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Knowledge-based system for method development of chiral separations with capillary electrophoresis using highly-sulphated cyclodextrins

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

Method development for chiral separations is not easy because it requires experience and many experimental possibilities can be chosen. In order to help the analyst, a knowledge-based system (KBS) for the rapid determination of experimental parameters, which allow a baseline separation of enantiomers, has been developed. On the basis of own laboratory knowledge, completed with literature data, rules were defined and a KBS was built. Five different techniques are considered in this KBS. This paper describes the capillary electrophoresis (CE) section, in which a strategy has been defined based on the use of highly-sulfated cyclodextrins as chiral selectors. A structured representation of the knowledge and its implementation in Toolbook software is presented.

Keywords: Highly-sulphated cyclodextrins; Capillary electrophoresis; Knowledge-based system; Chiral separation

1. Introduction

Chirality is an important property for many synthetic and biological compounds. Analysis of enantiomers requires the use of chiral separation techniques. Different techniques are used, such as (a) chromatographic techniques: reversed phase liquid chromatography (RPLC) [1–7], normal phase liquid chromatography (NPLC) [7–12] and supercritical fluid chromatography (SFC) [13,14]; (b) capillary electrophoresis (CE) [15–23]; and (c) capillary electrochromatography (CEC) [24–26]. Method development is not evident and requires experience among others because the enantioselective recognition mechanisms are not known in detail and therefore unpredictable.

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In CE, compared to chromatography, a chiral environment can easily be created by filling the capillary with an electrolyte solution containing a chiral selector. However, there are many possibilities to choose the initial experimental conditions. For example, many different chiral selectors can be added, such as cyclodextrins (CDs), crown ethers and antibiotics [15–18]. The most effective separation strategies in CE have been achieved with CDs as chiral selectors [18–23,27–30,32], especially with sulphated CDs [27–30,32].

To help the analyst in making decisions during method development, a knowledge-based system (KBS), the chiral KBS, is proposed. A KBS is a computer program, built similar to a decision tree, which contains the relevant knowledge about a specific domain. This knowledge is classified in such a way that the user (analyst) is guided through the program by answering questions and/or performing experiments.

The separation strategies included in the chiral KBS were derived from published knowledge [27–30] and our experience [22,23,31,32] about chiral separations. The chiral KBS will cover the whole method development of chiral separations for four of the five above-mentioned separation techniques. CEC will be considered last, since its application is not widespread at the present time. Method development is considered here to consist of two steps, (i) selection of initial experimental conditions (= fast screening) and (ii) optimisation of the most important parameters. A fast screening strategy allows chiral analysis of large numbers of novel molecules (including starting building blocks, intermediates and final products) provided, for instance, by conventional, automated parallel synthesis or in early drug discovery. For quality control of drug substances and drug products, in the later drug development and manufacturing stages, method optimisation (mainly method robustness) steps are needed, e.g. for determination of unwanted enantiomer impurity (distomer) in the presence of the active enantiomer drug (eutomer). Method optimisation (mainly method sensitivity) is also required for stereoselective metabolism and pharmacokinetic studies, in animal and human, to investigate possible racemisation or epimerisation of the chiral drugs in vivo.

The proposed structure of the chiral KBS is shown in Fig. 1. The two different development stages are presented: a screening (stage 1) and an optimisation level (stage 2). Before moving to the screening level, the KBS proposes a probably adequate technique of the four separation techniques included: NPLC, RPLC, SFC and CE, based on the properties of the solute and the context of the analysis.

In the first stage, the screening level, the aim of the proposed strategy is to achieve very rapid separations for large series of compounds with the proposed strategy. For drug discovery purposes,

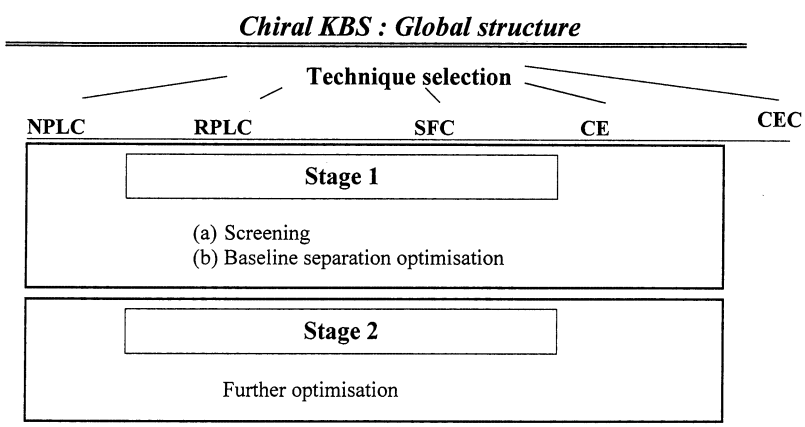

Fig. 1. General structure of the KBS.

an initial rapid optimisation may follow to improve resolution or for better and more accurate enantiomeric purity determinations. It is expected that in most cases, a baseline solution (e.g. $Rs > 1.5$ for CE) will be achieved after the first stage [30].

For quality control, a more stringent optimisation will be proposed to the user. This second stage is not always necessary. It may be used to optimise separations that require the distinguishing of one enantiomer as impurity in the presence of the other enantiomer (e.g. a concentration of 0.1% in the presence of 99.9% of the main compound). This situation could require a better separation (e.g. $Rs > 6$) than in the first stage where quantification is less stringent.

Simple charts, which may assist the analyst in chiral method development, in general [33] or chiral CE specifically [34,35], have been published previously. In the last years, the importance of highly-sulfated CDs (HS-CDs) in chiral separations has grown significantly [27–30,32]. An approach, based essentially on the use of HS-CD, has not been published yet. In this paper, the implementation in the chiral KBS of the first stage of a capillary electrophoresis strategy, based on the use of HS-CDs as chiral selectors, is described. The strategy used will be demonstrated for some molecules.

2. Experimental

2.1. Software and hardware

The chiral KBS is implemented using a software tool called Toolbook (Asymetrix Toolbook, Ver. 1.5, Bellevue, WA, DC). Toolbook is an object-oriented hypermedia tool, written in openscript, which runs under Microsoft Windows, version 3.0 or higher.

2.2. Capillary electrophoresis

The CE system consists of a SpectraPHORESIS Ultra capillary electrophoresis instrument (Thermo Separation Products, San Jose, CA) with a fast scanning UV-VIS detector. Separations were performed in a 6 cm (for short-end injection) or a 24 cm effective length (30 cm total length) uncoated fused silica capillary, 50 μm ID (Polymicro Technologies Inc., Phoenix, AZ). Sample injections were performed using a positive pressure of 0.5 psi for 3.5 s (injected volume 7.4 nl). Voltage, chiral selector concentrations, pH of background electrolyte, temperature and percent organic modifier can vary depending on the proposed experimental design or strategy requirements.

2.3. Chemicals

Ibuprofen, *N*-carboxybenzyl(CBZ)-L-proline and *N*-CBZ-D-proline were purchased from Sigma (Saint Louis, MO). Pindolol was a gift from Novartis (Basel, Switzerland). (*S*)-(+)-2-phenylglycinmethylester hydrochloride and (*R*)-(−)-2-phenylglycinmethylester hydrochloride were obtained from Aldrich (Steinheim, Germany).

Highly sulphated-α-CD, -β-CD and -γ-CD were purchased from Beckman (Fullerton, CA).

Water for preparation of separation buffers and samples was produced in-house by the Milli-Q System (Millipore, Milford, MA).

2.4. Electrolytes

All phosphate buffers with pH 2.5, 3.25 and 4, were prepared with orthophosphoric acid 85% (w/w) (Merck, Darmstadt, Germany) and Milli-Q-water and adjusted to the desired pH with triethanolamine.

2.5. Procedures

The chiral selector solutions were prepared by dissolving the required cyclodextrin in the appropriate buffer. All racemic sample solutions were prepared in Milli-Q water at a concentration of ≈ 240 μg/ml, except pindolol, which was dissolved in water/methanol (70/30).

After each run, the capillary was first washed with 0.1 N NaOH for 2 min. Then, it was rinsed for 1 min with phosphate buffer of the appropri-

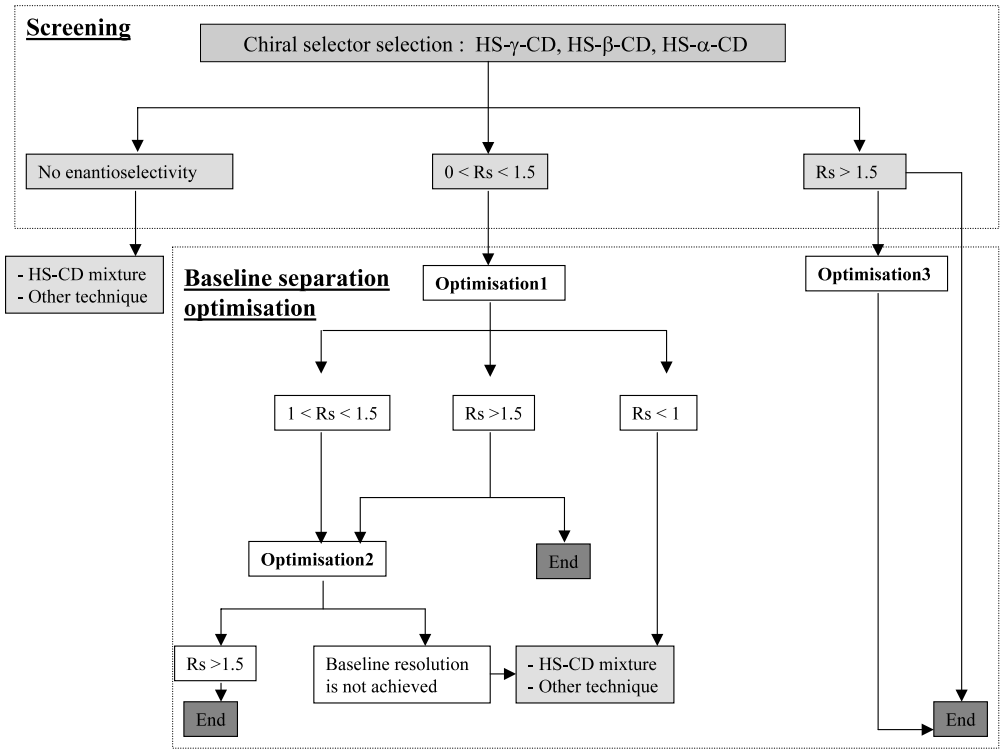

Fig. 2. General scheme of the CE method development strategy in stage 1 of the chiral KBS.

ate pH and equilibrated for 1 min with the chiral selector solution.

3. Results and discussion

3.1. Hypermedia tools

A hypermedia tool is used to represent and structure the knowledge into knowledge based systems. Some applications in the chemical domain are described [36–39]. In a hypermedia document, the information is structured as objects linked by means of object-oriented languages, which allow guiding the user through the information needed.

To develop the chiral KBS, the hypermedia tool Toolbook is used. Creating applications with Toolbook is relatively easy due to its graphical user interface and object-oriented programming features.

3.2. CE strategy implemented in the chiral KBS

The global structure of the chiral KBS is shown in Fig. 1. At the first stage, there are two different levels for each of the four different techniques: (a) screening, consisting of a few experiments to achieve an initial enantioseparation; and (b) baseline enantioseparation optimisation. In this paper, the strategy of the first stage will be elaborated only for CE methods.

The strategy for method development of CE methods, as defined for stage 1 of the chiral KBS, is shown in Fig. 2. A screening is applied in the early method development stage. The aim of the screening strategy is to achieve acceptable separations, if necessary for many molecules, in as few experiments as possible. Experimental design is applied in the baseline separation optimisation to improve the initial separation observed in the screening step.

3.2.1. Screening

The separation strategy (Fig. 2) is based on the use of highly-sulphated cyclodextrins (HS-CDs) because these selectors give, in general, very good results for chiral separations by CE [27–30,32]. Mixtures of a highly-sulphated and a neutral cyclodextrin sometimes yield to still better results, but in a general strategy, simplicity is important and therefore, the use of mixtures is considered here only when the use of HS-CDs alone does not lead to an acceptable separation.

Short-end injection is systematically applied to achieve short migration times (Mt). For this, the sample is injected at the end of the capillary closest to the detection window. Although the separation length is reduced, good separations are usually obtained due to the high selectivity of HS-CDs. The HS-CDs are fully negatively charged in the whole pH range and, in the absence of the EOF (electroosmotic flow), they migrate to the anode. Three HS-CDs are available, α-, β- and γ-HS-CD. In a first experiment (screening), a selection among those selectors is performed. It is recommended to test all three and select the one that yields the best results. However, when saving experiments is important, experiments should be carried out in the order: (1) HS-γ-CD, (2) HS-β-CD and (3) HS-α-CD. In many cases, only the HS-γ-CD will then be tested and in relatively few cases, it will be necessary to investigate the α derivative. Perrin et al. [32] found that for a series of 67 substances, the HS-γ-CD led to the best separations in 54% of the cases, followed by the HS-β-CD (33%) and HS-α-CD (12%).

The conditions for the first experiment are given in a table produced by the KBS: 50 mM phosphate buffer, pH 2.5, 5% cyclodextrin, 300 V/cm electric field, 20 °C (Fig. 3).

After this screening step, three results are distinguished (Fig. 2): (1) no enantioselectivity ($Rs = 0$) is shown with any of the three CDs; (2) a beginning separation ($0 < Rs < 1.5$) with at least one of the selectors is achieved; or (3) a good separation ($Rs \geq 1.5$) is obtained with one or more selectors.

To show the implemented strategy and the functioning of the KBS, a few examples will be followed throughout the paper, namely the development of methods for the chiral separation of

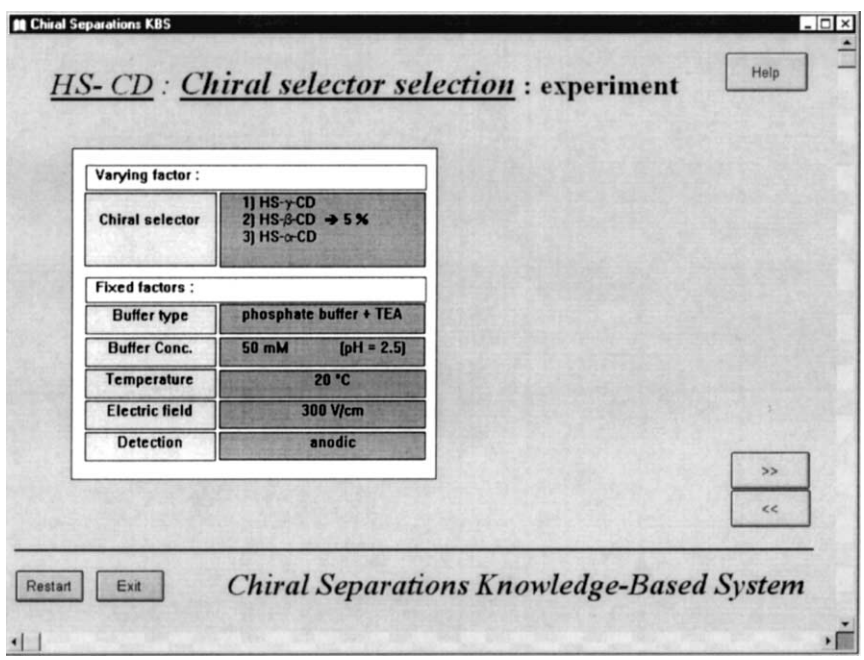

Fig. 3. First screening conditions screen in chiral KBS.

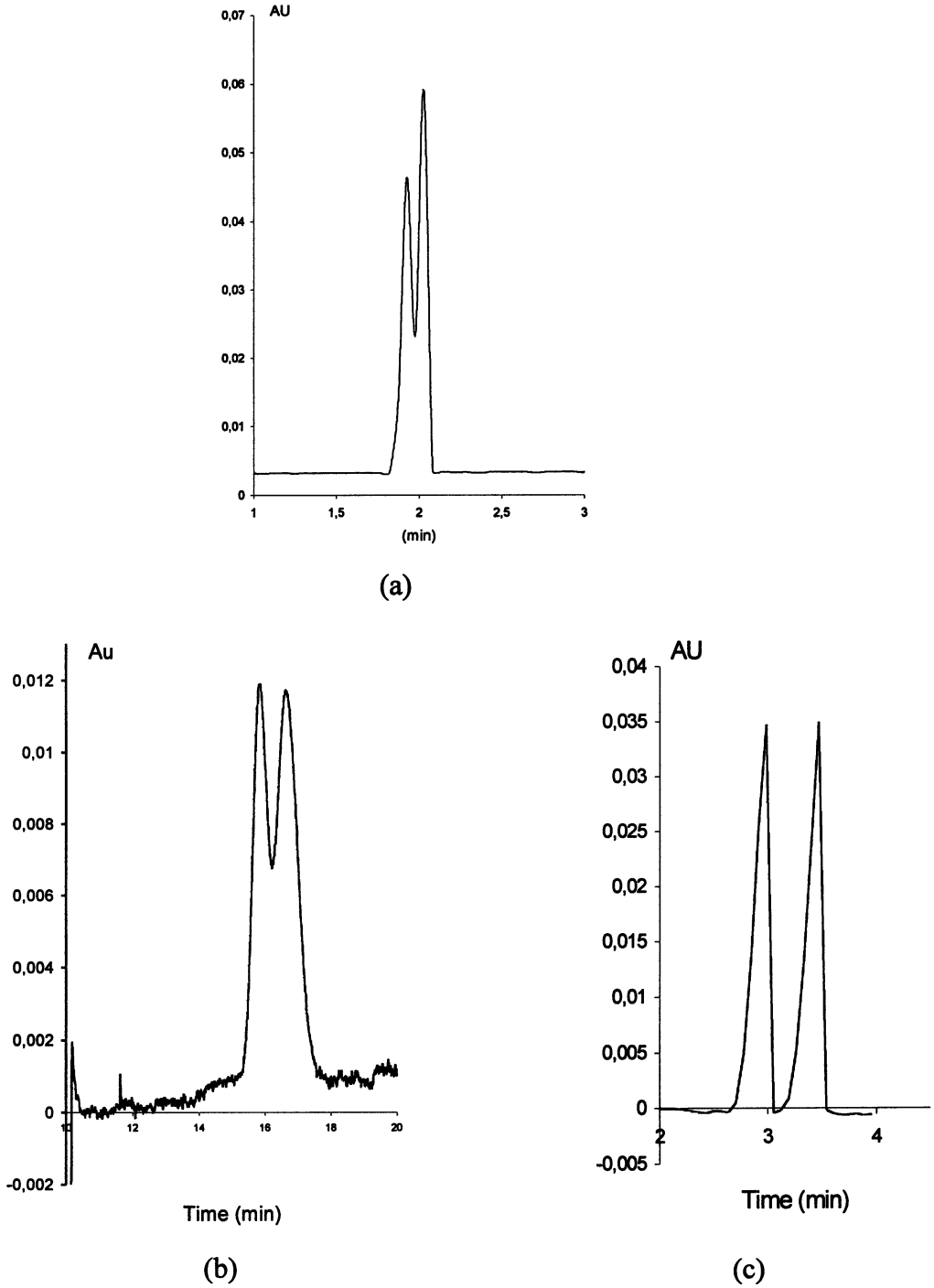

Fig. 4. Electropherograms obtained after the first screening: (a) pindolol (Rs = 0.77; chiral selector: HS-β-CD); (b) *N*-CBZ-proline (Rs = 0.60; chiral selector: HS-γ-CD); (c) phenylglycine methylester (Rs = 2.20; chiral selector: HS-α-CD).

Table 1
Best results of the first screening experiment

Compound	HS-CD	Rs	Mt (mm)
N-CBZ-proline	γ	0.60	16.6
Pindolol	β	0.77	2.0
Phenylglycine methyl ester	α	2.20	3.5
Ibuprofen	α,β,γ	0.00	10.8*

* Longest Mt for Ibuprofen (observed with HS-α-CD). Rs, resolution; Mt, migration time. Conditions as defined in the text and Fig. 3.

ibuprofen, pindolol, *N*-CBZ-proline and 2-phenylglycin methylester hydrochloride.

For these substances, the results shown in Table 1 were obtained in the screening step. A beginning of separation ($0 < Rs < 1.5$) is obtained for pindolol (Fig. 4a) and *N*-CBZ-proline (Fig. 4b), whereas good resolution is obtained for 2-phenylglycine methylester (Fig. 4c).

Ibuprofen did not show enantioselectivity with any of the three HS-CDs. The recommendations of the KBS are to switch to another recommended alternative technique, e.g. RPLC, or to try a mixture of HS-CD and neutral CDs. We found that, indeed, enantioselectivity is obtained in RPLC using the Chiralcel OJ-R column (Daicel Chemical Industries, Sakai City, Osaka, Japan), a polysaccharide based (cellulose Tris (4-methylbenzoate)) column. For a simplistic and time-saving reason, it is more economical to switch to a second choice recommended in the KBS.

3.2.2. Baseline separation optimisation

Three different optimisation strategies are available (Fig. 2). Optimisation 1 is performed when the compound is not fully resolved with the HS-CD that gave the best separation in the previous rapid screening step. This first optimisation (optimisation 1) can be followed by a second optimisation (optimisation 2) to further improve efficiency and peak shape. The third optimisation (optimisation 3) is proposed when the compound is fully resolved after the screening step, but the optimisation of other criteria (i.e. peak shapes, analysis times) seem useful. Optimisation 1 is used when necessary for drug discovery analysis objectives, whereas optimisations 2 and 3 are optional and recommended for drug development and manufacturing analysis purposes. For all optimisations, an experimental design methodology is applied.

3.2.2.1. Optimisation 1: beginning separation in first screening experiment. To achieve baseline separation, the factors to vary are concentration of CD, pH and percent of organic modifier (Fig. 5). Two different optimisation approaches are available, depending on the migration time obtained in the first experiment. When a long migration time is obtained (e.g. > 15 min), no organic modifier is included in the experimental design.

Experimental design is used here only as a way of efficiently mapping the experimental domain. The best result is selected from the design. No statistical computations are carried out to determine the variables that have an influence or to obtain response functions.

Methanol is considered as a variable in the experimental design because, in some cases, organic solvents can improve resolution [40–42].

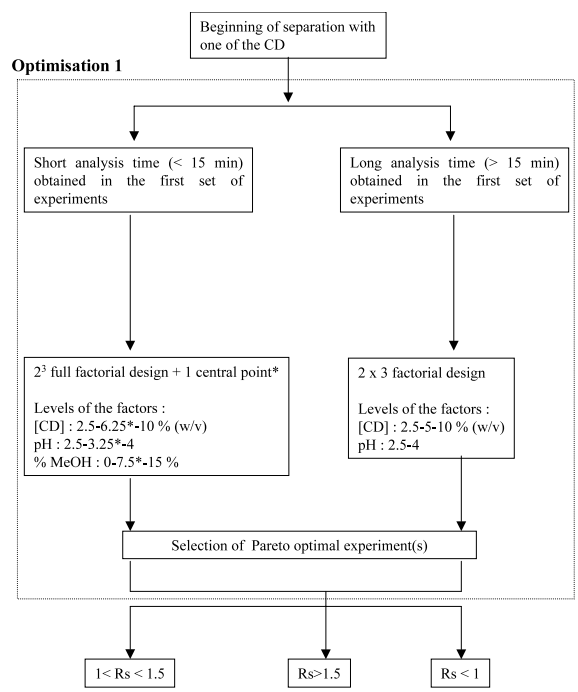

Fig. 5. Optimisation 1: optimisation strategy for compounds with a beginning separation after screening.

Table 2
Experimental conditions for optimisation 1, long migration times

Experiment	% CD	PH
1	2.5	4
2	2.5	2.5
3	5	4
4	5	2.5
5	10	4
6	10	2.5

The effect of organic solvents on the separation is rather unpredictable because it interacts in different ways. Wren et al. [40] and Wan et al. [41] state that an organic modifier behaves as a competitor because the solvent can be included in the cyclodextrin cavity. The less interacting enantiomer is then discriminated even more. Consequently, migration times increase and resolution sometimes improves.

Optimisation of pH is essential to achieve ionic states which best favour the interactions governing enantioselectivity because ionisation of the analyte is controlled by the operating pH. In our case, triethanolamine (TEA) is added to the buffer to adjust pH. TEA has been used in chiral separations because it tends to reduce the adsorption of basic analytes to the capillary walls and consequently, reduces peak tailing [43]. The CD concentration is included at three levels (or two levels + central point) in both designs because resolution as a function of the CD concentration may pass through a maximum, depending on the type of CD and the analyte [40,44].

The designs recommended for long and short migration times are, respectively, a 2×3 factorial design and a 2^3 full factorial design with one centre point. The investigated factors in the 2×3 full factorial design are the CD concentration and pH of the background electrolyte (BGE), respectively at three and two levels, as given in Table 2. In the centred 2^3 full factorial design, the same factors as in the previous design plus the percentage of methanol are investigated (Table 3).

To interpret the results of this first optimisation, the user has to determine what is meant with a satisfying baseline resolution. This depends on the objective of the separation. For separation of racemic mixtures, a resolution of 1.5 is sufficient. For the quantification of enantiomeric impurities, the resolution should be, for instance, 3 or more, depending on whether the impurity occurs in the front or after the main peak. In this paper, the emphasis is placed on rapidly separating enantiomeric mixtures (screening), thus baseline resolution will be defined as 1.5.

After performing the experimental design, the conditions that give the best results for Rs and Mt (Pareto optimal experiments) are determined. An experiment is Pareto optimal if there is no other experiment which has a better result on one criterion without having a worse result on another [45].

If these conditions still give insufficient resolution ($Rs < 1$), the user is recommended to try a mixture of HS-CD and neutral CD or to switch to another recommended technique (Fig. 2). However, when the user investigates only HS-γ-CD, it is preferred to first investigate the β- and α-HS-CD before switching to another technique. When baseline resolution is obtained, the user can go to the end of the separation module.

Of the four example substances mentioned previously, pindolol and *N*-CBZ-proline give a beginning separation in the first screening experiment and therefore, the user is directed towards optimisation 1 for these substances. In the screening step, pindolol and *N*-CBZ-proline show, respectively, a short Mt (2.0 min) and a long Mt (16.6 min). For pindolol, the experimen-

Table 3
Experimental conditions for optimisation 1, short migration times

Experiment	[CD]%	PH	% MeOH
1	2.5	2.5	0
2	2.5	2.5	15
3	2.5	4	0
4	2.5	4	15
5	10	2.5	0
6	10	2.5	15
7	10	4	0
8	10	4	15
9	6.25	3.25	7.5

Table 4
Results obtained for pindolol after optimisation 1 (short Mt), performed with a 2^3 full factorial design with one center point

Experiment	Rs	Mt (mm)
1	0.98	3.0
2	1.08	4.7
3	0.91	3.4
4	1.22	7.2
5	1.41	1.8
6	1.31	4.3
7	1.30	2.6
8	1.38	5.0
9	**1.50**	**3.8**

The conditions of each experiment are given in Table 3.

tal design of Table 3 is used, whereas for *N*-CBZ-proline, the design of Table 2 is used.

For both compounds, the resolution improves without a large increase in migration time. For pindolol, an increase in the resolution from 0.77 to 1.50 is obtained (Table 4, Fig. 6a) and for *N*-CBZ-proline, from 0.60 to 1.70. At the same time, for *N*-CBZ-proline a decrease in Mt is achieved, from 16.6 to 8.2 min (Table 5, Fig. 6b).

The optimal conditions to separate pindolol are selected by a multicriteria method based on Pareto optimality. Of the nine experiments, experiments 5 and 9 (Table 4) are determined by the KBS to be Pareto optimal compared to all other experiments. To do this, all experiments are compared and each time an experiment is found to be Pareto optimal towards another, the non-optimal experiment is eliminated, until only Pareto optimal experiments remain. For instance, experiment 1 is compared with the results of experiment 2. They are not Pareto optimal compared to each other because experiment 1 shows a shorter migration time and experiment 2, a higher resolution than experiment 1. However, experiment 1 is Pareto optimal to experiment 3 because for both responses, better results, a higher resolution and a shorter migration time, are obtained in experiment 1. Consequently, the third experiment is eliminated. It is found for pindolol that experiment 5 is Pareto optimal to all experiments, except to experiment 9. This means that experiment 5 shows a shorter migration time in combination with a higher resolution than the non-Pareto optimal experiments. The same is true for experiment 9. The two experiments are not Pareto compared to each other because experiment 5 has a shorter migration time and experiment 9, a higher resolution. The choice between these two Pareto optimal experiments is up to the user and will depend on the requirements.

For pindolol, we decided that the optimal conditions were situated at the centre point (experiment 9) of the design (6.25% of HS-β-CD, 50 mM phosphate buffer pH 3.25, 7.5% MeOH, electric field strength 300 V/cm and temperature of 20 °C), because it showed the highest resolution and still an acceptable Mt.

For *N*-CBZ-proline the optimal conditions were found at 10% HS-γ-CD concentration, 50 mM phosphoric acid buffer pH 2.5, 20 °C and 300 V/cm. Both compounds achieved baseline resolution ($Rs \geq 1.5$). Further peak shape and efficiency optimisation (optimisation 2) could be performed, if considered beneficial by the user.

3.2.2.2. Optimisation 2: peak shape and efficiency optimisation. For the peak shape and efficiency optimisation, a 2^3 full factorial design was applied (Fig. 7). The factors to vary were temperature, ionic strength and voltage (Fig. 8).

Responses to control during the peak shape and efficiency optimisation depended on the user's requirements. Resolution was always considered as a response. Other possible responses were Mt, plate number (N) and asymmetry factor (As). Multicriteria decision methods, such as Pareto optimality were then used to select the best combination. There are other multicriteria decision methods possible, such as the Derringer method and the Promothee method [46]. We will, in a later stage, investigate which of these is to be preferred, but actually the Pareto approach is used in the KBS. In many cases the requested baseline resolution ($Rs > 1.5$) was already achieved when entering optimisation 2 and, if not, it is usually the case at this stage. When it is not, the user can try a mixture of HS-CD and neutral CDs or switch to another recommended technique.

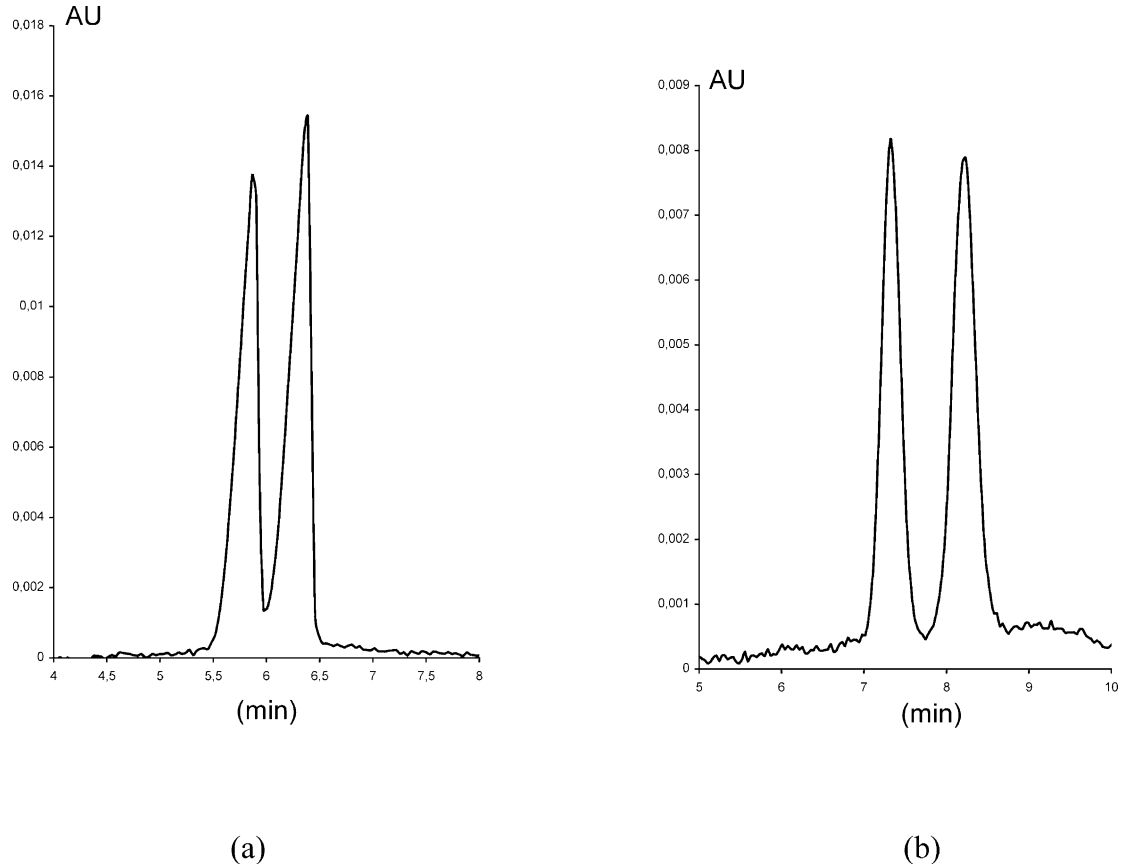

Fig. 6. Electropherograms after performing optimisation 1, (a) pindolol and (b) *N*-CBZ-proline.

3.2.2.3. Optimisation 3: migration time optimisation and global optimisation. After the first screening experiments, a good separation ($Rs \geq 1.5$) was obtained for 82% of the compounds investigated by Perrin et al. [32]. The user can stop the method development at this stage. However, it is possible, if the user requires it, to include a migration time optimisation and a global optimisation (Fig. 9), which are relatively similar to the peak and efficiency optimisation of Section 3.2.2.2. The most important difference is that in optimisation 3, the selector concentration was included as a factor, which was not the case in optimisation 2, because it was already optimized in optimisation 1, which precedes optimisation 2.

Resolution is not an important response anymore. The aim is to improve peak shape, efficiency or/and migration time without loosing resolution by changing experimental factors in an experimental design.

Two different optimisations are proposed: a migration time optimisation and a more general

Table 5
Results obtained for *N*-CBZ-proline after optimisation 1 (long Mt), performed with a 2×3 experimental design

Experiment	Rs	Mt (mm)
1	0.00	12.4
2	NP	NP
3	0.00	7.2
4	0.60	16.6
5	0.74	4.2
6	**1.70**	**8.2**

NP, no peak was observed within 40 mm. The conditions of each experiment are given in Table 2.

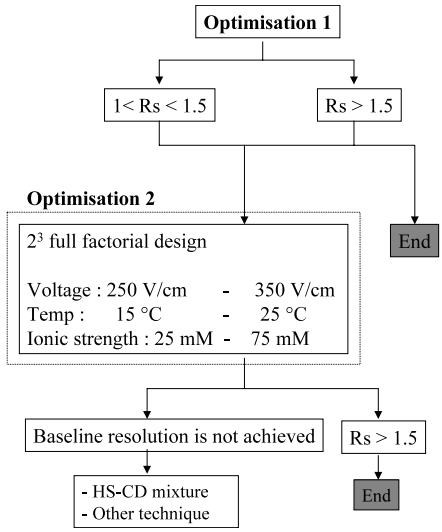

Fig. 7. Optimisation 2: peak shape and efficiency optimisation.

optimisation. Optimisation of migration time is proposed when reducing migration time is of most interest. It is recommended for migration times >15 min. When peak shape and efficiency are not acceptable, the second type of optimisation could be performed.

When optimising migration time, efficiency also improves because changing the factors influence migration time and efficiency in the same way. The factors: temperature, voltage and CD concentration are varied in a 2^3 full factorial. Levels are taken above the nominal conditions used in the first screening experiment because increasing temperature, voltage and/or CD concentration will lower migration time ((a) voltage (V/cm): 350–400; (b) T (°C): 25–30; and (c) [CD]%: 7.5–10).

For the global optimisation, four factors are included: temperature, ionic strength, voltage and concentration of cyclodextrin. To reduce the number of experiments a $2^{(4-1)}$ fractional factorial design. The levels are now taken around the conditions used previously because migration time was considered acceptable. The levels of the factors included are: (a) voltage (V/cm): 250–350, (b) T (°C): 15–25, (c) ionic strength (mM): 25–75 and [CD]% 2.5–10).

Possible responses included are: plate number (N), asymmetry factor (As), migration time (Mt) and resolution (Rs). After performing the eight

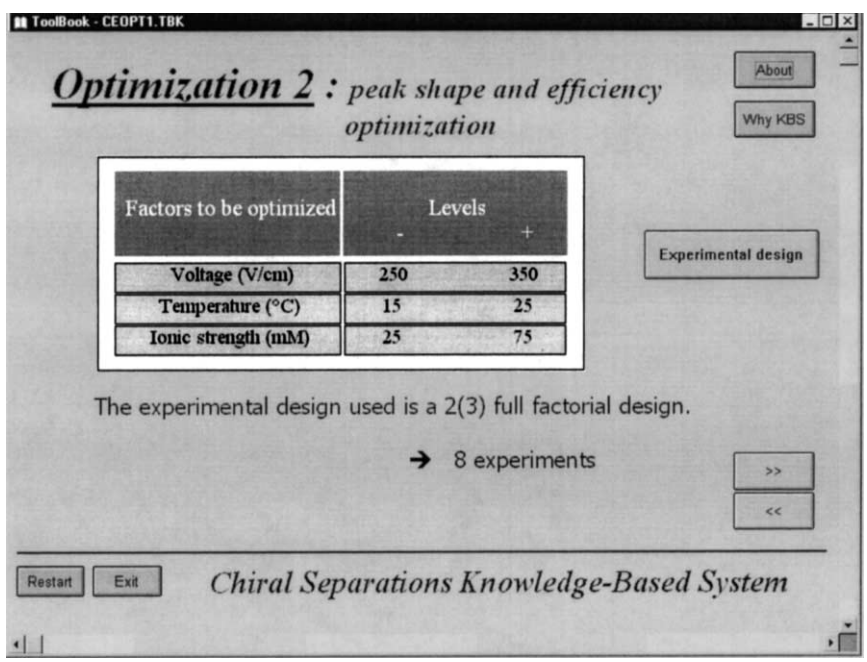

Fig. 8. Levels of factors used for the peak shape and efficiency optimisation (optimisation 2).

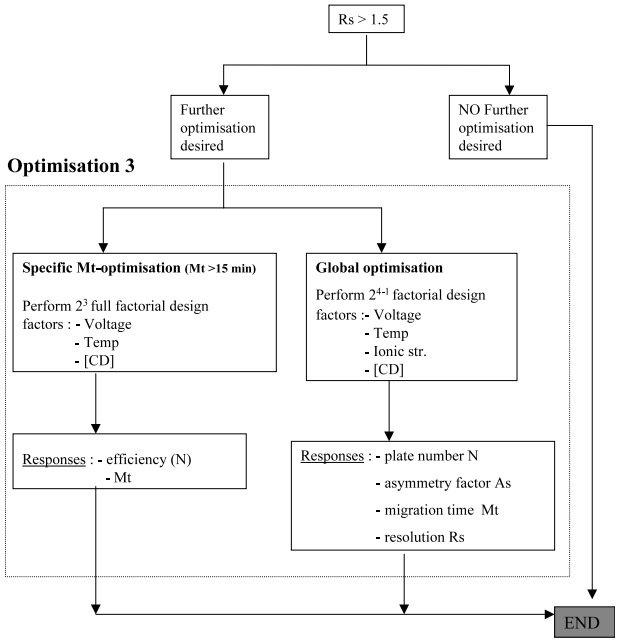

Fig. 9. Optimisation 3: migration time optimisation and global optimisation.

experiments, Pareto optimal experiments are selected by the KBS, as explained in Section 3.2.2.1. The selection of the final experiment out of the Pareto optimal experiments is performed by the user, dependent on the requirements.

Of the four substances chosen for illustration purposes, 2-phenylglycin methylester had $Rs > 1.5$ after the first screening (see Table 1). A global optimisation is performed for 2-phenylglycin methylester hydrochloride, since in the first screening, a good Rs and Mt were obtained (Table 1). Both, plate number and asymmetry factor improved after performing the $2^{(4-1)}$ fractional factorial design, respectively from 3067 to 6115 and from 0.58 to 0.64 (results are shown in Table 6 and Fig. 10).

4. Structuring 'the chiral KBS' in Toolbook

A Toolbook application consists of different books, which are built by creating pages and linking them by scripts. A script is a series of statements in Openscript, Toolbook's programming language. On the pages, objects are placed which contain information (text, graphics) or which make it possible for the user to execute an action (button). A script defines how an object behaves. By clicking a button the user can, for example, switch to another page.

Toolbook has two working levels: Reader and Author level. At the reader level, the user can use commands to flip through and even add pages, to type, edit and format text in fields. The Author level contains all the commands available at the Reader level plus the possibility to create new books and scripts.

Table 6
Results for two phenylglycine methylester after optimisation 3, general optimisation performed with a 2^{4-1} experimental design

Experiment	Rs	Mt (mm)	As	N (EP)
1	1.52	4.903	0.54	934
2	1.40	3.694	0.71	3383
3	**2.37**	**3.111**	**0.64**	**6115**
4	2.27	7.887	0.55	1025
5	1.84	2.271	0.50	5651
6	2.52	6.190	0.56	1267
7	1.65	3.009	0.56	1324
8	3.01	3.943	0.65	3173

The conditions of each experiment are given in Fig. 12. As, asymmetry factor; N (EP), efficiency calculated by the method given in the European Pharmacopoeia.

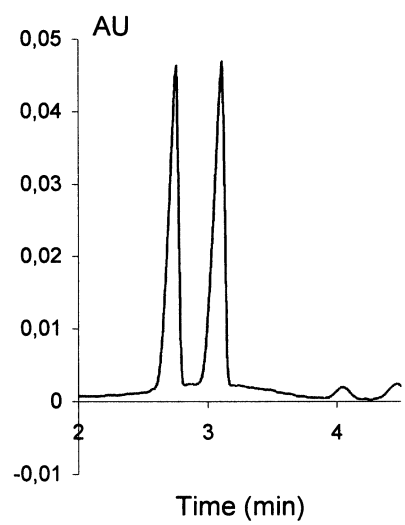

Fig. 10. Electropherogram for 2-phenylglycin methylester after performing optimisation 3.

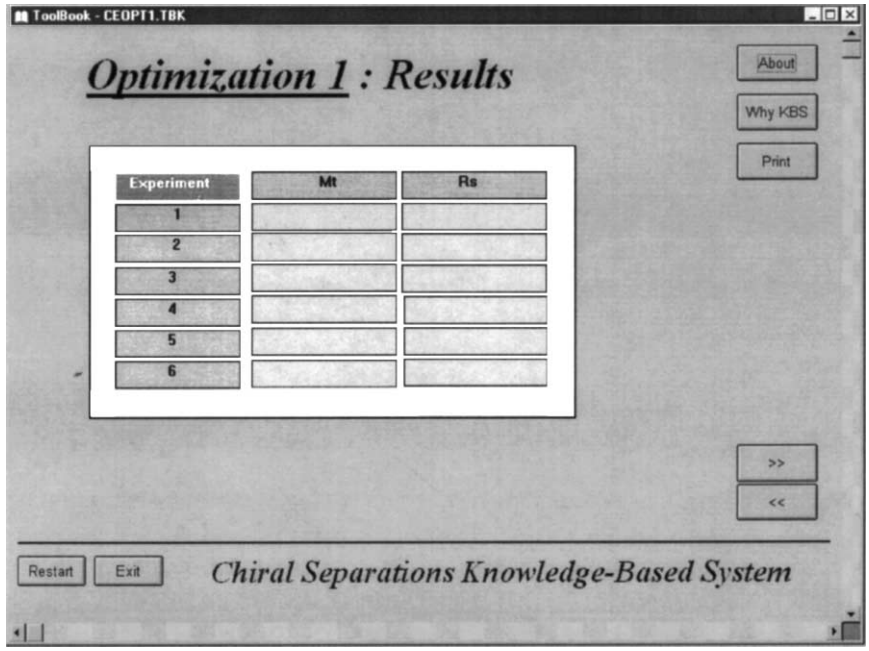

Fig. 11. Toolbook user interface of the chiral KBS. (A page on which the results, from a previously required experimental set-up, can be entered and printed.)

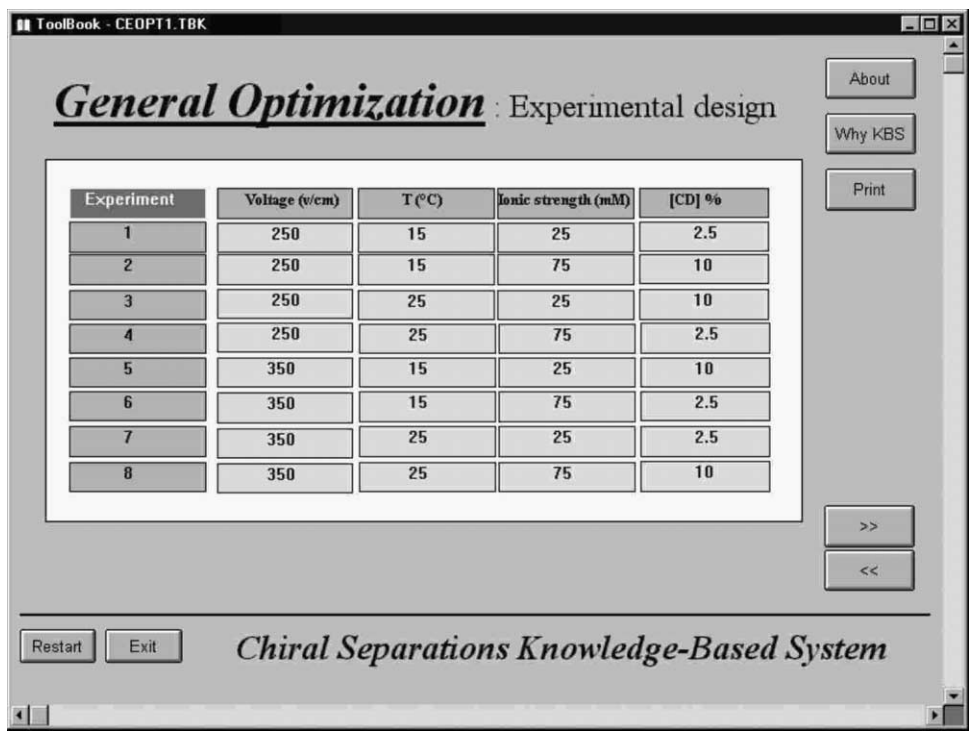

Fig. 12. 2^{4-1} fractional factorial design used for the global optimisation (optimisation 3).

The implementation of the knowledge in Toolbook was performed in different stages, at the Author level. First, different pages were constructed on which the objects were placed. Practical protocols are available to create pages and objects, such as text, fields and buttons. Later, some specific tools were included, such as blank pages on which the user could add comments, save and print options. The pages included in the CE part of the chiral KBS contain general information and explanations about the different steps and experiments (Fig. 3), experimental set-ups (Fig. 8) and also the possibility to input and print the experimental results (Fig. 11). Depending on the result received after performing experiments, a specific path will be followed in the decision tree.

5. Conclusion

Method development for chiral separations in CE is time-consuming and requires extensive experimental and theoretical expertise. Therefore, it was seen as a challenge to develop a knowledge-based system that can assist the user in the selection of initial conditions and in optimising the separation parameters. A KBS is built up by structuring knowledge in such a way that the user is guided to a solution for the problem with which he/she is faced. Some of this knowledge may be textbook or published knowledge. The basic rationale of this study is to combine such existing knowledge with the knowledge obtained in developing the present method development scheme and with generic experimental design for parameter optimisation.

Toolbook was found to be a good tool for the implementation of this knowledge and presents the structured knowledge in a clear and easily consultable way. The attractive user interface, the possibility given to the user to add comments and the easy implementation, make it a powerful tool for the building of small knowledge-based systems.

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References

[1] H. Kanazawa, Y. Kunito, Y. Matusushima, S. Okubo, F. Mashige, J. Chromatogr. A 871 (2000) 181–188.
[2] R.C. Williams, J.H. Miyawa, R.J. Boucher, R.W. Brockson, J. Chromatogr. A 844 (1999) 171–179.
[3] I. Fitos, J. Visy, M. Simonyi, J. Hermansson, J. Chromatogr. A 709 (1995) 265–273.
[4] M. Hoshino, K. Yajima, Y. Suzuki, A. Okahira, J. Chromatogr. B 661 (1994) 281–289.
[5] A. Ishikawa, T. Shibata, J. Liq. Chromatogr. 16 (4) (1993) 859–878.
[6] M. Kummer, G. Werner, J. Chromatogr. A 825 (1998) 107–114.
[7] J.P. McCarthy, J. Chromatogr. A 685 (1994) 349–355.
[8] V.L. Herring, J.A. Johnson, J. Chromatogr. A 612 (1993) 215–221.
[9] H.Y. Aboul-Enein, S.A. Bakr, J. Liq. Chromatogr. Rel. Technol. 21 (8) (1998) 1137–1145.
[10] K. Balmér, A. Persson, P. Lagerström, B. Personn, G. Schill, J. Chromatogr. A 553 (1991) 391–397.
[11] S.C. Sharma, M.B. Evans, S.J. Evans, J. Pharm. Biomed. Anal. 13 (2) (1995) 129–137.
[12] C.B. Ching, B.G. Lim, E.J.D. Lee, S.C. Ng, Chirality 4 (1992) 174–177.
[13] M. Maftouh, Spectra Anal. 199 (1997) 25–28.
[14] R.M. Smith, in: K. Anton, et al. (Eds.), Supercritical Fluid Chromatography with Packed Columns: Technique and Applications, Dekker, New York, 1997, pp. 223–244.
[15] B. Chankvetadze, J. Chromatogr. A 792 (1997) 269–295.
[16] G. Gübitz, M.G. Schmid, J. Chromatogr. A 792 (1997) 179–225.
[17] K. Verleysen, P. Sandra, Electrophoresis 19 (1998) 2798–2833.
[18] M. Nielen, Anal. Chem. 65 (1993) 885–893.
[19] T.E. Peterson, J. Chromatogr. A 630 (1993) 353–361.
[20] S. Pálmarsdóttir, L.-E. Edholm, J. Chromatogr. A 666 (1994) 337–350.
[21] S. Fanali, J. Chromatogr. A 875 (2000) 89–122.
[22] M.G. Vargas, Y. Vander Heyden, M. Maftouh, D.L. Massart, J. Chromatogr. A 855 (1999) 681–693.
[23] C. Perrin, M.G. Vargas, Y. Vander Heyden, M. Maftouh, D.L. Massart, J. Chromatogr. A 883 (2000) 249–265.
[24] S. Maver, X. Briand, E. Francotte, J. Chromatogr. A 875 (2000) 331–339.
[25] M. Lämmerhofer, F. Svec, J.M.J. Fréchet, W. Lindner, TRAC 19 (2000) 676–698.
[26] D. Wistuba, V. Schurig, J. Chromatogr. A 875 (2000) 255–276.
[27] A.F.-T. Chen, P/ACE Setter, vol. 3 (September), (1999) 9–10.
[28] K. Verleysen, T. Van den Bosch, P. Sandra, Electrophoresis 20 (1999) 2650–2655.
[29] K. Verleysen, S. Sabah, G. Scriba, A. A.F.-T. Chen, P. Sandra, J. Chromatogr. A 824 (1998) 91–97.
[30] J. Chapman, A.F.-T. Chen, LC-GC Eur. 14 (1) (2001) 33–37.
[31] V.A. Vu, Development of a fast separation strategy for chiral substances using normal phase liquid chromatogra-

phy, Master degree thesis in medical and pharmaceutical research, Vrije Universiteit Brussels, 1999–2000.
[32] C. Perrin, Y. Vander Heyden, M. Maftouh, D.L. Massart, Electrophoresis 22 (2001) 3203–3215.
[33] P. Piras, C. Roussel, J. Pierro-Sanders, J. Chromatogr. A 906 (2001) 443–458.
[34] N. Roos, K. Ganzler, J. Szemán, S. Fanali, J. Chromatogr. A 782 (1997) 257–269.
[35] M. Fillet, P. Hubert, J. Crommen, Electrophoresis 19 (1998) 2834–2840.
[36] M. Farkas, M. Cadish, E. Pretsch, in: E.J. Karjalainen (Ed.), Scientific Computing and Automation, Elsevier, Amsterdam, 1990.
[37] B. Bourguignon, P. Vankeerberghen, D.L. Massart, J. Chromatogr. A 592 (1992) 51–57.
[38] P.J. Schoenmakers, N. Dunand, A. Cleland, G. Musch, Th. Blaffert, Chromatographia 26 (1998) 37–44.
[39] W. Penninckx, P. Vankeerberghen, D.L. Massart, J. Smeyers-Verbeke, J. Anal. Atom. Spectrom. 10 (1995) 207–214.
[40] S.A.C. Wren, R.C. Rowe, J. Chromatogr. A 609 (1992) 363–367.
[41] H. Wan, A. Engström, L.G. Bloberg, J. Chromatogr. A 731 (1996) 283–292.
[42] S.F.Y. Li, Capillary electrophoresis principles, practice and applications, in: Journal of Chromatography Library 52, Elsevier, Amsterdam, 1992.
[43] M. Fillet, J. Crommen, Séparation énantiomérique de médicaments par électrophorèse capillaire à l'aide de cyclodextrines, Ph.D. Thesis in Pharmaceutical Science, Université de Liège, 1997–1998.
[44] S.A.C. Wren, R.C. Rowe, J. Chromatogr. A 603 (1992) 235–241.
[45] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, A. Smeyers-Verbeke, Data handling in science and technology, in: Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.
[46] H.R. Keller, J.P. Brans, D.L. Massart, Chemom. Intell. Lab. Sys. 11 (1991) 175–189.