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Optimization of the Chiral Separation of Some 2-Arylpropionic Acids on an Avidin Column by Modeling a Combined Response

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ABSTRACT The enantiomeric separation of some nonsteroidal antiinflammatory drugs was investigated on an avidin column. An experimental design approach (central composite design) was used to evaluate the effects of three method parameters (pH, concentration of organic modifier, and buffer concentration) on the analysis time and the resolution, as well as to model these responses. This revealed that the organic modifier concentration and sometimes the pH are significant parameters to control because of their influence on both analysis time and resolution. Furthermore, the central composite design results were combined in a multicriteria decision-making approach in order to obtain a set of optimal experimental conditions leading to the most desirable compromise between resolution and analysis time. *Chirality 13:556–567, 2001.*

KEY WORDS: Derringer's desirability function; multicriteria decision making approach; protein chiral stationary phase; method optimization; chiral separation

A substantial number of pharmaceuticals contain an asymmetric center. These drugs are generally administered as racemates. As the literature[1,2] illustrates, the individual enantiomers often differ in pharmacological action. It is thus important that good, robust separation methods are developed to determine both enantiomers in racemic mixtures. Moreover, regulatory agencies such as the International Conference on Harmonisation (ICH) require the enantioselective determination of chiral drug substances.[3]

Methods to check enantiomeric purity using HPLC have been extensively developed. To separate a racemic mixture by HPLC, diastereoisomeric derivatives of the enantiomers can be prepared, chiral discriminating agents can be added to the mobile phase, or, most easily, a chiral stationary phase (CSP) can be utilized.[1] After Pirkle et al.[4] introduced the first CSP, numerous CSPs of various natures have been developed, among them protein-based ones. Early versions of protein-bonded CSPs suffered from a lack of robustness and longevity. In the present study, a commercially available avidin column (Bioptic AV-1) was used as protein CSP to investigate its enantiomeric resolving power towards some 2-arylpropionic acids (2-APAs).

The 2-APAs represent an important group of nonsteroidal antiinflammatory drugs (NSAIDs), characterized by a chiral carbon atom near the carboxylic acid group (Fig. 1). They are administered for general relief of inflammatory conditions, more specifically, chronic rheumatoid arthritis

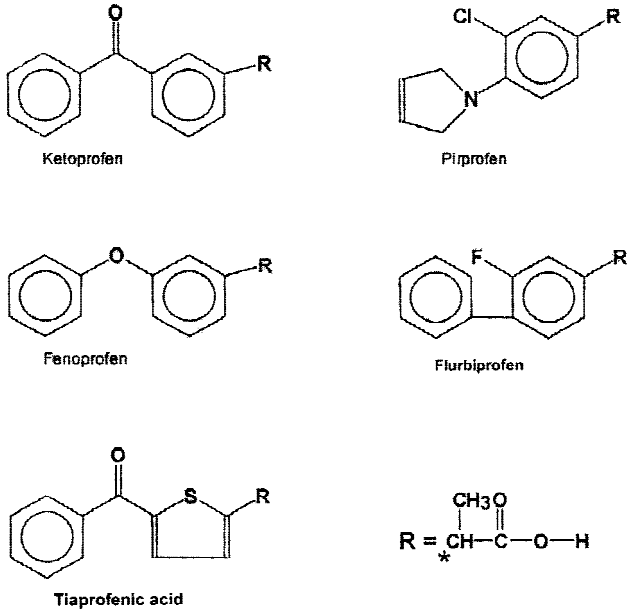

Fig. 1. Structures of the nonsteroidal antiinflammatory drugs studied (*chiral carbon center).

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TABLE 1. Levels of the method parameters

	Decoded value		
Coded value	pH	Conc. acetonitrile (%)	Conc. buffer (M)
–1.68	5.5	4	0
–1	6	5	0.05
0 (nominal value)	6.75	6.5	0.125
+1	7.5	8	0.2
+1.68	8	9	0.25

and osteoarthritis. Based on the knowledge that NSAIDs bind extensively to plasma proteins, numerous bioprotein CSPs were developed and tested for their enantioselectivity towards profens, including human serum albumin,[5] alpha 1-acid glycoprotein,[6–11] ovomucoid,[12–15] bovine serum albumin, avidin, and flavoprotein.[16]

The avidin column investigated has proven to be a powerful chiral selector for a wide variety of acidic, basic, and neutral compounds.[17–21] Avidin-bonded silica was developed by Miwa et al.[22] Avidin, a basic protein (molecular mass 68,300, isoelectric point 10), is well known because of its strong binding properties with biotin. Oda et al.[23] compared avidin and ovomucoid as chiral selectors for the resolution of drug enantiomers with respect to the effects of pH, organic modifier, and buffer salts. They also investigated the retention behavior of some racemic drugs on avidin and modified avidin columns.[24] Mano et al.[25] compared the retention behavior on avidin, ovomucoid, conalbumin, and flavoprotein phases.

The avidin-based CSP has been studied for its enantioselectivity toward 2-APAs (ibuprofen, ketoprofen, flurbiprofen, fenoprofen, and pranoprofen).[26] The effect of base material, binding characteristics between protein and base material, and protein modification on enantioseparation was investigated. These studies were performed using a univariate optimization, by changing one factor at a time. This approach has the disadvantage of being time-consuming and of examining only a limited part of the experimental domain. To overcome these problems, multivariate (chemometric) approaches using experimental design were developed and are becoming increasingly important to optimize separations of different natures using different separation techniques.[27–36] In the past, chiral optimizations were mostly performed envisaging separation quality as the only goal, not considering analysis time as a second optimization criterion. However, good resolving methods with extreme analysis times need to be avoided. Thus, a multicriteria decision-making problem (MCDM) appears, i.e., one has to find a compromise between separation quality and analysis time.

The first aim of the present study was to investigate the influence of some chromatographic variables on the enantiodiscrimination employing a chemometric approach. Second, a search for combinations of variables resulting in an acceptable enantioselective separation of the studied 2-APAs by simultaneously considering analysis time and separation quality was aimed at. Therefore, a three-factor central composite design associated with an MCDM approach was used. Derringer's desirability function (DDF)[37] and pareto optimality plots[38] as MCMD approaches are discussed and compared.

MATERIALS AND METHODS

Chemicals

The chemical structures of the 2-APA analytes are shown in Figure 1. Racemic (rac) flurbiprofen was obtained from Upjohn (Kalamazoo, MI), rac-calcium fenoprofen from Eli Lilly (Indianapolis, IN), rac-tiaprofenic acid from Erfa (Brussels, Belgium), rac-pirprofen from Profarma (Oud-Turnhout, Belgium), and rac-ketoprofen from Sigma Aldrich (Bornem, Belgium). Potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide, used to prepare the buffer solutions were from Merck (Darmstadt, Germany). HPLC-grade acetonitrile (ACN) was purchased

TABLE 2. Experimental conditions for the central composite design (expressed in coded values) and results from the different runs (bold = pareto-optimal points)

	Factor levels			Ketoprofen		Pirprofen		Fenoprofen		Tiaprofenic acid		Flurbiprofen	
Exp.	ACN (x_1)	Buffer (x_2)	pH (x_3)	tr_2	Rs	tr_2	Rs	tr_2	Rp	tr_2	Rp	tr_2	Rp
1	0	0	0	13.70	1.52	13.36	1.41	**12.18**	**0.89**	14.24	0.32	17.51	—
2	–1	–1	–1	19.60	1.54	23.10	2.08	23.52	0.96	28.18	0.48	31.80	—
3	1	1	–1	11.40	1.02	13.77	1.02	13.81	0.38	13.67	0.09	21.33	—
4	–1	1	1	14.40	2.05	12.06	1.48	**15.71**	**0.98**	**18.61**	**0.76**	21.22	—
5	1	–1	1	**6.70**	**1.31**	**7.77**	**1.31**	**8.64**	**0.79**	**9.08**	**0.36**	11.63	—
6	0	0	0	12.70	1.69	**13.38**	**1.77**	12.15	0.78	**14.53**	**0.43**	17.60	—
7	–1	–1	1	**13.90**	**2.29**	**14.62**	**2.88**	12.05	0.63	**16.89**	**0.71**	19.08	—
8	1	1	1	**9.00**	**1.47**	8.73	0.61	9.53	0.50	9.55	0.00	13.10	—
9	–1	1	–1	21.60	1.92	22.91	2.01	20.86	0.87	25.71	0.60	30.55	—
10	1	–1	–1	15.40	1.18	17.58	1.68	15.08	0.48	16.69	0.06	22.09	—
11	1.68	0	0	8.90	1.00	9.16	0.70	9.16	0.02	9.26	0.00	12.68	—
12	0	0	–1.68	21.00	1.19	25.26	1.70	20.60	0.09	23.04	0.14	31.21	—
13	–1.68	0	0	23.00	2.23	23.08	2.58	22.31	0.90	27.95	0.49	28.43	—
14	0	–1.68	0	16.80	1.60	13.09	1.10	15.37	0.68	9.48	0.31	18.79	—
15	0	1.68	0	13.40	1.52	11.16	1.28	13.51	0.42	15.07	0.15	18.88	—
16	0	0	1.68	**10.40**	**1.80**	9.48	1.28	10.25	0.58	11.54	0.22	13.49	—

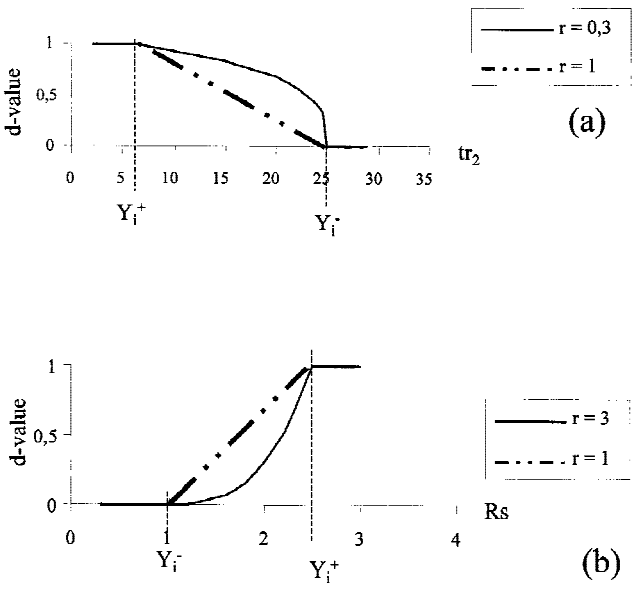

Fig. 2. One-sided transformations of the responses of ketoprofen into desirability values; (**a**) analysis time (tr2); (**b**) resolution (Rs).

from Pancreac-Química (Spain). Deionized water was used throughout. Methanolic test solutions of the chiral compounds with a concentration of about 1.0 mg ml^{-1} were used for all experiments.

Chromatographic Conditions

Chromatography was performed with a Varian 9010 SDS pump (Varian Associates, Walnut Creek, CA) using a Rheodyne 7125 injector with a 20 µl loop. Detection was performed with a Hewlett Packard series 1050 diode array detector (Hewlett Packard, Waldbronn, Germany). Integrations of the chromatograms were made with the Hewlett Packard software package. The analyses were carried out on a Bioptic AV-1 avidin column (150 × 4,6 mm i.d., 5 µm) (GL Sciences, Tokyo, Japan). The mobile phase was pumped at a flow-rate of 0.8 ml min^{-1}. Chromatography was carried out at 30°C. The column eluate was monitored at 254 nm.

Buffers were adjusted to the required pH using a Metrohm 691 pH-meter (Pleuger, Belgium) by means of a 0.1 M sodium hydroxide solution.

Calculations and Software

As a measure of the analysis time, the retention time of the last eluting peak (tr2) was recorded. To indicate separation quality, the resolution Rs (=1.18 $(t1-t2)/(w_1+w_2)$, with t the retention time and w the peak width at half height) or the Kaiser's peak separation index (Rp, the ratio of peak valley height between two peaks and the mean height of the two peaks) were calculated. The Kaiser's peak separation index was measured when for some design experiments the enantiomeric peaks were very incompletely resolved and the resolution could not be calculated. This was the case for fenoprofen and tiaprofenic acid.

Statgraphics (v. 3.1 for Windows; Manugistics, Rockville, USA) was used to model the chromatographic parameters and to generate response surfaces and contour plots.

Chemometric Approach

Investigated variables and their ranges. Several chromatographic variables may play a role in chiral separations on the avidin column: mobile phase pH, buffer type and concentration, type and concentration of organic modifier, flow-rate, and temperature. To limit the number of factors examined, it was decided to keep column temperature (30°C), buffer type (KH_2PO_4), type of organic modifier (ACN), and flow-rate (0.8 ml min^{-1}) constant. This is in agreement with Haque and Stewart's rationale,[20] which states that varying buffer pH and organic modifier has the strongest influence on resolution. In addition, the buffer concentration was also investigated. Phosphate buffer was used, based on literature data for this specific column.[23]

The experimental ranges of the method parameters obtained from preliminary experiments are tabulated in Table 1. Extra consideration has to take into account for pH; values above 8 are not recommended because of column lifetime limitations. Thus, the mobile phases consisted of various mixtures of ACN: KH_2PO_4 buffer with varying pH-values and buffer concentrations as required by the experimental design.

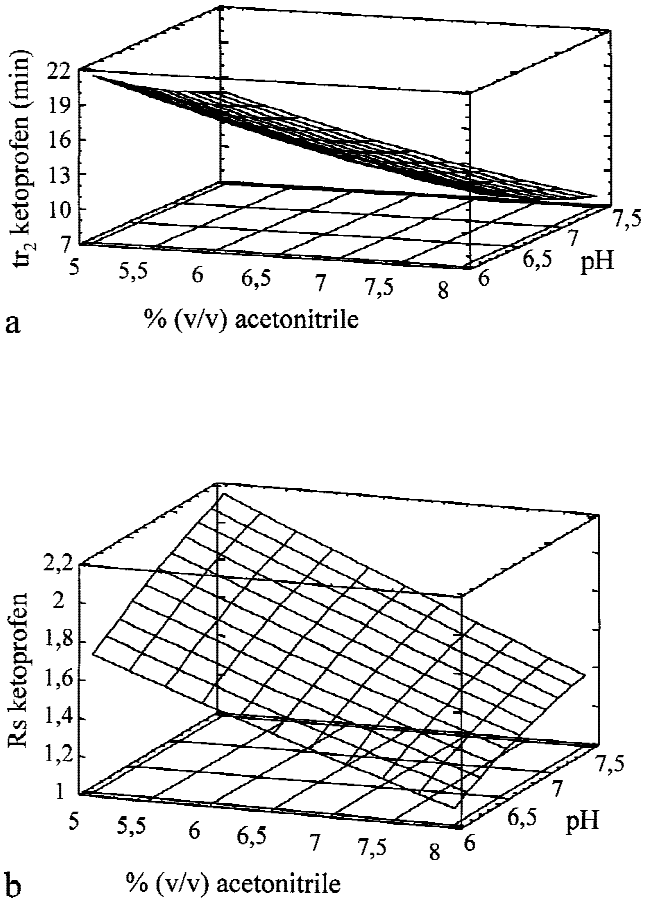

Fig. 3. Response surface plot of analysis time (**a**) and resolution (**b**) for ketoprofen as a function of percentage CH_3CN and pH (buffer concentration at nominal value (0.125 M)).

TABLE 3. Regression coefficients for Eq. 5 (bold coefficient = significant effect at the 5% significance level, —: not determined)

	Ketoprofen		Pirprofen		Fenoprofen		Tiaprofenic acid		Flurbiprofen	
Variable	tr_2	Rs	tr_2	Rs	tr_2	Rp	tr_2	Rp	tr_2	Rp
b_0	13.373	1.601	13.395	1.570	12.214	0.808	14.272	0.358	17.497	—
b_1	**–3.713**	**–0.358**	**–3.533**	**–0.512**	**–3.456**	**–0.203**	**–5.260**	**–0.210**	**–4.466**	—
b_2	–0.360	0.000	–0.648	–0.185	–0.184	–0.042	0.447	–0.031	0.128	—
b_3	**–3.063**	**0.182**	**–4.448**	–0.089	**–3.277**	0.076	**–3.622**	0.054	**–5.165**	—
b_1^2	0.570	0.014	0.914	0.065	**1.149**	–0.069	**1.757**	–0.006	**1.197**	—
b_{12}	–0.525	–0.018	–0.012	0.014	–0.172	–0.081	–0.225	–0.062	–0.022	—
b_{13}	0.225	–0.038	0.560	–0.131	0.738	0.081	0.832	–0.022	1.420	—
b_2^2	0.269	–0.006	–0.499	–0.094	0.692	–0.037	–0.481	–0.011	**0.589**	—
b_{23}	0.600	–0.038	0.300	–0.172	**1.060**	0.031	0.960	–0.058	**0.702**	—
b_3^2	0.481	–0.029	**1.359**	0.012	**1.040**	–0.113	1.292	–0.028	**1.832**	—

Choice of the design. To choose a response surface design for three variables, different alternatives are possible, such as the Box-Behnken,[39] Doehlert,[40] three-level full factorial,[32,41] and central composite design.[32,41] These response surface designs contain more than two levels for each factor and allow estimation of quadratic terms during modeling, i.e., curvature in the response surface. In the present work a central composite design was used. The center point was duplicated. The design, expressed in coded values, is presented in Table 2. Retention times of the last eluting peak and resolution or Kaiser's peak separation index were recorded for each run. The design experiments were carried out in a randomized order.

Optimization: MCDM approach. The optimization of (chiral) HPLC methods requires criteria to decide whether a given chromatogram is superior to another. Often a compromise between conflicting goals such as separation quality and analysis time needs to be found. Therefore, the responses can be considered in an MCDM process. Various strategies for handling such problems in the separation field have been published.[42–46] Until now, MCDM optimization methods have received little attention in enantiomeric separations. A number of MCDM procedures are useful for bicriteria optimizations, e.g., the threshold[48–51] and the pareto-optimality approach.[38] A pareto-optimal point is a point where no experiment yields better results on one criterion without having a worse on another. The threshold approach defines a threshold value for one criterion and optimizes the other for all situations in which the threshold for the first is reached. Presently, the pareto-optimality approach will be compared to Derringer desirability functions (DDF). In optimization procedures where more than two criteria are studied, desirability functions are more appropriate.[52] These functions were first presented by Harrington[53] and further developed by Derringer and Suich.[37] In HPLC, Bourguignon and Massart[52] were the first to use the DDF to optimize several performance goals. In Derringer's approach the measured responses are transformed to a dimensionless desirability (d_i) scale. Several responses, obtained with different desirability functions, are then combined. The desirability scale ranges between $d_i = 0$, a completely undesirable level of quality, and $d_i = 1$, the level of maximum quality.

Two types of transformations are possible: one-sided or two-sided.[52] A two-sided transformation is used when a given response value is optimal and deviating ones to either side are less desirable. A one-sided transformation is used to either minimize or maximize a response.

Consider the situation in which one wants to maximize a response Y, e.g., resolution, while Y_i^+ represents the smallest value of Y which is desirable and Y_i^- the largest value that can be considered undesirable (see Fig. 2b). Assuming that desirability decreases linearly from Y_i^+ to Y_i^-, the desirability function d_i is calculated as:

$$d_i = 0 \quad \text{if } Y_i \le Y_i^- \tag{1}$$

$$d_i = 1 \quad \text{if } Y_i \ge Y_i^+ \tag{2}$$

$$d_i = \left(\frac{Y_i - Y_i^-}{Y_i^+ - Y_i^-}\right)^r \quad \text{if } Y_i^- < Y_i < Y_i^+ \tag{3}$$

TABLE 4. Coefficient of multiple determination R^2 of the different modeled responses (—: not determined)

Substances	Responses	R^2
Ketoprofen	tr_2	0.932
	Rs	0.942
Pirprofen	tr_2	0.978
	Rs	0.827
Fenoprofen	tr_2	0.981
	Rp	0.700
Thiaprofenic acid	tr_2	0.967
	Rp	0.814
Flurbiprofen	tr_2	0.997
	Rp	—

TABLE 5. Y_i^+ and Y_i^- values as defined for each compound

	tr_2		Rs	
Substances	Y_i^-	Y_i^+	Y_i^-	Y_i^+
Ketoprofen	25	6	1	2.5
Pirprofen	26	8	0.5	3
Fenoprofen	25	8	0 (Rp)	1 (Rp)
Tiaprofenic acid	29	9	0 (Rp)	1 (Rp)

The factor *r* offers the flexibility to model the desirability function. The r-factor is set at a fixed value (Fig. 2) depending on the desirability wanted for a certain result. The *r*-factor is equal to 1 when the function is linear.

When a minimization of a specific response is required (Fig. 2a), the transformation becomes:

$$d_i = 0 \quad \text{if } Y_i \geq Y_i^- \tag{1'}$$

$$d_i = 1 \quad \text{if } Y_i \leq Y_i^+ \tag{2'}$$

$$d_i = \left(\frac{Y_i - Y_i^-}{Y_i^+ - Y_i^-}\right)^r \quad \text{if } Y_i^+ < Y_i < Y_i^- \tag{3'}$$

with now Y_i^+ the largest Y_i that is considered desirable and Y_i^- the smallest undesirable value. Combination of the desirability (d_i) functions from the considered responses leads to the overall desirability, D, a combined response, defined as the geometric mean of the different d_i-values:

$$D = (d_1 d_2 \ldots d_k)^{1/k} \tag{4}$$

The D-values can be modeled as a function of the examined factors. If one of the d_i-values equals zero, the overall desirability will be zero, which causes an abrupt change in the response surface. Therefore, when modeling D it can be recommended to select Y_i^- and Y_i^+ in such a way that the d_i-values corresponding to the measured Y_i-values are always $0<d_i<1$.

RESULTS AND DISCUSSION

Modeling the Responses

For each compound, the responses *tr*2 and Rs or Rp were measured (Table 2). During the entire design no switch in elution order appeared, which allows modeling the resolution or Kaiser's peak separation index. A quadratic model was built for the responses, separation quality, and analysis time, of each compound:

TABLE 6. d_i-Values and the overall desirability D (with various r-factors) for the different runs (bold = maximal D-value)

	Ketoprofen						Pirprofen					
Exp.	r = 0.3 d(Tr)	r = 3 d(Rs)	D	r = 1 d(Tr)	r = 1 d(Rs)	D	r = 0.3 d(Tr)	r = 3 d(Rs)	D	r = 1 d(Tr)	r = 1 d(Rs)	D
1	0.856	0.043	0.191	0.595	0.350	0.456	0.970	0.066	0.253	0.900	0.404	0.603
2	0.686	0.046	0.177	0.284	0.357	0.318	0.578	0.252	0.382	0.161	0.632	0.319
3	0.905	0.000	0.001	0.716	0.013	0.097	0.890	0.009	0.090	0.679	0.208	0.376
4	0.839	0.338	0.533	0.558	0.697	0.624	0.926	0.060	0.236	0.775	0.392	0.551
5	0.989	0.008	0.092	0.963	0.204	0.444	1.004	0.034	0.185	1.013	0.324	0.573
6	0.878	0.099	0.294	0.647	0.462	0.547	0.899	0.131	0.343	0.701	0.508	0.597
7	0.851	0.631	**0.733**	0.584	0.858	**0.708**	0.872	0.863	0.867	0.632	0.952	**0.776**
8	0.950	0.030	0.170	0.842	0.312	0.512	0.988	0.000	0.009	0.959	0.044	0.206
9	0.597	0.230	0.370	0.179	0.612	0.331	0.589	0.220	0.360	0.172	0.604	0.322
10	0.815	0.002	0.037	0.505	0.119	0.245	0.796	0.105	0.289	0.468	0.472	0.470
11	0.952	0.000	0.000	0.847	0.002	0.040	0.980	0.000	0.022	0.936	0.080	0.274
12	0.627	0.002	0.035	0.210	0.124	0.162	0.381	0.111	0.205	0.040	0.480	0.139
13	0.509	0.545	0.526	0.105	0.817	0.293	0.580	0.576	0.578	0.162	0.832	0.367
14	0.777	0.063	0.221	0.432	0.397	0.414	0.905	0.014	0.112	0.717	0.240	0.415
15	0.862	0.041	0.188	0.610	0.345	0.459	0.944	0.030	0.169	0.824	0.312	0.507
16	0.924	0.150	0.372	0.768	0.531	0.639	0.975	0.030	0.172	0.918	0.312	0.535

	Fenoprofen						Tiaprofenic acid					
Exp.	r = 0.3 d(Tr)	r = 3 d(Rs)	D	r = 1 d(Tr)	r = 1 d(Rs)	D	r = 0.3 d(Tr)	r = 3 d(Rs)	D	r = 1 d(Tr)	r = 1 d(Rs)	D
1	0.888	0.047	0.204	0.674	0.360	0.492	0.913	0.033	0.173	0.738	0.320	0.486
2	0.481	0.885	0.652	0.087	0.960	0.289	0.384	0.111	0.206	0.041	0.480	0.141
3	0.882	0.055	0.220	0.658	0.380	0.500	0.923	0.001	0.242	0.766	0.086	0.257
4	0.834	0.941	**0.887**	0.546	0.980	0.732	0.822	0.439	**0.601**	0.520	0.760	0.628
5	0.989	0.493	0.698	0.962	0.790	**0.872**	0.999	0.047	0.216	0.996	0.360	0.599
6	0.919	0.475	0.660	0.756	0.780	0.768	0.907	0.080	0.269	0.723	0.430	0.558
7	0.922	0.250	0.480	0.762	0.630	0.693	0.860	0.358	0.555	0.606	0.710	**0.656**
8	0.972	0.125	0.349	0.910	0.500	0.674	0.992	0.000	0.000	0.973	0.000	0.000
9	0.655	0.659	0.656	0.244	0.870	0.460	0.582	0.216	0.354	0.164	0.600	0.314
10	0.851	0.111	0.307	0.584	0.480	0.529	0.864	0.000	0.015	0.616	0.063	0.197
11	0.979	0.000	0.003	0.932	0.020	0.136	0.996	0.000	0.000	0.987	0.000	0.000
12	0.667	0.001	0.022	0.259	0.089	0.152	0.695	0.003	0.044	0.298	0.140	0.204
13	0.575	0.729	0.648	0.158	0.900	0.377	0.413	0.118	0.221	0.052	0.490	0.160
14	0.843	0.314	0.515	0.566	0.680	0.621	0.993	0.030	0.172	0.976	0.310	0.550
15	0.889	0.074	0.257	0.676	0.420	0.533	0.897	0.003	0.055	0.696	0.150	0.323
16	0.958	0.195	0.432	0.868	0.580	0.709	0.960	0.011	0.101	0.873	0.220	0.438

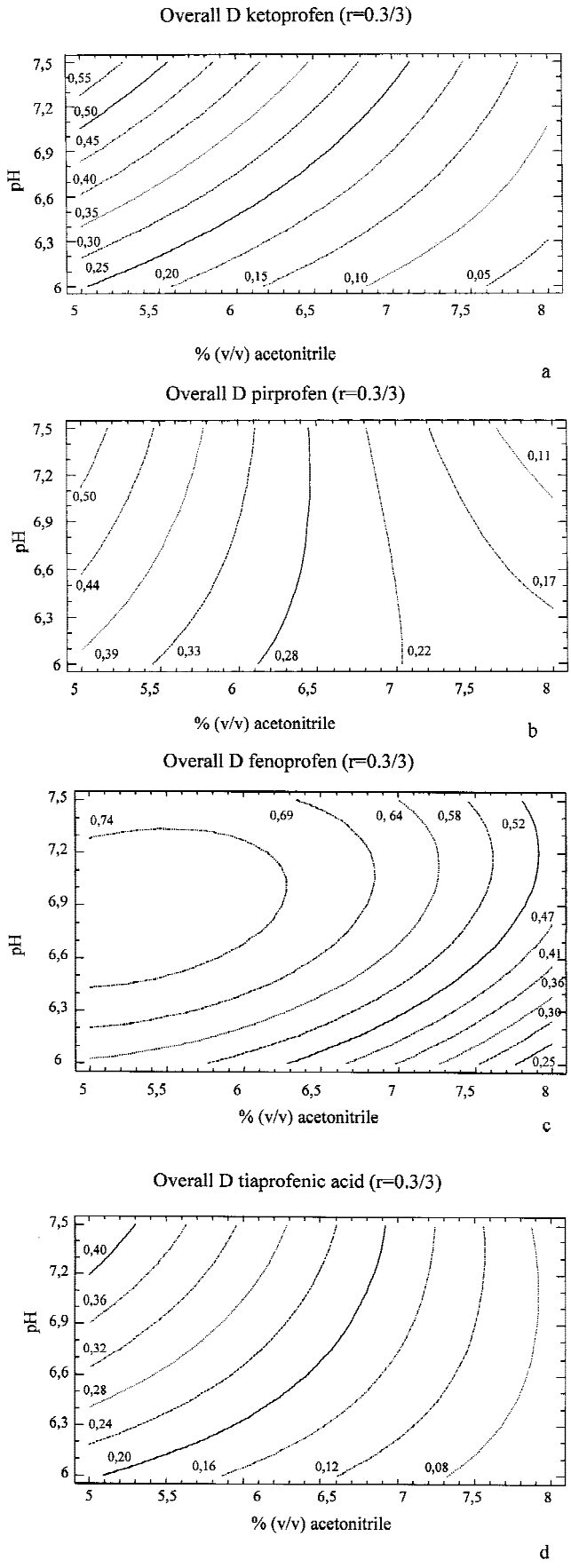

Fig. 4. Contour plots of the overall desirability with an r-factor of 0.3 for the analysis time and r = 3 for the separation quality (**a**) ketoprofen, (**b**) pirprofen, (**c**) fenoprofen, and (**d**) tiaprofenic acid. Buffer concentration in all cases kept at nominal value (0.125 M).

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_{12} + b_{13}x_{13} + b_{23}x_{23} + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (5)$$

where Y is the considered response, x_1 represents the percentage ACN, x_2 the pH, x_3 the buffer concentration, and b_i a multiple regression coefficient. No (nonsignificant) coefficients are excluded in the finally applied models. The same modeling procedure was applied to the overall desirability, D. The resulting equations (Table 3) enabled predictions for resolution and analysis time in the studied domain and could be used to produce response surface plots. As an example, for ketoprofen response surface plots of the resolution and the analysis time as a function of the mobile phase composition (percentage ACN and pH) are presented in Figure 3.

The goodness of fit of the model was evaluated by means of R^2, the coefficient of multiple determination.[54] This coefficient R^2 (Table 4) indicates the proportion of variation of Y explained by the model. From Table 4 it could be concluded that a quadratic model fits analysis time and separation quality to the design results with good statistical reliability.

Evaluation of the Models for Separation Quality

As flurbiprofen usually was not or very badly separated, it is not considered further. For tiaprofenic acid and fenoprofen, Kaiser's peak separation index was considered the criterion for separation quality.

The regression coefficients (Table 3) were evaluated for their significance by an ANOVA at the 5% significance level.[54] This reveals that for the four NSAIDs investigated, the percent organic modifier (x_1) significantly influences the separation quality in the domain studied. The negative coefficient indicates that separation quality (resolution or Kaiser's peak separation index) decreases as the ACN percentage increases. This effect is common in reversed phase LC and is in agreement with the findings of Haginaka et al.[26] on ketoprofen and fenoprofen separations. This effect is reported to be due to a hydrophobic interaction competition of ACN with the drugs for hydrophobic regions of the protein, such as the ($-CH_2$) stretches of the phenyl rings.

The pH of the buffer (x_3) was also a significant variable, but only for the separation of ketoprofen. Resolution of ketoprofen enantiomers increase while pH increases. The influence of the buffer concentration (x_2) in the ranges investigated was not significant for any of the four NSAIDs. Neither interactions nor quadratic terms were found to be of any significance. This results in fairly planar, not-twisted response surfaces (Fig. 3).

Evaluation of the Models for Analysis Time

From Table 3, the importance of two variables on the analysis time of the compounds studied (including flurbiprofen) can be seen: percentage ACN (x_1) and pH (x_3). While increasing the pH and the percentage of ACN in the

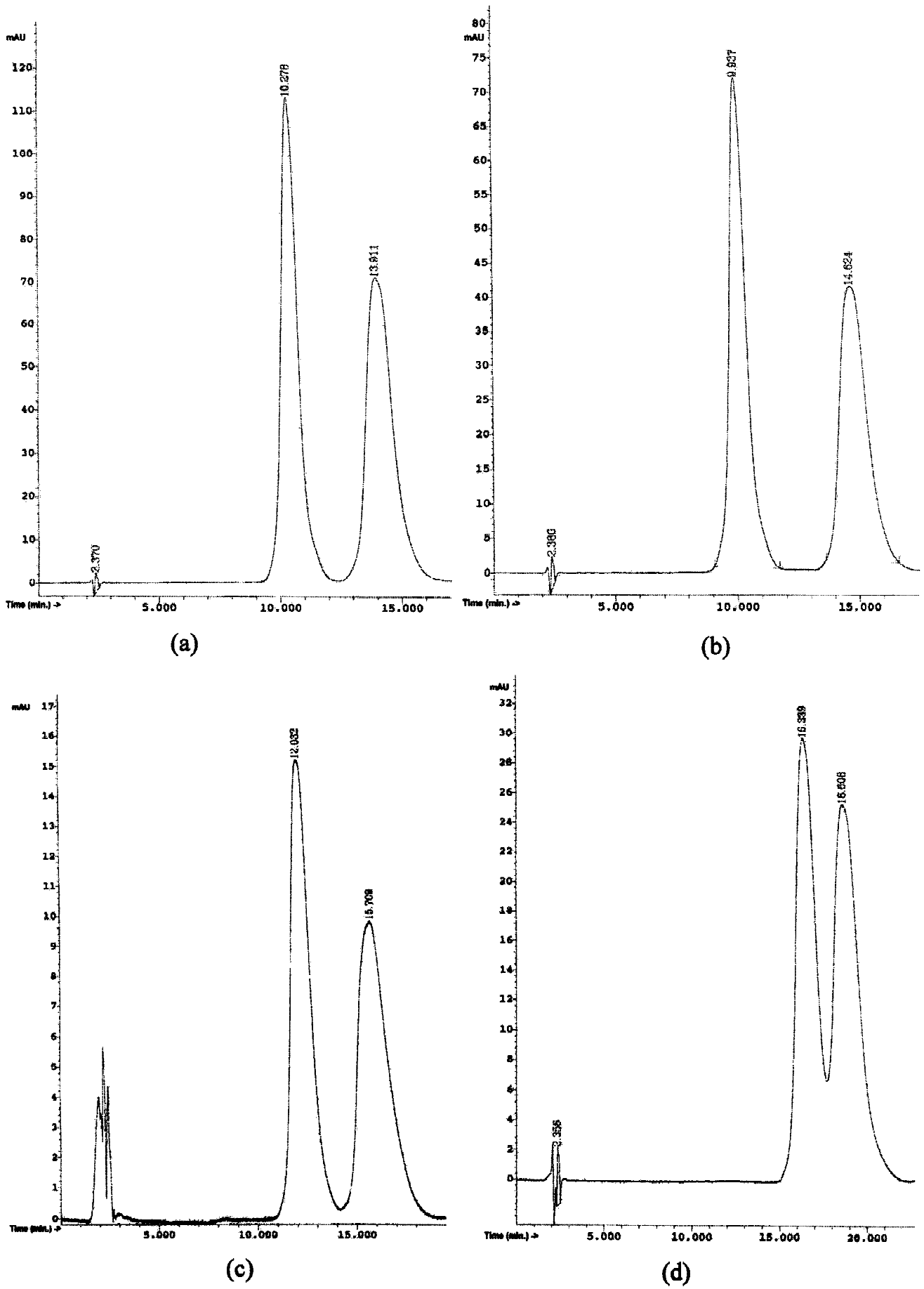

Fig. 5. Chromatogram of each compound at the optimal conditions obtained by using the DDF approach, with r = 0.3 as r-factor for the analysis time and r = 3 for the separation quality. (**a**) Ketoprofen (1 mg ml^{-1}); CH_3CN 5%, pH 7.5; (**b**) pirprofen (1 mg ml^{-1}); CH_3CN 5%, pH 7.5; (**c**) fenoprofen (1 mg ml^{-1}); CH_3CN 5%, pH 7.5; (**d**) tiaprofenic acid (1 mg ml^{-1}); CH_3CN 5%, pH 7.5.

mobile phase, analysis time is shortened. From the magnitude of the regression coefficients, it can be concluded that tiaprofenic acid was most influenced by the change in percentage ACN in the mobile phase. Buffer concentration (x_2) did not play a significant role in the analysis time. Some quadratic terms are also significant (mainly those for ACN, x_1^2, and pH, x_3^2), indicating curvature in the corresponding response surface.

Simultaneous Optimization of Two Criteria by Means of an MCDM Approach

First, Derringer's desirability approach was applied to select the experimental conditions with the most desirable analysis time and separation quality combination. Derringer's method requires the definition of the Y_i^- and Y_i^+ value for each response. Those used are described in Table 5. Both Y_i^+ and Y_i^- were chosen in such a way that for none of the runs the d_i-value became equal to zero. For the transformation into desirability values of values between Y_i^- and Y_i^+, an r-exponent is to be defined. The most obvious way of transforming responses into d_i-values is by drawing a straight line between the Y_i^- and Y_i^+, i.e., $r = 1$. The transformations for retention time and resolution when r = 1 are illustrated, for ketoprofen, in Figure 2. Sometimes, however, it is more reasonable that a result higher or lower (depending on if a response needs to be maximized or minimized) than Y_i^- rapidly becomes more desirable. This is illustrated in the curve in Figure 2a when r = 0.3. On the contrary, in some cases the choice is made that responses lower or higher (depending on if a response needs to be maximized or minimized) than Y_i^+ makes the separation rapidly less desirable, leading to a curve such as that obtained with r = 3 (Fig. 2b).

In this work, the d_i-values were calculated in two ways. To transform the resolution, or Rp, Eq. 3 was used, once with an r-value of 1 and once with r = 3. For the retention time, the one-sided transformation of Eq. 3′ is used, once with r equal to 0.3 and once with r = 1. The d_i-values obtained with r = 3 for Rs (or Rp) and those with r = 0.3 for the retention time were combined to the overall desirability D according to Eq. 4. These curved transformations result in a D-function that gives a higher importance to separation and a lower to analysis time. The same procedure was followed to obtain the overall desirability D with r = 1 for both responses. In this latter situation, both criteria have equal importance. The results obtained using these desirability functions are shown in Table 6.

Figure 4 shows the contour plots of the models for the overall desirability when the r-values for analysis time and separation quality are equal to 0.3 and 3, respectively. Since the buffer concentration does not contribute significantly to either analysis time or separation quality, it was kept at

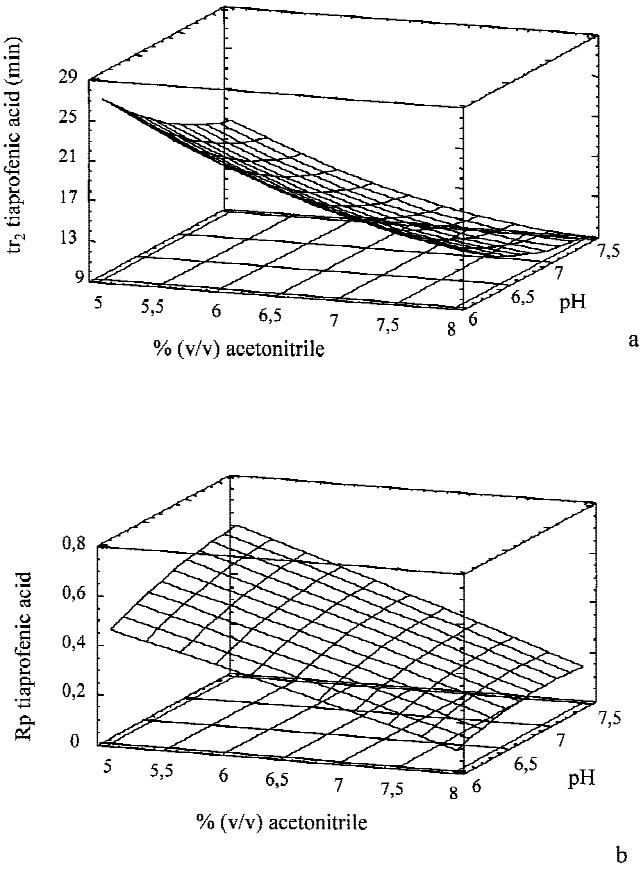

Fig. 6. Response surface plot of (**a**) analysis time and (**b**) resolution, for tiaprofenic acid as a function of percentage CH_3CN and pH (buffer concentration at nominal value (0.125 M)).

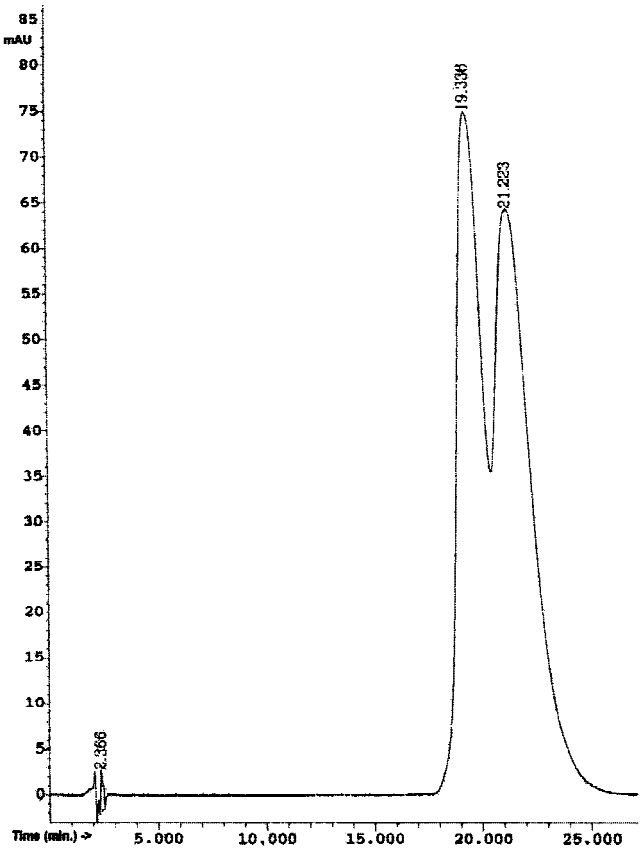

Fig. 7. Best chromatogram of flurbiprofen (1 mg ml^{-1}), out of all design runs (mobile phase composition: pH 7.5; CH_3CN 5%; buffer 0.2 M).

nominal level in the contour plots of Figure 4. The highest overall desirability is obtained in the region with low percentage ACN and high pH. The experimental runs with the highest D (Table 6) fulfil this requirement. For each compound, with the exception of fenoprofen, a relatively small area with maximal overall desirability can be selected. For fenoprofen, high D-values (above 0.7) are obtained in a broader region. A chromatogram of each compound at optimal conditions obtained using this DDF approach is given in Figure 5. It should be noted that due to column failure shortly after finishing the experimental design, no confirming experiment could be performed for fenoprofen in the region with predictions above 0.74 for D.

Excellent separations within a short analysis time were obtained on the avidin column for ketoprofen, pirprofen, and fenoprofen (Fig. 5). Tiaprofenic acid and flurbiprofen were not baseline separated in the examined domain. One could note that for those two compounds the experimental range of some factors was perhaps badly chosen. From the response surface plot for tiaprofenic acid it can be seen that Rp can be increased by lowering the content of ACN (Fig. 6b), but then the analysis time becomes too long (Fig. 6a). The best chromatogram of all runs for flurbiprofen is given in Figure 7. It can be observed that the separation is very incomplete.

The contour plots of the overall desirability models when the desirability functions had a linear path (r = 1) for both responses are presented in Figure 8. Again, optimal conditions can be predicted, showing for all compounds larger optimal zones than in the situation of r = 0.3/3. Almost all optimal zones found here include the zone selected in the situation of r = 0.3/3. Examining Table 6 reveals that for both approaches, with the exception of fenoprofen, maximal D-values are noticed in runs 7 or 4, corresponding with pH 7.5 and percentage of ACN 5%.

Where the r-exponent is equal to 3 for Rs or Rp and 0.3 for tr2, the difference between what is desirable and what is not is greater. It favors good separations and disadvantages long analysis times more than the situation in which the desirability function has a linear path. This is also the approach an expert usually would follow and can therefore be preferred.

Another MCDM technique used here is the pareto-optimality approach. Figure 9 shows pareto-optimality plots for the separation quality and the analysis time. Pareto-optimal points were derived for the situation where sepa-

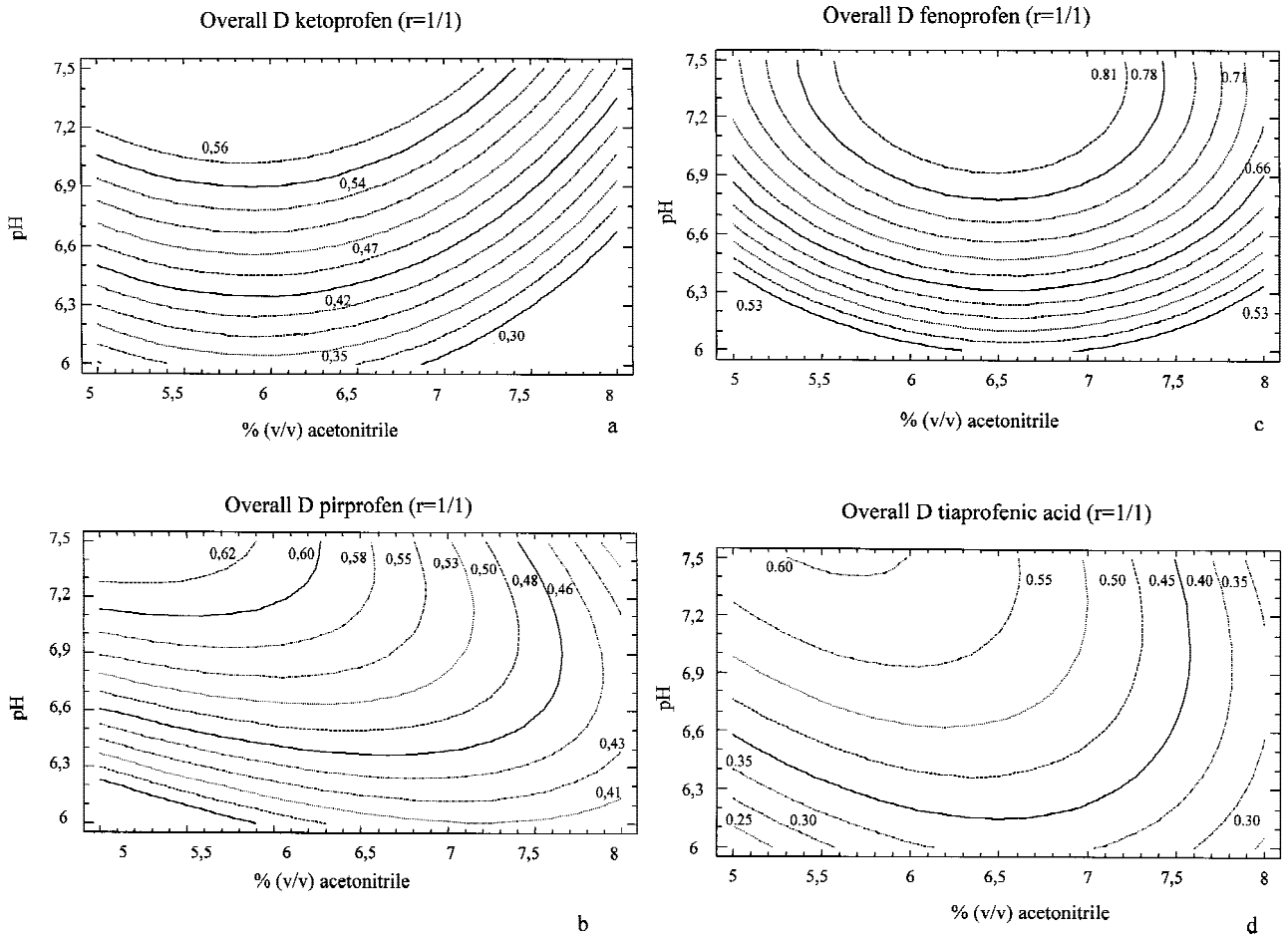

Fig. 8. Contour plots of the overall desirability with r = 1 for the analysis time and the separation quality. Ketoprofen (**a**), pirprofen (**b**), fenoprofen (**c**), tiaprofenic acid (**d**). Buffer concentration in all cases kept at nominal value (0.125 M).

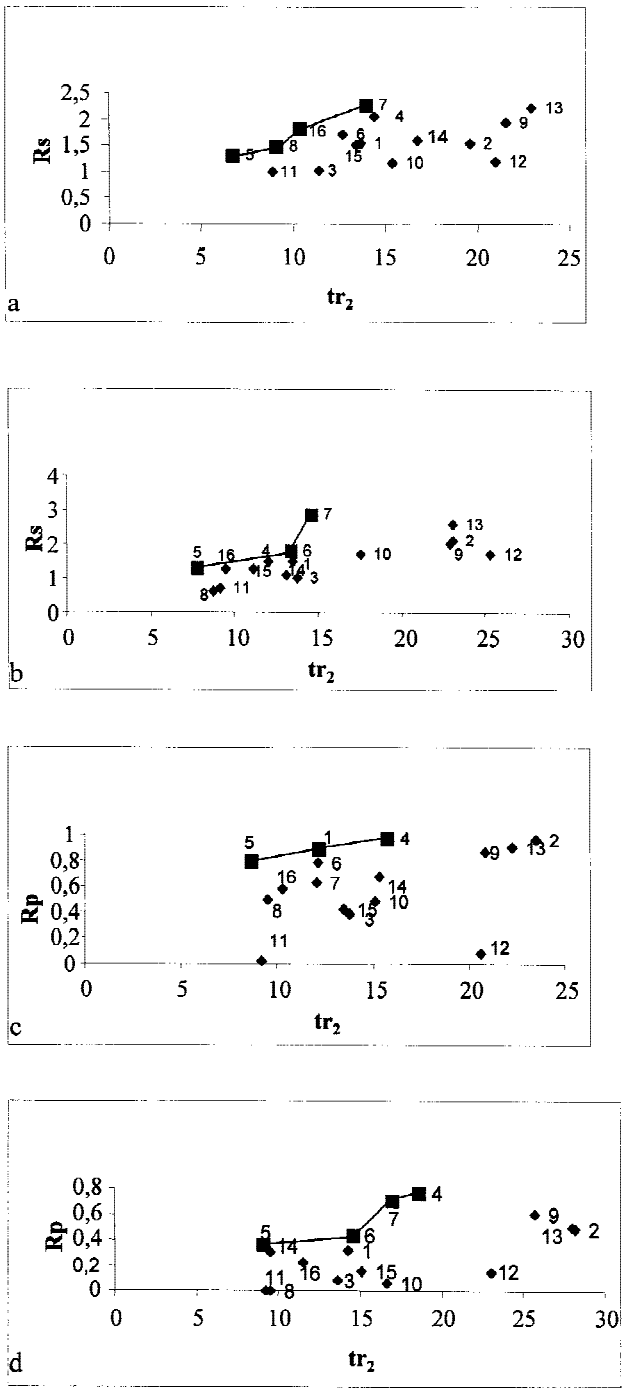

Fig. 9. Pareto-optimality plots for the separation quality and the analysis time: (**a**) ketoprofen, (**b**) pirprofen, (**c**) fenoprofen, and (**d**) tiaprofenic acid (solid square = pareto-optimal point; the indicated numbers are the corresponding experiment numbers from Table 2).

ration quality is to be maximized and analysis time minimized. Pareto-optimal points are indicated in Figure 9. One of the pareto-optimal points corresponds with the run having the maximum D-value in Table 6, i.e., for all compounds either run 7 or 4 is indicated as pareto-optimal. Therefore, the results obtained from the pareto-optimality approach or from Derringer's desirability functions lead to similar experimental optimal conditions. In practice, the best pareto-optimal point is selected by the researcher after inspection of the chromatograms at the different pareto-optimal conditions. For ketoprofen, the chromatographer will, for instance, prefer good separation (Rs: 2.29, run 7), with an acceptable analysis time of 13.9 min (chromatogram similar to Fig. 5a) while the pareto-optimal point with a much worse separation (Rs = 1.31, run 5) but with a shorter analysis time (6.7 min) is undesirable. In this MCDM approach, no a priori definition of desirable values has to be made. This approach is easy to use in an optimization procedure where only two optimization criteria are involved. In cases where more criteria are to be optimized it rapidly becomes complex and DDF could be preferred.[43]

A disadvantage of the pareto-optimality approach is that only the results of the experiments performed are plotted and no predictions are made for the results of the experimental domain covered. A situation as, for example, in Fig. 4c, where somewhere in the experimental domain better results ($D > 0.74$) are predicted than those found for the design experiments do not occur with pareto-optimality.

In summary, it could be recommended to start with the pareto-optimality approach. It immediately allows evaluating the suitability of one or some of the pareto-optimal points for practical purposes. If none of the pareto-optimal points is acceptable, a DDF approach can be used to see if some better conditions can be predicted elsewhere in the domain covered by the experimental design.

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