Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 560 (2006) 57–63
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Enantioseparation and quality control of biperiden in pharmaceutical formulations by capillary electrophoresis

Roberto Mandrioli [a], Alessandro Musenga [a], Stefano Savino Lasaponara [a], Maria Addolorata Saracino [a], Salvatore Fanali [b], Maria Augusta Raggi [a,*]

[a] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Alma Mater Studiorum – University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Institute of Chemical Methodologies, National Council of Research, Research Area of Rome, 00016 Monterotondo Scalo, Rome, Italy*

Received 20 October 2005; received in revised form 12 December 2005; accepted 12 December 2005
Available online 18 January 2006

Abstract

An original capillary electrophoretic method has been developed and applied for the enantioselective analysis of the antiparkinson drug biperiden in pharmaceutical formulations, using a modified cyclodextrin as the chiral selector. Baseline enantioseparation of the racemic compound was achieved in less than 7 min using an uncoated fused silica capillary (50 μm i.d. and 48.5, 40.0 cm, total and effective length, respectively), filled with a background electrolyte consisting of a 50 mM phosphate buffer at pH 3.5 supplemented with 3% (w/v) β-cyclodextrin sulphate and applying a voltage of 20 kV, reversed polarity. Samples were injected by pressure (50 mbar, 90 s) at the cathodic end of the capillary and detection wavelength was 195 nm (bandwidth: 10 nm). A simple and fast pre-treatment procedure allowed the complete extraction of the drug from commercial formulations (sustained release tablets and ampoules for injections) without any interference from the matrix. Good linearity was found in the 1–50 μg/mL concentration range; the limit of quantitation was 1 μg/mL and the limit of detection was 0.4 μg/mL. Precision and accuracy were good, with R.S.D. values always lower than 2.8% and a mean recovery value of 101.1%. The method was suitable for the quality control of biperiden in commercial formulations.

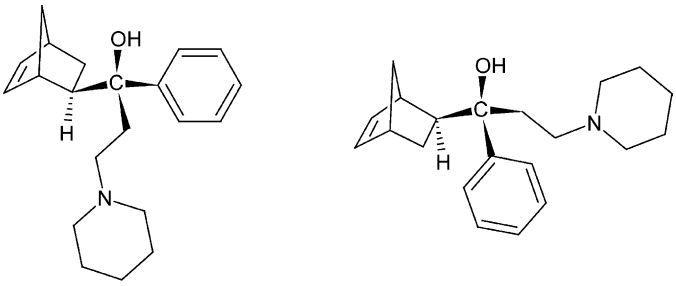
Keywords: Biperiden; Quality control; Enantioseparation; Capillary electrophoresis; Pharmaceutical formulations

1. Introduction

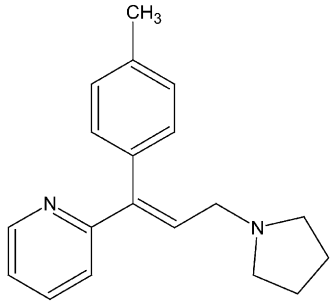
Parkinson's disease is a chronic degenerative syndrome which causes the progressive death of dopaminergic neurones in the brain which are necessary for a correct and precise control of voluntary movements. The key symptoms of Parkinson's disease are tremor, bradikinesia, dystonia and a curved stance. As the disease progresses, several muscles become progressively paralysed and death by breathing difficulty, pneumonia or lung embolism can occur [1]. Several other pathologies having symptoms similar to those of Parkinson's disease are collectively known as "parkinsonisms"; for example, treatment with neuroleptic drugs can cause side effects such as tremor and difficulty in movement, which are known as "extrapyramidal syndrome", and are caused by the inhibition of dopaminergic transmission. Treatment of Parkinson's disease and similar syndromes is based on a therapy of dopamine substitution, which can notably improve the quality of life of patients, at least in the first few years after the onset of the disease. Historically, the most widely used drug for this purpose is the physiological precursor of dopamine, (*l*)-3,4-dihydroxyphenylalanine (*l*-dopa or levodopa). Currently, however, the drugs of first choice are dopaminergic agonists such as ergot derivatives (e.g. bromocryptine, cabergoline), which tend to cause fewer undesired effects [1]. Anticholinergic agents, such as triesiphenydile, biperiden and orphenadrine, are also used for the treatment of Parkinson's disease. All these drugs have antimuscarinic activity: they correct the relative excess of cholinergic activity which is thought to happen as a consequence of dopamine deficit [2]. Among them, biperiden (1-[(1,2,4)-bicyclo[2.2.1]epta-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol, BPR, Fig. 1a) has a peculiar place, since it is selective toward central M1 muscarinic receptors, and is very suitable for the treatment of the extrapyramidal syndrome caused by the use of neuroleptic agents [3]. The drug is admin-

* Corresponding author. Tel.: +39 051 2099700; fax: +39 051 2099734.
E-mail address: mariaaugusta.raggi@unibo.it (M.A. Raggi).

0003-2670/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2005.12.027



(a) – Biperiden enantiomers



(b) – Triprolidine (IS)

Fig. 1. Chemical structures of (a) biperiden enantiomers and (b) triprolidine (IS).

istered orally at doses usually ranging from 2 to 12 mg/day. Side effects include nervousness, dizziness and gastro-intestinal problems, while cognitive processes can be impaired, especially in the elderly [4]. Chemically, BPR has three asymmetric carbon atoms and can thus exist as four enantiomeric pairs. However, since the active principle is synthesised in the *exo*-form, (1*RS*)-1-[(1*RS*,2*SR*,4*RS*)-bicyclo[2.2.1]epta-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol, only two enantiomers are present in the formulations (Fig. 1a), and all other diastereoisomers should only be found as impurities.

It has been found from in vitro functional studies that (+)-biperiden is able to discriminate between ileal M2β and cardiac M2α receptors and strongly between M1 and cardiac M2α receptors; in contrast, (−)-biperiden displays low but nearly indistinguishable affinity for all three receptors [5]. Thus, the spectrum of action of the two enantiomers can be quite different. These studies however have not reached any clear conclusion because no correlation has been observed between the potency and the affinity ratios of the two enantiomers [6]. This in part was due to the lack of sensitive stereoselective analytical methodologies for the determination of these enantiomers [7]. Thus, it would be important for this purpose to have a reliable analytical method which could be used for the enantioselective quality control of biperiden.

One paper can be found in the literature for the enantioseparation of BPR and its analysis in human plasma; it is based on liquid chromatography–mass spectrometry with chiral stationary phase and it also concerns other antiparkinson drugs [7]. Other papers report studies on the enantioseparation of many different drugs by capillary electrophoresis using cyclodextrins as the chiral selectors; these papers do not report any application to formulations; furthermore, most of them do not even obtain any enantioseparation of BPR [8–14]. Only a few literature sources report analytical methods for BPR determination in formulations, however none of them are chiral: one paper reports the use of spectrophotometry [15], while the United States Pharmacopeia uses a colorimetric assay [16]. The European [17] and British [18] Pharmacopoeias determine the active principle and impurities in bulk material only, by gas chromatography-flame ionisation detection. The aim of this study was the separation of BPR enantiomers and their analysis in commercially available pharmaceutical formulations such as Akineton® tablets and injections. A good strategy to obtain an enantioselective method suitable for this purpose is to use capillary electrophoresis (CE) with cyclodextrins as the chiral selectors. In fact, CE is an extremely efficient, versatile and selective technique, which allows very efficient separations in shorter run times with respect to other separation techniques such as liquid chromatography or gas chromatography [19–22]. Cyclodextrins are well-known chiral selectors, which can either be used in their native form or chemically modified to obtain selectivity toward different compounds or classes of compounds [23,24]; they are stable and quite efficient and can simply be added to the background electrolyte (BGE) to obtain enantioselectivity [25–27]. The fast and feasible method described herein has been validated in terms of linearity, precision and accuracy and has been successfully applied to the quality control of biperiden in commercial formulations.

2. Experimental

2.1. Chemicals

All chemicals were analytical grade. Biperiden was kindly provided by Abbott Italia S.p.A. (Campoverde, Italy). β-cyclodextrin sulphate (SβCD), γ-cyclodextrin and carboxymethyl-β-cyclodextrin were purchased from Fluka (Buchs, Switzerland); β-cyclodextrin was from Research Chemicals Ltd. (Heysam, Lancs., UK); sulphobutyl-β-cyclodextrin was from CyDex (Lenexa, KS, USA). Triprolidine used as the Internal Standard (IS, Fig. 1b) and HPLC-grade methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide, phosphoric acid (80%, w/w) and citric acid were from Carlo Erba (Milan, Italy). Ultrapure water (18.2 MΩ cm) was obtained using a MilliQ apparatus from Millipore (Milford, MA, USA).

2.2. Solutions

Stock solutions of BPR and triprolidine (IS), 1 mg/mL each, were separately prepared in methanol and were stable for at least 5 months when stored at −20 °C, as assessed by means of spectrophotometric assays. Working solutions were prepared daily from the stock solutions by sequential dilution with methanol; the last dilution was carried out in a pH 3.5, 5 mM phosphate buffer. The final concentration of the IS was always 10 μg/mL.

The background electrolyte (BGE) was a pH 3.5, 50 mM phosphate buffer containing 3% (w/v) SβCD. It was prepared as follows: 170 μL of 80% (w/w) phosphoric acid were mixed with about 45 mL of water; the solution was then brought to pH 3.5 with 1 M sodium hydroxide, transferred into a 50-mL volumetric flask and made to volume with water. An amount of 150 mg of SβCD was dissolved in 5 mL of this buffer. The BGE was filtered though a cellulose filter (pore size 0.2 μm, Stepbio, Bologna, Italy) before use.

2.3. Apparatus and electrophoretic conditions

All assays were carried out on a ^{3D}CE apparatus by Agilent Technologies (Waldbronn, Germany) equipped with a diode-array detector (DAD). A fused-silica uncoated capillary (50 μm i.d., 375 μm o.d., 48.5 cm total length, 40.0 cm effective length) was used, which was bought from Composite Metal Ltd. (Hallow, UK). Injection was carried out by pressure (50 mbar, 90 s) at the cathodic end of the capillary. The voltage applied was 20 kV reversed polarity; the capillary was thermostatted at 25.0 °C and the wavelength used for quantitative analyses was 195 nm (bandwidth: 10 nm).

New capillaries were sequentially washed and conditioned by pressure (5 bar) with water (5 min), 1 M sodium hydroxide (15 min), water (30 min) and BGE (10 min). The capillary was pre-conditioned with BGE (2 min, 2 bar) before each run and post-conditioned with BGE (1 min) after each run. At the end of the working day, the capillary was washed by pressure (5 bar) with water (5 min), 1 M sodium hydroxide (5 min) and water (10 min).

An external nitrogen cylinder was used as a pressure generator for all operations requiring a pressure application higher than 1 bar.

2.4. Analysis of standard solutions

Standard solutions were analysed by CE under the reported working conditions. The analyte/IS peak area ratios were plotted against the analyte concentrations and 10-point calibration curves were obtained for each single enantiomer by means of the least square method. Each concentration was injected in triplicate.

Precision assays were carried out at three different concentrations, corresponding to the lower limit, middle point and upper limit of the calibration curve. The solutions were prepared and injected six times at each concentration level to obtain the relative standard deviation (R.S.D.%) of both peak area ratios and migration times.

2.5. Sample treatment

Two commercial formulations were analysed: sustained release Akineton® tablets and Akineton® ampoules for injections (both from Abbott S.p.A., Campoverde, Italy).

Each tablet has a declared content of 4 mg of biperiden hydrochloride and the following inactive ingredients: maize starch, microcrystalline cellulose, carnauba wax, hydroxypropylcellulose, lactose, magnesium stearate, methylhydroxypropylcellulose, yellow iron oxide, polyethyleneglycol 400, polyethyleneglycol 600, polyvinylpyrrolidone, colloidal silica, sodium laurylsulphate, talc, titanium dioxide. Biperiden was extracted from tablets as follows. After weighing 20 tablets, they were finely ground in a mortar and thoroughly mixed. An aliquot of powder equivalent to 2 mg of biperiden free base (racemic nominal concentration) was weighed and a suitable amount of the IS was added. The powder was then transferred into a test tube with 5 mL of methanol, vortex-mixed for 5 min, then centrifuged (3000 rpm, 10 min). The liquid supernatant was separated and the remaining powder was treated thrice again with 5 mL of methanol. All the supernatant liquids were united, sonicated in an ultrasonic bath for 5 min and then filtered through paper. The resulting clear solution has a nominal concentration of 100 μg/mL. It was then diluted with a pH 3.5, 5 mM phosphate buffer to suitable nominal concentrations and injected into the CE apparatus.

Each 2-mL Akineton® ampoule has a declared content of 5 mg of biperiden lactate; it also contains water for injectables and sodium lactate. The content of one ampoule was quantitatively transferred to a volumetric flask and the IS added; the resulting solution was suitably diluted with a pH 3.5, 5 mM phosphate buffer and then injected into the CE apparatus.

2.6. Method validation

The formulation samples were treated as described above; linearity was assessed by injecting solutions at 10 different concentrations in triplicate. Sample solutions at three different concentrations (corresponding to the lower limit, middle point and upper limit of the calibration curve) were prepared and injected six times in the same day (to evaluate repeatability) and over six different days (to evaluate intermediate precision). The amount of drug found of declared was also calculated.

According to the United States Pharmacopeia guidelines [28], the limit of quantitation (LOQ) and limit of detection (LOD) were obtained as the analyte concentrations which gave rise to electrophoretic peaks whose height was 10 and 3 times the baseline noise, respectively.

Accuracy was assessed by means of recovery assays. Known amounts of the analyte and the IS were added to the formulations in order to obtain final spikings (after suitable dilution) corresponding to the lower limit, middle point and upper limit of the calibration curve. After sample pre-treatment, the resulting dilution was analysed and the recovery of the added analyte was calculated. This procedure was repeated six times for each concentration in order to obtain R.S.D.% values for recovery.

3. Results and discussion

3.1. Preliminary electrophoretic assays

Thanks to its tertiary amine function, biperiden is a weakly basic compound, whose conjugated acid has an approximate pK_a

of 9.8.[1] Thus, it should be at least partially positively charged at all pH values lower than 9, and in these conditions it should migrate as a cation toward the cathode. Preliminary assays were thus carried out to assess the electrophoretic behaviour of BPR at pH values between 2.5 and 7.5, using the normal polarity mode. Different buffers were used (phosphate, acetate, citrate), each at a 50 mM concentration. Higher pH values were not investigated because at these values BPR was not charged, and this made the analysis more difficult. As expected, a peak corresponding to BPR could be identified at all tested pH values. Migration times were shorter when higher pH values were used due to the effect of electroosmotic flow (EOF); in any case, an electrophoretic run would last 8 min at most.

3.2. Choice of the chiral selector

As already mentioned, cyclodextrins (CDs) are very versatile and efficient chiral selectors, widely used in the development of electrophoretic methods. Negatively charged CDs, in particular, are very attractive for the chiral separation of positively charged analytes (such as BPR), since in this case the electrostatic charge interactions can be quite strong and lead to very high selectivity toward the two enantiomers. Different kinds of native and charged CDs were tested for the enantioseparation of biperiden: β-CD, carboxymethyl-β-CD, sulphobutyl-β-CD and β-CD sulphate (SβCD), at concentrations in the 0.1–1.0% range. The cyclodextrins were dissolved in phosphate buffer BGEs under different conditions of pH (2.5 and 7.5) and concentration (25 and 50 mM) and the electrophoretic runs were carried out applying a constant voltage of 20 kV. While β-CD showed almost no interaction with the analytes, the use of charged CDs such as carboxymethyl-β-CD and sulphobutyl-β-CD lead to some slight enantioseparation, however it was not possible to obtain satisfactory results with these selectors. The CD which proved to interact the strongest with the analytes was β-CD sulphate: in fact, its use gave rise to quite long migration times (>20 min) or even, at concentrations of SβCD higher than 0.7%, to the migration of the analytes at the anodic end of the capillary: at these concentrations, it was impossible to detect the analytes in normal polarity mode. It was also noted that the higher pH values, while shortening migration times, impaired analyte resolution; for this reason, it was decided to work at a low pH value, namely 3.5. The strong interaction between SβCD and the analytes also meant that the resulting electrophoretic peaks lost efficiency and thus resolution; it was not possible to obtain baseline enantioseparation within reasonable times. For this reason, it was decided to further increase the concentration of SβCD and to carry out analyte detection at the anodic end of the capillary. Under these conditions, a strong EOF is detrimental because it drags the analytes toward the cathode; thus the BGE pH was kept at 3.5, which allows to have a negligible EOF. Complete baseline enantioresolution was achieved at all SβCD concentration values higher than 1%; increasing the SβCD concentration (up to 3%) decreased migration times, as well as increasing peak efficiency and thus resolution. When the SβCD concentration was increased above 3%, peak efficiency did not increase significantly, while the values of current were higher than 100 μA (85–90 μA were reached at 3% SβCD). For this reason 3.0% was chosen as the optimal SβCD concentration.

3.3. Choice of the internal standard

The use of an internal standard (IS) increases the reliability and precision of electrophoretic data. For this reason, compounds bearing some structural resemblance to BPR such as citalopram and triprolidine were tested as possible ISs. Since triprolidine has a migration time similar to that of the analytes under the final working conditions, it was chosen as the IS for all subsequent assays at a concentration equal to 10 μg/mL.

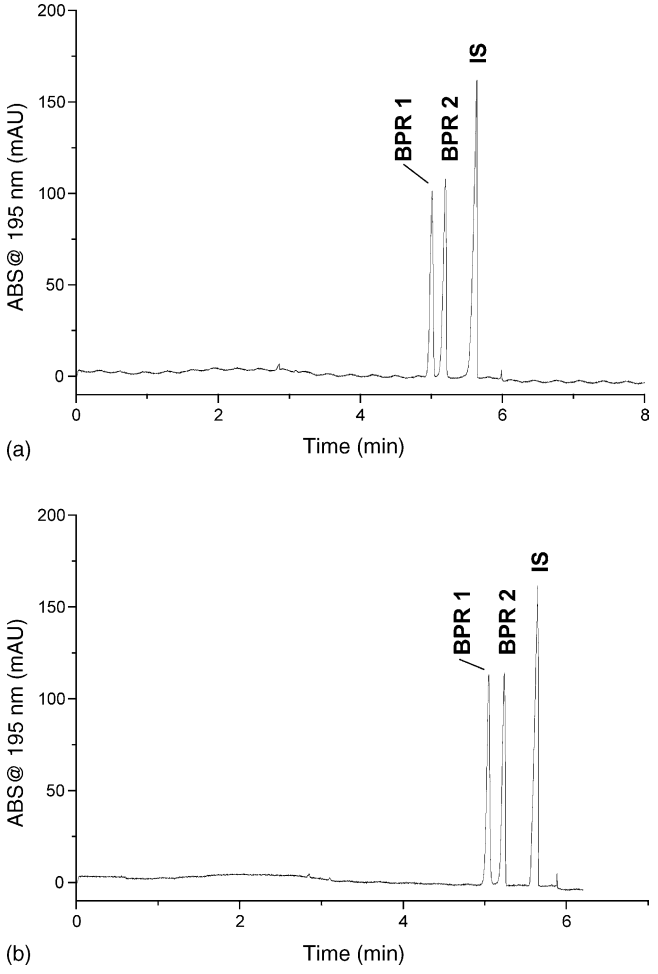


Fig. 2. Electropherogram of (a) a standard solution containing 20 μg/mL of racemic biperiden and 10 μg/mL of the IS; (b) a sample obtained from sustained release tablets containing a nominal racemic concentration of 20 μg/mL of biperiden. Electrophoretic conditions: uncoated fused silica capillary (48.5 cm total length, 40.0 cm effective length, 50 μm i.d., 375 μm o.d.); BGE: pH 3.5, 50 mM phosphate buffer containing 3% (w/v) SβCD; injection: pressure (50 mbar × 90 s) at the cathodic end of the capillary; applied voltage: 20 kV reversed polarity; detection wavelength: 195 nm (bandwidth: 10 nm); capillary temperature: 25.0 °C.

[1] Calculated using Advanced Chemistry Development (ACD) Software Solaris V8.14 (© 1994–2005 ACD).

3.4. Sample injection

Both voltage and pressure injections were tried. Reversed voltage polarity injection (20 kV, 5 s) gave rise to large peaks and allowed to detect concentrations as low as 0.1 μg/mL; repeatability, however, was poor. On the contrary, injection by pressure granted good repeatability even if much lower sensitivity was obtained. In order to increase sensitivity, the optimal injection time was determined by varying this parameter between 15 and 120 s (pressure was kept constant at 50 mbar). As expected, peak areas increased with increasing injection time; times longer than 90 s, however, caused efficiency and resolution to drop significantly. Thus, 90 s was chosen as the optimal injection time.

The electropherogram of a standard solution containing 20 μg/mL of racemic BPR and 10 μg/mL of the IS, obtained under the optimised electrophoretic conditions, is shown in Fig. 2a. As can be seen, peak asymmetry is quite low, analyte peaks are baseline separated and efficiency is good; a complete electrophoretic run lasts 7 min.

3.5. Analysis of standard solutions

The method thus developed was applied to standard solutions of BPR. Good linearity ($r_c > 0.9993$) was found over the 1–50 μg/mL concentration range (racemic concentration; 0.5–25.0 μg/mL for each enantiomer). The limit of quantitation (LOQ) was 1 μg/mL (racemic concentration), while the limit of detection (LOD) was 0.4 μg/mL (racemic concentration).

Precision assays at three different levels (1, 25 and 50 μg/mL) also gave good results, with R.S.D. (relative standard deviation) values always lower than 2% for both analyte/IS peak area ratios and migration times.

3.6. Extraction of the active principle from formulations

Preliminary assays allowed to ascertain that BPR could be quantitatively extracted from the sustained release tablets of Akineton® by a simple treatment with methanol (see Section 2). Dilutions with the same solvent, however, caused disruptive interactions with the electrophoretic system, so that no analyte peak was present in the electropherograms under these conditions. On the contrary, when the methanolic extract was diluted with a phosphate buffer (5 mM, pH 3.5), the resulting electropherograms were identical to those obtained injecting standard solutions prepared in the same solvent; no interference from inactive ingredients was found. Regarding the injectable formulation of Akineton, simple dilution with a phosphate buffer (5 mM, pH 3.5) proved sufficient for the purposes of quantitative analysis. In either case, the analytes were identified on the basis of both migration times of electropherograms and UV spectra obtained by means of the diode-array detector (DAD).

3.7. Application of the method to formulations

The extracts obtained from tablets and injectables were analysed by means of the developed capillary electrophoretic method. The electropherogram corresponding to an extract from sustained release tablets is shown in Fig. 2b. As can be seen, no interference from the matrix is present, the enantioseparation is complete. The electropherograms of samples from ampoules are superimposable to those obtained from tablets.

Table 1
Linearity parameters and LOD and LOQ values assessed on samples extracted from commercial formulations

Analyte	Linearity range (μg/mL)	Linearity equation, $y = a + bx$[a]		r_c	LOQ (μg/mL)	LOD (μg/mL)
		a[b]	b[b]			
BPR 1	0.5–25.0	−0.0131 ± 0.0021	0.0426 ± 0.0013	0.9994	0.5	0.2
BPR 2	0.5–25.0	−0.0120 ± 0.0025	0.0421 ± 0.0016	0.9998	0.5	0.2

[a] y is expressed as (analyte area)/(IS area), a pure number; x is expressed as μg mL^{-1}.
[b] Parameter value ± standard error.

Table 2
Validation parameters (amount found of declared, repeatability, intermediate precision) assessed on samples extracted from Akineton® tablets

Compound	Nominal concentration (μg/mL)	Amount found of declared (%)	Repeatability of analyte/IS area ratio (R.S.D.%)	Intermediate precision of analyte/IS area ratio (R.S.D.%)	Intermediate precision of migration times (R.S.D.%)
BPR 1	0.5	99	2.5	2.8	0.2
	10.0	102	1.9	2.1	0.4
	25.0	99	1.3	1.5	0.4
BPR 2	0.5	100	2.3	2.5	0.3
	10.0	101	2.1	2.2	0.4
	25.0	102	1.6	1.7	0.5
IS	10[a]	–	1.6[b]	1.8[b]	0.6

[a] Amount added.
[b] Precision of IS area.

Table 3
Validation parameters (amount found of declared, repeatability, intermediate precision) assessed on samples extracted from Akineton® ampoules

Compound	Nominal concentration (μg/mL)	Amount found of declared (%)	Repeatability of analyte/IS area ratio (R.S.D.%)	Intermediate precision of analyte/IS area ratio (R.S.D.%)	Intermediate precision of migration times (R.S.D.%)
BPR 1	0.5	101	2.5	2.6	0.3
	10.0	101	1.6	1.9	0.4
	25.0	99	0.6	0.8	0.3
BPR 2	0.5	99	2.5	2.7	0.3
	10.0	101	1.3	1.5	0.4
	25.0	102	0.8	1.1	0.4
IS	10[a]	–	1.7[b]	1.9[b]	0.7

[a] Amount added.
[b] Precision of IS area.

Table 4
Recovery and repeatability values for accuracy assays carried out on Akineton® tablets and ampoules

Compound	Concentration added (μg/mL)	Tablets		Ampoules	
		Recovery (%)	Repeatability (R.S.D.%)	Recovery (%)	Repeatability (R.S.D.%)
BPR 1	0.5	99	1.8	102	1.9
	5.0	101	1.5	101	1.8
	10.0	102	1.3	102	1.7
BPR 2	0.5	99	1.7	101	1.8
	5.0	102	1.6	100	1.7
	10.0	102	1.4	102	1.5

The method was validated in terms of linearity, precision and accuracy.

Good linearity was found on extracts from both formulations for both enantiomers ($r_c > 0.9994$) in the 1–50 μg/mL range (claimed racemic concentration). The LOD was found to be 0.2 μg/mL and the LOQ 0.5 μg/mL for each enantiomer. Linearity parameters can be found in Table 1. Precision assays carried out at three different nominal concentrations (1, 25 and 50 μg/mL, racemic) gave good results, with R.S.D. values always lower than 2.8% (analyte/IS area ratios) and 0.7% (migration times). The amounts of drug found of declared were in the 99–102% range for both tablets and injections. Thus, the formulations analysed are well within the limits given by the United States Pharmacopeia: 93–107% for tablets and 95–105% for injections [16]. The complete results of the assays on tablets are reported in Table 2 and those of the assays on ampoules are reported in Table 3.

Method accuracy was evaluated by means of recovery assays at three different concentration levels. The results of the recovery assays are reported in Table 4; they are very satisfactory, with mean recovery values ranging from 99 to 102%. The method is thus very accurate.

4. Conclusion

The electrophoretic method herein described has been used for the enantioseparation of BPR and the quantitative determination of its enantiomers in two different kinds of commercial formulations. The method uses SβCD as the chiral selector, detection at the anodic side and an acidic BGE to minimise the EOF. While method sensitivity is not outstanding (LOQ = 1 μg/mL of racemic BPR) due to the intrinsic limitations of the system (short optical path, injection by pressure), it is more than adequate for the purposes of the study. The method is quite rapid (simple extraction procedure, electrophoretic run shorter than 6 min) and feasible; furthermore, with respect to liquid chromatographic methods, it is also inexpensive and uses lower amounts of polluting and toxic organic solvents. Furthermore, precision and accuracy are satisfactory (R.S.D. < 2.8%, mean recovery 101.1%). Thus, the method seems to be suitable for the quality control of BPR enantiomers in commercial formulations. It should, however, be noted that only pure racemic BPR was available to us and not the single enantiomers; for this reason, it was not possible to assign a univocal identity to their electrophoretic peaks.

Since (+)-biperiden shows strong selectivity towards specific muscarinic receptor subtypes, enantioselective pharmacokinetic studies would be interesting, as well as the development of formulations containing an excess of one enantiomer, in order to obtain better therapeutical results. Thus, future developments of this study could involve the possible application of the method to the analysis of BPR enantiomers in biological fluids. We are currently developing original sample pre-treatment procedures for application to more complex matrices, such as plasma and urine.

Acknowledgements

Thanks are due to Abbott Italia S.p.A for providing pure biperiden for the development of this assay. This research

was financially supported by MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Italy – ex-60% funds).

References

[1] J.G. Hardman, L.E. Limbird, A. Goodman Gilman (Eds.), Goodman & Gilman's—The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, 2001, pp. 528–536.
[2] C. Medina, M.D. Kramer, A.A. Kurland, J. Am. Med. Assoc. 182 (1962) 1127.
[3] S. Avissar, G. Schreiber, Biol. Psychiatr. 26 (1989) 113.
[4] S.C. Sweetman (Ed.), Martindale: The Complete Drug Reference, 34th ed., Pharmaceutical Press, London, 2005, pp. 475–480.
[5] M. Eltze, V. Figala, Eur. J. Pharmacol. 158 (1988) 11.
[6] M. Waelbroeck, M. Tastenoy, J. Camus, R. Feifel, E. Mutschler, C. Strohmann, R. Tacke, G. Lambrecht, J. Christophe, Trends Pharmacol. Sci. 10 (1989) 353.
[7] V. Capka, Y. Xu, J. Chromatogr. B 762 (2001) 181.
[8] B. Koppenhoefer, A. Jakob, X. Zhu, B. Lin, J. High Res. Chromatogr. 23 (2000) 413.
[9] X. Zhu, B.C. Lin, A. Jakob, S. Wuerthner, B. Koppenhoefer, Electrophoresis 20 (1999) 1878.
[10] B.C. Lin, X. Zhu, U. Epperlein, M. Schwierskott, R. Schlunk, B. Koppenhoefer, J. High Res. Chromatogr. 21 (1998) 215.
[11] B. Koppenhoefer, U. Epperlein, R. Schlunk, X. Zhu, B.C. Lin, J. Chromatogr. A 793 (1998) 153.
[12] B.C. Lin, X. Zhu, B. Koppenhoefer, U. Epperlein, LC–GC 15 (1997) 40, 44–46.
[13] B. Koppenhoefer, U. Epperlein, B. Christian, B.C. Lin, Y. Ji, Y. Chen, J. Chromatogr. A 735 (1996) 333.
[14] B. Koppenhoefer, U. Epperlein, B. Christian, Y. Ji, Y. Chen, B.C. Lin, J. Chromatogr. A 717 (1995) 181.
[15] D. Zivanov-Stakic, O. Dzikinic, D. Agbaba, S. Vladimirov, Acta Pol. Pharm. 48 (3/4) (1991).
[16] The United States Pharmacopeia, 28th ed., United States Pharmacopeial Convention, Rockville, MD, 2005, pp. 261–262.
[17] European Pharmacopoeia, 5th ed., Council of Europe, Strasbourg, 2005, pp. 1101–1103.
[18] British Pharmacopoeia, The Stationery Office, London, 2004, pp. 260–261.
[19] A. Amini, Electrophoresis 22 (2001) 3107.
[20] S. Fanali, P. Catarcini, G. Blaschke, B. Chankvetadze, Electrophoresis 22 (2001) 3131.
[21] B. Chankvetadze, Capillary Electrophoresis in Chiral Analysis, John Wiley and Sons, New York, 1997.
[22] M.A. Raggi, R. Mandrioli, C. Sabbioni, C. Parenti, G. Cannazza, S. Fanali, Electrophoresis 23 (2002) 1870.
[23] S. Fanali, J. Chromatogr. A 875 (2000) 89.
[24] U. Schmitt, S.K. Branch, U. Holzgrabe, J. Sep. Sci. 25 (2002) 959.
[25] P.S. Bonato, Electrophoresis 24 (2003) 4078.
[26] C. Desiderio, S. Rudaz, M.A. Raggi, S. Fanali, Electrophoresis 20 (1999) 3432.
[27] R. Mandrioli, S. Fanali, V. Pucci, M.A. Raggi, Electrophoresis 24 (2003) 2608.
[28] The United States Pharmacopeia, 28th ed., United States Pharmacopeial Convention, Rockville, MD, 2005, pp. 2748–2751.