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Inter-instrumental method transfer of chiral capillary electrophoretic methods using robustness test information

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ABSTRACT

Capillary electrophoresis (CE) is an electrodriven separation technique that is often used for the separation of chiral molecules. Advantages of CE are its flexibility, low cost and efficiency. On the other hand, the precision and transfer of CE methods are well-known problems of the technique. Reasons for the more complicated method transfer are the more diverse instrumental differences, such as total capillary lengths and capillary cooling systems; and the higher response variability of CE methods compared to other techniques, such as liquid chromatography (HPLC). Therefore, a larger systematic change in peak resolutions, migration times and peak areas, with a loss of separation and efficiency may be seen when a CE method is transferred to another laboratory or another type of instrument. A swift and successful method transfer is required because development and routine use of analytical methods are usually not performed in the same laboratory and/or on the same type of equipment. The aim of our study was to develop transfer rules to facilitate CE method transfers between different laboratories and instruments. In our case study, three β-blockers were chirally separated and inter-instrumental transfers were performed. The first step of our study was to optimise the precision of the chiral CE method. Next, a robustness test was performed to identify the instrumental and experimental parameters that were most influencing the considered responses. The precision- and the robustness study results were used to adapt instrumental and/or method settings to improve the transfer between different instruments. Finally, the comparison of adapted and non-adapted transfers allowed deriving some rules to facilitate CE method transfers.

1. Introduction

Because capillary electrophoresis offers performance and automation at levels similar to HPLC, it is commonly used in the pharmaceutical drug analysis. The capillary electrophoretic separation mechanism differs from that of HPLC, making both techniques complementary. CE was a rapid developing technique but it did not met the high expectations and, unlike HPLC, it became a less widely used analytical technique due to several drawbacks. The best known are the lower precision and the more complicated method transfer compared to HLPC [1]. However, CE also has multiple advantages; it is a low cost, fast, flexible and highly efficient technique and is applicable for a whole range of compounds, including chiral separations. CE is a very flexible enantioselective technique due to the applicability of a large variety of chiral selectors, available at a lower cost than the chiral columns used in HPLC. The high peak efficiency, the possibility of performing direct chiral separations and the consumption of only small volumes of solvents and selectors, also render it an economically and environmental-friendly technique [2,3].

The common application of chiral analytical methods entails frequent method transfers. The transfer of an analytical method is a documented process that qualifies the receiving laboratory/instrument to successfully perform a method that was developed and validated elsewhere, and is an inevitable part of the method life-cycle [4]. The main aim of an analytical method transfer (AMT) is to guarantee that the receiving laboratory/instrument is able to implement the procedure and obtain similar results, with a similar experimental error, as the developing laboratory/instrument. According to the literature, prerequisites for a fast

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and successful AMT are a clear pre-approved protocol, containing detailed information about the participating laboratories and instruments, predefined system suitability test (SST) limits and a rationale for the chosen factors of the method [4–6]. Cornerstones for a successful AMT are well-thought pre-transfer studies, such as robustness tests and precision studies [7]. As mentioned higher, the AMT is more complicated for CE than for HPLC methods. This has several reasons, such as the existing instrumental differences of the commercially available CE instruments and the strong impact of the CE instrumental settings on the separation. For an HPLC method transfer, a broader analytical experience is available and a better knowledge of the instrumental differences is known. Moreover the number of factors influential on the separation is more limited [8,9]. This is resulting in a higher inter-laboratory study success rate [10]. This is not the case for CE, because this technique is more sensitive to small changes in instrumentation and separation characteristics are often combinations of different instrumental factors, such as the hydrodynamic injection volume that is defined by injection pressure and injection time and also depends on the capillary temperature and its total length [11]. The higher response variability of CE methods and the larger number of factors influencing the separation process are beside the instrumental differences elements complicating the AMT of CE methods [12].

This work focused on the investigation of the critical major instrumental differences and the use of robustness testing data as well as precision knowledge to facilitate AMT [13]. The CE instrumental differences are, among others, capillary cartridges, syringe pumps, sample trays, vial caps, capillary cooling systems, data collection and detectors and their settings [14].

The standard applied detector settings can have a large influence on the responses as our group has reported before [15]. However detector settings are not covered in this study exactly because several can be identified and studied [15]. Our intention is to examine them separately in a later study and then finally combine the knowledge from a prior study on the application of a constant current by De Cock et al. [13], from this study and from the study on the detector settings in one method transfer strategy. Consequently, most probably factors like sampling rate and bandwidth will have to be added to such transfer protocol. The actual study was, however, mainly focused on achieving similar electropherograms, maintaining the separation, by practically adapting the known critical parameters from our studies and the knowledge of some instrumental differences. The capillary cartridge determines the total and effective lengths of the used capillary. Effective lengths can be directly transferred between different types of CE instruments, while total capillary lengths cannot. Differences in total capillary length influence both the applied electric field and the injection volume [16,17]. The syringe pump creates the pressure used during sample injection, rinsing and capillary preconditioning steps. Injection and rinsing volumes are determined by both the time and pressure applied by the syringe pump. CE instruments have specific ranges of pressure application. In some cases the required pressure from the development instrument might be out of range of the receiving instrument or its applicable pressure is fixed. In the above cases, the time has to be adjusted adequately to obtain identical injection volumes [11]. Moreover, the ability of the instruments to reach exact and repeatable pressures may differ. A stable and identical injection volume is an absolute necessity for both good analytical practice and method transfer [12]. This fact also applies to the rinsing and preconditioning volumes. Another instrumental difference involves the capillary temperature control set-up; this can be performed either by forced air circulation or liquid cooling. Good temperature control is crucial to maintain a stable temperature inside the capillary and to obtain repeatable results [11]. The capillary temperature also determines the buffer viscosity, both affecting the migration times (MT) of the molecules and the injection volume and also influences reaction kinetics that may occur inside the capillary, such as the interaction with chiral selectors [14,18].

Identification of critical instrument-different parameters was performed by robustness testing in this work. Robustness is the ability of a method to remain unaffected by small but deliberate variations in experimental conditions without observing major differences in the considered response [19,20]. Robustness testing is part of the method validation process and was initially introduced at a late stage of method validation, since inter-laboratory transfer/reproducibility testing is only executed in the final stage. Nowadays it is performed much earlier, to avoid reoptimalisation and revalidation of a non-robust method [21,22]. The aim of the robustness test is to examine the sources of variability for one or several responses of the method. The sources/factors leading to critical effects are to be strictly controlled in an inter-laboratory transfer, and occasionally to be adapted if AMT fails. Therefore robustness testing should be performed to avoid problems in subsequent inter-laboratory studies. The different steps of a robustness test are: selection of the factors and their levels, selection of an experimental design and the considered responses, execution of the experimental work and finally calculation and interpretation of the factor effects. The particular selection of the factors examined during this study did included both instrumental differences, such as injection times, injection pressures, applied currents and capillary temperatures, and methodological parameters, such as chiral selector concentration and rinsing step volumes [23,24]. A (schematic) overview of the different steps performed during a robustness test is shown in Dejaegher et al. [19].

The aim of this study was to improve the transfer of CE methods by recognizing the critical instrumental differences and experimental factors that should be strictly controlled/adapted during AMT. The identification of the instrumental differences and critical factors was accomplished by transferring a CE method to two types of instruments and performing robustness tests on both instruments. The used test components were three racemic β-blockers; propranolol, sotalol and betaxolol. The chiral separation method was chosen to have a simple, 2 peak, test case with different degrees of separation and was based on a study by Fillet et al. [24] which was adapted during a preliminary study.

2. Materials 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 ultra pure water was made in-house by a Sartorius Arium® pro UV system (Sartorius Stedim Biotech, Goettingen, Germany). Sodium hydroxide (NaOH) 0.1 M and 1 M solutions were from Fisher Scientific (Leicestershire, UK). Sodium carboxymethyl β-cyclodextrine with a substitution degree of ±3.5 was acquired from Cyclolab (Budapest, Hungary).

The used sample substances were racemic mixtures of propranolol·HCl (Fluka, St. Gallen, Switzerland), sotalol·HCl (Sigma, Steinheim, Germany) and betaxolol·HCl (gift with unknown origin). The samples were daily prepared in ultra pure water at a concentration of 50 μg/ml 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 required amount of phosphate was measured by weighing to keep the ionic strength at the given conditions as constant as possible. The pH was measured with an Orion glass electrode (Ankersmid, Wilrijk, Belgium) and adjusted