Development and validation of a LC method for the enantiomeric purity determination of S-ropivacaine in a pharmaceutical formulation using a recently commercialized cellulose-based chiral stationary phase and polar non-aqueous mobile phase

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ABSTRACT

Ropivacaine is the first enantiomerically pure long-acting local anaesthetic used for surgical anaesthesia and post-operative pain relief.

A liquid chromatographic (LC) method using acetonitrile as the main solvent and cellulose tris(4-chloro-3-methylphenylcarbamate) coated on silica as chiral stationary phase was successfully developed and applied for the enantiomeric purity determination of S-ropivacaine in a pharmaceutical formulation (Naropin®). The key role played by the acidic additive (trifluoroacetic acid or formic acid) in the enantioseparation of basic drugs in these LC systems was demonstrated by the reversal of ropivacaine enantiomers elution order observed when both acids were compared. In order to elute the enantiomeric impurity (R-ropivacaine) before S-ropivacaine, formic acid (FA) was selected. The temperature and the percentages of acidic additive and hexane in the mobile phase were found to significantly influence the retention and resolution of these enantiomers. The optimized mobile phase consisted of ACN/0.1% DEA/0.2% FA/5% hexane (v/v/v/v). The temperature was set at 35 °C to avoid the interference from a peak system related to the presence of water in the sample on ropivacaine enantiomers.

The LC method was then fully validated applying the strategy based on total measurement error and accuracy profiles. The accuracy profile obtained by linear regression after square root transformation was selected, the acceptance limits being settled at ±10% for the intended use of this analytical method. The relative bias was lower than 1.5%, while the RSD values for repeatability and intermediate precision were both below 1.0%. The limit of detection (LOD) and the limit of quantification (LOQ) were found to be about 0.2 and 1.0 μg/mL, respectively, corresponding to 0.02 and 0.1% of the enantiomeric impurity in S-ropivacaine.

1. Introduction

Chirality has become a very important topic in pharmacology and analytical chemistry since a thorough assessment of potential chiral drug candidates is required in drug development by the Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) [1,2]. These guidelines recommend to test the new chiral drug molecules in their racemic form as well as single-isomers in the various stages of development. Both regulatory bodies recommend the use of validated methods to evaluate the enantiomeric purity of single-isomers.

Ropivacaine is the first enantiomerically pure local anaesthetic available for therapeutic use. It belongs to the same structural group (pipecoloxylidide) as bupivacaine and mepivacaine used for many decades as racemates. Even though (R)-(+) and (S)-(−)-1-propyl-2′,6′-pipecoloxylidide have similar nerve blocking properties, S-ropivacaine has shown less cardiotoxicity than the R-enantiomer as it is also the case for bupivacaine [3–5] Ropivacaine has then been developed as a pure enantiomer and is currently used in epidural anesthesia and post operative pain [6].

Sänger-van de Griend et al. have developed a capillary electrophoretic method for the enantiomeric purity determination of ropivacaine using heptakis (2,6-di-O-methyl)-β-cyclodextrin as chiral selector [7,8]. The method was found to be specific, linear, robust, accurate with a LOQ of 0.1% for the enantiomeric impurity. Nevertheless, the analysis time (about 25 min) as well as the LOD (0.05%) could be improved.

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Table 1
Preparation of standard solutions related to R-ropivacaine for validation.

Concentration level (% relative to 1.0 mg/mL of S-ropivacaine)	Concentration of R-ropivacaine (μg/mL)	
	Calibration standards	Validation standards
0.1	1.0	1.0
0.2	2.0	2.0
0.5	5.0	5.0
1.0	10.0	–
1.5	15.0	15.0
Total	10 samples/day	12 samples/day

In a pending monograph of ropivacaine from the United States Pharmacopeia (USP), the enantiomeric purity of ropivacaine is determined by a LC method [9]. The separation was achieved on a chiral-AGP column (150 mm × 4.0 mm i.d., 5 μm). The mobile phase consisted of isopropyl alcohol and monobasic sodium phosphate buffer (1:9, v/v). Under the prescribed experimental conditions, the run time was about 15 min and an enantioresolution of 1.8 between S-ropivacaine and its enantiomeric impurity was obtained. Eriksson Möller reported a very similar LC method for which a detection limit of 0.1% for the enantiomeric impurity was observed [10].

The main goal of the present work was to develop and validate a selective, precise, accurate and reliable LC method for the determination of R-ropivacaine in S-ropivacaine pharmaceutical formulation (Naropin®, 10 mg/mL solution). The method should be suitable for routine quality control of S-ropivacaine and the run time should be less than 15 min. To achieve this purpose, the newly commercialized chiral stationary phase (CSP), namely cellulose tris(4-chloro-3-methylphenylcarbamate) coated on silica (Sepapak-4), which showed a good enantioresolution for local anesthetics like bupivacaine and mepivacaine, was used [11,12]. Moreover, the developed method was fully validated according to the strategy proposed by a Commission of the Société Française des Sciences et Techniques Pharmaceutiques [13]. This strategy is based on the use of accuracy profiles which take into account the total error, i.e. estimation of systematic and random errors of measurement results.

2. Experimental

2.1. Chemicals and reagents

Ropivacaine hydrochloride monohydrate and ropivacaine impurity G were supplied by EDQM (Strasbourg, France). Ropivacaine chemical structure is presented in Fig. 1. Naropin® (10 mg/mL) was supplied by NV AstraZeneca SA (Brussel, Belgium). Acetonitrile (ACN) of HPLC grade and glacial acetic acid (AcA) pro
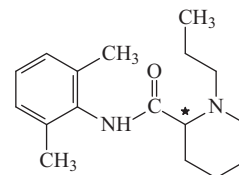
Fig. 1. Chemical structure of ropivacaine.

analysis were provided by Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA), diethylamine (DEA), and formic acid (FA) pro analysis were obtained from Acros Organics (Geel, Belgium). *n*-Hexane of HPLC grade was from BDH Hypersol (Poole, UK).

2.2. Instrumentation

The chromatographic system from Agilent Technologies (Waldbronn, Germany) consisted in a binary pump, a thermostated column compartment, a diode array detector and an automatic injector, all of 1200 series. The Chemstation software was used for system control and data acquisition. The chiral column Sepapak-4 (250 mm × 4.6 mm I.D.) was kindly provided by Sepaserve (Münster, Germany). The chiral selector adsorbed on aminopropylsilanized silica was cellulose tris(4-chloro-3-methylphenylcarbamate).

The statistical calculations for validation were performed by means of e.noval version 3.0 software (Arlenda, Liège, Belgium).

2.3. Standards solutions

2.3.1. Sample solutions used for method development

Different stock solutions of R- and S-ropivacaine were prepared by dissolving an accurately weighed amount of approximately 1 mg of each compound in 10 mL of ACN. The stock racemate solution was prepared by dissolving a weighed amount of approximately 1 mg for R ropivacaine and 1.1 mg for S-ropivacaine in 10 mL of ACN. This
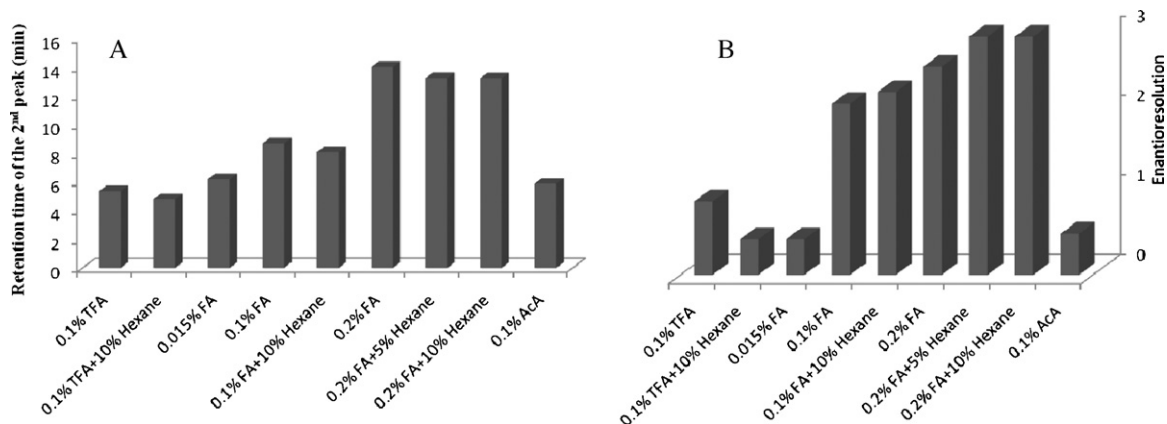
Fig. 2. Influence of the nature and the percentage of the acidic additive on the retention time of the second peak and on enantioresolution.
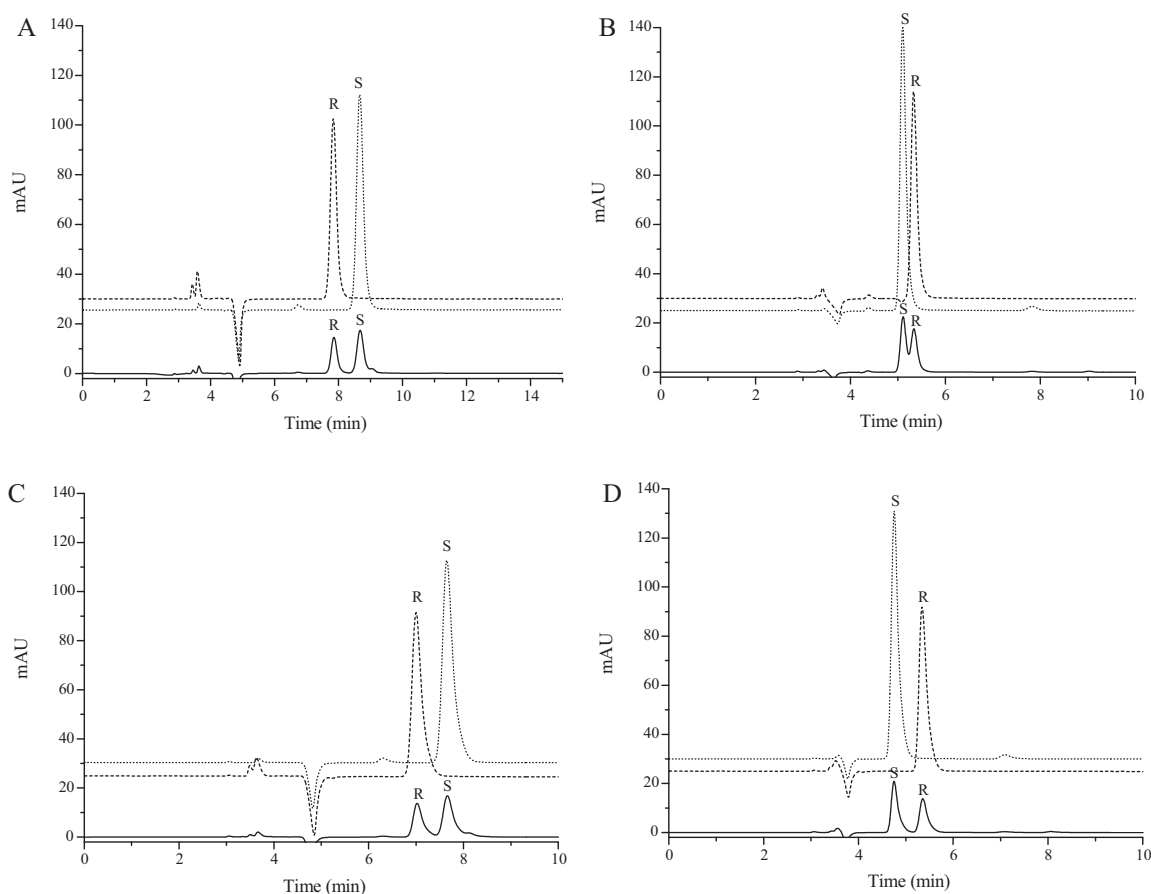
Fig. 3. Chromatograms showing the reversal of ropivacaine enantiomers elution order according to the nature of the acidic additive. Mobile phase: ACN/0.1% DEA/0.1% FA (A and C), ACN/0.1% DEA/0.1% TFA (B and D). Flow rate: 1 mL/min; temperature: 25 °C; UV detection: 240 nm; CSP: Sepapak-4 (A and B), sepapak-2 (C and D).

stock solution was then diluted 5-fold to obtain a final solution of 20 μg/mL.

2.3.2. Sample solutions used for validation

A stock solution of R-ropivacaine was prepared by dissolving an accurately weighed amount of approximately 5 mg of this compound in 100 mL of the LC mobile phase. Then subsequent dilutions were achieved in order to obtain five calibration standards ranging from 1.0 to 15 μg/mL ($m = 5$) (cf. Table 1). Two replicates ($n = 2$) were prepared per concentration level. Each solution is injected one time. The number of concentration levels was sufficient to generate different regression models.

A stock solution of S-ropivacaine was prepared by diluting 10-fold the aqueous solution of Naropin (10 mg/mL) in the LC mobile phase. The final concentration of S-ropivacaine was 1 mg/mL. Then three independent series of validation standards were prepared by spiking the correct volume of the R-ropivacaine stock solution in a sufficient volume of S-ropivacaine stock solution in order to reach final concentrations of 1, 2, 5 and 15 μg/mL ($m = 4$) of the enantiomeric impurity (cf. Table 1). Three replicates ($n = 3$) were prepared per concentration level and each one was injected one time.

2.4. Chromatographic conditions

The mobile phases consisted in a mixture of acetonitrile, organic modifier (hexane), acidic additive and basic additive (v/v) and were pumped at a constant flow-rate of 1.0 mL/min. In all experiments,

the basic additive percentage in the mobile phase was settled at 0.1%. The optimal mobile phase was made up of ACN/0.1% DEA/0.2% FA/5% hexane. The injection volume was 10 μL. The analytes were detected photometrically at 240 nm.

3. Results and discussion

3.1. Method development

In a previous work, an experimental design was applied for the enantioresolution of ten basic drugs, including bupivacaine and mepivacaine [12]. The optimal mobile phases consisted of ACN/0.1% DEA/0.1% FA/5% hexane for mepivacaine and ACN/0.1% DEA/0.2% FA for bupivacaine. For both methods, the temperature was set at 25 °C. Moreover, this study highlighted the fact that the enantiomeric resolution of chiral pharmaceuticals on Sepapak-4 was influenced by the nature and the percentage of the acidic additive and hexane in the mobile phase [11,12]. Finally, a strategy for rapid method development using a mobile phase made up of 0.2% FA, 5% hexane with ACN and 0.1% DEA at 25 °C was proposed. Therefore, these experimental conditions were applied for the enantiomeric resolution of ropivacaine. As can be seen in Fig. 2, under these conditions, ropivacaine enantiomers were completely resolved (Rs-value: 3.0) in a relatively short analysis time (14 min).

In order to verify that the generic conditions were the best for ropivacaine enantiomers with respect to retention time and enantioresolution, experiments were performed using other acidic additives (TFA and AcA), different FA and n-hexane percentages
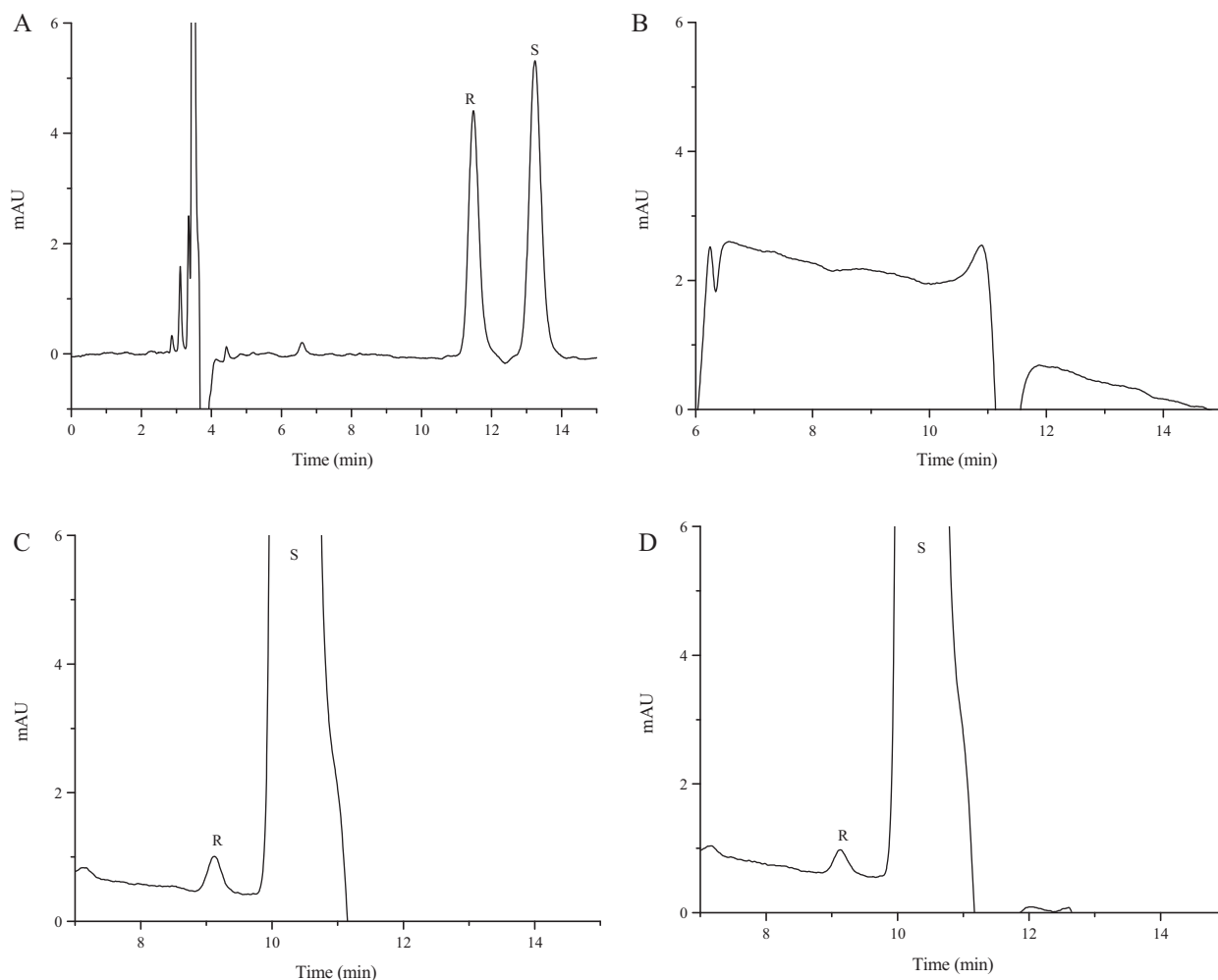
Fig. 4. Typical chromatograms of (a) a solution of racemic ropivacaine (20 µg/mL); (b) water diluted in the mobile phase; (c) a solution of S-ropivacaine (1 mg/mL of diluted Naropin®) containing R-ropivacaine (1 µg/mL); (d) a solution of S-ropivacaine (1 mg/mL of diluted Naropin®). Mobile phase: ACN/0.1% DEA/0.2% FA/5% Hexane, Flow-rate: 1 mL/min; Temperature: 25 °C (A) and 35 °C (B–D), UV: 240 nm; CSP: Sepapak-4. Other conditions see Section 2.

(cf. Fig. 2A and B). As expected, the nature of the acidic additive has a strong influence on the enantioresolution of ropivacaine. Indeed, only FA (at a percentage of at least 0.1%) leads to a complete separation of ropivacaine enantiomers. An increase of FA concentration up to 0.2% has a positive effect on the enantioresolution and retention of the enantiomers. Moreover, the presence of 5–10% of *n*-hexane in the mobile phase gives rise to a small decrease of the analysis time and also slightly improves enantioresolution.

It is noteworthy that the elution order of ropivacaine enantiomers depends on the nature of the acidic additive. Indeed, as can be seen in Fig. 3A and B, when TFA was used as acidic additive, S-ropivacaine was the first eluting peak whereas an opposite elution order was observed with FA under these conditions. Therefore, FA was kept as acidic additive since the impurity eluted before the main compound. Indeed, to avoid possible interference in the enantiomeric purity determination caused by the tailing of the main enantiomer present in high concentration, the elution order is of utmost importance, especially when the enantioseparation is minimal [14,15]. Here, it is clearly an advantage of these LC systems to enable a reversal of the elution order by changing the nature of the acidic additive.

As can be seen in Fig. 3C and D, the same elution order was observed using Sepapak-2 (chiral selector: cellulose tris(3-chloro-

4-methylphenylcarbamate)) with similar changes in the mobile phase. Interestingly, this reversal of elution order only occurred using these two CSPs which have both electron-withdrawing (chlorine) and electron-donating (methyl) groups on the phenylcarbamate moiety. Indeed, no reversal of ropivacaine enantiomers elution order was observed using Chiralcel ODH or Sepapak-5 with either two electron-donating or two electron-withdrawing groups in positions 3 and 5 of the phenylcarbamate moiety (data not shown). Therefore, the key role of the acidic additive in the reversal of ropivacaine enantiomers elution order could be related to the simultaneous presence of methyl and chlorine groups on the phenylcarbamate moiety of Sepapak-2 and -4.

Finally, taking into account the desired elution order, a short analysis time without jeopardizing enantioresolution, a mobile phase made up of ACN/0.1% DEA/0.2% FA/5% hexane was selected for this application. Fig. 4A illustrates the chromatogram of ropivacaine enantiomers using this mobile phase at 25 °C.

Nevertheless, when analyzing a validation sample, a peak system related to the presence of water in the sample appeared between ropivacaine enantiomers, which disturbed the quantification of the targeted impurity. To overcome this problem, the temperature was set at 35 °C and, as can be seen in Fig. 4B and C, the peak system was then located after S-ropivacaine peak
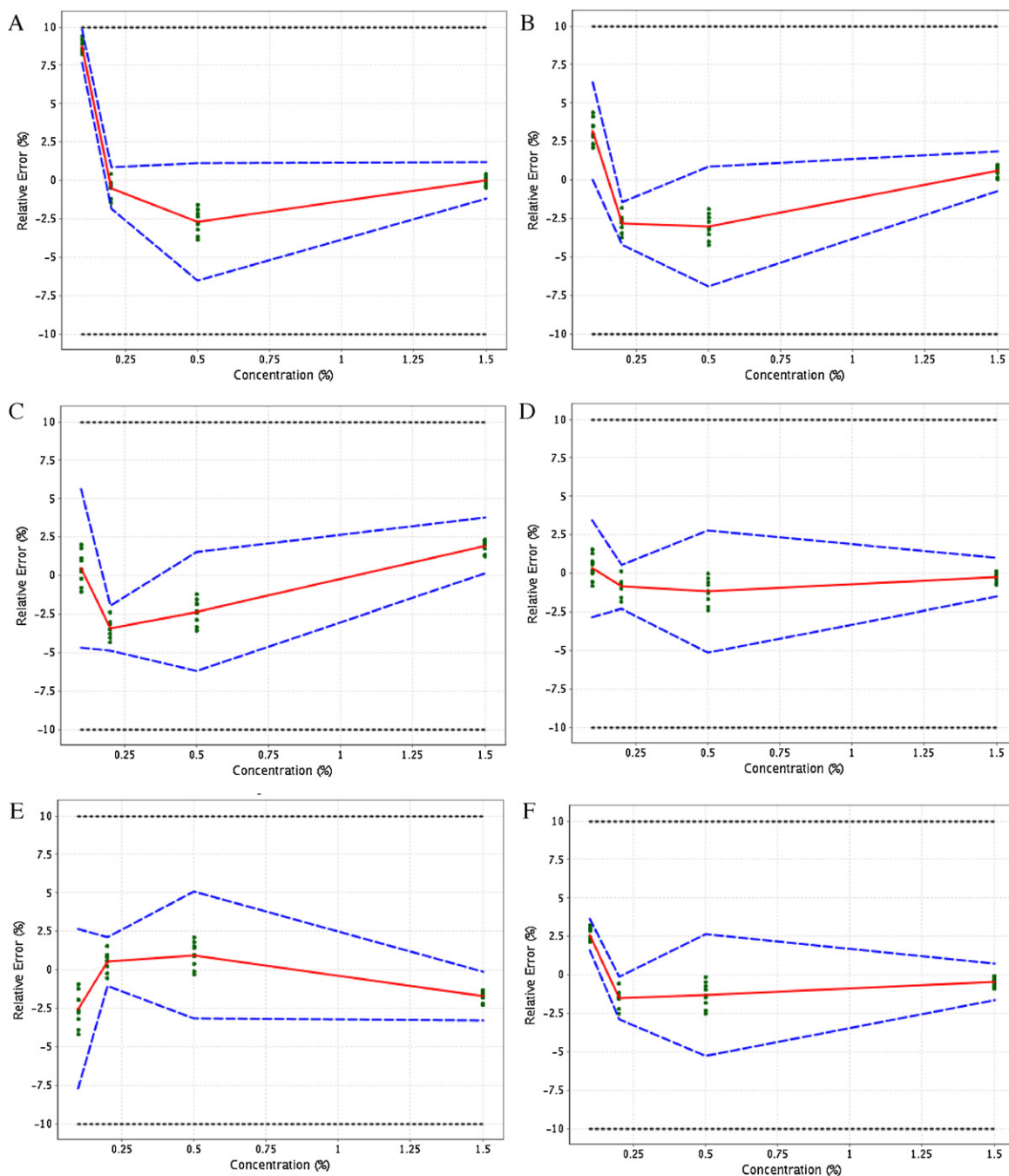
Fig. 5. Accuracy profiles obtained using (a) a linear regression model. (b) A weighted linear regression model with a weight equal to $1/X$. (c) A weighted linear regression model with a weight equal to $1/X^2$. (d) A linear regression model after square root transformation. (e) A linear regression model after logarithm transformation. (f) A quadratic regression model. The plain line is the relative error (%), the dashed lines correspond to the accuracy profile, i.e., to the β-expectation tolerance limits expressed in relative error, and the dotted curves represent the acceptance limits (±10%).

and therefore did no longer interfere in the determination of the enantiomeric impurity. However, as already observed earlier, this change of temperature had no significant effect on enantioresolution [11,12].

3.2. Method validation

The validation approach, based on total measurement error and accuracy profiles as decision tool, was applied to demonstrate that the developed method is suited for its intended purpose [13,16,17]. This strategy was elaborated by a SFSTP (Société Française des Sciences et Techniques Pharmaceutiques) Commission. The concept

of accuracy profile was also used to select the most appropriate calibration model, to determine the lower limit of quantification and the range over which the method can be considered as valid.

3.2.1. Selectivity

To evaluate method selectivity, the chromatograms obtained by analyzing water diluted in the mobile phase and a sample of S- and R-ropivacaine, were compared in order to check the absence of compounds likely to interfere in the quantification of R-ropivacaine. As can be seen in Fig. 4A and B, no interference was observed at the retention time of the peak corresponding to R-ropivacaine. It is worth noting that even though the peak cor-

responding of water interferes slightly with S-ropivacaine peak, it does not compromise the quantification of the enantiomeric impurity.

Moreover, a solution of S-ropivacaine (1 mg/mL), obtained by diluting 10-fold Naropin®, was analysed (cf. Fig. 4D). As can be seen from this figure, a small peak, identified as R-ropivacaine by comparison of the retention times, was observed. Therefore, three independent injections of this solution were carried out daily and, at each validation day, the mean peak area of R-ropivacaine was subtracted from the responses of the validation standards.

3.2.2. Selection of the calibration model

Several regression models were fitted to the calibration standards. From each regression line obtained, the concentrations of the validation standards were back-calculated in order to determine, at each concentration level, the mean relative bias, the relative standard deviation for intermediate precision as well as the upper and lower β-expectation tolerance limits at 95% [13].

Taking into account the validation data, several accuracy profiles were plotted to select the most suitable regression model for the intended use of the analytical method, as illustrated in Fig. 5. Six response functions, namely the simple linear regression, the weighted $(1/X)$ and $(1/X^2)$ linear regression models, the linear regression models after square root and logarithm transformation as well as the quadratic regression model, were tested. The acceptance limits were set at ±10% according to the regulatory requirements [18–20].

All these response functions allowed to demonstrate the capability of the method over the concentration range considered, since the tolerance intervals were totally included inside the acceptance limits as shown in Fig. 5. However, the linear regression model after square root transformation seems to be the best. Indeed, the relative error is the lowest especially at the two first concentration levels. The variability of the results is also weak. Table 2 presents the validation results obtained by applying this linear regression model.

3.2.3. Trueness

Trueness refers to the closeness of agreement between a conventionally accepted value or reference value and a mean experimental one. It gives information on systematic error and is expressed in term of relative bias (%). As can be seen in Table 2, the relatives biases assessed from the validation standards at four

Fig. 6. Linearity profile of the developed method. The plain line is the identity line: $Y = X$, the dashed lines correspond to the accuracy profile, i.e., to the β-expectation tolerance limits expressed in absolute values. The dotted curves represent the acceptance limits (±10%) expressed in the concentration unit.

Table 2
Validation results for R-ropivacaine using the linear regression model after square root transformation.

Validation criteria	R-Ropivacaine		
Response function	Slope	Intercept	r^2
($k = 3$; $m = 5$; $n = 2$) (0.1–1.5%)			
Day 1	6.47	−0.341	0.9999
Day 2	6.50	−0.356	0.9999
Day 3	6.48	−0.337	0.9999
Trueness ($k = 3$; $n = 3$)		Relative bias (%)	
0.1%		0.3	
0.2%		−0.85	
0.5%		−1.2	
1.5%		−0.25	
Precision ($k = 3$; $n = 3$)	RSD (%)		
	Repeatability	Intermediate precision	
0.1%	0.5	0.9	
0.2%	0.6	0.6	
0.5%	0.3	0.9	
1.5%	0.2	0.3	
Accuracy ($k = 3$; $n = 3$)	Relative β-expectation tolerance limits (%)		
0.1%	[−2.79, 3.46]		
0.2%	[−2.27, 0.57]		
0.5%	[−5.12, 2.79]		
1.5%	[−1.50, 0.99]		
Linearity ($k = 3$; $m = 4$; $n = 3$) (0.1–1.5%)			
Slope	0.9979		
Intercept	−0.0015		
r^2	1		
LOD (%)	0.02		
LOQ (%)	0.1		

concentration levels were found quite acceptable according to the regulatory requirements, since their values are largely below the maximum of 10%, irrespective of the concentration level.

3.2.4. Precision

Precision is the closeness of agreement among measurements from multiple sampling of homogeneous sample under the recommended conditions. The precision of the analytical method was estimated by calculating repeatability and time-dependent intermediate precision at each concentration level. The relative standard deviation (RSD) values were calculated from the estimated concentrations. As can be seen from Table 2, the RSD values were found to be very low (between 0.2 and 0.9%), illustrating the excellent precision of the proposed method.

3.2.5. Accuracy

Accuracy refers to the closeness of agreement between the test result and the accepted value, namely the conventionally true value. The accuracy of the analytical method takes into account the total error, i.e. both systematic and random errors, related to the test result. The upper and lower β-expectation tolerance limits expressed in relative bias (%) as a function of the introduced concentrations are presented in Table 2. The different tolerance limits of the mean relative bias did not exceed the acceptance limits for each concentration level. Therefore, the developed method can be considered as accurate over the whole concentration range investigated.

3.2.6. Linearity

The linearity of an analytical method is the ability within a definite range, to obtain results directly proportional to the con-
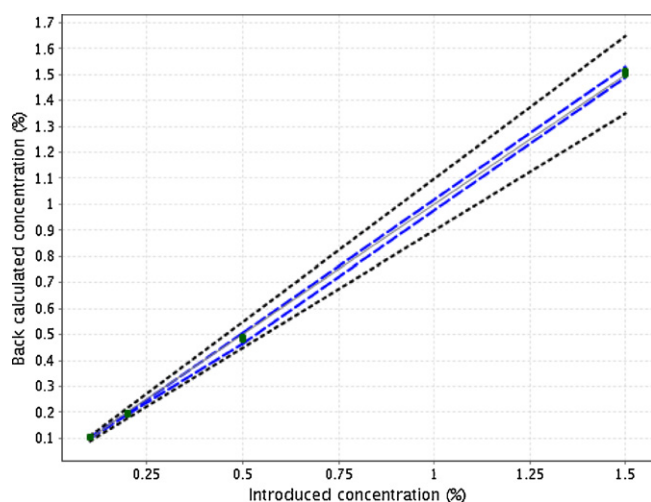
centration (quantity) of the analyte in the sample. For all series, a regression line was fitted on the back-calculated concentrations of validation standards as a function of the introduced concentrations by applying the linear regression model based on least squares method.

The results attesting the method linearity, namely the regression equation corresponding to that relationship with its coefficient of determination, are presented in Table 2.

Moreover, in order to demonstrate the method linearity, the approach based on absolute β-expectation tolerance limits was applied. As can be seen in Fig. 6, the linearity of the present method was demonstrated since the absolute β-expectation tolerance limits were within the absolute acceptance limits.

3.2.7. Detection and quantification limits

The limit of detection (LOD) is the smallest quantity of the targeted substance that can be detected, but not accurately quantified in the sample. In the present study, the LOD was estimated to be the response corresponding to three times the signal to noise ratio. By applying this method, the LOD of the developed method was found to be equal to 0.02%. The limit of quantification (LOQ) of an analytical method is the lowest amount of the targeted substance which can be quantitatively determined under the experimental conditions prescribed with a well-defined accuracy, i.e., taking into account the systematic and random errors [13,16]. As the accuracy profile was included inside the acceptance limits over the whole concentration range investigated, the first concentration level (0.1%) was considered as the LOQ, according to the selected regression model. Indeed, precision and trueness were demonstrated at this concentration level.

4. Conclusion

In this paper, a chiral LC method with Sepapak-4 as CSP in polar organic solvent chromatography mode, was developed to determine the enantiomeric purity of S-ropivacaine in an aqueous formulation. The method development shows the reversal of ropivacaine enantiomers elution order according to the acidic additive (TFA or FA).

The method was then fully validated according to the strategy based on the accuracy profiles. Good performance with respect to selectivity, trueness, precision and accuracy, were obtained. The limits of quantification (0.1%), detection (0.02%) and the analysis time make the method suitable for rapid quality control of the enantiomeric purity of ropivacaine hydrochloride in an aqueous pharmaceutical formulation in comparison with the existing methods.

References

[1] FDA Policy Statement for Development of New Stereoisomeric Drug, http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122883.htm (accessed 20.05.10).
[2] E. Francotte, W. Lindner, in: R. Mannhold, H. Kubinyi, G. Folkers (Eds.), Chirality in Drug Research, Wiley-VCH, Weinheim, 2006.
[3] A. Markham, D. Faulds, Ropivacaine: a review of its pharmacology and therapeutic use in regional anaesthetisia, Drugs 52 (1996) 429–449.
[4] G. Aberg, K.G. Dhuner, G. Sydnes, Studies on the duration of local anaesthesia: structure/activity relationships in a series of homologous local anaesthetics, Acta Pharmacol. Toxicol. 41 (1977) 432–434.
[5] B. Akerman, I.B. Hellberg, C. Trossvik, Primary evaluation of the local anaesthetic properties of amino amide agent ropivacaine (LEA 103), Acta Anaesthesiol. Scand. 32 (1988) 571–578.
[6] J.M. Mcclure, Br. Ropivacaine, J. Anaesth. 76 (1996) 300–307.
[7] C.E. Sänger-van de Griend, K. Gröningsson, Validation of a capillary electrophoresis method for the enantiomeric purity testing of ropivacaine, a new local anaesthetic compound, J. Pharm. Biomed. Anal. 14 (1996) 295–304.
[8] C.E. Sänger-van de Griend, H. Wahlström, K. Gröningsson, M. Widahl-Näsman, A chiral capillary ellectrophoresis method for ropivacaine hydrochloride in pharmacutical formulations: validation and comparison with chiral liquid chromatography, J. Pharm. Biomed. Anal. 15 (1997) 1051–1061.
[9] Ropivacaine authorised USP pending Monograph, Version 1 (2010); http://www.ups.org (accessed 20.05.10).
[10] L. Eriksson Möller, Astra Pain Control Report.
[11] K.S.S. Dossou, P. Chiap, B. Chankvetadze, A.-C. Servais, M. Fillet, J. Crommen, Enantioresolution of basic pharmaceuticals using cellulose tris(4-chloro-3 methylphenylcarbamate) as chiral stationary phase and polar organic mobile phases, J. Chromatogr. A 1216 (2009) 7450–7455.
[12] K.S.S. Dossou, P. Chiap, B. Chankvetadze, A.-C. Servais, M. Fillet, J. Crommen, Optimization of the LC enantioseparation of chiral pharmaceuticals using cellulose tris(4-chloro-3-methylphenylcarbamate) as chiralselector and polar non-aqueous mobile Phases, J. Sep. Sci. 33 (2010) 1699–1707.
[13] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Validation of quantitative analytical procedure: harmonization of approaches, STP Pharma Pratiques 13 (2003) 101–138.
[14] T.J. Wozniak, R.J. Bopp, E.C. Jensen, Chiral drugs: an industrial analytical perspective, J. Pharm. Biom. Anal. 9 (1991) 363–382.
[15] R. Cirilli, R. Ferretti, B. Gallinella, L. Zanitti, F. La Torre, A new application of stopped-flow chiral HPLC: inversion of enantiomer elution order, J. Chromatogr. A 1061 (2004) 27–34.
[16] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal – part I, J. Pharm. Biomed. Anal. 36 (2004) 579–586.
[17] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Quantitative analytical procedures: Harmonization of the appraoches – Part II, STP Pharma Pratiques 16 (2006) 30–60.
[18] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Workshop/conference report – quantitative bioanalytical methods validation and implementation: Best practices for chromatographic and ligand binding assays, AASP J. 9 (2007) E30–E42.
[19] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Jacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Analytical methods validation: biovailability, bioequivalence and pharmacokinetic studies, J. Pharm. Sci. 81 (1992) 309–312.
[20] V.P. Shah, K.K. Midha, J.W.A. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.I. Powell, A. Tonelli, C.T. Viswanathan, A. Jacobi, Bioanalytical method validation – a revisit with a decade of progress, Pharm. Res. 17 (2000) 1551–1557.