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Development and validation of a capillary zone electrophoretic method for the determination of atenolol in presence of its related substances in bulk and tablet dosage form

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Abstract

The development of a capillary zone electrophoresis method for determination of the drug atenolol in the presence of its related substances in bulk and in a tablet dosage form is described. The method was fully validated in terms of repeatability (RSDs for migration time and peak area of atenolol at 0.05 mg ml^{-1} were 0.25% and 0.52%, $n = 10$, respectively), reproducibility (RSD of peak area 0.84%, $n = 5$), linearity at two ranges of atenolol concentration, limits of detection and quantitation, ruggedness and robustness. The method was applied to the determination of the drug in a commercial tablet preparation (recovery 99.4%, m/m). The method proved to be fast and reliable for the quantitative analysis of atenolol in the presence of its related substances in bulk and pharmaceutical forms.

Keywords: Atenolol; Capillary zone electrophoresis; Drug analysis; Bulk and dosage forms; Method development; Method validation

1. Introduction

There is growing interest in the use of capillary electrophoresis (CE) [1] for the analysis of bulk drugs and pharmaceutical preparations [2–5]. High separation efficiency, selectivity, large separation capacity, flexibility and relatively low operational cost are the attractions of the technique [5]. Overall, CE has provided reproducible assays over the range from small molecules, including drugs, to macromolecules such as polypeptides, proteins and nucleic acids [6]. However, the quantitative aspects of CE, particularly in pharmaceutical applications, need to be explored more fully owing to the wide spread of parameters which can influence the analytical results. However, when these parameters are taken into account and optimized, the advantageous aspects as described above can be utilized in resolving complex component mixtures. The β-blocker drug atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy) phenyla-

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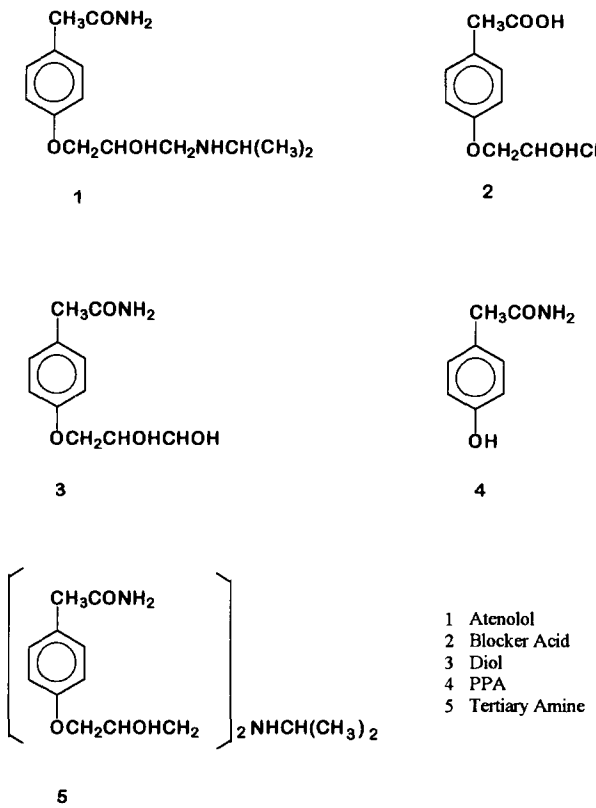

Fig. 1. Molecular structure of atenolol (**1**) and its principal related impurities (**2**–**5**).

cetamide (Fig. 1, structure **1**), is widely used in the management of hypertension, angina pectoris, cardiac dysrhythmias and myocardial infarction [7]. The commercial drug represents a typical mixture when taken alongside its four major related substances, which include the amino alcohol, acid and neutral species. There is, however, a pharmacopoeial method for assay of the bulk drug or pharmaceutical preparation, which is based on an HPLC assay which makes an allowance for not fully resolving all the components [8]. There has also been a recent addition to the available methods in the format of an ion-pairing reversed-phase HPLC method, which resolves atenolol and the principal related substances in bulk drug and dosage forms [9] and this is suggested to be more efficient than the pharmacopoeial method.

In the present work, the potential utility of CE was studied as an efficient alternative method for determination of atenolol in bulk drug and tablet dosage formulation by capillary zone electrophoresis (CZE). In order to achieve a robust, validated assay for atenolol, close attention was paid to the capillary conditioning, the optimization of buffer type, ionic strenth, pH sample stacking effects and sample introduction.

2. Experimental

2.1. CE conditions

An Applied Biosystems (ABI) Model 270A capillary electrophoresis instrument, equipped with a Hewlett-Packard Model HP3396A integrator (Hewlett-Packard, Waldbronn, Germany) was used throughout with uncoated fused-silica capillaries of 720 mm total length (500 mm to the detector) × 0.05 mm i.d. (Composite Metal Services, Worcester, UK). The capillary was kept at

a relatively constant temperature (25 ± 1°C) using a thermostated oven. In all experiments the detector was set at 0.05 aufs and with a rise time of 1.0 s. In addition, to show that the method was applicable (robust) on a different instrument, a Beckman P/ACE 2210 capillary electrophoresis instrument was connected to Beckman System Gold chromatography software on a PS/2 IBM PC, and used with an uncoated fused-silica capillary from Composite Metal Services of 570 mm total length (500 mm to the detector) and 0.05 mm i.d. Again, the capillary was kept at a constant temperature (25 ± 1°C), in this case using a thermostated liquid around the capillary. The detector range used was 0.01 aufs.

In all experiments, samples were introduced by hydrodynamic injection (either vacuum or pressure injection) at the anodic end of the capillary and detected by UV measurement at 226 nm (214 nm on the Beckman instrument where a wide bandpass filter was used). A mixture of sodium dihydrogenphosphate and sodium borate (50:50, v/v), each at 20 mM concentration, was used to prepare buffers in the pH range 6.0–10.0 in order to cover a wide pH range in the optimization experiments. Other conditions were as follows: applied voltage, 15 kV; temperature, 25°C; and optimum injection time, 2 s (except for examining the impurities, where the injection time was 5 s). Buffers were prepared freshly on a daily basis and filtered through a 0.45 μm membrane filter before use.

2.2. Materials and dosage form

Atenolol powder and its related substances were kindly provided by the British Pharmacopoeia Commission Laboratories (BPC, Middlesex, UK). Atenolol tablets (50 mg) were supplied by Evans Medical (Langhurst, Horsham, UK). Atenolol in the bulk form was kindly provided by Berk Pharmaceuticals (East Sussex, UK). All reagents used in this work were of analytical grade (BDH Poole, Dorset, UK). Methanol used for standard and sample solutions was of HPLC grade and obtained from Fisons (Fisons Scientific Equipment, Loughborough, UK). The four main related impurities of atenolol shown in Fig. 1 are present in the composite British Pharmacopoeial standard, and are blocker acid (**2**), diol (**3**), *p*-hydroxyphenylacetamide (PPA) (**4**) and tertiary amine (**5**).

2.3. Standard and sample solutions

2.3.1. Standard solutions

A standard solution of atenolol was prepared by dissolving 10 mg of BPC atenolol in 1 ml of methanol and adding the appropriate buffer to make 10.0 ml (concentration 1.0 mg ml^{-1}). Other atenolol standard solutions were prepared in the range 0.01–0.07 mg ml^{-1} by diluting this solution with buffer to the required nominal concentration. Standard solutions of each impurity was similarly prepared at 1 mg ml^{-1}.

2.3.2. Preparation of tablet sample solutions

Twenty atenolol tablets were weighed, powdered and ground with a mortar and pestle. An amount equivalent to one tablet was accurately weighed and transferred to a flask, 10 ml of methanol were added and the mixture was shaken for 10 min on a sonic bath. The mixture was then filtered into a 50 ml volumetric flask and buffer was added to volume to give a concentration of 1.00 mg ml^{-1} (one extraction was sufficient to give a 99.4% (m/m) recovery). To 5 ml of this solution in a 100 ml volumetric flask, buffer was added to volume, to obtain a nominal drug concentration of 0.05 mg ml^{-1}.

2.4. Optimization of capillary conditioning procedures

Initially a new capillary was washed for 1 h with 1 M NaOH and then for 0.5–1 h with distilled water, prior to use. The optimized procedures for routine capillary conditioning were as follows:

Daily wash cycles before starting experiments:

(1) 1 M NaOH	20 min
(2) distilled water	20 min

Wash cycles before each injection:

(1) 0.1 M NaOH	2 min
(2) running buffer	3 min

Daily wash cycles after finishing experiments at end of day:

(1) 1 M NaOH	5 min
(2) distilled water	5 min

For capillaries which would be stored for a period of time:

(1) 1 M NaOH	20 min
(2) distilled water	20 min
(3) flushed with nitrogen	10 min

3. Results and discussion

3.1. Method development

Accurate and precise quantitative results in CE depend on many parameters. These parameters include instrumental factors and operational variables, such as buffer composition and concentration, pH, voltage, temperature, injection parameters, sample concentration and composition, plus such technical matters as the need to level the buffer solution at the ends of the capillary to reduce further siphoning and stabilize migration times. In order to achieve robust and reliable methods with CE for subsequent validation and application to the analysis of pharmaceutical on a regular basis, the analyst has to consider these parameters and their effects on the analytical responses and results. In terms of the separation, preliminary experiments in this work showed that pH is crucial for the resolution of all five sample components. It was also shown that the choice of an inappropriate buffer at the optimized pH may affect the reproducibility of the method owing to ionic depletion [10]. In the method development experiments, a two-buffer system was used to allow as wide a range of pH as possible to be explored. The effect of pH (6.5–10.0) on the migration times of atenolol and its principal impurities is shown in Fig. 2. The experiments were completed with a constant buffer concentration and the increase in electroosmotic flow (EOF) was due to the addition of acid or base for pH adjustment, which leads to an increase in ionic strength and a slight increase in the EOF. From the data in Fig. 2, it can be seen that the best resolution was found at pH 9.7, using the mixed buffer of phosphate–borate (20 mM; 50:50, v/v) and applying a voltage of 15 kV (Fig. 3a). The peak efficiency of the atenolol peak at this optimum was found to be 3×10^5 theoretical plates for the capillary (ABI system).

The CZE method developed on the ABI CE unit was then validated and as part of the robustness testing the assay was transferred to the Beckman system (which uses pressure injection) with only a small number of changes in the operating parameters, associated with the shorter capillary length (applying a voltage of 20 kV and using a shorter capillary of length 570 (500) mm. From this, it was possible to demonstrate that a similar baseline resolution of the sample components was obtained after the instrument transfer and with a different method of sample introduction (Fig. 3b).

3.2. Mechanism of separation

Resolution of atenolol and its related substances is difficult by HPLC because of the similarity of their chemical structures (Fig. 1).

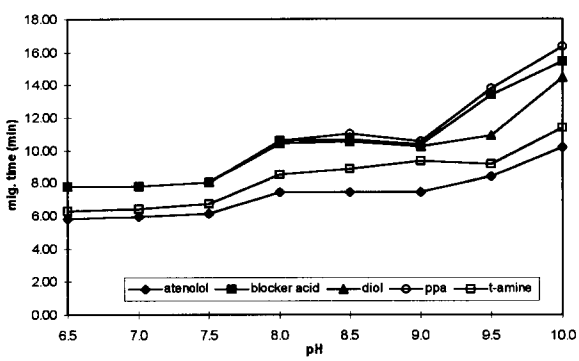

Fig. 2. Effect of pH on migration times of atenolol and its principal related impurities in the CZE method with the voltage and buffer concentration kept constant. Conditions: sodium phosphate (20 m*M*)–borate (20 m*M*) buffer; voltage, 15 kV; temperature, 25°C; detection wavelength, 226 nm (on the ABI CE unit).

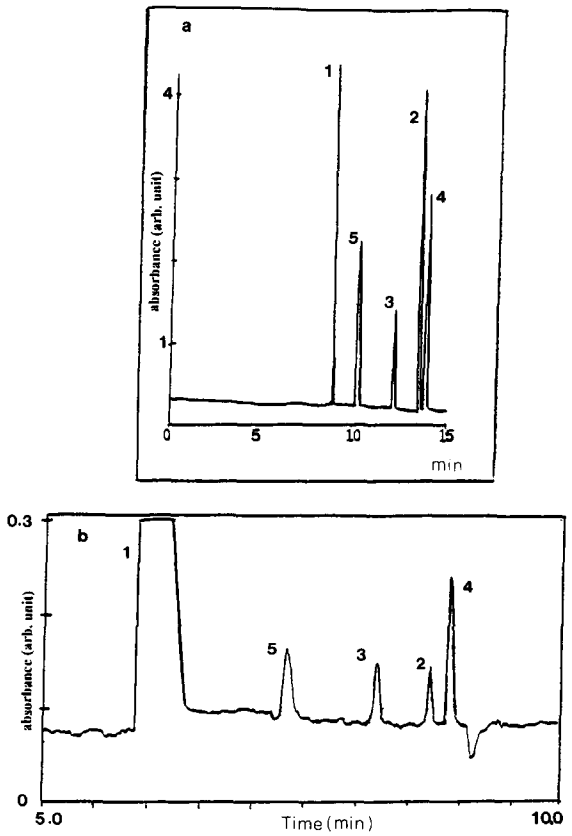

Fig. 3. Resolution of atenolol and its related substances (a) on the ABI CE unit, where the concentration of atenolol and the principal impurities is 50 μg ml^{-1}, and (b) on the Beckman CE unit, where the atenolol (Berk Pharmaceuticals) concentration is 50 μg ml^{-1} and the principal impurities (**2**–**5**) were spiked into the sample at ca. 5 μg ml^{-1}. The detection wavelength in the Beckman CE unit was 214 nm and the voltage was 20 kV. Other conditions as in Fig. 2.

However, small differences in their acidic and basic properties provide a basis for their resolution using a CZE method. Atenolol and the tertiary amine (**5**) are both weakly basic compounds and are therefore positively charged at pH values below 10. The PPA impurity (**4**) is a phenolic compound and is therefore negatively charged at high pH. The blocker acid (**2**) is, in fact, a zwitterion and is positively charged at low pH and negatively charged at high pH. The diol (**3**) is neutral over the pH range examined (6.5–10.0). The migration behaviors of these compounds are shown in Fig. 2. As noted above, the best resolution was achieved at pH 9.7 (Fig. 3a and b), where atenolol (**1**) and the tertiary amine (**5**) are positively charged, the diol (**3**) is neutral and the blocker acid (**2**) and PPA (**4**) are negatively charged.

3.3. Validation of the method

Although there is no official guideline for the validation of a CE method, the criteria for a valid chromatographic method have regularly been employed to validate CE methods [3,10]. The CZE method for quantitation of atenolol was validated in terms of repeatability, linearity (over two consecutive ranges of atenolol concentration), limit of detection (LOC) limit of quantitation (LOQ), reproducibility, ruggedness and robustness.

3.3.1. Repeatability

Ten consecutive injections of a standard solution of atenolol at a concentration of 0.05 mg ml^{-1} were preformed. The relative standard deviations (RSDs) of migration time and peak area were 0.25% and 0.52%, respectively (area normalization was not required in this assay, as there was considerable stability in both the migration times and peak areas). This excellent precision for peak area was only obtained in this work when great care was observed with the many aspects of sample introduction, capillary preparation and peak integration. This included regular checks on the sealing of all parts of the vacuum/pressure injection system, in order to reduce the possibility of errors in sample introduction. Alongside this is the initial choice of the hardware for CE assays, where some differences in injection mechanism exist. Optimization of the injection time should also be considered, since too short an injection time can cause problems due to viscosity feedback adjustments, while too large an injection can give solubility problems when stacking effects are involved. Another aspect is to ensure that differences in the heights of the collection and originating buffer reservoirs are kept to a minimum. This further reduces siphoning and therefore gradient effects which could otherwise lead to migration differences from run to run.

3.3.2. Linearity

The linearity of peak area measurement for atenolol was assessed over ranges of the sample concentration based on a target concentration of 0.05 mg ml^{-1}. In the first series, the concentration range was between 20 to 140% of the target concentration (0.01–0.07 mg ml^{-1} at 0.01 mg ml^{-1} intervals) with an absorbance range of 0.01 aufs. In the second series, the linearity of peak area measurement over a wider range of atenolol concentration (100–600% of the target concentration with an absorbance range of 0.1 aufs) was examined (0.05–0.30 mg ml^{-1} at 0.05 mg ml^{-1} intervals). Duplicate injections were made at each concentration in each case with the following results: first series, $A = 0.95x + 0.97$ ($r^2 = 0.9980$; $n = 7$); and second series, $A = 0.07x + 0.16$ ($r^2 = 0.9990$; $n = 6$).

3.3.3. Limits of detection and quantitation

The LOD for atenolol, on the basis of a signal-to-noise ratio of 3, was determined to be 1.0 μg ml^{-1} (the sample injection time was 5 s). In order to assess the background signal, initially ten consecutive replicates of buffer injection were made. In addition, the LOQ, based on a signal-to-noise ratio of 10, was found to be 3.5 μg ml^{-1}. For the four principal impurities, the LOD values were as follows: **2**, 1.2; **3**, 0.8; **4**, 0.9; and **5**, 1.4 μg ml^{-1}.

3.3.4. Reproducibility

In order to demonstrate the reproducibility of the method for the assay of a tablet pharmaceutical preparation, five tablet extracts were injected into the capillary in duplicate. The resultant RSDs for migration time and peak area were 0.25% and 0.63%, respectively, for atenolol.

3.3.5. Recovery assessment

The five tablet extracts and a standard solution of atenolol for single-point standard bracketing (prepared similarly) were analysed sequentially and the recovery was calculated (with respect to the nominal label strength) from the standard solution concentrations. The mean recovery of atenolol from tablets was found to 99.4% (m/m) ($n = 5$), assuming the label strength to be 50.0 mg per tablet.

3.3.6. Ruggedness and robustness

Preliminary experiments revealed that amongst the many operating parameters involved, the buffer pH is the most influential parameter on the repeatability of the method, when suitable precautions have been taken with regard to instrumental aspects of injection and capillary conditioning. the method was employed for two periods at 2 month intervals, using two different instruments and with two different operators. In these experiments, five standard solutions of atenolol (at 0.05 mg ml^{-1}) were assessed on each of two occasions and the results showed no significant statistical differences between operators or between instruments. The RSD values for migration time and peak area for the initial start time were 0.35% and 0.87% ($n = 5$), respectively, and for measurements at 2 months the RSDs were 0.28% and 1.07%, respectively.

3.3.7. Determination of the main drug in bulk and tablet dosage form

Five solutions of atenolol prepared from the bulk drug were compared with an equimolar solution of the BPC primary standard drug. In a similar experiment, five tablet extracts were compared with an equimolar BPC standard solution of atenolol which was carried through the same extraction procedure as for the tablets. The complete results are given in Table 1.

3.3.8. Detection of the principal impurities

In the solution of the bulk drug (Berk Pharmaceuticals) at 1.0 mg ml^{-1}, the major impurities, **3**

Table 1
Results of quantitation of atenolol in bulk and dosage form using the CZE method

Sample	Expected value (μg ml^{-1})	Observed value[a] (μg ml^{-1})	Error (%)
Bulk	50.00	50.10 ± 0.28	1.02 ± 0.56
Tablet	50.00	49.83 ± 0.40	0.997 ± 0.80

[a] The ranges were based on 95% confidence intervals (the significance level in this work was ± 5% and the confidence interval for the y intercept was 0.97 ± 0.06).

and **4**, were detectable above the limit of detection using the method on the ABI instrument. Assuming approximately equal response factors for atenolol and the impurities, the levels of each impurity relative to the integrated area count for the atenolol peak at 226 nm were 0.16% (m/m) for **3** and 0.18% (m/m) for **4**. Thus the proposed CE method can be used both to detect these impurities and in principle to quantitate them in the bulk drug at levels corresponding to those required for control purposes by the international compendia.

Three extracts of 50 mg tablets, corresponding to ca. 0.2 mg ml^{-1} in buffer, were injected into the Beckman CE system (injection time 5 s). No impurities were found in these extracts, indicating that, if present, the four principal impurities were at lower than the median LOD, i.e. less than ca. 0.2% (m/m).

3.4. Quantitative analysis

Quantitative calculations in this work were based on direct peak area measurements (without area/migration time t_m normalization). The peak area reproducibility in CE is critical for quantitative analysis and, typically, RSD values better than 1.5% ($n = 10$) can be obtained under well controlled conditions. Many of the factors discussed earlier can be controlled by the user, while few are completely instrument dependent (e.g. detector sensitivity). In this work, quantitation of atenolol in analytical samples was based on a calibration curve. For the related substances, however, the results were calculated on the basis of areas relative to the atenolol peak, because the amount of impurities available was not sufficient to make the requisite standard solutions for full validation.

An interesting aspect of quantitative analysis based on peak area in CE involves the different migration velocities of the solutes [11]. In such cases, normalization of peak areas with migration times in CE is required in order to quantitate impurity levels. The normalization procedure is relatively simple to perform and involves the division of each peak area in the electropherogram by its corresponding migration time. This peak area normalization is proposed as a method to be considered for the measurement of the impurities in cases where (unlike the present work) the variation of the migration time is relatively high.

4. Conclusions

The CZE method developed for the separation and quantitation of atenolol in the presence of its related impurities is rapid, reliable and robust and can be used as a routine method for the determination of this drug both in bulk drug and in tablet dosage forms. It has been demonstrated, however, that complete method optimization is essential in order to obtain a fully validated procedure which gives acceptable quantitative results, and that this should include consideration of instrumental and operational parameters. It has been shown that the fully developed and validated CZE method can be applied to the assay of atenolol in bulk drug and pharmaceutical preparations. In this case, the robustness and stability of the migration times in the developed method allows the assay to be carried out without the requirement of area/migration time normalization, or the need for an internal standard to aid statistical acceptance. However, when the robustness of the method is not satisfactory, or the method requires extraction from a difficult matrix, then area normalization and internal standardization may have to be considered to obtain adequate robustness.

Moreover, further validation data will be required to confirm the potential applicability of this highly selective method for the control of the principal related substances, and possibly additional impurities, in the bulk drug and dosage forms for compendial purposes.

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