1 + A_{S,j})\sqrt{N_i N_j}}{4A_{S,i}t_i(1 + A_{S,j})\sqrt{N_j}\sqrt{1 + \frac{1}{2}\ln\left(\frac{h_i}{h_j}\right)} + 4t_j(1 + A_{S,i})\sqrt{N_i}} \quad (2)$$

where t is the retention time, A_S is the asymmetry factor, N is the number of theoretical plates and h is the peak height.

However, using effective resolution implies that not only retention data, but also data on efficiency (or peak areas), peak heights and peak symmetry need to be recorded. Schoenmakers et al. [16] recently demonstrated the feasibility of simultaneously optimizing separations with regard to selectivity, efficiency and peak shape. The optimization procedure requires the modelling of retention time, peak height, peak area and peak asymmetry. From these chromatographic characteristics, effective resolution can be calculated at each point in the parameter space.

2.2. Ruggedness criteria

Considering the optimization of two independent parameters such as pH and solvent composition (volume fraction of methanol = φ_{MeOH}), robustness can be evaluated by using the numeri-

cal estimates of the derivatives of a selected criterion (basically minimum effective resolution, $R_{l,\min}$) with respect to the optimized parameters. Robustness criteria [$R_u(\varphi)$ and $R_u(\mathrm{pH})$] are then described by the equations

$$R_u(\varphi) = \Delta_\varphi \cdot \frac{dR_{l,\min}}{d\varphi} \quad (3)$$

$$R_u(\mathrm{pH}) = \Delta_{\mathrm{pH}} \cdot \frac{dR_{l,\min}}{d\mathrm{pH}} \quad (4)$$

where $dR_{l,\min}/d\varphi$ and $dR_{l,\min}/d\mathrm{pH}$ are the variation of the minimum effective resolution with solvent composition and with pH, respectively, Δ_φ and Δ_{pH} are the permitted variations of solvent composition (e.g., 0.01) and pH (e.g., 0.05), respectively, which are defined by the user according to the instrumental errors. Notice that $dR_{l,\min}/d\varphi$ and $dR_{l,\min}/d\mathrm{pH}$ provide information on the impact of small variations in the optimization parameters on the separation. These values can be used to determine limits between which these parameters must be maintained in order to obtain reproducible results. R_u (pH) [or $R_u(\varphi)$] gives an indication of how much the minimum effective resolution will vary if we are able to control pH (or φ) within Δ_{pH} (or Δ_φ) units.

By adding Eq. 3 to Eq. 4, we obtain a global robustness criterion. The following equation is then obtained:

$$R_u = \Delta_\varphi \cdot \frac{dR_{l,\min}}{d\varphi} + \Delta_{\mathrm{pH}} \cdot \frac{dR_{l,\min}}{d\mathrm{pH}} \quad (5)$$

R_u has to be minimized during the optimization process. However, greater values of the robustness criterion can be accepted when the minimum resolution increases. Similarly, when the minimum resolution value is only marginally acceptable, the requirements for the ruggedness of the method will be much stricter. Another ruggedness criterion (R_u^*), which is related to the actual resolution value, is then expressed by

$$R_u^* = \frac{R_u}{R_{l,\min}} \quad (6)$$

Inversing Eq. 6 leads to

$$[R_u^*]^{-1} = \frac{R_{l,\min}}{R_u} \quad (7)$$

$[R_u^*]^{-1}$ has to be maximized. Using this last criterion avoids the occurrence of mathematical problems when the minimum resolution is zero. However, in a computer program, a provision is needed to deal with the case in which R_u is zero (when the minimum resolution is essentially constant).

Similarly, Eqs. 3 and 4 can also be modified separately to give

$$[R_u^*(\varphi)]^{-1} = \frac{R_{l,\min}}{R_u(\varphi)} \quad (8)$$

$$[R_u^*(\mathrm{pH})]^{-1} = \frac{R_{l,\min}}{R_u(\mathrm{pH})} \quad (9)$$

Considering the definition of $R_u(\varphi)$ and $R_u(\mathrm{pH})$, $R_u^*(\varphi)$ and $R_u^*(\mathrm{pH})$ are indications of the relative error at a given value of the minimum resolution.

3. Experimental

3.1. Instrumentation

The HPLC system consisted of two Waters Model 6000A pumps (Millipore–Waters, Milford, MA, USA), which were controlled by a Waters system controller. The system was equipped with an injection valve (Model 7125; Rheodyne, Cotati, CA, USA) fitted with a 20-μl injection loop and a variable-wavelength UV–visible detector from Waters (Model 481). The apparatus was connected to an IBM-compatible computer and chromatographic data (retention times, peak heights, peak areas and asymmetry factors) were collected by chromatographic integration software (PC Integration Pack; Kontron Instruments, Milan, Italy).

3.2. Chromatographic conditions

A reversed-phase system was chosen for this study. We used a 5-μm C_{18} LiChrospher column (125 × 4 mm I.D.) and a 5-μm C_{18} LiChrospher

precolumn (4 × 4 mm I.D.) from Merck (Darmstadt, Germany) at ambient temperature (the laboratory temperature is maintained at 22 ± 1°C by the ventilation system). The hold-up time (t_0) was estimated to be 1.28 min, by using replicate injections of 10^{-4} *M* KI. The flow-rate was 1.0 ml/min and UV detection was set at 254 nm.

Mixtures of methanol (MeOH) and citrate–phosphate buffers constituted the different mobile phases. Methanol was of HPLC grade (UCB, Leuven, Belgium). The volume fraction of MeOH was varied between 0.30 and 0.40. Water was obtained from a Milli-Q purification system (Millipore, Milford, MA, USA). Citrate–phosphate buffers (pH ranging from 2.76 to 6.83) were prepared by mass at a total ionic strength of 0.05 *M* according to Ref. [17]. Constant ionic strength was obtained by the addition of the appropriate amount of potassium chloride. Potassium chloride, citric acid and disodium hydrogenphosphate (all of the highest purity) were purchased from Merck. Reported pH values are those of the aqueous solution, before mixing with methanol. The mixture injected into the HPLC system consisted of four acids: salicylic acid (0.2 mg/ml), benzoic acid (0.1 mg/ml), 3,5-dinitrobenzoic acid (0.02 mg/ml) and *m*-nitrobenzoic acid (0.02 mg/ml). Salicylic acid was purchased from UCB. The other solutes were supplied by Merck. All solutes were of the highest available purity. Stock standard solutions of the investigated compounds were prepared in methanol and then diluted as required in water–methanol (90:10). Peak recognition was performed by the injection of each individual solute.

3.3. Software

Different in-house software programs were developed or improved to model the different chromatographic parameters (retention time, peak height, peak area and asymmetry factor) and to generate response surfaces. All the programs were written in Pascal (Turbo Pascal 7.0; Borland International, Scotts Valley, CA, USA) and implemented on an IBM-compatible computer. Data generated by Pascal programs were imported directly in Excel software (version 4.0) in a Windows environment (Microsoft).

3.4. Optimization procedure

A 4 × 3 experimental design (three levels of methanol volume fraction and four levels of pH) was used to realize the simultaneous optimization of pH and solvent composition (Fig. 1). Eq. 10 was used to model the capacity factor (k) as a function of pH and φ (volume fraction of organic modifier):

$$k = \frac{\left[k^0_{HA}\, e^{S_{HA}\varphi + T_{HA}\varphi^2} \cdot 10^{-pH} + k^0_{A^-} K^0_a\, e^{(S_{A^-}+Q_1)\varphi + (T_{A^-}+Q_2)\varphi^2} \right]}{10^{-pH} + K^0_a\, e^{(Q_1\varphi + Q_2\varphi^2)}} \quad (10)$$

where k^0_{HA} and $k^0_{A^-}$ are extrapolated capacity factors of the protonated and the dissociated forms, respectively, of the solute in pure water, K^0_a is the extrapolated acid-dissociation constant in pure water, S_{HA} and T_{HA} are parameters describing the variation of retention with φ for protonated species, S_{A^-} and T_{A^-} are corresponding parameters for dissociated species and Q_1 and Q_2 are coefficients describing the variation of the acid-dissociation constant with φ.

The use of a 4 × 3 experimental design and the use of Eq. 10 were recommended by Lopes Marques and Schoenmakers [18] in a previous study on the modelling of retention as a function

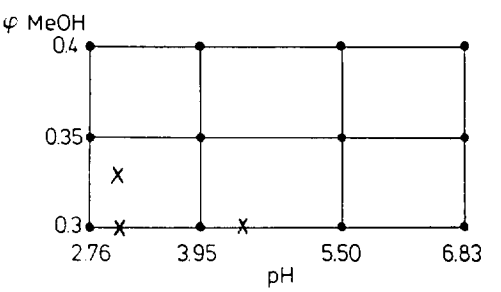

Fig. 1. The 3 × 4 experimental design used for the separation of a mixture of four acids; × refers to additional experiments.

of pH and solvent composition. Eq. 10 is also suitable to model peak height and peak area [16]. For the asymmetry factor (A_S), the following quadratic equation was found to give a reasonable description (see also Ref. [16]):

$$A_S = A_{S,HA}\, e^{S_{HA}\varphi + T_{HA}\varphi^2} + A_{S,A}\, e^{(S_A\varphi + T_A\varphi^2)}\text{pH} + K_a^0\, e^{(Q_1\varphi + Q_2\varphi^2)}(\text{pH})^2 \quad (11)$$

4. Results and discussion

The values for pH and solvent composition corresponding to the experimental design in Fig. 1 are listed in Table 1. Experiments 1–12 are the initial experiments defined by the experimental design; 13–15 are three additional experiments that were added to the data set to yield an improved description of the response surface. Table 2 gives retention times, peak heights, peak areas and asymmetry factors for the four solutes at the fifteen experimental locations. The coefficients describing these data for retention (capacity factors), peak height and peak area in terms of Eq. 10 and asymmetry factors in terms of Eq. 11 are listed in Table 3. Table 4 provides some idea on the accuracy of the model descriptions. Two criteria are listed, the sum of squares (SSQ) of the absolute deviations between calculated (by the model) and experimental data and the average relative deviation between calculated and experimental data (ARD). Generally, Eq. 10 provides accurate descriptions of experimental data. ARD values are between 2.5% and 5.3% for capacity factors (average absolute deviation ≤ 0.1 capacity factor units), between 2.7% and 9.5% for peak heights and between 0.4% and 2.7% for peak areas. Asymmetry data are described reasonably well by Eq. 11 (average absolute deviation ≤ 0.1), except for salicylic acid at $\varphi_{MeOH} = 0.30$, where larger deviations were observed (average absolute deviation 0.18; maximum absolute deviation 0.34). Note that large SSQ values for the height and area models are largely caused by the magnitude of the parameters (see Table 2). Conversely, large values for ARD occur for parameters with low values (k, A_S).

Table 1
pH and volume fraction of methanol (φ_{MeOH}) corresponding to the fifteen experimental locations

No.[a]	pH	φ_{MeOH}
1	2.76	0.30
2	2.76	0.35
3	2.76	0.40
4	3.95	0.30
5	3.95	0.35
6	3.95	0.40
7	5.50	0.30
8	5.50	0.35
9	5.50	0.40
10	6.83	0.30
11	6.83	0.35
12	6.83	0.40
13	3.07	0.30
14	3.07	0.33
15	4.39	0.30

[a] Nos. 1–12 are the initial experiments defined by the experimental design; Nos. 13–15 are three additional experiments.

Fig. 2 shows the response surface of the minimum effective resolution obtained during the simultaneous optimization of pH and mobile phase composition for a mixture of four acidic solutes. This response surface is complex owing to the occurrence of peak cross-overs during the optimization process. Consequently, this practical example is appropriate to study the use and usefulness of robustness criteria.

Figs. 3 and 4 give the response surfaces of the individual robustness criteria [$R_u(\varphi)$ and $R_u(\text{pH})$] calculated by Eqs. 3 and 4, respectively. The response surface of the global robustness criterion (R_u) expressed by Eq. 5 is shown in Fig. 5.

In this study, the permitted variations of solvent composition and pH were set at 0.01 and 0.05, respectively. For each point in the parameter space, minimum effective resolution and ruggedness factors are provided by the optimization software. These values constitute the response surfaces.

MCDM procedures combining minimum effective resolution and robustness criteria were ap-

Table 2
Experimental data for 3,5-dinitrobenzoic acid, benzoic acid, salicylic acid and *m*-nitrobenzoic acid at fifteen experimental locations indicated in Table 1

Acid	No.	Retention time (min)	Area (mV s)	Height (mV)	Asymmetry
3,5-Dinitrobenzoic	1	12.49	1697	53.1	1.53
	2	8.76	1685	71.8	1.41
	3	6.59	1759	88.5	1.55
	4	5.32	1966	97.7	1.25
	5	4.17	1956	103.6	1.19
	6	3.36	1946	139.9	1.46
	7	4.43	1882	122.7	1.28
	8	3.55	1899	149.4	1.43
	9	2.93	1889	164.1	1.59
	10	4.36	1858	125.7	1.39
	11	3.41	1866	145.8	1.53
	12	2.83	1859	164.1	1.60
	13	8.59	1875	75.9	1.37
	14	7.02	1939	97.0	1.45
	15	4.57	1950	119.0	1.35
Benzoic	1	14.55	1219	36.2	1.30
	2	9.94	1186	48.9	1.29
	3	6.89	1144	61.1	1.42
	4	10.47	1095	36.7	1.07
	5	7.67	1102	38.9	1.04
	6	5.68	1091	59.0	1.16
	7	2.47	1038	96.6	1.61
	8	2.15	1045	102.1	1.73
	9	1.92	1022	107.3	1.92
	10	2.00	980	93.6	1.84
	11	1.78	938	93.1	1.96
	12	1.67	991	101.5	2.08
	13	13.74	1109	32.5	1.29
	14	10.87	1153	46.5	1.30
	15	6.69	1060	50.8	0.97
Salicylic	1	13.89	1558	48.3	1.59
	2	9.57	1500	60.9	1.45
	3	6.84	1520	79.2	1.43
	4	4.08	920	60.4	1.22
	5	3.23	908	61.1	1.22
	6	2.64	907	72.5	1.34
	7	2.62	879	70.7	1.83
	8	2.24	857	75.8	1.89
	9	1.94	858	82.8	1.98
	10	2.56	868	67.2	1.80
	11	2.14	863	77.2	1.95
	12	1.88	854	85.9	2.06
	13	9.24	1266	51.7	1.13
	14	7.54	1217	61.0	1.09
	15	3.02	896	69.8	1.81

Table 2 (*continued*)

Acid	No.	Retention time (min)	Area (mV s)	Height (mV)	Asymmetry
m-Nitrobenzoic	1	13.89	1161	34.2	1.57
	2	9.53	1301	52.5	1.52
	3	6.68	1204	61.6	1.59
	4	5.45	1132	55.7	1.41
	5	4.18	1147	61.0	1.33
	6	3.31	1143	81.5	1.49
	7	2.67	1110	98.9	1.53
	8	2.28	1129	111.6	1.72
	9	2.00	1157	121.5	1.86
	10	2.57	1118	97.9	1.65
	11	2.17	1111	105.5	1.82
	12	1.93	1125	120.3	1.92
	13	11.22	1132	36.6	1.48
	14	8.87	1192	50.7	1.53
	15	3.57	1163	81.2	1.54

plied to a selection of 115 grid points, each representing a specific combination of pH and φ_{MeOH}. The number of grid points selected can easily be increased, but some clarity will be lost in the presentation. Fig. 6 shows the pareto-optimality plot for the minimum effective resolution, $R_{l,min}$, and the overall ruggedness criterion, R_u (Eq. 5). Resolution has to be maximized and R_u has to be minimized. Pareto-optimal (PO) points are given in Table 5. The MCDM plot (Fig. 6) visualizes directly the pay-off between the two criteria. Information with respect to both criteria is available, so we can decide which of the points is preferable. No preselection of weighting factors or threshold values for the criteria is needed. The PO points 11, 12 and 13 (from Table 5) represent favourable choices. Indeed, good resolution is achieved at acceptable values of the ruggedness criterion. Each PO point in Table 5 represents a potential optimum. The chromatographer may opt for a good separation with $R_{l,min} = 1.74$ and $R_u = 0.21$ (corresponding to conditions of pH 4.31 and $\varphi_{MeOH} = 0.30$; see Table 5) or one may prefer a worse separation with a greater robustness (i.e., lower R_u value), such as $R_{l,min} = 1.4$ and $R_u = 0.09$ (at pH 4.27 and $\varphi_{MeOH} = 0.34$). In MCDM procedures using PO plots, this kind of decision can be made after inspection of the figure and predicted chromatograms at the various PO points. Fig. 7 represents a chromatogram obtained at one of the PO points (i.e., at pH 4.31 and $\varphi_{MeOH} = 0.30$).

The combination of the pareto-optimality method with a robustness coefficient appears to be a powerful tool for selecting the "best" conditions in pH optimization studies. As mentioned by Bourguignon and Massart [9], the pareto-optimality method loses much of its simplicity when more than two criteria are optimized.

Although total ruggedness factors, in which the total variation of the minimum resolution is taken into account, appear to be more relevant than partial ruggedness coefficients, it can be interesting to consider separate ruggedness criteria [R_u(pH) and $R_u(\varphi)$]. Indeed, by calculating the global ruggedness criterion R_u, a high value of R_u(pH) can be compensated by a low value of $R_u(\varphi)$ (or vice versa). Derringer's desirability function can easily be applied to the optimization of more than two criteria. The application of this method requires the definition of the minimum [$Y^{(-)}$] and maximum [$Y^{(+)}$] acceptable values of the response criteria. $Y^{(-)}$ and $Y^{(+)}$ have to be defined according to the objectives of the chromatographer. By way of example, to transform the minimum effective

Table 3
Coefficients describing retention (capacity factor, k), peak height (h), and peak area (A) in terms of Eq. 10 and asymmetry factors (A_S) in terms of Eq. 11

Acid	Parameter	k^0_{HA} [a]	k^0_A	K^0_a	S_{HA}	S_A	Q_1	T_{HA}	T_A	Q_2
3,5-Dinitro-benzoic	k	$5.50 \cdot 10^{7}$	12.07	$5.44 \cdot 10^{-2}$	−59.66	−4.00	13.98	49.45	−4.21	−57.86
	h	$7.84 \cdot 10^{4}$	11.98	$3.75 \cdot 10^{19}$	63.60	11.71	$-3.16 \cdot 10^{2}$	−87.67	−12.92	$4.58 \cdot 10^{2}$
	A	17.26	$1.33 \cdot 10^{3}$	$3.40 \cdot 10^{13}$	25.45	2.24	$-2.13 \cdot 10^{2}$	35.80	−3.34	$3.00 \cdot 10^{2}$
	A_S	$4.79 \cdot 10^{5}$	$-3.69 \cdot 10^{10}$	$6.48 \cdot 10^{5}$	−68.89	$-1.38 \cdot 10^{2}$	88.88	94.26	$1.81 \cdot 10^{2}$	$1.14 \cdot 10^{2}$
Benzoic	k	$2.53 \cdot 10^{3}$	49.19	$2.01 \cdot 10^{-2}$	−12.19	−21.92	−30.01	5.32	21.44	35.92
	h	2.10	$2.61 \cdot 10^{2}$	$2.90 \cdot 10^{4}$	11.75	−6.20	17.64	−8.83	9.93	27.74
	A	$5.98 \cdot 10^{2}$	$2.68 \cdot 10^{3}$	$1.15 \cdot 10^{1}$	4.26	−5.61	−31.91	−6.58	7.88	32.67
	A_S	$2.36 \cdot 10^{7}$	$-2.41 \cdot 10^{12}$	$2.45 \cdot 10^{7}$	−89.84	$-1.60 \cdot 10^{2}$	$-1.08 \cdot 10^{2}$	$1.22 \cdot 10^{2}$	$2.15 \cdot 10^{2}$	$1.45 \cdot 10^{2}$
Salicylic	k	$5.00 \cdot 10^{2}$	1.50	$2.73 \cdot 10^{-5}$	−6.13	4.54	38.04	−11.80	−18.58	−72.69
	h	3.65	51.01	$5.80 \cdot 10^{7}$	11.46	$2.72 \cdot 10^{1}$	$-1.25 \cdot 10^{2}$	9.69	2.63	$1.24 \cdot 10^{2}$
	A	$2.38 \cdot 10^{5}$	$1.30 \cdot 10^{3}$	$1.18 \cdot 10^{1}$	−24.51	−2.18	18.76	35.82	2.83	28.89
	A_S	$6.96 \cdot 10^{2}$	$-6.70 \cdot 10^{-3}$	1.42	−30.62	39.34	−15.86	37.21	−88.49	13.85
m-Nitro-benzoic	k	75.44	1.28	$8.21 \cdot 10^{7}$	−3.74	4.72	42.01	−7.68	−17.64	−67.56
	h	$8.50 \cdot 10^{4}$	60.72	$3.38 \cdot 10^{11}$	56.60	1.44	$-2.08 \cdot 10^{2}$	−71.83	78.05	$2.94 \cdot 10^{2}$
	A	1.30	$2.18 \cdot 10^{3}$	$2.14 \cdot 10^{11}$	39.56	−3.91	$-1.74 \cdot 10^{2}$	−56.04	5.70	$2.29 \cdot 10^{2}$
	A_S	$2.37 \cdot 10^{4}$	$-2.05 \cdot 10^{10}$	$8.77 \cdot 10^{4}$	−51.82	$-1.35 \cdot 10^{2}$	−80.05	69.89	$1.76 \cdot 10^{2}$	$1.05 \cdot 10^{2}$

[a] Or equivalent parameters, i.e., h^0_{HA} and h^0_A, A^0_{HA} and $A^0_{A^-}$, or $A^0_{S,HA}$ and $A^0_{S,A}$.

Table 4
Accuracy of model descriptions

Parameter	3,5-Dinitrobenzoic acid		Benzoic acid		Salicylic acid		*m*-Nitrobenzoic acid	
	SSQ	*ARD* (%)	*SSQ*	*ARD* (%)	*SSQ*	*ARD* (%)	*SSQ*	*ARD* (%)
k	0.13	2.5	0.23	5.3	0.14	4.4	0.35	4.0
h	660.9	5.5	612.4	9.5	79.9	2.7	143.3	3.1
A	49752.9	2.7	12192.9	2.1	363.2	0.4	2780.0	1.0
A_s	0.049	3.4	0.32	9.1	0.61	12.4	0.083	4.0

SSQ = sum of squares of the absolute deviations between calculated and experimental data; *ARD* = average relative deviation between calculated and experimental data.

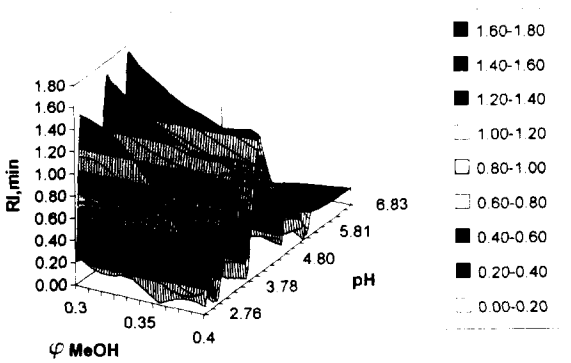

Fig. 2. Response surface of the minimum effective resolution obtained during the optimization of the separation of four solutes.

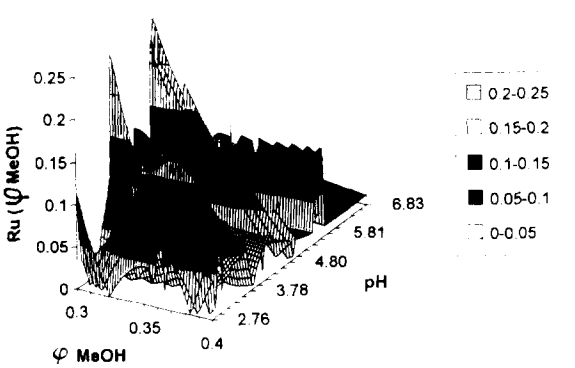

Fig. 3. Response surface of the individual robustness criterion $R_u(\varphi)$ obtained during the optimization of the separation of four solutes.

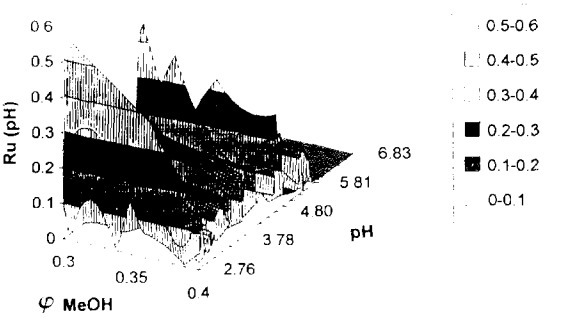

Fig. 4. Response surface of the individual robustness criterion R_u(pH) obtained during the optimization of the separation of four solutes.

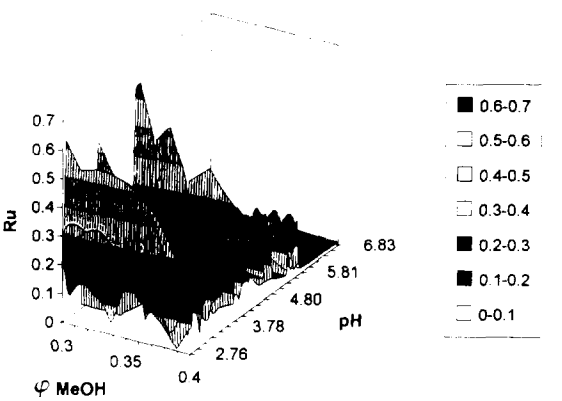

Fig. 5. Response surface of the global robustness criterion R_u obtained during the optimization of the separation of four solutes.

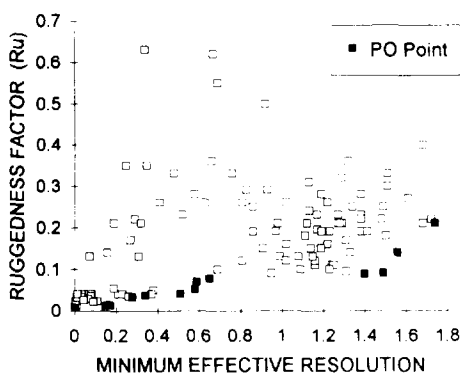

Fig. 6. Pareto-optimality plot for the minimum effective resolution and the ruggedness criterion R_u.

resolution into desirability values, $Y^{(-)}$ is set to 0.5 and $Y^{(+)}$ to 1.5. For the global ruggedness factor (R_u), we define a target value of 0.2 [for individual ruggedness factors $R_u(\varphi)$ and R_u(pH), this value is set to 0.1]. Values $\geq$0.4 [or 0.2 for R_u(pH) and $R_u(\varphi)$] have a desirability equal to 0. Reasonable target values of 0.1 for $R_u(\varphi)$ and R_u(pH) were defined by assuming that the variation of the minimum effective resolution with solvent composition and with pH should not exceed 0.1 for a variation of φ of 0.01 and for a variation of pH of 0.05 (Δ_φ and Δ_{pH} are set at 0.01 and 0.05, respectively). R_u, $R_u(\varphi)$ and R_u(pH) have to be minimized during the process. However, as mentioned in the theoretical

Table 5
Pareto-optimal points and corresponding pH and φ_{MeOH} values

No.	φ_{MeOH}	pH	$R_{l,min}$	R_u
1	0.30	3.30	0.003	0.010
2	0.40	6.83	0.15	0.012
3	0.40	6.14	0.17	0.014
4	0.36	5.20	0.28	0.033
5	0.40	5.20	0.34	0.037
6	0.30	4.88	0.51	0.040
7	0.36	4.75	0.58	0.052
8	0.34	4.75	0.59	0.070
9	0.40	4.75	0.65	0.078
10	0.34	4.27	1.40	0.089
11	0.32	3.65	1.49	0.090
12	0.32	4.27	1.56	0.14
13	0.30	4.31	1.74	0.21

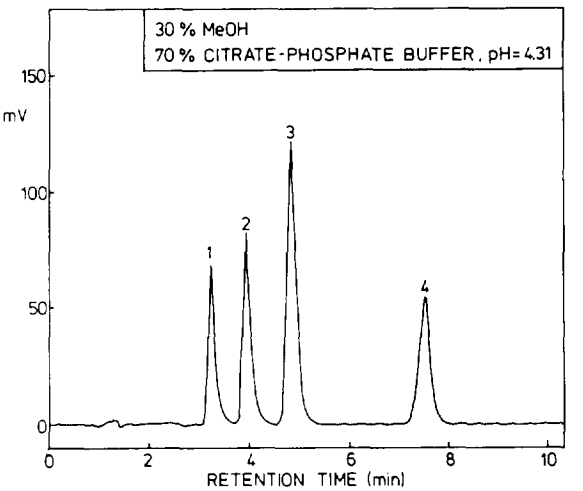

Fig. 7. Chromatogram selected as a possible optimum (φ_{MeOH} = 0.30; pH = 4.31). Peaks: 1 = salicylic acid; 2 = *m*-nitrobenzoic acid; 3 = 3,5-dinitrobenzoic acid; 4 = benzoic acid.

section, higher values of the robustness criterion can be accepted when the minimum resolution increases. Conversely, when the minimum resolution value is (very) low, the requirements for the ruggedness factor are stricter. To this end, we developed a total ruggedness criterion corrected according to the resolution value, $[R_u^*]^{-1}$, calculated from Eq. 7. The corresponding individual ruggedness criteria are $[R_u^*(\varphi)]^{-1}$ and $[R_u^*(\text{pH})]^{-1}$ expressed by Eqs. 8 and 9. These three criteria have to be maximized.

By analogy with the values defined for R_u, the target value is set to 5 for $[R_u^*]^{-1}$. The minimum acceptable value for this criterion is 2.5. Minimum and maximum acceptable values for the corresponding individual ruggedness criteria are set to 5 and 10, respectively.

One-sided transformations of the response criteria into desirability values (d) are shown in Fig. 8. Four different global desirability values (D_1–D_4) are calculated by using the geometric mean of the desirability values on all criteria. These four global desirabilities correspond to the combination of the minimum effective resolution ($R_{l,min}$) with four different expressions of the robustness factor. D_1 combines the minimum effective resolution ($R_{l,min}$) with $[R_u^*]^{-1}$. D_2

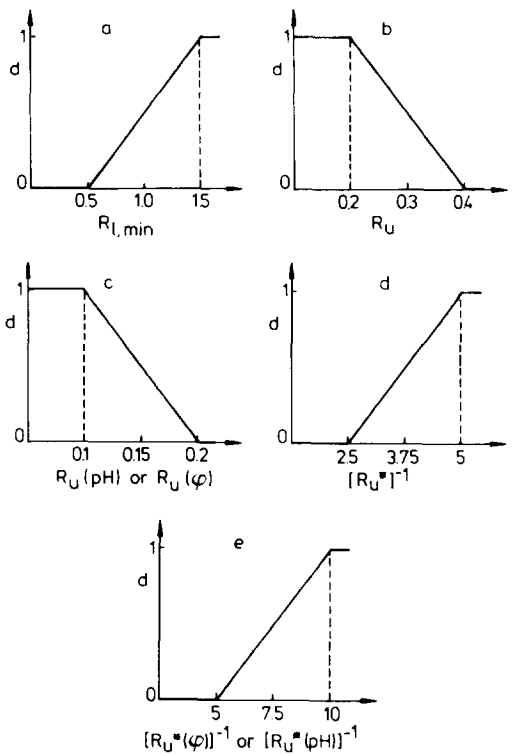

Fig. 8. One-sided transformations of response criteria into desirability values, d. (a) Minimum effective resolution; (b–e) robustness criteria.

takes into account the desirability of $R_{l,min}$ and separate desirabilities for the individual robustness criteria $[R_u^*(pH)]^{-1}$ and $[R_u^*(\varphi)]^{-1}$. D_3 is the combination of $R_{l,min}$ and the robustness factor R_u. Like D_2, D_4 considers three criteria: $R_{l,min}$ and the individual robustness criteria $R_u(pH)$ and $R_u(\varphi)$ separately. Table 6 gives the experimental conditions yielding the best values of the overall desirabilities D_1, D_2, D_3 or D_4, from the 115 selected sets of data. Some of them lead to an overall desirability of 1. This ideal value is obtained when the target value is reached for each criterion. The optimization can then be considered to be completely successful with regard to the objectives defined by the chromatographer.

The overall desirabilities including separate robustness factors in terms of pH and φ_{MeOH} (D_2 or D_4) can be lower than those considering a global robustness criterion (D_1 or D_3). For example, considering experiment 1 from Table 6, the value of D_3 is 0.71, but $D_4 = 0$ because $R_u(pH)$ exceeds 0.2 ($d = 0$; see Fig. 8c). In contrast, R_u has an intermediate value between the minimum and maximum acceptable values (see Fig. 8b). However, the overall ruggedness factors appear to be more relevant, because

Table 6
Experimental conditions and corresponding values of the criteria [$R_{l,min}$; R_u: $R_u(pH)$; $R_u(\varphi)$] yielding the best values of the overall desirabilities D_1, D_2, D_3 or D_4

No.	pH	φ_{MeOH}	k_{max}	$R_{l,min}$	R_u	$R_u(\varphi)$	$R_u(pH)$	D_1	D_2	D_3	D_4
1	3.61	0.30	8.47	1.51	0.30	0.092	0.21	1	0.77	0.71	0
2	4.27	0.30	5.14	1.72	0.22	0.085	0.135	1	1	0.95	0.87
3	4.31	0.30	4.90	1.74	0.21	0.14	0.072	1	1	0.97	0.85
4	4.35	0.30	4.67	1.61	0.27	0.12	0.15	1	1	0.81	0.74
5	4.39	0.30	4.45	1.49	0.25	0.11	0.14	0.99	0.99	0.86	0.81
6	4.43	0.30	4.22	1.38	0.23	0.098	0.13	0.94	0.96	0.86	0.84
7	2.92	0.30	10.04	1.50	0.18	0.030	0.15	1	1	1	0.79
8	3.65	0.30	8.31	1.68	0.21	0.10	0.11	1	1	0.97	0.97
9	4.27	0.32	4.63	1.56	0.14	0.076	0.064	1	1	1	1
10	4.31	0.32	4.43	1.48	0.22	0.11	0.11	0.99	0.99	0.94	0.93
11	4.35	0.32	4.23	1.38	0.22	0.10	0.12	0.94	0.96	0.89	0.89
12	3.65	0.32	7.17	1.49	0.090	0.078	0.012	0.99	0.99	0.99	0.99
13	4.27	0.34	4.12	1.40	0.089	0.063	0.026	0.95	0.97	0.94	0.97

these values refer to the total variation of the minimum resolution.

For seven experimental conditions, D_1 reaches the ultimate value of 1. This value is only obtained twice for D_3. This fact can be easily explained because D_1 takes into account the global ruggedness factor corrected for the resolution, $[R_u^*]^{-1}$. "Higher" values of R_u are then accepted when the minimum resolution increases. In our opinion, using $[R_u^*]^{-1}$ is recommended when Derringer's desirability method is used. In contrast to the pareto-optimality procedure, this method does not permit the visualization of the pay-off between the two types of criteria (minimum resolution and robustness).

Selecting D_1 as the best overall desirability function gives seven optimum experiments (see Table 6). We can choose experiment 3 which corresponds to the best resolution and leads to a short analysis time (7.56 min). This experiment is also a pareto-optimal point (see Table 5) and the corresponding chromatogram (at pH 4.31 and $\varphi_{MeOH} = 0.30$) is shown in Fig. 7.

A multiple-threshold approach (MTA) can also be applied for optimizing several chromatographic goals. Fixed thresholds for any given criterion can be visualized as a step function (see Fig. 9a and b). A single number to assess optimization quality is then obtained by maximizing or minimizing a final criterion, the value of which is not required to meet a specified target. Analysis time is an obvious choice for this purpose. The hyperbolic curve in Fig. 9c for retention (capacity factor of the last peak, k_{max}) corresponds to the use of $1/k_{max}$ as the final optimization criterion. The total desirability can be expressed as the product of all individual values. This function is equal to the value of the final criterion if all thresholds are reached and equal to zero if the latter is not the case. From the analogy between Figs. 8 and 9, the MTA can be seen as a special case of the Derringer method. The advantage of using firm thresholds is the transparency and the obvious correctness of the method. There is no compromise involved in the MTA process, so that the chromatographer may trust the results more easily

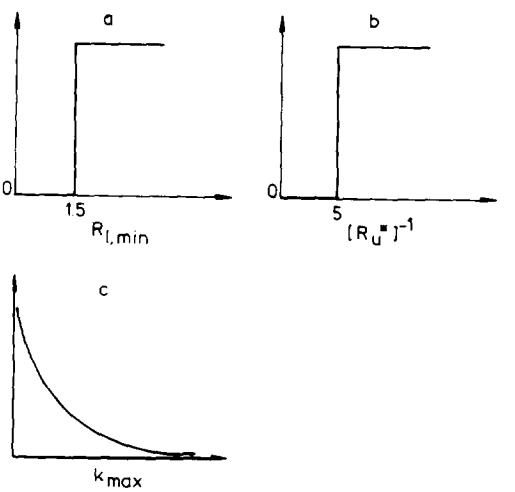

Fig. 9. Example of multiple-threshold approach: (a) and (b) step functions for the minimum effective resolution and $[R_u^*]^{-1}$; (c) hyperbolic curve for retention (k_{max}).

than those obtained in a less transparent manner. A disadvantage is the black-and-white nature of firm thresholds. If the thresholds are not reached, all information on the separation is lost and "grey" (almost optimum) areas on a response surface are discarded. A typical approach in practice is to set ideal targets and lower these stepwise if all targets cannot be met simultaneously. Although this method is not unattractive in case of a single threshold, potential problems arise when several thresholds need to be established and iteratively optimized.

Conventionally, a compromise between time and separation quality is achieved with this strategy. When the threshold resolution value is reached, the optimum analysis time is selected. If the threshold value is not reached, the criterion value is zero. In this study, this method was used for optimizing the minimum effective resolution and a ruggedness criterion. Other combinations of (multiple) threshold criteria are obviously possible. The threshold value of the minimum effective resolution is set at 1.5. Among the experiments reaching this minimum resolution (Nos. 1, 2, 3, 4, 7, 8 and 9 in Table 6), the highest ruggedness factor, $[R_u^*]^{-1}$, is selected. Experiment 9 then represents the optimum (pH 4.27 and $\varphi_{MeOH} = 0.32$) with $[R_u^*]^{-1} = 11.1$.

A multiple-threshold approach can also be used. For example, target values for the minimum resolution (typically 1.5) and for the ruggedness factor $[R_u^*]^{-1}$ (typically 5) can first be defined. Subsequently, and only if both threshold values are reached, the analysis time can be minimized. In this case, experiment 9 is again selected (see Table 6).

Having established the possibility of including ruggedness criteria in selectivity optimization procedures, many other possibilities arise. We have concentrated in this study on the ruggedness of chromatographic resolution, but various other ruggedness criteria can be envisaged. Particularly relevant may be a concentration ruggedness parameter ($R_{u,c}$). In the case in which concentration is obtained from the area (A_i) of a solute peak relative to that of an internal standard (A_{st}), a definition may read

$$R_{u,c} = \sum_x \left(\Delta_x \cdot \frac{\mathrm{d}(A_i/A_{st})}{\mathrm{d}x} \right) \qquad (12)$$

where x denotes the optimization parameters (solvent composition, pH, etc.).

Analogous equations for peak height and other quantification methods can be readily derived. This will be part of future work.

5. Conclusions

It is of great potential benefit to consider the ruggedness of chromatographic separations at an early stage of method development. This greatly reduces the risk of major disappointments when seemingly good methods fail a ruggedness test.

Ruggedness criteria can be elegantly defined as partial or total derivatives of resolution-based criteria with respect to the parameters to be optimized. However, ruggedness cannot be a goal in itself and it must be incorporated in multi-criteria decision making (MCDM) strategies. Three such approaches have been applied in the present study, each showing certain advantages.

Pareto-optimality plots are a highly informative and convenient tool, provided that a trade-off is sought between not more than two different criteria (e.g., resolution and ruggedness). Derringer's desirability function allows the incorporation of many different criteria to yield a single number for the overall quality of a separation. However, the chromatographer sacrifices control of the optimization process, as various compromises between the different criteria will lead to identical desirability values. Using a multiple-threshold approach (MTA), well defined targets can be achieved. MTA is the most easily applied MCDM method, provided that the initial targets can be met. When this is not the case and several thresholds need to be reconsidered, a more complex situation arises, in which other MCDM methods become more attractive.

Acknowledgements

P.F.V. thanks the academic authorities of the Catholic University of Louvain for making possible her working period in Amsterdam and Shell Research for financial support.

References

[1] M. Mulholland and J. Waterhouse, *Chromatographia*, 25 (1988) 769.
[2] M. Mulholland, P.J. Naish, D.R. Stout and J. Waterhouse, *Chemometr. Intell. Lab. Syst.*, 5 (1989) 263.
[3] J.A. van Leeuwen, L.M.C. Buydens, B.G.M. Vandeginste, G. Kateman, P.J. Schoenmakers and M. Mulholland, *Chemometr. Intell. Lab. Syst.*, 10 (1991) 337.
[4] C. Vandenbosch, C. Vannecke and D.L. Massart, *J. Chromatogr.*, 592 (1992) 37.
[5] Y. Vander Heyden, M.S. Khots and D.L. Massart, *Anal. Chim. Acta*, 276 (1993) 189.
[6] S.N. Deming, *J. Chromatogr.*, 550 (1991) 15.
[7] M.W.B. Hendriks, J.H. de Boer, A.K. Smilde and D.A. Doornbos, *Chemometr. Intell. Lab. Syst.*, 16 (1992) 175.
[8] A.K. Smilde, A. Knevelman and P.M.J. Coenegracht, *J. Chromatogr.*, 369 (1986) 1.
[9] B. Bourguignon and D.L. Massart, *J. Chromatogr.*, 586 (1991) 11.
[10] J.H. de Boer, A.K. Smilde and D.A. Doornbos, *Chemometr. Intell. Lab. Syst.*, 7 (1990) 223.
[11] J.H. de Boer, A.K. Smilde and D.A. Doornbos, *Chemometr. Intell. Lab. Syst.*, 10 (1991) 325.

[12] J.H. de Boer, A.K. Smilde and D.A. Doornbos, *Chemometr. Intell. Lab. Syst.*, 15 (1992) 13.
[13] J.H. de Boer, *Ph.D. Thesis*, University of Groningen, Groningen, 1992, Ch. 11.
[14] J.H. de Boer, *Pharm. World Sci.*, 15 (1993) 180.
[15] P.J. Schoenmakers, J.K. Strasters and A. Bartha, *J. Chromatogr.*, 458 (1988) 355.
[16] P.J. Schoenmakers, N. Mackie and R.M. Lopes Marques, *Chromatographia*, 35 (1993) 18.
[17] P.J. Elving, J.M. Markowitz and I. Rosenthal, *Anal. Chem.*, 28 (1956) 1179.
[18] R.M. Lopes Marques and P.J. Schoenmakers, *J. Chromatogr.*, 592 (1992) 157.