CAPILLARY ELECTROPHORESIS IN THE ANALYSIS OF HYPOLIPIDEMIC AGENTS

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ABSTRACT: Hypolipidemic agents are a diverse group of pharmaceutical substances used in the treatment of high levels of lipids and lipoproteins in the blood, used currently for the prevention of cardiovascular diseases. Capillary electrophoresis is a relatively new separation technique which is gaining more and more momentum in the analysis of pharmaceutical substances, including hypolipidemic agents like statins and fibrates. This review presents the applicability of different capillary electrophoresis techniques in the analysis of certain hypolipidemic agents.

Keywords: hypolipidemic agents, fibrates, statins, capillary electrophoresis, pharmaceutical analysis

INTRODUCTION:
Coronary heart disease remains one of the major causes of mortality in the world. Epidemiological studies have repeatedly shown that coronary heart disease tends to be associated with hyperlipemia, more precisely with elevated levels of low-density lipoprotein (LDL) cholesterol and triglycerides and with low levels of high-density lipoprotein (HDL) cholesterol (Epstein, 1996).

Dependence between cholesterol levels and mortality, and the positive influence of cholesterol-lowering effects on decreased progression of cardiovascular disease, are both clearly established; therefore, cholesterol-lowering therapy is essential for the prevention of the progression of cholesterol-loaded plaques in vessel (Gordon et al., 1989).

Hyperlipidemias may be elevated as a result of an individual’s lifestyle, by lack of exercise and consumption of a diet containing excess saturated fatty acids, but also can be related to an inherited gene defect in lipoprotein metabolism or, more commonly, to a combination of genetic and lifestyle factors (Scirica et al., 2005).

Appropriate lifestyle changes in combination with drug therapy can lead to a decline in the progression of coronary plaque, regression of preexisting lesions, and reduction in mortality due to coronary heart disease.

The clinically important lipoproteins, listed in decreasing order of atherogenicity, are LDL, very-low-density lipoprotein (VLDL) and chylomicrons, and HDL. The occurrence of coronary heart disease is strongly associated with high total cholesterol, and even more strongly with elevated LDL cholesterol in the blood. In contrast to LDL cholesterol, high levels of HDL cholesterol have been associated with a decreased risk for heart disease. Recommendations for the reduction of LDL cholesterol to specific target levels are influenced by the coexistence of coronary heart disease and the number of other cardiac risk factors. Patients with LDL levels higher than 160 mg/dL and with one other major risk factor, such as hypertension, diabetes, smoking, or a family history of early coronary heart disease, are candidates for drug therapy (Berger et al., 2015).

There are several classes of hypolipidemic drugs, but the most frequently used in modern therapy are the HMG-CoA inhibitors (statins) and fibric acid derivatives (fibrates). Other drugs with hypolipidemic properties are: niacin, bile acid sequestrants (fibrates) or cholesterol absorption inhibitors (ezetimibe). Clinically, the choice of an agent or another will depend on the patient’s cholesterol profile, cardiovascular risk, and the liver and kidney functions, evaluated against the balancing of risks and benefits of the medications (Luc, 2000).

HYPOLIPIDEMIC DRUGS
Statins are widely used in the treatment of patients with pure hypercholesterolaemias and mixed dyslipidaemias while fibrates are used to treat hypertriglyceridaemias and mixed hyperlipidaemias (Fruchart et al., 2004).

Statins also known as HMG-CoA reductase inhibitors act by competitively inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, a key enzyme which catalyzes the conversion of HMG-CoA to mevalonol, a critical intermediary in the cholesterol biosynthesis. Because statins exhibit structural similarities with HMG-CoA on a molecular level, they will fit into the enzyme’s active site and compete with the native substrate; this competition reducing the rate by which HMG-CoA reductase is
able to produce mevalonate, the next molecule in the cascade that eventually produces cholesterol (Duriez, 2000).

Statins can be grouped into fermentation-derived (native) and chemically synthetized (synthetic) derivatives. Lovastatin, the first statin introduced in therapy, was obtained after fungal fermentation from the fungus Aspergillus terreus. Simvastatin and pravastatin are semisynthetic derivatives; as simvastatin was obtained by synthesis from lovastatin, while pravastatin was produced by microbial hydroxylation of mevastatin. Native and semisynthetic statins have in common the hexahydronaphtalene ring which is substituted by a α-methylbutyric acid ester and a β-hydroxy-δ-lactone, connected by an ethylene bridge. The compounds differ from one another by the positions of the methyl groups on the hexahydronaphtalene ring or side chain. Fluvastatin, atorvastatin, and rosuvastatin are completely synthetic compounds. The only structural part common to all statins is the lactone ring, which binds to HMG-CoA reductase responsible for the inhibitory action; the common elements of the synthetic statins are the fluorophenyl side chain, an isopropyl substituent and a residue of heptanoic acid (Endo, 2010, Sirtori, 2014).

Although all statins share a common mechanism of action and structural component that is very similar to the HMG portion of HMG-CoA reductase, they differ in terms of their chemical structures. The structural characteristics of the statins currently used in therapy are presented in Figure 1.

Fig. 1 Chemical structures of the statins used currently in therapy

Statins exist in two forms, lactone and open-ring hydroxy acid forms. Lovastatin and simvastatin are prodrugs administered as lactone prodrugs and subsequently transformed to active metabolites while all the other statins, are formulated in the pharmacologically active β-hydroxy acid form (Pedersen et al., 2001).
Fibrates activate PPAR (peroxisome proliferator-activated receptors), a class of intracellular receptors that modulate carbohydrate and fat metabolism and adipose tissue differentiation, inducing the transcription of a number of genes that facilitate lipid metabolism. Fibrates reduce plasma triglyceride levels by altering the expression of numerous genes coding for proteins involved in fatty acid metabolism (fatty acid transport protein, acyl-CoA synthetase, etc.) and also by increasing the lipoprotein lipase synthesis and decreasing the apolipoprotein C-III synthesis (Duriez, 2000).

Fibrates present phenoxy-2-methylpropionic acid or 5-phenoxy-2,2-methylpropionic acid structure, being derivatives of fibrac acid derivatives. Fibrates are amphipatic molecules having a hydrophobic end and a hydrophilic substituent characterized by a terminal carboxyl group (-COOH) which in the case fenofibrate and clofibrate is esterified with an alkyl group (Tenenbaum, 2012).

The structural characteristics of the fibrates currently used in therapy are presented in Figure 2.

**Fig. 2 Chemical structures of the fibrates used currently in therapy**

**CAPILLARY ELECTROPHORESIS**

Capillary electrophoresis (CE) is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage. There are six types of capillary electroseparation available: capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MEKC), capillary electrochromatography (CEC), capillary isoelectric focusing (IEF), and capillary isoelectric focusing (IEF). Taking in consideration the characteristics of the substances used in pharmaceutical practice, usually CZE and MEKC are the most frequently used techniques in drug analysis (Anastasos et al., 2005).

CZE, also known as free solution CE, is the most commonly used technique; in this technique the separation is based on the differences in electrophoretic mobility of the analytes. The velocity at which the ion moves is directly proportional to the electrophoretic mobility and the magnitude of the electric field (Suntornsuk, 2007).

MEKC is a separation technique that is based on solutes partitioning between micelles and the solvent; in this technique a surfactant is added to the background electrolyte in a concentration higher than the critical micellar concentration; hydrophobic molecules will spend the majority of their time in the micelle, while hydrophilic molecules will migrate quicker through the solvent. In CZE neutral molecules will migrate with the electroosmotic flow and no separation will occur, and MEKC will extend the applicability of CZE to neutral molecules (Suntornsuk, 2007).

Compared with high performance liquid chromatography (HPLC), CE has a few distinct advantages, including small sample size, minimal sample preparation, use of very small amounts of organic solvents and inexpensive chemicals, ease of buffer change and method development, and low cost of capillary columns (Siddiqui, 2013).

**CAPILLARY ELECTROPHORESIS IN THE ANALYSIS OF STATINS**

Determination of lovastatin in the presence of its oxidation products after exposure to an oxidative atmosphere has been carried out using CE technique. Since the molecule of lovastatin in its lactone form is unchanged and is only slightly soluble in water, base hydrolysis was used to open the lactone ring and...
transform the compound into a water-soluble acid form, which is negatively charged. Different solvents, different amounts of NaOH added, different hydrolysis times and different temperatures for sample preparation were tested. The CE and HPLC methods are compared in terms of susceptibility, precision, linearity and accuracy. HPLC method was found to be more susceptible and more precise (Javernik Rajh et al., 2003).

CE was used for the rapid quantitation of atorvastatin in tablets. Method development included studies of the effect of applied voltage, buffer concentration, buffer pH, and hydrodynamic injection time on the electrophoretic separation. The method was validated with regard to linearity, precision, specificity, LOD, and LOQ. The optimum electrophoretic separation conditions were 25 mM sodium acetate buffer at pH 6, with a separation voltage of 25 kV using a 50 μm capillary of 33 cm total length. Sodium diclofenac was used as an internal standard. Analysis of atorvastatin in a commercial tablets gave quite high efficiency, coupled with a short analysis time of less than 1.2 min in comparison to HPLC methods. Once the separation was optimized on capillary, it was further miniaturized to a microchip platform, with linear imaging UV detection using microchip electrophoresis. Linear imaging UV detection allowed for real time monitoring of the analyte movement on chip, so that the optimum separation time could be easily determined. This microchip electrophoretic method was compared to the classic CE method with regard to speed, efficiency, precision, and LOD. Miniaturization of CE offers a sizeable reduction in the volumes of sample and buffer required. This is accompanied by speed, efficiency, portability, and compatibility with many types of detectors such as MS (Guihen et al., 2006).

A MEKC method has been also developed for simultaneous quantitation of atorvastatin and its related substances. The separation was carried out in an extended light path capillary at applied voltage of 30 kV using a background electrolyte consisting of 10 mM sodium tetraborate buffer pH 9.50, 50 mM sodium dodecyl sulphate and 20% (v/v) methanol. The addition of methanol to the running buffer resulted in a very effective choice to achieve resolution between the peaks of charged substances adjacent to atorvastatin as well as the peaks of neutral drug-related substances. Linear calibration curves were established over the concentration range 100–1200 μg mL\(^{-1}\) for atorvastatin and 1.0–12.5 μg mL\(^{-1}\) for related substances. The proposed MEKC procedure has been validated with respect to selectivity, precision, linearity, limits of detection, and quantitation, accuracy and robustness. The method has been successfully applied to the determination of atorvastatin and purity evaluation of bulk drug and formulated products (Nigović et al, 2009).

CE was applied for determination of fluvastatin in capsule and serum; a fused-silica capillary of 86 cm length (58 cm effective length) and 75 μm ID and a background electrolyte consisting of 10mM borate at pH 8 was used. The optimum capillary electrophoretic and analytical parameters were investigated and method validation studies were performed. The separation was performed by current-controlled system applying 41 μA, UV detection at 239 nm and 0.5 s vacuum injection. Phenobarbital was used as internal standard. LOD and LOQ values were found to be 1×10\(^{-6}\) M and 2.89×10\(^{-6}\) M, respectively. Linearity in the range of 1.03×10\(^{-3}\) –5.15×10\(^{-3}\) M was examined employing intra-day and inter-day studies and well-correlated calibration equations were obtained. The developed method was applied for the determination of fluvastatin in Lescol capsules containing 40 mg, the amount of active substance was found to be 41.9±0.4mg. Furthermore, fluvastatin was determined in serum applying standard addition technique; good repeatability and no interference from the matrix were observed (Dogrukol–Ak et al., 2001).

A chiral CE method was developed for the enantiomeric purity determination of fluvastatin enantiomers. Fluvastatin is marketed as a racemic mixture of the (+)-3R,5S and (-)-3S, 5R enantiomers; however, the therapeutic activity is 30-fold higher for the (+)-3R, 5S-FV than (-)3S, 5R-FV enantiomer. Fluvastatin enantiomers were separated on an uncoated fused silica with 100 mM borate solution running buffer containing 30 mg/mL of (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) as chiral selector and fenoprofen as an internal standard. The linearity was observed within a 400-700 μg/mL concentration range for both fluvastatin enantiomers. The repeatability expressed as coefficient of variation of the method were 0.96 and 0.92% for (+)-3R, 5S and (-)3S, 5R-fluvastatin, respectively. The limit of detection and quantification for both fluvastatin enantiomers were 1.5 μg/mL and 2.5 μg/mL, respectively. Compared to chiral HPLC separations, CE analyses are cheaper (no chiral column, no solvent, low consumption of chiral selector) and offers higher peak efficiencies (Trung et al., 2008).

A CE method was developed for the determination of pravastatin in pharmaceutical tablet formulations. Pravastatin and lansoprazole used as internal standard migrated in a background electrolyte of 10 mM borate buffer at pH 8.5 and 10% acetonitrile as organic additive. The separation was achieved by applying 27.5 kV voltage, UV detection at 200 nm and injecting the sample with a 0.5 s pressure. The results were precise and repeatable for areas of the peaks and peak normalization ratio. Linearity was found in the concentration range of 1.56–7.78 × 10\(^{-5}\) M. Intra-day and inter-day assays were performed and reliable results were obtained. Limit of detection and limit of quantification were 8 × 10\(^{-6}\) and 2.4 × 10\(^{-5}\) M, respectively. The method proved simple, precise and fast since the analysis can be performed in less than 5 min (Kircali et al., 2004).

MEKC has been applied for determination of pravastatin in fermentation broth in order to optimize its production in bioreactors. The method successfully separated pravastatin from interfering matrix, mevastatin and 6-epi pravastatin. Its determination in production media was also performed using two distinct HPLC methods. The analyses were performed
on particle column, monolithic column and silica capillary filled with borate buffer pH 9.3 containing 20 mM SDS. All three methods successfully separate pravastatin from interfering compounds (matrix, mevastatin and 6-epi pravastatin) and runtimes were shorter than 1 min. However the two HPLC methods, had superior sensitivity compared to MEKC, with LOD around 0.01 ng/mL, 0.2 ng/mL and 20 ng/mL, respectively (Kocijan et al., 2005).

CZE was applied also for pravastatin determination in pharmaceutical dosage form. Rapid migration of negatively charged pravastatin molecule was obtained in alkaline buffer by the application of of a 30 kV voltage. Influence of the pH value and ionic strength of running buffer, applied voltage and capillary temperature on mobility and sensitivity was evaluated. Detection wavelength was set to 237 nm. Pravastatin is a 6-hydroxy acid, which is prone to lactonize and epimerize in a pH-dependent manner. MEKC approach was chosen to develop a method able to separate pravastatin and its degradation products in acidic media. The proposed method allows baseline separation of hydroxy acid and neutral lactone forms of the drug that appear as interconversion products depending on the pH value. The above mentioned interconversion compounds of pravastatin represent its related impurities defined in Ph. Eur. and are also potential biotransformation products (Nigović et al, 2008).

The acid dissociation constant of pravastatin was determined under degraded conditions using a CE method. pravastatin was degraded in an acidic solution (pH = 2.0) for 5 hours , and the degradation solution was subjected to the measurement of the effective electrophoretic mobility by CZE. Although the amount of pravastatin decreased by the acid degradation, its acid dissociation constant was successfully determined with the residual pravastatin through its effective electrophoretic mobility. The determined acid dissociation constant value agreed well with the one obtained with freshly prepared solution and with some reported values (Takayanagi, 2015).

A MEKC method was developed for the quantification of lovastatin and simvastatin in pharmaceutical dosage forms. Lovastatin and simvastatin were separated using an electrolyte system consisting of 12% acetonitrile (v/v) in 25 mM sodium borate buffer pH 9.3 containing 25 mM sodium dodecyl sulphate (SDS) with an extended light path capillary (48.5 cm length x 50 μm i.d, 40 cm effective length). The method has been validated and proven to be rugged. Calibration curves were linear over the studied ranges with correlation coefficients greater than 0.996. A limit of detection of 3.2 μg mL\textsuperscript{-1} and a limit of quantification of 10.6 μg mL\textsuperscript{-1} were estimated for both the drugs. The proposed method was found to be suitable and accurate for the determination of these drugs in commercial formulations (Srinivasu et al., 2002).

Analytical methods for determination of statins were developed individually as expected from their different structural properties. This approach to the analysis was chosen most probably because statins are not used in combination with other statin molecules during therapy. However, the development of a rapid analytical procedure that will not be limited to the analysis of only one statin could be very useful.

A universal MEKC method was developed for the simultaneous and short-time analysis of lovastatin, simvastatin, pravastatin, fluvastatin and rosuvastatin. Base hydrolysis was used to open lactone ring of lovastatin and simvastatin, administered as lactone prodrugs, in order to transform these compounds to the corresponding β-hydroxyl acid forms before MEKC analysis. This approach offered shorter analysis time due to a decrease of the migration times of negatively charged statin drugs in comparison to neutral lactone forms. Optimized conditions were found to be a 25 mM borate buffer pH 9.5 with 25 mM sodium dodecyl sulphate and 10% methanol added as an organic modifier, an applied voltage of 23 kV and a separation temperature of 30 °C. Ketoprofen was used as an internal standard. The linearity of the detector response for each statin was within the concentration range from 10 to 100 μg mL\textsuperscript{-1} with a correlation coefficient greater than 0.9994. Analyses of six statin drugs in pharmaceutical samples were carried out in only 5 min. The interference of the tablet sample matrix was not observed. The recovery values were in the range of 98.04–100.80% (Damic et al., 2010).

Another study report the development of a simple, rapid and efficient capillary electrophoresis method for the simultaneous determination of atorvastatin, fluvastatin, lovastatin and simvastatin. CZE proved to be efficient for the simultaneous separation of atorvastatin and fluvastatin, but could not resolve the determination of lovastatin and simvastatin. The simultaneous separation of all four statins was achieved by applying a micellar electrokinetic chromatographic method, after transforming lovastatin and simvastatin in β-hydroxyl acid forms through alkaline hydrolysis. The optimum electrophoretic conditions and analytical parameters were investigated and the analytical performances of the method were verified with regard to linearity, precision, accuracy, LOD and LOQ. The optimum electrophoretic separation conditions were: 25 mM sodium tetraborate with 25 mM sodium dodecyl sulphate buffer electrolyte at pH 9.5, applied voltage +25 kV, separation temperature 25 °C, injection pressure/time 50 mbar/1 minutes, UV detection at 230 nm. Using the optimized electrophoretic conditions. The simultaneous determination of the four statins was resolved in approximately 3 minutes, the order of migration being: atorvastatin, fluvastatin, lovastatin, simvastatin. The proposed method has been applied to the determination of the analytes in pharmaceutical tablets formulations (Mircia et al, 2016).

**CAPILLARY ELECTROPHORESIS IN THE ANALYSIS OF FIBRATES**

A CE method for achiral separation of the racemic ciprofibrate and its main metabolite ciprofibrate glucuronide was developed. The glucuronide was isolated from urine by chromatographic procedures and characterized by alkaline as well as enzymatic
hydrolysis and mass spectrometric and nuclear magnetic resonance experiments. Chiral discrimination of the ciprofibrate enantiomers and their diastereomeric glucuronides by CE was achieved by the use of γ-cyclodextrin as buffer additive. The fractionated crystallization of ciprofibrate yielded the R-(+)-enantiomer as less soluble diastereomeric salt with (+)-1-phenylethylamine. This allowed the identification of the enantiomers of ciprofibrate as well as the diastereomeric glucuronides of ciprofibrate by CE. In a study with three volunteers inter- and intra-individual differences of ratios of both ciprofibrate glucuronides in urine were observed. After oral administration of a single dose of the racemate the S-ciprofibrate glucuronide was mainly excreted in the first time intervals, in the last time intervals the R-glucuronide dominated (Hüttemann et al., 1999).

A CE method has been developed to measure fenofibrate in capsules based on MEKC with detection at 280 nm using a borate buffer containing sodium dodecyl sulfate. However, the metabolite of this drug (fenofibric acid) in serum and whole blood was analyzed by CZE in a borate-carbonate buffer using acetonitrile stacking. MEKC is well suited for the analysis of the neutral drug fenofibrate while CZE is more suited for the analysis of the ionizable fenofibric acid. The analysis was rapid, in 7 minutes with no interferences. Incubation of fenofibrate in whole blood caused hydrolysis of the ester bond with the release of fenofibric acid. The advantage of the CE for fenofibrate analysis is the elimination of the need for expensive organic solvents and columns and elimination of the need for sample preparations often used in previously reported HPLC methods (Shihabi, 2004).

The enantiomeric separation of gemfibrozil chiral analogues was performed by chiral CE. Resolution of the enantiomers was achieved using heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) as chiral selector dissolved into a buffer solution. In order to optimize the separation conditions, type, pH and concentration of running buffer and chiral selector concentration were varied. For each pH value, the optimum chiral selector concentration that produced the resolution of the isomers was found. The migration order of labile diastereoisomers formed was valued at the optimum experimental conditions by adding a pure optical isomer to the racemic mixture. Data from 1H NMR studies confirmed host-guest interaction between TM-beta-CD and 5-(2,5-dimethylphenoxy)-2-ethylpentanoic acid sodium salt. The hypothesized stoichiometry host-guest was 1:1. An apparent equilibrium constant (Ka) was estimated monitoring the chemical shift variation as a function of TM-beta-CD concentration. Salt effect on complexation equilibrium constant was also investigated (Ammazzalorso et al., 2005).

Six fibrate derivatives: bezafibrate, ciprofibrate, clofibrate, clofibric acid, fenofibrate and gemfibrozil were separated by means of CE, using unmodified fused silica tubing of 75 μm internal diameter and 87 cm length (65 cm to the UV detector at 227 nm). Migration time and selectivity were examined in differing pH of separation buffer, varying separation voltage and differing temperature. Optimal separation was achieved using 1/15 M phosphate buffer pH 10, 240 V/cm at 25 °C. The optimal separation conditions were then used to elaborate the method of quantitation of bezafibrate, ciprofibrate and gemfibrozil in pharmaceuticals. The clofibric acid was used as internal standard. The calibration curve was constructed from 0.2 to 0.8 mg/ml of each compound and 0.5 mg/ml of internal standard. The calibration data were proved to be linear by Mandel and Lack-of-fit tests. Statistical evaluation of results proved proper recovery of elaborated method (102.42, 97.32 and 101.51%, respectively) and good repeatability (9.51, 5.52 and 11.15%, respectively). The linearity of recovery was also tested by analyzing increasing amount of the samples. Three fortified samples of each drug were also analyzed to perform additional accuracy validation (Komsta et al., 2006).

CONCLUSIONS:

The use of hypolipidemic drugs especially statins and fibrates has augmented in recent years and is expected to increase further in the years ahead because high cholesterol and cardiovascular diseases are being diagnosed more frequently. Therefore, the development of new analytical methods for commercially available hypolipidemic drugs as well is a necessity and a challenge for the analysts.

Development of the analytical methods for identification, purity evaluation and quantification of hypolipidic drugs has received a great deal of attention in the field of pharmaceutical analysis in recent years and CE became an alternative and also a complementary method to the more frequently used HPLC techniques. CE has become a useful tool in pharmaceutical analysis because of its advantages related to its high resolution, high selectivity, simplicity, short analysis time, cost efficiency and low consumption of solvents and reagents.

This review includes trends and advances in CE methods developed for the analysis of statins and fibrates in pharmaceutical dosage forms and biological samples.

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