ABSTRACT

Capillary Electrophoresis (CE) is an official separation technique in the 9th edition of the European Pharmacopoeia (EurPh9), in which separation takes place inside a thin glass capillary filled with an electrolyte solution; it can be used to separate both ionized and non-ionised analytes by introducing proper additives into the electrolyte solution, or by using only the electrosmotic migration. Among the CE techniques the most commonly used in drug analysis are: **capillary zone electrophoresis (CZE)** and **micellar electrokinetic capillary chromatography (MEKC)**.

The **advantages** of using CE in the analysis of pharmaceutical substances are related to the rapid method development, complete automation of the analysis cycle, relatively low cost of consumables, low consumption of reagents and analytes and to the possibility of using different detection systems.

In the present, EC is considered to be a viable alternative and a complementary method to the more widely used high performance liquid chromatography (HPLC) techniques, in the analysis of pharmaceuticals.

The habilitation thesis presents aspects related to the use of CE in the analysis of substances of pharmaceutical interest, and is structured in three distinct chapters based on the addressed research directions:

1. CE applications in chiral analysis of pharmaceutical substances;

2. CE applications in drug analysis of fixed combinations;

3. CE applications in the analysis of chemically-related drug substances.

CE represents a valuable method in the chiral analysis of pharmaceutical substances with advantages related to the high chiral resolution, relatively short analysis time, rapid development of analytical method, low consumption of reagents, analytes and chiral selectors, and especially with the high flexibility in choosing and changing the chiral selector. Also, in CE, a direct method of analysis is generally used, by simply dissolving the chiral selector in the electrolyte solution. Among many chiral selectors usable in CE, by far the most efficient are cyclodextrins (CD), cyclic oligosaccharides, having a hydrophilic external surface and a hydrophobic internal cavity in which they can incorporate different analytes by hydrophobic interactions.

Chiral CE analysis was applied to achieve chiral resolution of compounds of various classes of pharmaceuticals with demonstrated enantioselective properties: β-blockers (carvedilol, sotalol), H1 antihistamines (brompheniramine, chlorpheniramine, cetirizine, promethazine), proton pump inhibitors (omeprazole, pantoprazole). A comparative study of these compounds has been carried out in order to understand the analyte-chiral selector complex interaction mechanisms responsible for the enantiomeric discrimination. We also performed chiral separation in the case of serotonin reuptake inhibitors

(fluoxetine), non-thiazide diuretics (indapamide), anorexigenic amphetamines (sibutramine), calcium channel blockers (amlodipine) and antipsychotics (asenapine).

As a chiral selectors CD derivatives were used, performing a complex CD screening of several native and derivatized, neutral and ionized CDs in order to establish the optimal chiral selector for each enantioseparation. The optimization of the analytical conditions was done using the "one factor at time" technique or in some cases by experimental design. We evaluated the analytical performances of the optimized methods, verifying the repeatability, precision, linearity, accuracy, robustness and by determining LOD and LOQ of the enantiomers. The developed methods have been applied for the determination of the enantiomers of the studied substances from pharmaceutical forms.

The chiral separation of optically active drugs has become a necessity in both pharmaceutical industry and bioanalysis; the development of new chiral separation techniques is and will continue to be an important research topic; therefore the developed methods can be used successfully in the preliminary chiral analysis of the selected substances.

Modern trends in therapy imply more and more frequently the use of fixed dose combinations, offering potential benefits regarding the increasing patient compliance with drug treatment, improving therapeutic effect and diminishing individual adverse effects.

CE analysis was applied mainly for the determination of analytes from combinations used in cardiovascular medication: calcium channel blocker (amlodipine) + angiotensin II receptor antagonist (telmisartan); angiotensin II receptor antagonist (telmisartan) + thiazide diuretic (hydrochlorothiazide); calcium channel blocker (amlodipine) + HMG-CoA inhibitor (atorvastatin); HMG-CoA inhibitor (atorvastatin) + cholesterol absorption inhibitor (ezetimibe). We also performed separations of β-lactam antibiotics (amoxicillin + clavulanic acid), antituberculotic agents (isoniazid + rifampicin) and analgesics (paracetamol + tramadol).

In the case of fixed combinations, separation generally targets two substances having completely different electrophoretic behaviors, the challenge being to establish optimal electrophoretic conditions in which both substances are ionized and exhibit different electrophoretic mobilities. The developed methods were optimized, validated and were applied to the analysis of the studied substances from fixed dose combination pharmaceutical forms.

The utility of developing methods for the simultaneous determination of compounds from the same therapeutic class is questionable, since in therapy the co-administration of two or more chemically related substances is rare. But lately this approach is gaining more and more ground; the advantages of developing generic methods applicable to a large number of compounds without the need to develop individual methods for each compound being more than obvious.

CE analysis was applied for the simultaneous determination of β-lactam antibiotics (penicillins,

cephalosporins), fluoroquinolones, H1 antihistamines and hypolimenants (statins, fibrates). In this type of separations, the substances have very similar physico-chemical properties and therefore similar electrophoretic behaviors. In many cases, analytes could not be separated by CZE due to similar electrophoretic mobilities, but MEKC has proven its utility in separating chemically related substances. The developed methods have been optimized and validated, being applied to the determination of certain substances from pharmaceutical forms.

The results presented in this habilitation thesis demonstrate the applicability and the potential of CE methods in the modern analysis of pharmaceuticals.