Capillary electrophoresis method for the determination of the $\beta 3$ adrenergic receptor agonist mirabegron in pharmaceutical formulations

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Abstract

A capillary electrophoresis method was developed for the determination of mirabegron in pharmaceutical formulations. Separation was effected by stacking on a 50cm capillary (I.D 50µm) using 50mM acetate buffer, pH 4.0 with UV detection at 249nm. In calibration, the area under the curve of mirabegron was corrected with migration time (approx. 3min) and the calibration curve is found to be linear over the concentration range 5.00-45mg/L. The purpose method was validated as per the ICH guidelines. The method was accurate, precise, specific and found to be suitable for the quantitative analysis of the drug in dosage form. Moreover, in contrast with liquid chromatography, the presented method does not make use of organic solvents such as methanol and acetonitril meeting todays requirements for low-cost and sustainable assays. Combined with mass spectrometer detector, the method has potential for clinical analysis of mirabegron in blood.

Keywords: mirabegron, tablets, buffer, capillary electrophoresis

Introduction

Mirabegron ((R)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide) is a potent and selective human β3-adernoceptor agonist and is the first of a new class of compound for the symptomatic treatment of overactive bladder[1]. Additionally, studies carried out at the Capitol Region of Denmark, Cardiac Center suggest that the drug also has potential for cardiovascular stimulation; clinical trials are ongoing[2]. Mirabegron is sold in Europe as Betmiga in 25mg and 50mg prolonged-release tablets. A literature survey reveals that several methods for mirabegron determination and its metabolites in human plasma using LC-DAD and LC-MS/MS but to our knowledge, a method using capillary electrophoresis has yet to be published[3]. The presented study is for the determination of mirabegron in 25mg tablets although same procedure may be extended to cover 50mg dosage.

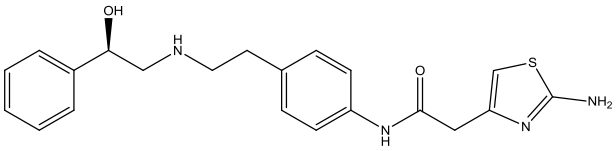

Figure 1: Structure of mirabegron. In buffered solution (pH 4) the mayor specie is constituted by the protonated secondary amine and the imine (reference see text).

The use of capillary electrophoresis (CE) methods for pharmaceutical analysis has become increasingly more popular in recent years. The wide range of applications for which its use has proved successful includes assay of drugs, determinations of drug-related impurities, physicochemical measurements of drug

molecules and the analysis of pharmaceutical excipients[4].

In CE, analytes migrate through an electrolyte solution under influence of an electric field. The separation takes place in a glass capillary of fused silica (quartz) with a typical length of 30-60 cm and an inner diameter of 50-75μm. The ends of the capillary are placed in separate buffer chambers containing the same buffer – running buffer. Separation takes place by applying a current in the range of 5-30kV. At the detector side, a small optical window in the capillary makes it possible to detect the analytes - usually by UV-VIS. Positive charged ions will migrate towards the cathode, while negative charged ions will move to the opposite site. Normally, the cathode is placed on the detector side even though it may be turned around if appropriate.

The separation principle in CE is based on the difference in size-to-charge and promotes the analytes to move with different rate in an electric field. The rate (V_{ep}) is dependent on the charge (q) and the hydrodynamic radius (r) and the applied field (E) and the viscosity (η) of the running buffer.

The majority of drugs are basic and therefore ionized at low pH. Free solution capillary electrophoresis is popular and involves the use of simple buffered aqueous electrolytes to separate a range of basic drugs based on the analyte size and the number of positive charges whereas neutral compounds do not migrate through the detector. At low pH phosphate buffer has been used to analyse more than 550 different basic drugs[5] and a similar method has been validated for the analysis of basic drugs, excipients and raw materials[6].

Pharmaceutical analysis is dominated by liquid chromatography (LC). Other techniques include IR and UV spectrometry which are often used for identity testing of pharmaceutical and a range of flask-based method such as titration. The advantages of CE for pharmaceutical analysis include its speed and cost of analysis, reduction of solvents and possibility of a rapid method development. CE instruments like LC can be coupled to a variety of detector types including mass spectrometry.

Materials and Method

The mirabegron reference was bought from Cayman Chemical and stored at -20^{o}C until use. Acetic acid and sodium acetate trihydrate (Ph. Eur. grade) were bought from Sigma-Aldrich. All solution was constituted from MilliQ (MilliPore). Mirabegron tablets (Betmiga, 25mg, Astellas Pharma Europe) were a gift from Capitol Region of Denmark.

Buffers
50mM acetic acid was mixed with 50mM sodium acetate making the running buffer with pH 4.0. The running buffer is diluted 10 times and used to dissolve and dilute stock, standards and tablets.

Stock and standard solutions
Stock solution was made from 100mg mirabegron reference and dissolve in 100mL 5mM buffer by sonication. Stock solution is stored at 4°C (1 week); Standards are made by dilution of 5mM buffer and filtrated (0.45µm) on the day of use.

Analysis
Twenty tablets of mirabegron were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 25mg mirabegron was transferred into 100mL volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume with 5mM buffer. The mixture is filtrated through 0.45µm membrane filter. From the above solution 500µL aliquot was taken into a separate 5mL volumetric flask and diluted addendum with 5mM buffer and mixed well. Running conditions are collected in table 1.

BUFFER	50mM acetate, pH 4.0, filtered 0.45µm
CAPPILLARY	50cm (48cm to detector), 50µm I.D. Before each run the capillary was flushed with 0,1M NaOH and rinsed with running buffer.
INJECTION	Split flow injector
VOLTAGE	15kV (60-70µA)
POLARITY	Positive at injection end (inlet)
TEMPERATURE	Ambient
DETECTION	UV-DAD 249nm
INSTRUMENT	AGILENT CE systems G1600AX with ChemStation

Table 1: CE Running conditions

Results

The goal of this study was aimed at developing a sensitive, precise and accurate CE method for the analysis of mirabegron in pharmaceutical dosage form as per the ICH guidelines[vii] although we would like to have open window to extent the method for clinical use in blood. We therefore sought not only to develop a rapid method but make separation of matrix compounds more likely. It is possible to shorten the run by almost two minutes by applying a higher current (figure 2, supplementary data).

Parameters	**Values**
Concentration range [mg/L]	5.00 – 45.00
Slope (a)	1.035±0.004
Correlation coefficient (r^2)	0.9996
Intercept (b)	-0.038±0.13
LOD [mg/L]	0.9
LOQ [mg/L]	3.1
Effectivity, N	129.000

Table 2: Optical and system suitability parameter. LOD and LOQ is based on the slope of the calibration curve

The calibration curve with 95% confidence interval is depicted in figure 3, supplementary data.

API	**Mirabegron**
Labeled doses	25 mg
Amount proposed	25.2±0,2mg
Recovery	101%

Table 3: Amount proposed by the method is calculated from 6 determinations with 95% confidence interval.

Concentration [mg/L]	**5,000**	**25,00**	**45,00**
Cor. area [mAU/min]	5,213	26,03	46,68
%RSD	0,55	0,43	0,34

Table 4: Repeatability is measured by 9 determination covering the specific range for the procedure (3 concentrations/3 replicates).

Discussion

At pH 4.0, the calculated Bjerrum diagram suggests that the mayor specie is constituted by the protonated secondary amine and imine in the thiazol ring (>76%)[viii]. Initially, we have tried at higher pH and different buffer concentrations, but migration times ware significantly altered between runs. Depletion of buffer is a common problem in CE and tends to change sufficiently after several runs to alter migration times, especially when buffers at low ionic strength are used. At pH 4 migration times are consistently ca. 3 min.

Mirabegron exhibits stereoisomerism due to the presence of one chiral center. The R enantiomer has been used in the manufacture of the finished product. The enantiomeric purity cannot be controlled by CE under normal conditions. However, using separation buffer containing chirale additives such as sulphated cyclodextrines (HSCD's)[ix], it is likely to distinguish between the two forms. To our knowledge, no paper has yet been published about the topic yet no doubt remain that the enantiomeric purity is controlled routinely by the producer - most likely using chiral LC.

Conclusion

Our research adds mirabegron to the pallet of application for CE. Here we present a method that offers a number of advantages over LC and other analytical techniques: analysis and method development speed, reduced consumable and solvent expenses, simplicity of operation. The results indicate that the described method can be used for quantitative analyses of the compound in dosage forms. Goal is now to continue our research to expand the method to make it applicable for clinical blood samples.

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Supplementary data

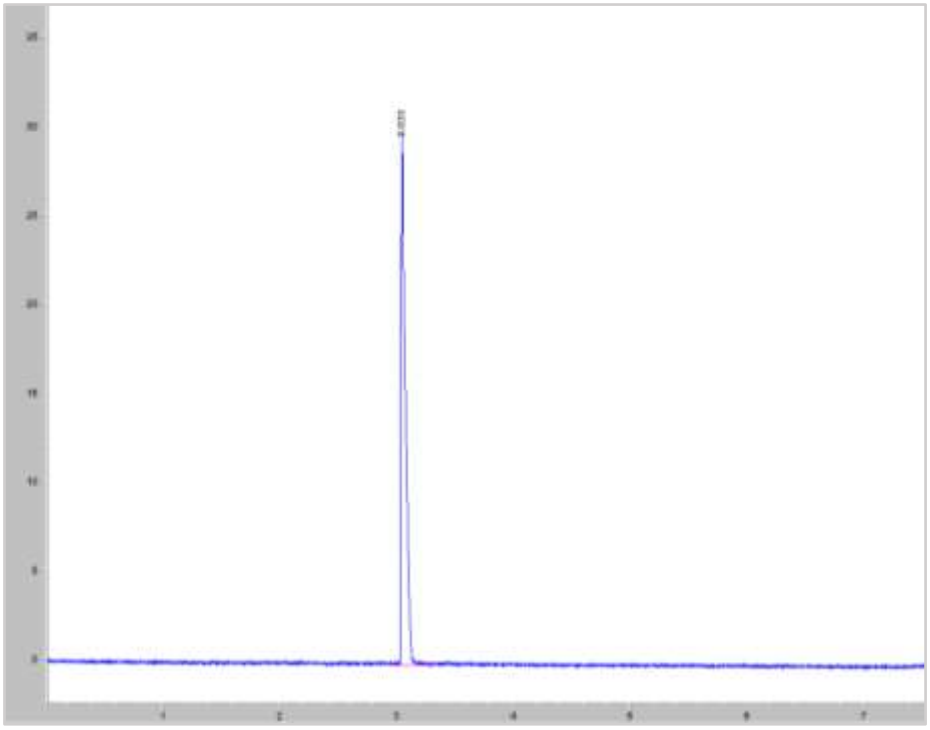

Figure 2: Electropherogram of sample of mirabegron (tablet (25mg) depicting good specificity of the method. No impurities or degradation products are like vice detected.

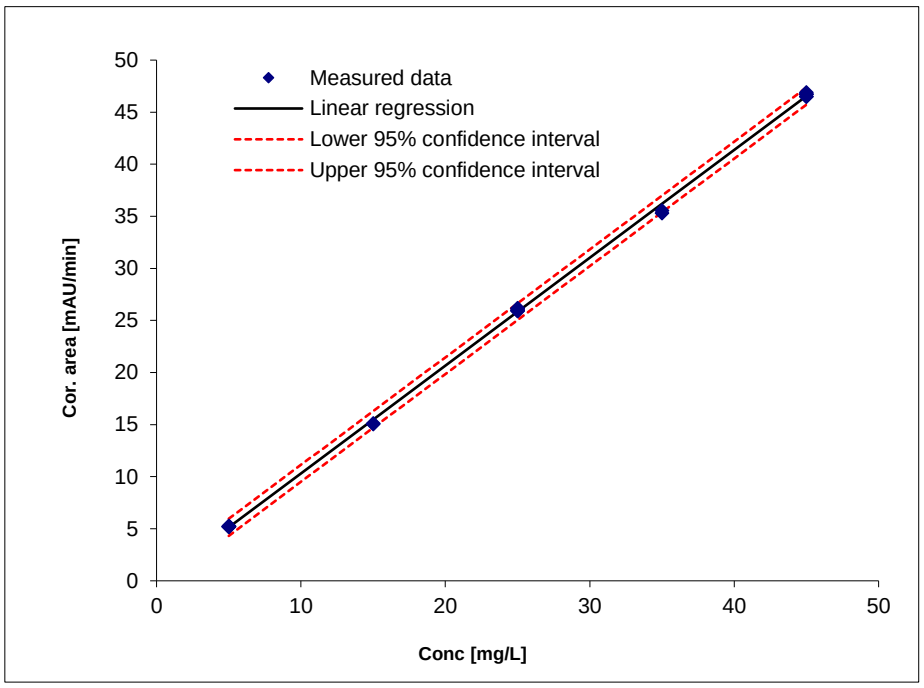

Figure 3: Calibration plot including 95% confidence intervals. Areas are corrected in respect to migration times.

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