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The use of capillary electrophoresis in the simultaneous determination of fixed-dose combination drugs for cardiovascular diseases

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Abstract

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide and are usually multifactorial medical conditions. Usually cardiovascular patients must follow a complicated treatment scheme, containing several drugs. Fixed dose combinations (FDCs) are pharmaceutical formulations containing two or more active ingredients in a one pill. FDCs can add multiple benefits to the treatment of CVDs including increased patient compliance, elimination of some side effects and the simultaneous blockage of multiple pathogenic links. The great prevelance of FDCs in modern therapy brings the necessity of developing new analytical methods for the simultaneous analysis of their components. Capillary electrophoresis (CE) was shown to be as a complementary and attractive alternative to the more frequently used chromatographic methods. CE advantages relate to the high efficiency of separation, rapid method developement, short analysis time and relatively low operational costs. The most frequently used CE techniques in the analysis of FDCs are capilllary zone electrophoresis (CZE) for ionized analytes and micellar electrokinetic chromatography (MEKC) for neutral ones. The current article reviews application of CE in the analysis of FDCs used in the treatment of CVDs.

Keywords: Capillary Electrophoresis, Cardiovascular Ciseases, Fixed Dose Combination

1. Introduction

Cardiovascular diseases (CVDs) are the world's leading cause of death and disability, as more people die from CVDs annually than from any other cause, however control of the risk factors and secondary prevention rates are relatively low (1).

CVDs are usually multifactorial; consequently, adopting a multifactorial approach to their management, where multiple risks factors are treated at the same time is more effective than treating single risk factors separately (1, 2).

Cardiovascular patients have in many cases several associated pathologies including hypertension, coronary heart diseases, obstructive ischemic diseases, hyperlipidaemia, or diabetes. These patients have in their treatment scheme association of several active substances (which may rise to 5 or even more), if we are just thinking to an antihypertensive, a diuretic, a statin, an antiplatelet drug, or an oral antidiabetic. Thus, treatment regimens can become slightly complicated and can be neglected by patients, considering that they must be followed throughout their life (1, 2).

Optimising risk factors reduces overall CV risks and thereby reduces CVD mortality and morbidity; patients with both hypertension and dyslipidaemia have a greater risk of CVD than those with either hypertension or dyslipidaemia

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alone, highlighting the importance of considering overall CVD risk as opposed to individual risk factors. Therefore, adopting a multi-factorial approach to CVD risk management, where multiple risk factors are modified simultaneously, is more effective in reducing CVD events than focusing on single risk factors (1,2).

Fixed dose combinations (FDCs) are defined as medication which contains two or more active pharmaceutical ingredients in a fixed dose ratio. In modern therapy FDC are viable alternatives with double therapeutic impact-disease control and increased adherence to treatment (3).

The complexity of the prescribed drug regimen negatively influences adherence to treatment. In a recent study, adherence to treatment was highly influenced by the number of pills prescribed to hypertensive patients. Non-adherence was <10% for monotherapy, increasing to ≈20% for dual therapy, ≈40% for therapy with three pills and even higher rates of partial or total non-adherence in patients who received five or more pills (4).

FDC can bring many benefits in CVD treatment, including increased patient compliance (several tablets become one), elimination of side effects (peripheral oedema caused by calcium channel blockers can be eliminated in combination with ACE inhibitors), thus making it possible to combine substances with synergistic or additive mechanisms at lower therapeutic doses (5).

Drug combinations target several mechanisms, such as blocking renin-angiotensin system and inducing vasodilatation and/or diuresis, reducing the heterogeneity of the hypertension initial treatment response, resulting in a more efficient response than in the case of increasing the dose of the active substance in monotherapy treatment (6).

Cohort studies in large populations have shown that initiation of combination therapy is associated with a lower rate of discontinuation of treatment, with patients at a lower risk of having a cardiovascular events compared to the initial administration of monotherapy, followed by gradual increase in the number of administered drugs (7).

Taking in consideration the high prevalence of FCDs in the modern therapy of CVDs the development of new analytical methods for the simultaneous quantification of active pharmaceutical ingredients became a necessity.

Screening the published articles, we can conclude that the large majority are using high performance liquid chromatography (HPLC) methods followed by the ones using UV-spectrophotometry. In the last 25 years, capillary electrophoresis (CE) has emerged as an alternative and a complementary technique for the more frequently applied chromatographic techniques.

The current article reviews the use of CE techniques in the analysis of FCDs for the treatment of CVDs.

2. Fixed-dose combination used in treatment of cardiovascular diseases

Several FDCs are currently used in the treatment of CVDs, among these we can mention: angiotensin-II receptor+antagonist (ARA)+thiazide diuretics, angiotensin-converting enzyme inhibitor (ACEI)+thiazide diuretics, ARA+calcium channel blocker (CCB), ACEI+CCB, CCB+HMG-CoA reductase inhibitor (statin) (1, 2).

Also triple fixed-dose combinations are sometimes used, like: ARA+CCB+thiazide diuretics or ACEI+CCB+thiazide diuretics (1, 2).

Recommended and not recommended combinations in the treatment of hypertension are presented in Table 1.

FDCs containing an ACEI or an ARA, in combination either with CCB or with a thiazide / thiazide-like diuretic are complementary, as both CCB and diuretics activate the renin-angiotensin system, an action which will be counteracted by their combination with an ACEI or ARA. These combinations will restrict potential adverse reactions linked with diuretic or CCB monotherapy, minimizing the risk of hypokalemia due to diuretic administration, and the prevalence of CCB related peripheral edema (8).

The advantages of using FDCs are related to the synergy between the mechanism of action of the active substances, possibility of cancelling adverse effects, simplification of therapy scheme, increased compliance to treatment, lowering medical costs and reduction of medical errors. There are also arguments against the use of FDCs, these may include: the need for individualization of

Table 1. Recommended and not recommended combinations in the treatment of hypertension (1).

Preferred combinations	Acceptable combinations	Unacceptable combinations
ACEI + diuretic	β-blocker + diuretic	ACEI + ARA
ARA + diuretic	CCB + β-blocker	ACEI + β-blocker
ACEI + CCB	Renin inhibitor + diuretic	ARA + β-blocker
ARA + CCB	thiazide diuretic + K saving diuretic	CCB (non-dihydropyridine) + β-blocker

doses, unfavourable pharmacokinetic interactions between components, dosage adjustment for certain populations (elderly patients, renal or hepatic dysfunction), large physical size of the product (9).

Combination therapy is used in the treatment of hypertension, ischaemic heart disease, and other CV diseases however the definition of "polypill" is still being studied and benefits in many cases have not been clearly identified (10).

In conclusion, fixed-dose pharmaceuticals are currently an attractive option, presenting efficiency and safety at least comparable with the individual components along with a series of advantages, being more and more used to treat serious health problems.

3. Capillary electrophoresis in the analysis of pharmaceuticals

Most pharmaceuticals are either acidic and/or basic water-soluble substances; the basis for their separation in CE is the exploitation of the differences between the electrophoretic mobilities of the analytes, which are related to the substance charge and size (11).

The most frequently used electrophoretic techniques used in the analysis of pharmaceuticals are capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). CZE may be used for the separation of charged analytes but is not useful in its native form for the separation of neutral molecules. This problem can be resolved using MEKC, by adding surfactants to the background electrolyte (BGE), in a concentration above their critical micelle concentration (CMC) whereby they form a pseudo-stationary phase analogous to the stationary phase in HPLC (12). A relatively new interesting method is capillary electrochromatography (CEC), a hybrid separation technique that combines the high separation efficiency of CZE with the selectivity of HPLC using the application of an electric field rather than hydraulic pressure to drive the mobile phase through a chromatographic stationary phase packed capillary (13).

The advantages of using CE methods for the separation of pharmaceuticals is being connected to its high separation efficiency, relatively short analysis time, rapid method development and especially with the low consumption of analytes and reagents which leads to low operational costs. The disadvantage when directly compared with HPLC can be poorer sensibility and limited preparative options (14).

4. Electrophoretic methods in the analysis of fixed-dose combinations

CZE was used for the simultaneous determination of the β-blocker, atenolol, and the thiazide diuretics, bendroflumethiazide, hydrochlorothiazide and amiloride in urine samples. A 50 mM sodium tetraborate BGE at pH 9.0 was used in the determination. The samples were prepared through solid phase extraction (SPE); atenolol and amiloride have basic characteristics while bendroflumethiazide and hydrochlorothiazide are acids; consequently, the combinations required different SPE cartridges, pH levels at which urine was buffered and eluents. The order of migration was atenolol, followed by amiloride, bendroflumethiazide, and hydrochlorothiazide The method was applied for the simultaneous quantitative determination of the studied compounds in urine samples collected after administration of atenolol (100 mg) - bendroflumethiazide (5 mg) and atenolol (50 mg) - amiloride (2.5 mg) - hydrochlorothiazide (25 mg) in combined pharmaceutical formulations (15).

A generic CZE method was used for the simultaneous determination of hydrochlorothiazide and six structurally related ACEI (cilazapril, enalapril, fosinopril, lisinopril, quinapril, ramipril). A

Table 2. CE techniques for the determinations of fixed-dosed combination for cardiovascular pathologies.

CE method	Experimental conditions	Analytes	Matrices	Ref
CZE	78 cm x 75 µm capillary, 50 mM borate BGE, pH 9.0, 25 kV, 25°C, 214 nm	atenolol – bendroflumethiazide atenolol – amiloride - hydrochlorothiazide	urine	15
CZE	52 cm x 75 µm capillary, 100 mM phosphate BGE, pH 7.25, 20 kV, 20°C, 214 nm	hydrochlorothiazide – ACE inhibitors (cilazapril, enalapril, fosinopril, lisinopril, quinapril, ramipril)	pharmaceutical formulations	16
CZE CEC	CZE: 48.5 cm x 50 µm capillary, 100 mM borate BGE, pH 9.0, 6 mM TM-β-CD, 15 kV, 25°C, 214 nm CEC: 33 cm x 100 µm capillary, LiChrospher 100 RP-18 (5 µm), mobile phase 50 mM sodium acetate buffer pH 7:water:acetonitrile (10:15:75), 20 kV, 25°C, 214 nm	losartan - hydrochlorothiazide	pharmaceutical formulations	17
CZE MEKC	CZE: 85 cm x 50 µm capillary, 60 mM phosphate BGE, pH 2.50, 30 kV, 30°C, 214 nm MEKC: 85 cm x 50 µm capillary, 55 mM phosphate BGE, 15 mM SDS, pH 6.50, 30 kV, 30°C, 214 nm	hydrochlorothiazide – angiotensin-II-receptor antagonists (candesartan, eprosartan, irbesartan, losartan, telmisartan, valsartan)	pharmaceutical formulations	18
CZE	48.5 cm x 75 µm capillary, 50 mM sodium carbonate BGE, pH 10.3, 20 kV, 25°C, 226 nm	losartan – hydrochlorothiazide losartan - chlortalidone	pharmaceutical formulations	19
CZE	48 cm x 50 µm capillary, 40 mM borate BGE, pH 9.5, 30 kV, 30°C, 210 nm	olmesartan - hydrochlorothiazide	pharmaceutical formulations	20
CZE MEKC	CZE: 38 cm x 50 µm capillary, 25 mM phosphate BGE, pH 2.50, 25 kV, 25°C, 230 nm MEKC: 38 cm x 50 µm capillary, 25 mM borate BGE, 15 mM SDS, pH 9.50, 25 kV, 25°C, 230 nm	telmisartan - hydrochlorothiazide	pharmaceutical formulations	21
CZE	50 cm x 50 µm capillary, 50 mM borate BGE, pH 9.20, 25 kV, 25°C, 210 nm	indapamide erbumine - indapamide	pharmaceutical formulations	22
CZE	65 cm x 75 µm capillary, 20 mM phosphate BGE, pH 9.00, 25 kV, 25°C, 198 nm	atenolol - chlortalidone	pharmaceutical formulations	23
CZE	52 cm x 75 µm capillary, 150 mM acetic acid BGE, 25 kV, 28°C, C4D detector	atenolol - amiloride	pharmaceutical formulations	24
CZE	52 cm x 75 µm capillary, 25 mM phosphate BGE, pH 9.00, 25 kV, 25°C, 198 nm	atenolol – amiloride atenolol - chlortalidone	pharmaceutical formulations	25
CZE	50.2 cm x 50 µm capillary, 50 mM phosphate BGE, pH 9.50, 25 kV, 25°C, 240 nm	metoprolol - hydrochlorothiazide	pharmaceutical formulations	26

Continued Table 2.

CZE	48 cm x 50 µm capillary, 25 mM phosphate BGE, pH 8.00, 25 kV, 25°C, 214 nm	amlodipine - valsartan	human plasma, pharmaceutical formulations	27
CZE	94 cm x 75 µm capillary, 100 mM borate BGE, pH 8.00, 20 kV, 30°C, 237 nm	amlodipine - valsartan	pharmaceutical formulations	28
CZE	48 cm x 50 µm capillary, 50 mM phosphate BGE, pH 4.50, 25 kV, 25°C, 210 nm	amlodipine - telmisartan	pharmaceutical formulations	29
CZE	67 cm x 75 µm capillary, 25 mM phosphate BGE, pH 4.50, 20% methanol, 15 kV, 25°C, 210 nm	amlodipine - atorvastatin	pharmaceutical formulations	30
CZE	50.2 cm x 50 µm capillary, 25 mM phosphate BGE, pH 6.50, 25 kV, 25°C, 210 nm	amlodipine - atorvastatin	human plasma, pharmaceutical formulations	31
CZE	48 cm x 50 µm capillary, 50 mM phosphate BGE, pH 7.00, 25 kV, 25°C, 210 nm	amlodipine - atorvastatin	pharmaceutical formulations	32
CZE	57 cm x 75 µm capillary, 40 mM phosphate BGE, pH 6.00, 17 kV, 25°C, 245 nm	aliskiren – amlodipine - hydrochlorothiazide	pharmaceutical formulations	33
MEKC	47 cm x 50 µm capillary, 40 mM phosphate BGE, 20 mM SDS, pH 9.50, 10% acetonitrile, 25 kV, 30°C, 220 nm	amlodipine – hydrochlorothiazide - olmesartan	pharmaceutical formulations	34
CZE	57 cm x 75 µm capillary, 40 mM phosphate BGE, pH 7.50, 15 kV, 25°C, 230 nm	amlodipine – hydrochlorothiazide - valsartan	pharmaceutical formulations	35
CZE	78.5 cm x 75 µm capillary, 40 mM phosphate BGE, pH 7.50, 30 kV, 25°C, 210, 225 nm	amlodipine – benazepril - hydrochlorothiazide	pharmaceutical formulations	36

100 mM phosphate BGE at pH 7.25 was used in the determination. The migration order was hydrochlorothiazide followed by the ACEIs. The method was used for the determination of analytes from the corresponding fixed dose pharmaceutical formulations (16).

CE and CEC were used for the determination of hydrochlorothiazide and an ARA, losartan. A 100 mM sodium tetraborate BGE at pH 9.0 containing trimethyl-β-cyclodextrin (TM-β-CD) was used in the CE determination; CD was added to the BGE to form inclusion complexes. In the CEC determination a capillary packed with a RP-18 stationary phase and a mobile phase containing 50 mM ammonium acetate pH 7.0: water: acetonitrile (1:1.5:7.5) was used. The migration order was hydrochlorothiazide followed by losartan in both methods. In the CE method, LOD and LOQ of two analytes were, 0.032 and 0.096 ng for losartan, 0.04 and 0.12 ng for hydrochlorothiazide. In the CEC method, LOD and LOQ of two analytes were, 0.028 and 0.084 ng for losartan, 0.025 and 0.075 ng for hydrochlorothiazide. CEC seemed to be more selective than CE, combining the high sensitivity of HPLC with the separation efficiency of CE (17).

CZE and MEKC methods were used for the simultaneous determination of hydrochlorothiazide and six ARAs (candesartan, eprosartan, irbesartan, losartan, telmisartan, valsartan). In the CZE method a 60 mM phosphate BGE at pH 2.50 while in the MEKC a 55 mM sodium phosphate containing 15 mM SDS at pH 6.50 was used. In CZE the migration order was telmisartan, irbesartan, eprosartan, losartan and hydrochlorothiazide; while in MEKC the migration order was hydrochlorothiazide, eprosartan, losartan, valsartan. Both methods proved to be suitable for the determination of the

analytes from corresponding fixed dose pharmaceutical formulations. Depending on the nature of the ARA one of the methods can be used for its simultaneous determination with hydrochlorothiazide. The development of a generic method for the quantification of several ARAs in combination with hydrochlorothiazide, without the development of methods for each ARA has the advantage of reducing considerably analytical work (18).

In another CZE method losartan was determined in combination with thiazide diuretics, chlortalidone or hydrochlorothiazide. A 50 mM sodium carbonate BGE at pH 10.3 was used in the determination. The migration order was losartan followed by chlortalidone and hydrochlorothiazide, analysis time was less than 5 minutes. The method was applied for determination of analytes in capsules (19).

CZE was used for the simultaneous determination of another ARA, olmesartan and hydrochlorothiazide. A 40 mM sodium tetraborate BGE at pH 9.5, was used in the determination. The migration order was hydrochlorothiazide followed by olmesartan, analysis time was less than 3 minutes. The methods were applied for the determination of the analytes in tablets (20).

Simultaneous determination of telmisartan and hydrochlorothiazide was achieved with both CZE and MEKC methods. The CZE separation was achieved using a 25 mM phosphate BGE at pH 2.50, while in the case of MEKC a 25 mM borate BGE, 25 mM SDS at pH 9.50 was used. The migration order was telmisartan followed by hydrochlorothiazide when CZE method was applied, while in the MEKC method migration order was reversed, hydrochlorothiazide migrating first. Migration times were fast, below 4 minutes, in both methods. In the CZE method, LOD and LOQ were, 0.017 and 0.051 mg/mL for telmisartan, 0.08 and 0.24 mg/mL for hydrochlorothiazide; while in the MEKC method, LOD and LOQ were, 0.036 and 0.108 mg/mL for telmisartan, 0.062 and 0.185 mg/mL for hydrochlorothiazide. The LOD and LOQ values were slightly higher in the MEKC method. The method was applied for the determination of the analytes in tablets (21).

HPLC and CE methods were developed for the simultaneous determination of perindopril, an ACEI and indapamide, a thiazide-like diuretic. A 50 mM phosphate BGE at pH 9.20, was used in the determination. Difficulties were encountered because of low UV absorptivity of perindopril. The migration order in CE was indapamide followed by perindopril. The LOD values of CE method was much higher by comparison with HPLC results. Analysis time were below 5 minutes for both methods. Both methods proved to be reliable solutions for the analysis of the analytes from pharmaceutical combinations (22).

A CZE method was used for the simultaneous determination of atenolol and chlortalidone. A 20 mM phosphate BGE at pH 9.0 was used in the separation. The migration order was atenolol followed by chlortalidone; migration times of the analytes were below 5 minutes. The method was used for the determination of analytes in tablets (23).

CZE coupled with a capacitively coupled contactless conductivity detector (CE-C4D) was used for the simultaneous determination of atenolol and amiloride. C4D is a conductometric measurement approach based on the differences in conductivity between the sample zones and the BGE. A 150 mM acetic acid BGE was used in the separation. The migration times of the analytes was below 7 minutes. The method was used for the determination of the analytes in tablets (24).

CZE with UV detection was used for the simultaneous determination of atenolol, amiloride and chlortalidone. A 25 mM phosphate BGE at pH 9.0 was used in the separation. The migration order was atenolol followed by amiloride and chlortalidone; the migration times of the analytes was below 4 minutes. The method was used for the determination of analytes in various pharmaceutical formulations (25).

CZE was used for the simultaneous determination of the β-blocker, metoprolol, and hydrochlorothiazide. In method development a univariate approach was used for screening purposes followed by a factorial design with response surface plots, as multivariate approach method optimization of significant parameters. A 50 mM phosphate BGE at pH 9.50 was used in the determination. The migration order was metoprolol followed by hydrochlorothiazide; the migration times

were below 4 minutes (26).

A CZE method was used for the simultaneous determination of amlodipine, a dihydropyridine CCB and valsartan, an ARA in human plasma and pharmaceuticals. A 25 mM phosphate BGE at pH 8.0 was used in the determination. The drugs were extracted from plasma using liquid–liquid extraction (LLE) technique. The migration order was valsartan followed by amlodipine; the migration times were below 6 minutes. The LOD for both analytes was 0.03 mg/L, which allows determination of drugs in human plasma (27).

Thin layer chromatography (TLC) and CZE methods were used for the simultaneous determination of amlodipine and valsartan. A 100 mM sodium tetraborate BGE at pH 8.0 was used in the determination. The migration order was valsartan followed by amlodipine; however, the migration time of valsartan was around 15 minutes. The method was applied for the determination of the analytes in tablets (28).

A CZE method was used for the simultaneous determination of amlodipine and telmisartan. A 45 mM phosphate BGE at pH 4.50 was used in the determination. The migration order was amlodipine followed by telmisartan; migration times were below 3 minutes. The LOD and LOQ values were 0.66 and 2.18 µg/mL for amlodipine and 1.14 and 3.42 µg/mL for telmisartan. The method was applied for the determination of the analytes in tablets (29).

A CZE method was used for the simultaneous determination of amlodipine and an HMG-CoA reductase inhibitor, atorvastatin. A 25 mM phosphate BGE at pH 6.50 containing 20% methanol was used in the determination. The migration order was amlodipine followed by atorvastatin; migration times were around 10 minutes. The two drugs were subjected to hydrolytic, oxidative, photolytic, and thermal stress conditions and the samples were analysed. The method proved to be adequate for the separation of the analytes and their degradation products, and can be used to measure the analytes in the presence of their degradation products and associated organic impurities which can be present in the formulation (30).

Another CZE method was used for the simultaneous determination of amlodipine and atorvastatin in pharmaceutical preparations and human plasma. A 25 mM phosphate BGE at pH 6.50 was used in the determination. The drugs were extracted through LLE technique. The LOD and the LOQ values were 0.05 mg/mL and 0.10 mg/mL for both analytes, respectively. The migration order was amlodipine followed by atorvastatin; migration times were below 5 minutes (31).

A CZE method was used for the simultaneous determination of amlodipine and atorvastatin. A 50 mM phosphate BGE at pH 7.0 was used in the determination. The migration order was amlodipine followed by atorvastatin; the migration times were below 5 minutes. The LOD and LOQ values were 0.64 and 2.10 µg/mL for amlodipine and 0.47 and 1.53 µg/mL for atorvastatin. The method was applied for the determination of the analytes in tablets (32).

A CZE method was used for the simultaneous determination of aliskiren, a direct renin inhibitor, amlodipine, and hydrochlorothiazide in their triple mixture fixed dose combination. A 40 mM phosphate BGE at pH 6.0 was used in the determination. The migration order was amlodipine followed by aliskiren and hydrochlorothiazide; the migration times were below 6 minutes. LOD and LOQ values were 0.11, 0.33, and 5.83 µg/mL respectively 0.33, 1.01, and 17.65 µg/mL for amlodipine, hydrochlorothiazide and aliskiren. The method was applied for the determination of analytes in synthetic mixtures and co-formulated tablets (33).

A MEKC method was used for the simultaneous determination of amlodipine, hydrochlorothiazide and olmesartan. A 40 mM phosphate BGE, 20 mM SD at pH 6.0 and acetonitrile (90:10, v/v) was used in the determination. The migration order was hydrochlorothiazide, followed by amlodipine and olmsertan; migration times were below six minutes. LOD values of 1.25, 0.44, 1.25, and 3.81 µg/mL were obtained for amlodipine, olmesartan and hydrochlorothiazide, respectively. The results were compared with a previously reported RP-HPLC method, the results were in good agreement between the two methods. The method was applied for the determination of analytes in formulations (34).

A CZE method was used for the simulta-

neous determination of amlodipine, hydrochlorothiazide, and valsartan. A 40 mM phosphate BGE at pH 7.50 was used in the determination. The migration order was amlodipine followed by hydrochlorothiazide and valsartan; migration times were around 12 minutes. LOD values of 0.38, 065, and 1.82 µg/mL and LOQ values of 1.17, 1.96 and 5.51 µg/mL were obtained for amlodipine, hydrochlorothiazide, and valsartan, respectively. The method was applied for the determination of analytes in co-formulated tablets (35).

A CZE method was used for the simultaneous determination of amlodipine, benazepril, and hydrochlorothiazide. A 40 mM phosphate at pH 7.50 was used in the determination. The migration order was amlodipine followed by hydrochlorothiazide and valsartan; migration times were around 7 minutes. LOD values of 0.896, 1.00 and 1.22 µg/mL and LOQ values of 2.75, 3.12 and 3.73 µg/mL were obtained for benazepril, amlodipine, and hydrochlorothiazide, respectively. The method was applied for the determination of analytes in two combined pharmaceutical tablets (36).

Applications of CE in the analysis of CV drugs are summarized in Table 2.

4. Conclusion

In the last 20 years several CE techniques have been published for the simultaneous quantification of analytes from different FDCs. However, there are still many combinations available on the market, with no CE method published yet. CE have great potential in the quantitative analysis of analytes from FDC especially from their pharmaceutical forms, and probably many such articles will be published in the years to come.

The difficulty in developing such separation methods is related to the fact that FDC contain analytes with synergic pharmacodynamic properties but with totally different structural characteristic and electrophoretic properties; consequently, the analyst has to establish the optimum electrophoretic conditions in which analytes can be detected properly.

Most of the studies already published in the literature employ CZE with DAD detection for the separation of analytes, however there are also several MEKC methods have been published for the determination of neutral analytes. Many of the published articles deal with the determination of the analytes from their combined pharmaceutical formulations, however there are a few which describe analysis from biological matrices (urine, plasma).

Another interesting option can be the development of a generic method of analysis for the simultaneous determination of several structurally related active substances (ACEI or ARA) which are used in FDC in a combination with a certain analyte (thiazide diuretics).

When applying CE methods satisfactory results were obtained for method validation according to the ICH guidelines with respect to selectivity, linearity, accuracy, and precision.

CE usually has acceptable performances with respect to all validation parameters and can be considered an inexpensive and precise method for the determination of analytes in FDC. The main advantages of CE over HPLC are related to the low operational costs: low price of the capillary which has a long lifetime and low consumption of analytes and electrolytes. Furthermore, CE mainly uses aqueous BGE and can be considered a "green" method in comparison with HPLC techniques which consume large volume of organic solvents.

CE techniques can be used as an alternative for HPLC methods, being a convenient solution for quality control routine analysis in pharmaceutical industry laboratories.

Conflict of Interest

None declared.

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