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Precision evaluation of chiral capillary electrophoretic methods in the context of inter-instrumental transfer: Constant current versus constant voltage application

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ABSTRACT

Capillary electrophoresis (CE) is an electrophoretic separation technique that was rapidly increasing in popularity some years ago and that led to high expectations. Because of their different separation mechanisms, CE and HPLC are alternative and complementary separation techniques. Chiral molecules can be directly separated with CE by simply adding a chiral selector to the running buffer solution, leading to flexible and cheap methods. Major drawbacks of capillary electrophoretic separation methods are, however, the lower precision compared to HLPC methods and a more problematic analytical method transfer. Both above stated disadvantages limit the generalized use of CE methods in the pharmaceutical industry. Multiple solutions have been suggested to improve the precision of CE methods. In this work the application of a constant current during the electrophoretic separation instead of the more commonly used application of a constant voltage was studied on two CE instruments with different cooling mechanisms. This was done in the context of optimizing method transfer conditions. A constant current may reduce the generation of heat in the capillary and the consequentially radial and axial temperature fluctuations that both negatively influence the precision of the peak areas, migration times and resolutions of a CE method. The repeatability and time-different intermediate precision of both electrophoretic separation modes were compared on two different CE instruments after a successful analytical method transfer. The chiral separations of three beta-blockers, propranolol, sotalol and betaxolol, were used as test cases. A constant current led to a general improvement of the repeatability and time-different intermediate precision of the relative Area Under the Curve of all three beta-blockers, while that of the migration times remained rather constant. It also led to more similar electropherograms than the application of a constant voltage.

1. Introduction

Capillary electrophoresis (CE) is regularly used nowadays for the separation of a broad range of compounds, especially large entities or macromolecules, such as viruses and proteins, besides drug molecules. CE is an alternative and complementary technique to liquid chromatography (LC). In contrast to LC, CE has not been used that widely in the analytical chemistry for several reasons. The major drawback to use CE to a broader extent is that it is a less precise and less robust technique than LC [1]. Precision evaluation is a part of analytical method validation and according to the ICH guidelines it "expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample". Precision is considered at three levels: repeatability, intermediate precision (time-, equipment-, analyst-, and calibration-different) and reproducibility [2].

Another reason for the less generalized use of CE methods is that their analytical method transfer is far more complex. This results

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from larger instrumental differences and the fact that compared to LC, where the used mobile phase and column play a key role in the separation process, much more factors influence the separation in a CE method. Although successful analytical method transfers between multiple laboratories have been reported [3,4] several inter-laboratory studies have experienced precision problems or suffered from drop outs caused by instruments not complying with the system suitability limits specified by the protocol [5,6]. An improvement of the precision, combined with well-defined transfer protocols, which take into account potential instrumental differences, could enhance the wider use and improve the successful analytical transfer of CE methods [7].

Many studies have focused on the low precision, high response variability of CE methods and defined optimization solutions [1,5,8,9]. As described by Mayer [8] the global precision of a CE method is determined by the variability on migration times (MT) and peak areas (AUC). The variability of the MT is caused by the variability of the electroosmotic flow (EOF), the electrophoretic mobility of the compounds, the capillary re-equilibration and conditioning processes and the capillary temperature. The variability of the AUC is determined by injection-volume, diffusion, wall-interaction, peak-integration, MT and temperature fluctuations. Thus one of the major factors, both influencing AUC and MT variability, is the capillary temperature. The capillary temperature control is considerably affected by a phenomenon called Joule heating. This occurs when the heat generation inside the capillary is higher than the heat transfer to the environment. Countering the Joule heating is the main challenge when controlling the capillary temperature.

Heat generation, dH, is caused by the current which goes through the capillary. The higher the electro-conductivity of the buffer ions and/or the applied voltage, the higher the heat generation will be [10].

Heat dissipation on the other hand happens mostly by diffusion, which is higher at the capillary walls than in the center of the capillary. When the diffusion of heat is lower than the heat generation, increased temperatures, a decreased buffer viscosity (resulting in a reduced electrical resistance) and an increased ionic mobility will be present in the center of the capillary. This leads to a more parabolic flow profile and bandbroading [11]. Joule heating thus eliminates the initial benefit of the flat flow profile of the EOF. Besides the radial fluctuation there is also axial temperature fluctuation because heat dissipation at the capillary ends is larger than in the middle of the capillary. Increasing heat dissipation and avoiding excessive heat generation are therefore prerequisites to reduce the temperature variability and/or gradients and hence to improve the global precision of a CE method [12].

Multiple solutions have been suggested to improve the precision of CE methods or to correct for it such as the use of an internal standard, the reduction of the injection volume variability and the use of optimal rinsing steps to avoid wall interaction, to maintain a constant mobility of the analytes and a constant EOF. These measures [1,5,8,9] led to a general improvement of the precision of CE methods. However, an adequate capillary temperature control remains necessary to avoid temperature fluctuations and less precise MT, AUC, peak widths and resolutions.

A capillary electrophoretic separation can be conducted by either applying a predetermined and constant voltage, current or power. As stated by Altria and Fabre [5], the precision of a CE method can be improved by applying a constant current instead of a constant voltage. The advantage of working with a constant current is that the increase of the current, and consequently the heat generation, in the capillary, which occurs when applying a constant voltage, is disabled. A drawback of this approach that has been reported is the lower time-different intermediate precision of the MT and AUC caused by a higher between-day variability of the applied voltage when keeping the current constant.

The aim of this study was to examine the repeatability $\left(S_r^2\right)$ and the time-different intermediate precision $\left(S_{I(t)}^2\right)$ of a constant current and an equivalent constant voltage method. In a second phase, the study was repeated on an instrument with among other instrumental differences another type of capillary cooling. An analysis of variance (ANOVA) was used to estimate S_r^2 and $S_{I(t)}^2$. Comparison and analysis of the time-different intermediate precisions, repeatability and between-day variances of both approaches was done. Three beta-blockers, propranolol, sotalol and betaxolol, were chirally separated by a CE method with carboxymethyl β-cyclodextrine as chiral selector and used as test cases [13,14]. However, it should be noted that improvement of the initial precision of the test cases by for instance reducing the applied current or voltage, working with longer capillaries or lower conductivity BGE were not investigated in this study.

This study is part of a broader one in which it is tried to define method transfer guidelines which should increase the successful analytical method transfer rate, both between laboratories using a same instrument and between different instruments. These method transfer rules we will try to derive will originate from studying and optimizing the method precision on the one hand and the method robustness on the other. Transfer rules derived from a robustness study will be described elsewhere [15]. In the actual paper we focus on the method precision.

2. Material and methods

2.1. Chemicals used

Phosphoric acid (H_3PO_4) (85% m/m) was from Acros Organics (Thermo Fisher scientific, Geel, Belgium), triethanolamine (TEA) was purchased at Laboratoria Flandria (Gent, Belgium). The ultrapure water was made in-house by a Sartorius Arium® pro UV system (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Sodium hydroxide (NaOH) 0.1 M and 1 M solutions were purchased from Fisher Scientific (Leicestershire, UK). Sodium carboxymethyl β-cyclodextrine with a substitution degree of ±3.5 was acquired from Cyclolab (Budapest, Hungary).

The samples substances used were racemic mixtures of propranolol·HCl (Fluka, St. Gallen, Switzerland), sotalol·HCl (Sigma, Steinheim, Germany) and betaxolol·HCl (gift with unknown origin). The sample concentrations were 50 μg/ml. The samples were dissolved in ultra-pure water and placed in a Branson 5210 ultra-sonic bath (Danbury, USA) during 10 min for sonication.

Phosphate solutions with a concentration of 100 mM were prepared in ultra-pure water. The pH of the phosphate solution was measured with an Orion glass electrode (Ankersmid, Wilrijk, Belgium) and adjusted to 5.2 by addition of TEA. The solution was filtered through a 2 μm Supor® 200 polyethersulfon filter (Pall Corporation, Zaventem, Belgium) and sonicated for 10 min.

The BGE used during the electrophoretic separation consisted of 10 mM sodium carboxymethyl β-cyclodextrine dissolved in the above prepared phosphate solution. The BGE was mixed well, sonicated for 10 min and stored cool (<4 °C). The BGE vials were replenished after 3 runs to avoid electrolysis.

2.2. Instrumental set-up

Experiments were performed on a P/ACE capillary electrophoresis system from Beckman Coulter (Fullerton, USA) equipped with a diode-array detector. The detection wavelength was set at 210 nm. An uncoated fused silica (TSP, Molex, USA) capillary with an internal diameter of 50 μm, an effective length of 40 cm and a total length of