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Optimization of the direct enantiomeric separation by high-performance liquid chromatography of the D-2 dopamine agonist N-0437 using response surface methodology and the multi-criteria decision-making method

DIRK T. WITTE*, JAN BOSMAN and ROKUS A. DE ZEEUW
Research Group Bioanalysis and Toxicology, University Centre for Pharmacy, A. Deusinglaan 2, 9713 AW Groningen (Netherlands)
and
JAN H. DE BOER and DURK A. DOORNBOS
Research Group Chemometrics, University Centre for Pharmacy, A. Deusinglaan 2, 9713 AW Groningen (Netherlands)
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ABSTRACT

The applicability of a Chiralcel OD column for the separation of the enantiomers of the racemic dopamine agonist 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin (N-0437) was studied. The resolution between the enantiomers was described by means of the response surface methodology and optimum chromatographic conditions were found using Smilde's multi-criteria decision-making technique. The variables studied were eluent composition, flow-rate and temperature. The effects of these variables on the retention time of the last-eluting enantiomer and the resolution between the enantiomers were examined. The optimum result was considered to be the highest resolution possible within a short retention time for the last-eluting enantiomer. It appeared that a decrease in temperature gave higher resolutions. With hexane–ethanol (95:5, v/v) as eluent at 10°C and a flow-rate of 0.5 ml/min, a resolution of 2.5 and a retention time of the last-eluting enantiomer of 23 min were obtained. Under these conditions calibration graphs for the (+)- and (−)-enantiomers were prepared using racemic N-0437.

INTRODUCTION

N-0437 (Fig. 1) is a potent racemic dopamine agonist. Evidence has been reported for differences in pharmacological behavior between the two possible enantiomers, (−)-, indicated as N-0923, and (+)-, indicated as N-0924 [1–4]. The agonistic D-2 dopaminergic activity was ascribed to N-0923. For pharmacological studies with N-0923 the enantiomeric purity had to be established. Until now, the enantiomers could only be separated by reversed-phase high-performance liquid chromatography (RP-HPLC) after precolumn derivatization with D-(+)-glucuronic acid [5]. This method, however, is very time consuming.

With the development of new chiral stationary phases other approaches became

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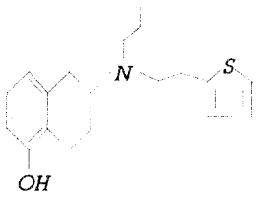

Fig. 1. Structure of N-0437.

available. The applicability of a Chiralcel OD column, containing chiral cellulose carbamates, for the separation of the N-0437 enantiomers was studied. This column was chosen because it already proved its applicability for drugs containing amines and hydroxyl groups [6]. Our preliminary studies with this column and the N-0437 enantiomers showed that an eluent consisting of hexane and ethanol gave better results than an eluent consisting of hexane and isopropanol with or without ethanol. Our first results with the column seemed promising, so optimization of the separation was carried out chemometrically, using response surface methodology (RSM) [7] and multi-criteria decision making (MCDM) [8]. Variables used during this optimization were the hexane concentration in the binary eluent (Hex), temperature (Temp) and flow-rate (Fl).

The effects of these variables on the chromatographic parameters, resolution (R_s) and retention time (t_R), were studied. The aim of this study was to have a high R_s between the enantiomers within the shortest retention time possible for the second-eluting enantiomer (t_{R2}). Chromatograms were obtained at different flow-rates, temperatures and eluent compositions. The settings of these variables were fixed in a factorial experimental design. In the starting design four different temperatures from 25 to 40 °C were studied. From the data obtained with this design, an increase in R_s at lower temperatures became clear. However, with the temperatures used in this design no R_s values greater than 2 could be obtained. With these results in mind, lower temperatures were studied. The total design (Table I) therefore covered temperatures from 0 to 40 °C.

Statistical polynomial models were used to describe the effects of the variables on R_s and t_{R2} within the factor space covered by the design. In theory the models to describe the effects of the variables on R_s and t_{R2} could contain linear and quadratic terms and maybe even terms of higher order. Effects caused by interactions between the variables should also be taken into account. In practice, in the model describing the effects of the variables on R_s, only significant linear effects of all variables were found. In the model describing the logarithm of t_{R2}, all variables showed linear effects but in addition Fl and Hex gave quadratic effects. With the models (Table II) and the application of MCDM, the pareto-optimum (PO) points were selected for the two responses R_s and t_{R2}. The MCDM methodology eliminates the necessity for making preliminary assumptions about the relative importance of objectives used in the optimization of HPLC separations. In this approach it is not necessary to preselect acceptable values of minimum resolution or maximum retention time. In MCDM the predicted values of R_s and t_{R2} were calculated from the models, using a small grid for each variable inside the whole factor space covered by the design. From the calculated

values of the R_s and t_{R2} the PO points were selected. A point is called a PO point if there exists no other point in the region which yields an improvement in one response without causing a degradation in another response. In the MCDM plot the PO points for R_s and t_{R2} are shown. In this plot the relationship between R_s and t_{R2} in the PO points is shown. Each PO point corresponds to a combination of variable settings.

Under conditions near one of the PO points thus found, calibration graphs for the two enantiomers were obtained using racemic N-0437. The influence of different concentrations N-0437 on R_s and t_R was investigated. Also, the molar absorptivities of the enantiomers at 225 nm were verified. Using the same conditions enantiomeric purity determinations of several batches N-0923 were made [9].

EXPERIMENTAL

Apparatus

The HPLC system utilized a (250 × 4.6 mm I.D.) Chiralcel OD column (Daicel Chemical Industries, Tokyo, Japan). For temperatures above 20°C the temperature of the column was regulated with a laboratory-made water jacket, thermostated with a Thermomix 1442 D (Braun, Rijswijk, Netherlands). For temperatures between 20 and 0°C a cooling device from HETO (Birkerod, Denmark) was used in combination with the thermostat. The solvent-delivery system was a Model 2150 HPLC pump (Pharmacia LKB, Uppsala, Sweden). Injections of 10 μl were made by a Model 710 A WISP (Waters Assoc., Milford, MA, USA) and detection was carried out with a Model 770 spectrophotometric detector at 225 nm (Spectra-Physics, Santa Clara, CA, USA). For integrating the chromatograms a C-R3A Chromatopac (Shimadzu, Kyoto, Japan) and for recording a BD 40 recorder (Kipp, Delft, Netherlands) were used. The models describing the influences of the variables Fl, Temp and Hex on R_s and t_{R2} were calculated by means of the SAS 6.04 package (SAS Institute, Cary, NC, USA).

Chemicals

The eluent contained hexane and ethanol, both of analytical-reagent grade, obtained from E. Merck (Darmstadt, Germany) and filtered through a 0.45-μm membrane filter (Schleicher & Schüll, Dassel, Germany). Mixtures were prepared by volume and degassed in a Sonicor (Farmingdale, NY, USA) ultrasonic bath before using the eluent. Racemic N-0437 · HCl and the enantiomers N-0923 · HCl and N-0924 · HCl were obtained from Whitby Research (Richmond, VA, USA). Stock solutions of N-0437 · HCl, N-0923 · HCl and N-0924 · HCl were prepared in ethanol and diluted to the desired concentrations with the eluent. Direct dissolution in the eluent was difficult because of solvation problems with the salts in the non-polar eluent.

HPLC conditions used for optimization

The eluent compositions tested were mixtures of hexane and ethanol, the temperature was varied from 0 to 40°C and the flow-rates were between 0.1 and 1 ml/min. The sample used during the optimization contained 390 μg/ml of N-0437. From this sample 10-μl injections were made. The HPLC conditions tested to establish the effect of the variables on the chromatographic parameters are summarized in Table I.

TABLE I

FLOW-RATES (ml/min) USED UNDER THE DIFFERENT HPLC CONDITIONS IN THE OPTIMIZATION EXPERIMENTS

Eluent, hexane–ethanol (v/v)	Temperature (°C)					
	0	10	25	30	35	40
80:20	0.25 0.50[a] 0.60 0.75	0.50	0.10 0.25[a] 0.50[b] 0.75[c] 1.00	0.50[a]	0.10 0.25 0.50 0.75 1.00	0.50
85:15	0.50	0.50	0.10 0.25 0.50[a] 0.75 1.00	0.50	0.10 0.25 0.50 0.75 1.00	0.50
90:10	0.25[a] 0.50[a] 0.75	0.50	0.25 0.50 0.75	0.50	0.25 0.50 0.75	0.50
95:05	0.10 0.25[a] 0.50[c]	0.50[a]	0.50	0.50	0.50	0.50

[a] Indicates two runs under these conditions.
[b] Indicates eight runs under these conditions.
[c] Indicates three runs under these conditions.

If each set of conditions (four eluent compositions, five flow-rates, six temperatures) is tested once, $4 \times 5 \times 6 = 120$ runs are necessary. Information was obtained by carrying out 70 runs under 51 different conditions. To study the reproducibility of the measurements under some conditions several runs were carried out. From each chromatogram t_R and R_S were calculated.

Calibration graph for the enantiomers of N-0437

For the calibration graph, samples with concentrations of N-0437 in the range 500–0.25 μg/ml were prepared. From these samples 10 μl were injected in the HPLC system. The eluent was hexane–ethanol (95:5). The temperature was 10°C and the flow-rate 0.5 ml/min. The graph was measured twice with new eluent each time. This calibration graph was made with racemic N-0437. With racemic N-0437 the absorptivities of the enantiomers at 225 nm were compared. The peak areas, peak widths at half-height and t_R of the enantiomers were measured. Each enantiomer was identified by comparing the retention times in the racemic samples with samples of authentic N-0923 and N-0924.

RESULTS

Optimization

The models describing the effect of the variables on the chromatographic

TABLE II

MODELS DESCRIBING THE CHROMATOGRAPHIC PARAMETERS

$R_s = a + b\text{Hex} + c\text{Temp} + d\text{Fl}.$
$\text{Ln}\ t_{R2} = e + f\text{Hex} + g\text{Temp} + h\text{Fl} + k\text{Hex}^2 + m\text{Fl}^2.$

Regression parameter	S.D.	Regression parameter	S.D.
a 1.31	0.48	e 23.3	3.1
b 0.016	0.005	f −0.48	0.07
c −0.051	0.002	g −0.0050	0.0007
d −0.47	0.14	h −5.23	0.15
		k 0.0030	0.0004
		m 2.69	0.15

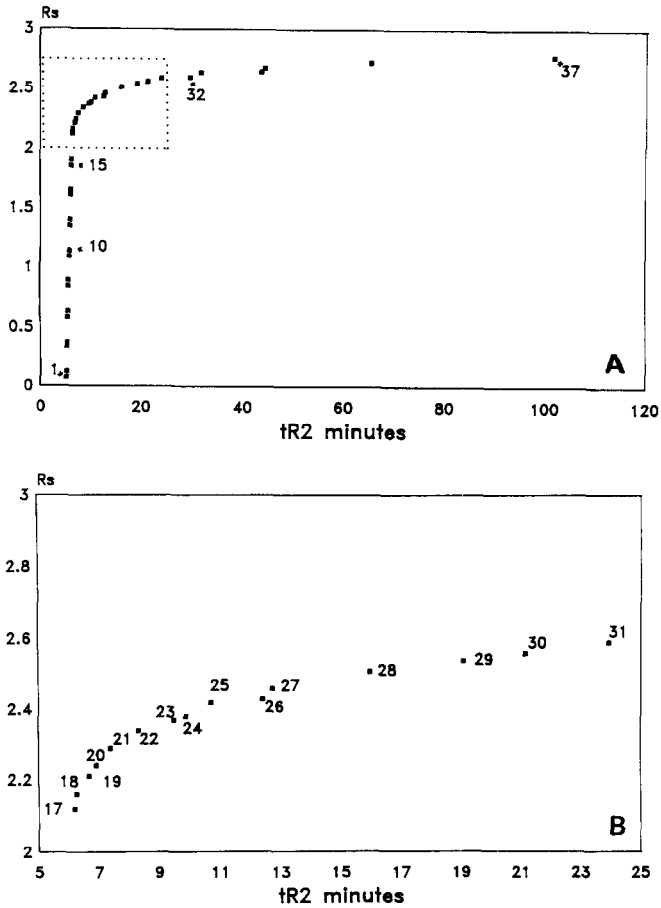

Fig. 2. (A) PO points for maximum resolution and minimum retention time of the last-eluting enantiomer. (B) Enlargement of the part in (A) in the dotted box, which contains the most relevant PO points.

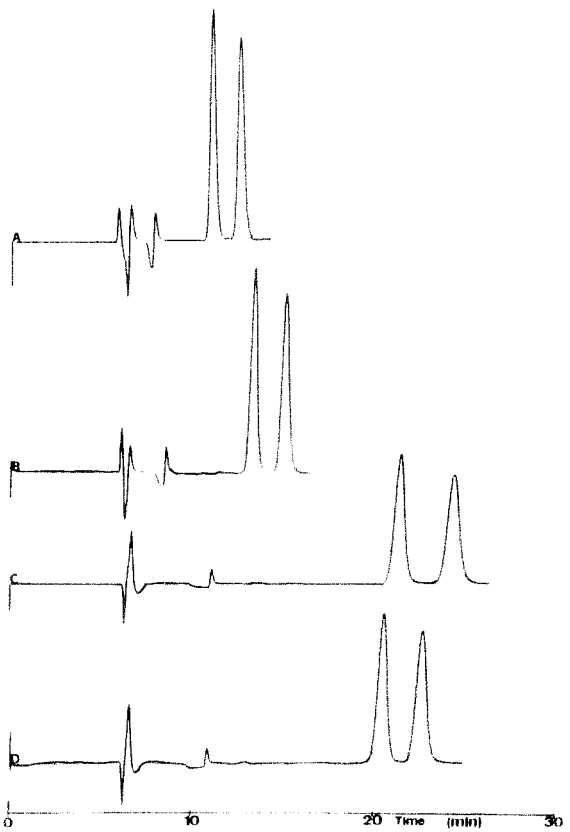

Fig. 3. Chromatograms of N-0437 (390 μg/ml) obtained under various conditions: (A) eluent, hexane–ethanol (85:15), temperature 0 °C, flow-rate 0.5 ml/min, PO point 26; (B) eluent, hexane–ethanol (90:10), temperature 0 °C, flow-rate 0.5 ml/min, PO point 28; (C) eluent, hexane–ethanol (95:5), temperature 0 °C, flow-rate 0.5 ml/min, PO point 31; (D) eluent, hexane–ethanol (95:5), temperature 10 °C, flow-rate 0.5 ml/min.

parameters are given in Table II. With aid of the models describing the effect of flow-rate, temperature and percentage of hexane in the eluent on R_s and t_R, the calculations used in the MCDM technique were made. The objectives were for R_s to be as high and t_{R2} as short as possible.

In Fig. 2A the MCDM plot is given and Fig. 2B shows an enlargement of that part of the MCDM plot containing the most relevant PO points. The variable settings corresponding to the PO points are given in Table III.

Calibration graph for N-0437

The calibration graphs were obtained at a temperature of 10 °C instead of 0 °C for the practical reason that 10 °C is easier to control. The eluent was hexane–ethanol (95:5) and the flow-rate 0.5 ml/min. Under these conditions resolutions of 2.5 were obtainable in practice within 23 min. A chromatogram obtained under these

TABLE III

VARIABLE SETTINGS OF THE PO POINTS

PO point	Eluent (hexane, %)	Flow-rate (ml/min)	Temperature (°C)	R_s	t_{R2} (min)
1	80	1.0	40	0.07	5.03
2	80	0.9	40	0.12	5.09
3	80	1.0	35	0.33	5.16
4	80	0.9	35	0.37	5.22
5	80	1.0	30	0.58	5.29
6	80	0.9	30	0.63	5.35
7	80	1.0	25	0.84	5.42
8	80	0.9	25	0.89	5.49
9	80	1.0	20	1.09	5.56
10	80	0.9	20	1.14	5.63
11	80	1.0	15	1.35	5.71
12	80	0.9	15	1.40	5.78
13	80	1.0	10	1.61	5.85
14	80	0.9	10	1.65	5.92
15	80	1.0	5	1.86	6.00
16	80	0.9	5	1.91	6.08
17	80	1.0	0	2.12	6.16
18	80	0.9	0	2.16	6.23
19	80	0.8	0	2.21	6.65
20	85	0.9	0	2.24	6.90
21	85	0.8	0	2.29	7.37
22	85	0.7	0	2.34	8.31
23	90	0.8	0	2.37	9.50
24	85	0.6	0	2.38	9.88
25	90	0.7	0	2.42	10.70
26[a]	85	0.5	0	2.43	12.40
27	90	0.6	0	2.46	12.73
28[a]	90	0.5	0	2.51	15.97
29	95	0.6	0	2.54	19.07
30	90	0.4	0	2.56	21.15
31[a]	95	0.5	0	2.59	23.93
32	90	0.3	0	2.60	29.56
33	95	0.4	0	2.64	31.69
34	90	0.2	0	2.65	43.59
35	95	0.3	0	2.68	44.29
36	95	0.2	0	2.73	65.32
37	95	0.1	0	2.78	101.64

[a] Fig. 3 shows chromatograms obtained under these conditions.

conditions is shown in Fig. 3D. The curve for the total area [(+)- + (−)-] is given in Fig. 4. The absorbance ratio, N-023/N-0924, at 225 nm was found to be 1.02 with a relative standard deviation (R.S.D.) of 9% over the whole concentration range used. The calibration graph was measured twice. The correlation coefficient of the mean N-0437 curve was 0.9998. For this curve, the N-0923 and N-0924 peak areas were calculated. The retention times of the enantiomers were measured and the resolutions were calculated for each point on the calibration graph. The mean retention times were 20.40 min with an R.S.D. of 0.3% ($n = 20$) for N-0924 and 22.94 min with an R.S.D. of

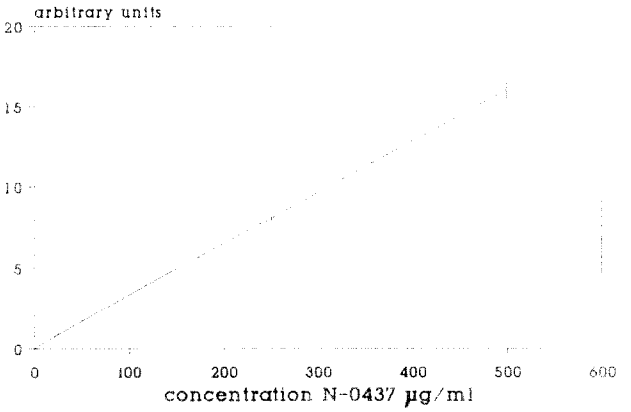

Fig. 4. Calibration graph for N-0437. Bars indicate the range.

0.3% ($n = 20$) for N-0923. The mean resolution between the enantiomers was 2.50 with an R.S.D. of 6.6% ($n = 20$). A sample of 25 µg/ml of N-0437 was injected eight times. The mean R_s and t_R values were calculated: $R_s = 2.57$ with an R.S.D. of 2.3% ($n = 8$); $t_{R1} = 20.9$ min with an R.S.D. of 0.4% ($n = 8$), t_R of the first-eluting enantiomer; and $t_{R2} = 23.2$ min with an R.S.D. of 0.6% ($n = 8$), t_R of the second-eluting enantiomer.

DISCUSSION

The models given in Table II give a good description of the effects that the variables have on the chromatographic parameters. The logarithmic transformation of t_{R2} is carried out because the error in the measurement of the retention time increases as the retention time increases. The assumption made in RSM is that the error in the response is constant over the whole range of the response [7]. R^2_{adj} for the model describing the resolution was 0.9104 and for the model describing the retention time of the last-eluting enantiomer R^2_{adj} was 0.9844. This $R^2_{adj} = 1$ if all the variance in the response is explained by the model. If the variance in the response cannot be explained by the model, this R^2_{adj} becomes smaller.

Table IV gives the differences between the predicted and observed values of the chromatographic parameters for five PO points and under the conditions used in the experiments for the calibration graphs. The effect of temperature on resolution was remarkable. Lowering the temperature gave a better resolution. This phenomenon may make the Chiralcel OD column more widely applicable than has been appreciated so far, if used at even lower temperatures. The manufacturer advises not to use temperatures below 0 °C. Experiments at lower temperatures may be done in the future after consultation with the manufacturer. A temperature below 11 °C was essential for the baseline separation of the N-0437 enantiomers. Lowering the flow-rate and increasing the proportion of hexane in the eluent also led to higher resolutions.

With the present objectives it is clear that there is not just one optimum set of conditions but that it depends on the relative importance of each objective which optimum is chosen. With these PO points, each representing a setting of the variables,

TABLE IV

RESULTS OF MEASUREMENTS UNDER PO CONDITIONS AND THE CONDITIONS USED FOR THE CALIBRATION GRAPHS

PO point[a]	Eluent (hexane, %)	Flow-rate (ml/min)	Temperature (°C)	Predicted		Observed	
				R_s	t_R (min)	R_s	t_R (min)
3	80	1.0	35	0.33	5.16	0.00	4.63
26[b]	85	0.5	0	2.43	12.40	2.51	12.50
28[b]	90	0.5	0	2.51	15.97	2.45	15.95
31[b]	95	0.5	0	2.59	23.93	2.72	23.50
37	95	0.1	0	2.78	101.64	2.78	104.76
Cal[b,c]	95	0.5	10	2.08	22.65	2.50	23.20

[a] The PO points correspond to those in Table III.
[b] Fig. 3 shows chromatograms obtained under these conditions.
[c] Conditions used for the preparation of the calibration graphs.

the balance between the chosen criteria is seen. It is easy to see in Fig. 2A what an improvement in resolution costs with respect to the retention time. An increase in resolution from 1.61 to 2.60 (PO points 13 to 32) is, according to the models, linked with an increase in retention time from 5.85 to 29.56 min. An increase in resolution is impossible without an increase in retention time along the curve formed by the PO points. It depends on the requirements of the user and on the availability of cooling instrumentation which variable settings are chosen. Determination of enantiomeric impurity, where a vast difference in concentration between the two enantiomers may occur, requires the highest resolution possible within workable limits. As the number of batches to be analysed is normally small, the retention time is of minor importance. In practice, a temperature of 10°C is more easily controllable than 0°C and 10°C was therefore selected for further experiments. The decrease in resolution caused by working at a 10°C instead of 0°C is to overcome by using a higher proportion of hexane in the eluent and working at a lower flow-rate. With higher temperature (> 10°C), the resolution decreases to an unacceptable value.

For quantitative work and enantiomeric impurity determinations, the variable settings used in this study appeared to be applicable. The reproducibility under these conditions was high and the calibration graph showed good linearity. Both enantiomers had the same absorption at 225 nm. The minimum amount that could be determined was 0.25 μg/ml of N-0437, which resulted in a minimum concentration of 0.125 μg/ml for each of the enantiomers. With an injection volume of 10 μl this corresponds to an absolute amount of 1.25 ng of each enantiomer. There was no effect of the concentration of N-0437 on the chromatographic parameters studied.

CONCLUSIONS

For the separation of N-0437 enantiomers the Chiralcel OD column is a good choice. At low temperatures (< 11°C) the separation between the N-0437 enantiomers is good, $R_s > 2.0$, under the conditions used. For the clean synthetic samples used in this study the normal-phase character of the column is not a disadvantage. However, if

one wishes to use this column for biological samples, it should be noted that the column cannot withstand aqueous solutions, so that extraction and removal of traces of water are required.

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