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Generic, highly selective and robust capillary electrophoresis method for separation of a racemic mixture of glitazone compounds

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Abstract

A generic, highly selective, and robust capillary electrophoresis (CE) method was developed for separation of a racemic mixture of three available glitazone compounds (also known as thiazolidinediones) in active pharmaceutical ingredients (API) and tablets. The method separated the R and S enantiomers of balaglitazone, pioglitazone and rosiglitazone, and showed that the samples contained an equal (50:50) quantity of the enantiomers as a mixture. After a simple extraction of samples with acetonitrile:water (80:20), separation was performed using a combination of two cyclodextrins: sulfobuthylether-β-cyclodextrin (SB-β-CD) and dimethyl-β-cyclodextrin (DM-β-CD) in the electrolyte at pH 8.0. The method showed a very good specificity, and all separations were achieved with a resolution (Rs) over 3.0. The developed CE method was then validated. The Rs for the separations were 3.5 for balaglitazone enantiomers, 3.5 for pioglitazone enantiomers, and 3.7 for rosiglitazone. The squared correlation coefficients (r^2) were found to be 0.999 for all three compounds. The range of the CE method (injection volume was approximately 4 nl) was demonstrated to be from 1.0 to 2.4 ng. The R.S.D. in the repeatability study was found to be less than 0.5 for area/area ratio (and 3.0% for area) for all three compounds. The R.S.D. in the intermediate precision study was found to be less than 0.7 for area/area ratio (and 4.5% for area) for all three compounds. Generally, the method showed good robustness. Resolution between the enantiomers peak was maintained acceptable throughout the small variations around the pH value of the buffer, different capillary, CE instrument and electrolytes ion strength capacity, but changes in concentration of cyclodextrins and acetonitrile showed significant effects on separations and affected the resolution. The validation results showed that the CE method was suitable for separation of the racemic mixtures of the three glitazone drugs. The CE method was then applied for routine test during the drug and formulation development work of balaglitazone. Due to the achieved results from this work, it is the authors' belief that this method can easily separate other glitazone racemic mixtures.

Keywords: Balaglitazone; Pioglitazone; Rosiglitazone; Glitazone; Enantiomer separation

1. Introduction

During the development of a new drug there are a number of parameters which must be studied to ensure the quality of the drug and the product. The development of the active pharmaceutical ingredients (API), the pharmaceutical formulation, and the stability testing requires a great number of analyses. Capillary electrophoresis (CE) is recognized as a good analytical technique for pharmaceutical and biological applications [1–6]. The chiral separation of pharmaceutical drugs is one of the most important and frequently cited application areas of CE where good separations with high selectivity are routinely achieved. CE chiral method development can be rapid, and the methods tend to be more robust than their HPLC counterparts. Generally, the chiral separations of ionic solutes are achieved in free solution capillary electrophoresis (FSCE) by the addition of chiral selectors such as cyclodextrins (CDs) into the electrolyte. Recent reviews [7–10], and an entire volume of *Journal of Chromatography A* [11] have comprehensively covered the background theory and applicable equations required for chiral separation by CE.

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Chiral separations are based on the formation of diastereomeric complexes between the enantiomeric analytes and chiral selector added to the electrolyte solution. The mechanism of the complexes can be explained as follows: drug enantiomers can fit inside the cavity of the CD through a complexation and have an individual binding constant. If the enantiomers of a compound have different binding constants, then it is possible to chirally resolve them using CD addition into the running electrolyte. In high pH electrolytes the complexation of the drug enantiomers with the CD reduces the migration time of the negatively charged drug and the enantiomer with the strongest interaction with the uncharged CD will be detected first [12].

This paper describes development and performance (as validation) of a selective CE method using two cyclodextrins as chiral selectors for separation and determination of enantiomers of three available glitazone drugs, namely balaglitazone, pioglitazone and rosiglitazone. The glitazones (also called thiazolidinediones) are a class of drugs which reverse the insulin resistance seen in type 2 diabetes. The effect of the thiazolidinediones is mediated by the activation of a transcription regulator called peroxisome proliferator-activated receptor gamma (PPAR-gamma). This action modulates adipogenesis and carbohydrate metabolism in adipocytes and skeletal muscle. There are currently two licensed drugs in this class, pioglitazone (Actos®) and rosiglitazone (Avandia®). Balaglitazone is a new thiazolidinedione drug candidate and is under development by Dr. Reddys Research Foundation and Novo Nordisk A/S [13–15]. The structure of balaglitazone (5-[[4-[3-methyl-4-oxo-3,4-dihydro-2-quinazolinyl] methoxy]phenylmethyl]thiazolidine-2,4-dione), pioglitazone and rosiglitazone is shown in Fig. 1. As can be seen, all three compounds have an identical part in their chemical structure namely the glitazone (the right part of the molecule).

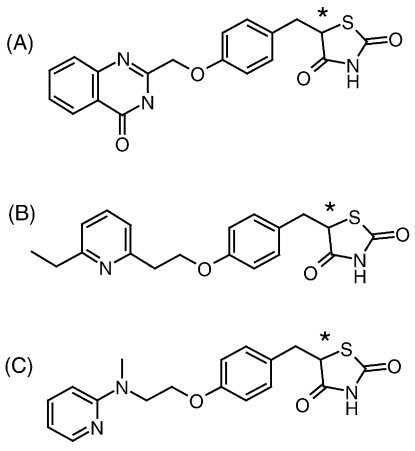

Fig. 1. Structure of the three glitazones: (A) balaglitazone, (B) pioglitazone and (C) rosiglitazone. The marked carbon atom with the star (*) shows the chiral center.

During the development of a synthesis method, the formulation of the tablets and stability studies for balaglitazone, a test was need to control the quantity of the R and S enantiomers as the chemistry of the drug points to a complete 50:50 equilibrium of the R and S enantiomers. To be able to separate and quantify the R and S enantiomers of balaglitazone (and later pioglitazone and rosiglitazone) in samples (API and tablets), a generic, highly selective, and robust CE method with UV detection was developed. As part of the documentation, the method was validated according to official guidelines [16,17]. The developed method was then applied as a routine method for demonstration of stable 50:50 equilibrium of the R and S enantiomers in samples from synthesis, formulation and stability testing during the drug and formulation development work.

There are very few publications on analytical work of the pioglitazone or rosiglitazone. These works only focus on quantitative determination of these compounds in pharmaceutical or bio samples, but none could be found that focus on separation of the enantiomers. The developed CE method showed a very good ability on separation of the tested glitazones. Due to the achieved results from these separations, it is the author's belief that this method can easily separate other glitazone racemic mixtures.

2. Experimental

2.1. Chemicals, materials, reagents, and solutions

Purified water (water) was obtained from a Milli-Q system, acetonitrile (ACN), HPLC grade (e.g. Rathburn), 0.01 M and 0.1 M sodium hydroxide, NaOH (e.g. diluted sodium hydroxide 1.0 M, Cat. No. LAB00330, from Bie & Berntsen, Copenhagen, Denmark), Sulfobutylether-β-cyclodextrin (Advasep, Purity 100% anhydrate, CyDex (SB-β-CD)), dimethyl-β-cyclodextrin (Cyclolab, Hungary, 98% purity (DM-β-CD)), Sodium dihydrogen phosphate-monohydrate (Merck), balaglitazone standard, API and tablets (CMC Development Novo Nordisk A/S, Maaloev, Denmark), pioglitazone API (as HCl, from Jiangsu Wujin Hutang Secondary Fine Chemical Plant, China) and tablets (Actos®), rosiglitazone API (as maleate, hydrate salt, from SUN Pharmaceutical Industries Ltd., India) and tablets (Avandia®).

2.1.1. Preparation of solutions

Buffer (25 mM phosphate buffer pH 8.0 for manufacture of buffer electrolyte): in a 200 ml measuring flask, 690 mg sodium dihydrogen phosphate-monohydrate was dissolved in approximately 180 ml water. The pH value was adjusted to 8.0 with sodium hydroxide (e.g. 40%) and filled to the mark with water.

Buffer-electrolyte (for manufacturing of running electrolyte): the buffer-electrolyte consisted of two cyclodex-

trins (2.0% SB-β-CD/0.7% DM-β-CD (w/v)) dissolved in 25 mM phosphate buffer pH 8.0. Preparation: 2.0% SB-β-CD + 0.70% DM-β-CD (w/v) was prepared as follows: 200 mg SB-β-CD and 70 mg DM-β-CD were dissolved in 10.0 ml 25 mM phosphate buffer pH 8.0. The solution was then filtered through a filter (approximately 0.45 μm).

Electrolyte for electrophoresis (running electrolyte); 10:90 (v/v), ACN/buffer-electrolyte (already containing 2.0% SB-β-CD/0.7% DM-β-CD (w/v)); 9.0 ml buffer-electrolyte added 1.0 ml ACN. The CE vials (e.g. 2.0 ml size HP 5181-3375) were filled (approximately 1.0 ml) with electrolyte. The working life of the electrolyte is 6 months when stored in a freezer at around −20 °C.

2.2. Instrumentation

Capillary electrophoresis was carried out using an Agilent Technologies ^{3D}CE system (Agilent Technologies). Data acquisition and signal processing were performed using Agilent Technologies ^{3D}CE ChemStation (rev. A.06.03, Agilent Technologies).

The capillary was an 80.5 cm (72.0 cm efficient length) 50 μm inner diameter "Extended Light Path Capillary" Agilent T. capillary HP part no. G1600-62232. UV detection was performed at 225 nm (16 nm Bw, Reference 350 nm and 80 nm Bw). The Auto sampler temperature was room temperature (approximately 21 °C).

Preconditioning (for new capillary): new capillaries were flushed with 1.0 M NaOH for 20 min followed by 20 min with 0.1 M NaOH. Preconditioning (for used capillaries): used capillaries were conditioned for 10 min with 0.1 M NaOH, then flushed with purified water for 10 min. Capillaries were rinsed after each sample run with 0.1 M NaOH and then water (flushed for 1 min with each). Hydrodynamic injection at 40 mbar for 5.0 s (approximately 4 nl) was used. The voltage was +30 kV (50 μA approximately). The capillary temperature was 30 °C, and runtime was 20 min.

2.3. Sample preparation

The analysis concentration for samples was approximately 500 μg/ml for all compounds calculated as the free acid. The API samples were dissolved in acetonitrile:water (80:20). The tablets were transferred to the measuring flask and acetonitrile:water (80:20) was added. The samples were stirred for approximately 10 min, centrifuged at 4000 rpm (with rotating radius of 15 cm) for 10 min, and were then transferred directly to a CE-vial (ready for the analysis).

3. Results and discussion

3.1. Development of the CE method

The original target of the task was separation of the R and S enantiomers of balaglitazone. Later, the target was expanded and separation of the R and S enantiomers of pioglitazone and rosiglitazone was also included.

Good and stable migration was achieved for all three compounds using high pH buffers as the electrolyte (running buffer), but there was no enantiomer separation. Phosphate buffer at concentration 25 mM at pH 8.0 as electrolyte showed a very nice peak shape, a stable baseline (and current) and could be reproduced injection after injection without any problems.

The effect of cyclodextrins type, cyclodextrins concentration, and the addition of organic modifier to the electrolyte was then investigated. A number of different cyclodextrins were tested, alone and in combination, to obtain the best separation. These studies showed that a combination of sulfobutylether-β-cyclodextrin and dimethyl-β-cyclodextrin gave an excellent separation of the R and S enantiomers. Then the concentration of the two cyclodextrins in the electrolyte was optimized, and found to be 2% for SB-β-CD and 0.7% for DM-β-CD. The next parameter which needed optimization was the long migration time of balaglitazone, pioglitazone and rosiglitazone enantiomers. During the development of the method, acetonitrile was used as the modifier but this needed more investigation and optimization. The effect of adding an organic modifier to the electrolyte was tested, and best results were achieved using acetonitrile. Acetonitrile at concentration of 10% (v/v) in electrolyte was found to be the best choice as organic modifier to make the migration times shorter.

It was interesting that the separation of the racemic mixture could not be achieved using only one of the cyclodextrins at the optimized concentration. It was clear that a combination of these two was necessary. The best explanation for this was founded in two published papers [18,19]. SB-β-CD is negative charged and DM-β-CD is neutral (no charge). It seems that there are two different enantioselective retention/migration mechanisms possible with CD. (A) In the reversed phase mode, migration is mainly due to the hydrophobic inclusion complexation, while enantioselectivity also requires hydrogen bond and steric interactions at the mouth of the CD cavity. (B) The polar-organic mode where acetonitrile occupies the hydrophobic cavity and the analyte is retained via a combination of hydrogen-bonding and dipolar interactions at the mouth of the CD. Steric interactions can also contribute to chiral (and racemic) separation. Knowing these mechanisms the conclusion was that each cyclodextrin must have a complex with each of the racemic compound and as result a separation of these was achieved.

During the development and pre-validation stage, it was demonstrated that both rinse and pre-conditioning of the capillary improved the resolution, precision, and robustness of the method.

The method was fully optimized and very good separation was achieved for the enantiomers of all three compounds. The API and tablet samples were then investigated for R and S enantiomers.

3.2. Validation

The validation was performed as a part of the development work with respect to selectivity, linearity, range, precision as repeatability and intermediate precision, and robustness of the method with focused on separation. A short review of the validation results is described below.

3.2.1. Selectivity

The method showed a significant degree of selectivity for all three separations. The enantiomers were beautifully separated with good resolution in all samples (Fig. 2). The enantiomers of all three compounds were chirally resolved but the sets of enantiomers co-migrated. The Rs for the separations were 3.5 for balaglitazone enantiomers, 3.5 for pioglitazone enantiomers and 3.7 for rosiglitazone enantiomers. The results also confirmed that balaglitazone, pioglitazone and rosiglitazone in the tested APIs and tablets contained a 1 to 1 mixture (50:50) of the R and S enantiomers. The peak heights were not equal, but the areas were identical. Unfortunately, it was not possible to identify the peaks because it was not possible to synthesis the R or S enantiomer of balaglitazone, and, the single R or S enantiomer of pioglitazone or rosiglitazone was not available commercially.

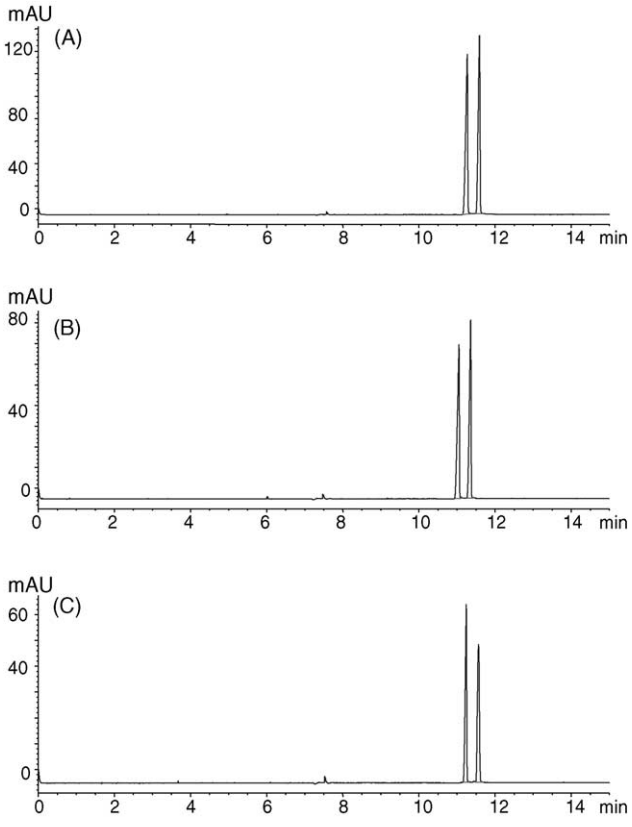

Fig. 2. Separation of the enantiomers of (A) balaglitazone, (B) pioglitazone and (C) rosiglitazone. The area of peaks on all electropherograms shows equilibrium (50:50).

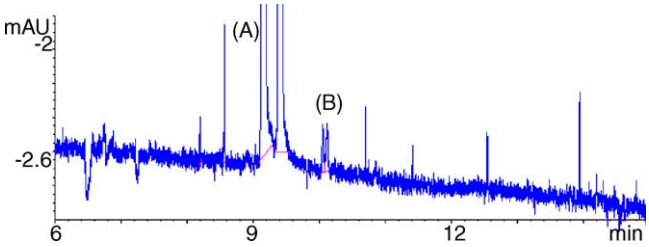

Fig. 3. Separation of the enantiomers of balaglitazone (A) and an impurity/degradation product (B) with glitazon structure. The area of peaks of double peaks for (A) and (B) shows equilibrium (50:50).

The performance of the method for separation and detection of known impurities/degradation products was shortly examined. A slightly degraded API sample of balaglitazone contained a know degradation product with glitazon structure was injected in the system. The result showed that the CE method have a good selectivity and resolution for detection of this impurity and could also separate the enantiomers of this impurity (Fig. 3). No further investigations were preformed as there was no interest for qualifying and using of this CE method as a purity method due two reasons: (1) a good purity method already exist for the API and the drug product and (2) in this method impurities with glitazone structure will appear as double peaks and others with non glitazone structure with one peak. This could make data process complicated.

3.2.2. Linearity and range

Calibration curves for balaglitazone, pioglitazone and rosiglitazone were prepared using a standard stock solution of each compound. Linearity was tested at five points from 50 to 120% of the nominal concentration of samples. Three injections were preformed at each point. The squared correlation coefficients (r^2) were found to be 0.999 for all three compounds. The linearity for the compounds was found to be good and acceptable. The range of the CE method (injection volume was approximately 4 nl) was demonstrated to be from 1.0 to 2.4 ng.

3.2.3. Precision as repeatability and intermediate precision

The precision of the method was investigated as repeatability and intermediate precision. Both studies were calculated using the total area of the enantiomers and the % area/area ratio. The repeatability was estimated as within the lab variation whereas the intermediate precision included the variation due to different groups in same lab conditions. The results obtained from different groups were totally independent (different lab technicians, running buffer, CE systems, another capillary, etc.).

The repeatability of the method was demonstrated by the relative standard deviations of the determinations and % area/area ratio. This was performed as six single determinations from one prepared solution (split in six portions/vials) at 100% level of the test concentration.

The intermediate precision of the method was studied as follows: on 2 different days, different lab technicians prepared a test solution (one new sample preparation at each day) and performed six single determinations from the prepared solution (split in six portions/vials) at 100% level of the test concentration. The analyses were performed on two different CE instruments using two different batches of capillary. The intermediate precision of the method was demonstrated by the relative standard deviations of the determinations performed in the repeatability and intermediate precision study.

The R.S.D. in the repeatability study for the determination of the area was found to be less than 3.0% and for % area/area ratio less than 0.5% for all three compounds. The R.S.D. in the intermediate precision study for the determination of the area was found to be less than 4.5% and for % area/area ratio less than 0.7% for all three compounds. The obtained repeatability and intermediate precision of the method was found to be good and satisfactory.

3.2.4. Robustness of the separation

The robustness of the method was examined during the development of the method and as a routine part of the validation focused on the resolution (Rs). Parameters such as the concentration of the cyclodextrins, acetonitrile, different capillaries, different instruments and electrolyte ion strength capacity were examined. The resolution between the enantiomers was determined in order to evaluate the separation.

Generally, the method showed good robustness. Resolution between the enantiomers peak was maintained acceptably throughout the small variations (±5%) around the pH value of the buffer, different capillary, CE instrument and electrolytes ion strength capacity, but changes in the concentration of cyclodextrins and acetonitrile showed significant effects on separations and affected the resolution.

4. Conclusion

A generic, highly selective, and robust capillary electrophoresis method was developed for separation of the racemic mixtures of three available glitazone (also known as thiazolidinediones) compounds in active pharmaceutical ingredients and tablets.

The method can separate the R and S enantiomers of balaglitazone, pioglitazone and rosiglitazone and shows that samples contain an equal quantity (50:50) of the enantiomers as a mixture. Good and acceptable method performance was obtained for all validation points. Enantiomers of balaglitazone, pioglitazone and rosiglitazone were separated with excellent resolution, and quantified with good precision. Unfortunately, it was not possible to identify the peaks because it was not possible to synthesis the R or S enantiomer of balaglitazone and single R or S enantiomer of pioglitazone or rosiglitazone was not available commercially. The developed method was then applied for demonstration of stable 50:50 equilibrium of the R and S enantiomers of balaglitazone in samples from drug and pharmaceutical formulation development.

The developed CE method showed a very good ability on separation of the tested glitazones. Due to the achieved results from these separations, it is the authors' belief that this method can easily separate other glitazone racemic mixtures.

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