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Review

Capillary electrophoresis of phytochemical substances in herbal drugs and medicinal plants

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ABSTRACT

This paper reviews the applications of electromigration methods in analysis of phytochemical substances in herbal drugs and medicinal plants.

A short description of the basic principles of capillary electrophoretic techniques is firstly given, then the overview deals with the applications of selected methods published in the period 2005–2010.

The phytochemical substances have been classified according to their chemical nature (e.g. alkaloids, polyphenols, carbohydrates, lipids, terpenes) and the applied CE approaches, namely CZE, NACE, MEKC, MEEKC and CEC, together with the different detection methods, are critically discussed for each of the considered classes of natural compounds.

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1. Introduction

In the last years a renewed and increasing interest has been observed towards bioactive phytochemical substances. Several reasons may be invoked to explain the attention towards substances from plant kingdom: their availability from almost inexhaustible sources; the relatively low-cost (in some cases the bioactive phytochemicals can be affordable if compared with compounds obtained from total chemical synthesis); individual plant species may contain an impressive number of chemical substances and only a minor fraction (of both plants and phytochemicals) have been studied for biomedical application; ethnopharmacology and Traditional Medicine offer the opportunity for the investigation of a wide range of potentially active principles.

The discovery of novel chemotypes from nature is an intriguing challenge in which bioactive assay plays a primary role. Further, separation techniques combined with mass spectrometry (MS) and nuclear magnetic resonance spectrometry (NMR) are applied as the most important tools for identification and structural elucidation of the new molecules [1].

Beside the phytochemical investigations on new bioactive plant metabolites, there is an increasing need for qualitative and quantitative analytical methods in quality control of herbal drugs and crude plant extracts.

The therapeutic efficacy of the phytopreparations results from multiple components at multiple targets, thus the quantitation of one or more active components does not always represent its quality. Nevertheless, in order to provide high efficacy and safe use of herbal medicines as well as in ensuring stability of their quality, the quantitation of active components undoubtedly represent the most direct and important issue [2,3].

Capillary electrophoresis (CE) meets the requirements for the quality control of herbal drugs because of its versatility and high separation power. The wide opportunity for selectivity tuning allows the analysis of molecules with a wide range of polarity and molecular weight [4,5]. Recently, Unger reviewed the current applications and the promising advances of CE in analysis of natural products by focusing on the different approaches and separation modes of electromigration methods [6]. Capillary zone electrophoresis (CZE) is the simplest CE mode in which the separation is based on differences in the charge-to-mass ratio of the analytes. This approach can be effective in a number of applications; in particular, quaternary alkaloids and anthocyans–anthocyanidins, owing to the permanent charge, are ideal solutes in CZE, regardless the pH of the running buffer. Furthermore, CZE is also suitable in analysis of other important classes of secondary plant metabolites and in particular alkaloids, phenolic acids and flavonoids. Li et al. reviewed the optimisation of CZE methods specifically applied in analysis of phytochemical bioactive compounds [7]. A particular mode of CZE is non-aqueous capillary electrophoresis (NACE), which employ non-aqueous buffer system. Use of organic solvents instead of water firstly helps in increasing the solubility of hydrophobic analytes but also improves selectivity. Actually NACE widened the set of physicochemical characteristics of the solvents, which are known to affect the electrophoretic behavior by influencing solute–solvent, solute–additive and ion–ion interactions. Most importantly, p*K*a values of basic analytes in organic solvents are notable different from those in water allowing separations which are difficult to be obtained in water. In addition, NACE appears to be ideally suited for online coupling with mass spectrometry due to the high volatility and low surface tension of many organic solvents [8–10].

Electrokinetic chromatography (EKC), and in particular micellar electrokinetic chromatography (MEKC) has been introduced in 1984 by Terabe and proved to be not only the method of choice in analysis of neutral compounds but also one of the most versatile separation approach among the electromigration methods. In MEKC a surfactant (often sodium dodecyl sulfate, SDS) is introduced into the background electrolyte (BGE) at concentration above the critical micelle concentration in order to generate a micellar pseudo-stationary phase. Separation mechanism is a combination of chromatographic partitioning of solutes between pseudo-stationary phase and continuous phase and the electrophoretic mechanism. In MEKC the separation selectivity can be modulated not only by variation of BGE type, pH and concentration, but also by profit of the proper selection of the surfactant as well as by optimizing its concentration. Because phytomarkers to be simultaneously analysed in plant materials are often acidic, basic and neutral compounds, MEKC is widely applied [11–13]. Microemulsion electrokinetic chromatography (MEEKC), similarly to MEKC utilizes as separation media, pseudo-stationary phases represented by microemulsions. Typically the microemulsions are composed of nanometer-sized oil droplets suspended in an aqueous buffer (oil-in-water microemulsions, O/W). These systems are stabilized by the presence of surfactant i.e. SDS and a co-surfactant, which is a short-chain alcohol such as butanol. The oil droplets are obtained by dispersion of n-octane or other types of hydrophobic solvents [14].

Capillary electrochromatography (CEC) as other electrokinetic techniques combines the electromigration of the charged solutes with chromatographic partition; the latter event, differently to MEKC and MEEKC, is established between a liquid mobile phase and a solid stationary phase packed in a fused-silica capillary (small particles, $\sim$3 μm) fixed by frits prepared by silica sintering. CEC can also be performed in monolithic stationary phases formed by the in situ (in capillary) polymerization of monomers. In CEC the mobile phase, driven through the stationary phase by electroosmotic flow (EOF), assumes a flat and homogenous profile that leads to high separation efficiency. Specific CEC applications on natural products have been recently extensively reviewed [15,16].

The present review focuses on the applications of electromigration methods to phytochemical analysis and quality control of herbal drugs in the last five years.

2. Alkaloids

Alkaloids are cyclic organic compounds containing nitrogen in a negative oxidation state; they are widely distributed in plant kingdom and are thought to play role in plant protection, germination and as plant growth stimulants. These compounds usually have marked pharmacological activity and alkaloids-containing plants are often used as traditional medicines [17]. Alkaloids are easily protonated to form water-soluble salts, thus aqueous acidic electrolytes are often used as the BGEs in CZE analysis of plant extracts.

Table 1
Application of electromigration techniques to the analysis of alkaloids.

Alkaloids	Sample	Separation conditions	Detection	Ref
Tropane alkaloids				
Calystegine A_3 and B_2	*Solanaceae*	20 mM histidine, 20 mM BES, 20 % methanol	Indirect UV at 300 nm	[18]
Atropine	*Cannabis sativa*	20 mM ammoinum acetate pH 8.5	CZE-ESI-MS	[25]
Tropine, belladonnine, norhyoscyamine, apoatropine, hyoscyamine, 6β-hyoscyamine, scopolamine	*Atropa belladonna*	60 mM ammonium acetate pH 8.5, 5% isopropanol	CE-ESI-TOF-MS	[23]
Atropine, scopolamine, anisodamine	*Flos daturae*	20 mM phosphate pH 7.0, 4 mM βCD	ECL	[21]
		20 mM phosphate pH 8.48	ECL	[20]
		NACE: 1 M acetic acid, 20 mM sodium acetate, 2.5 mM tetrabutylammonium perchlorate in acetonitrile:2-propanol (4:1, v/v)	Dual ECL/EC	[22]
3α-Senecioyloxy-7β-hydroxytropane 3α-Hydroxy-7β-senecioyloxytropane 3α-Hydroxy-7β-angeloyloxytropane 3α-Hydroxy-7β-tigloyloxytropane	*Schizanthus grahamii*	NACE: 1 M trifluoroacetic acid, 25 mM ammonium formate in methanol:ethanol (40:60, v/v) or methanol:tetrahydrofuran (80:20, v/v)	UV at 220 nm and ESI-MS	[24]
Phenylethylamine alkaloids				
(−)-Ephedrine, (+)-pseudoephedrine	*Ephedra* supplements	25 mM phosphate-triethanolamine, pH 2.5, 7.5% highly sulfated βCD	UV at 190 nm	[36]
(−)-Ephedrine, (+)-pseudoephedrine, (−)-*N*-methylephedrine	*Ephedra sinica* and phytopreparations	20 mM Tris–phosphate pH 2.5, 20 mg/mL DM-βCD, 5% tetrabutylammonium chloride	UV at 210 nm	[39]
(−)-Ephedrine, (+)-pseudoephedrine	*Ephedra* Herba (Ma Huang)	20 mM tris-phosphate (pH 4.5) containing 2.5 mM Cu(II)-L-lysine (molar ratio 1:2)	UV at 254 nm	[30]
(−)-Ephedrine, (+)-pseudoephedrine	SRM supplements	BGE: 25 mM phosphate buffer (pH 2.5) Method A: 2.8% sulfated βCD + 1.2% DM-βCD Method B: 4% DM-βCD Method C: 4% HP-βCD	UV at 210 nm	[38]
(−)-Ephedrine, (+)-pseudoephedrine	*Ephedra* herb phytopreparations	NACE: 80 mM ammonium acetate, 3% acetsic acid in methanol	LIF ex 488, em 520	[33]
		MEKC: 25 mM tetraborate pH 9.7, 20 mM SDS	LIF ex 488 em 520	[31]
		MEKC: 20 mM tetraborate pH 9.8, 20 mM SDS, 15% acetonitrile	LIF ex 488 em 520	[32]
(−)-Ephedrine; (+)-pseudoephedrine; (−)-methylephedrine; (−)-norephedrine; (+)-norpseudoephedrine; (+)-*N*-methylpseudoephedrine	SRM supplements	MEKC: 15 mM ammonium acetate, 35 mM polysodium *N*-undecenoxycarbonyl-L-leucinate, pH 6.0, 30% acetonitrile	UV and ESI-MS	[40,41]
(±)-Octopamine, (±)-synephrine, tyramine, *N*-methyltyramine, hornedine	*Citrus* species and related genera	70 mM phosphate pH 3.1, 40 mM HP-βCD, 9 mM βCD, 8.2 mM DM-βCD	UV at 210 nm	[37]
Aconitine alkaloids				
Aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine, benzoylhypaconine	*Aconitum* roots	200 mM Tris–150 mM perchloric acid, 40% 1,4-dioxane (pH 7.8)	UV at 214 nm	[46]
Aconitine, mesaconitine, hypaconitine	*Aconitum kusnezoffii A. carmichaeli*	30 mM phosphate, pH 8.4	ECL	[45]
		35 mM 1B-3MI-TFB (ionic liquid) pH 8.5	UV at 254 nm	[44]
		20 mM phosphate, 35% acetonitrile pH 9.5	UV at 235 nm	[43]
Quinolizidine alkaloids				
Matrine, sophoridine, sophocarpine, oxymatrine	*Sophora flavescens* roots and phytopreparations	60 mM borate pH 8.5	UV at 204 nm	[48]
Sophocarpine, matrine, lehmanine, sophoranol, oxymatrine, oxysophocarpine, cytisine	*Sophora tonkinensis*	50 mM phosphate pH 2.5, 1% HP-βCD and 3.3% isopropanol	UV at 200 nm	[49]

Table 1 (*Continued*).

Alkaloids	Sample	Separation conditions	Detection	Ref
Sophoridine, matrine, oxymatrine	*Sophora flavescens*	50 mM phosphate, pH 8.4	ECL	[50]
Matrine, oxymatrine	*Sophora flavescens* roots, phytopreparations	NACE: 70 mM ammonium acetate, 7.0% acetic acid, 10% acetonitrile in methanol	UV at 205 nm	[51]
Sparteine, lupanine, angustifoline, 13α-hydroxylupanine	*Lupinus* species	NACE: 100 mM ammonium formate in methanol/acetonitrile/water 70/20/10, 1% acetic acid	UV at 210 nm and ESI-MS	[52]
Matrine, sophoridine, oxymatrine, oxysophcarpine, cytisine	*Sophora flavescens* roots	MEEKC: 98.2% 1 mM tetraborate-2 mM phosphate pH 6.5, 21 mM sodium cholate, 4 mM Mg^{2+}, 1.2% 1-butanol and 0.6% ethylacetate	UV-FASS at 200 and 214 nm	[53]
Isoquinoline alkaloids				
Berberine, hydrastine	*Hydrastis Canadensis*	CZE: 100 mM ammonium acetate–acetic acid pH 3.4 and methanol (20:80, v/v).	UV at 225 nm	[58]
Berberine, palmatine, jatrorrhizine, columbamine, epiberberine, coptisine, tetradehydroscoulerine, tetrahydrocheilanthifolinium	*Rhizoma coptidis*	NACE (I): 50 mM ammonium acetate pH* 6.8 in acetonitrile–methanol (20:80, v/v)	UV at 230, 265, 350 nm	[63]
		CZE (II): 50 mM ammonium acetate pH 6.8 in acetonitrile–water (50:50, v/v)	ESI-MS	
Berberine, jatrorrhizine, palmatine	*Rhizoma coptidis*, *Caulis mahoniae*, *Cortex berberidis*, *Cortex phellodendri*, *Herba chelidonii*	NACE: 50 mM ammonium acetate, 0.5% acetic acid in acetonitrile–methanol (10:90, v/v)	UV at 214 nm	[56]
Berberine, jatrorrhzine, palmatine	*Rhizoma coptidis, Caulis mahoniae*	NACE: 35 mM ammonium acetate, 0.25% acetic acid in acetonitrile–methanol (5:95, v/v)	LIF (native fluorescence) em 488 nm em 520 nm	[59]
Berberine, coptisine, palmatine, tetrandrine	*Coptidis rhizoma*	MEKC: 3 mM borate-10 mM phosphate pH 7.3, 50 mM sodium deoxycholate, 30% acetonitrile	UV at 270 nm	[64]
Berberine, coptisine, palmatine,	*Coptidis chinensis*	MEKC: 100 mM phosphoric acid, 15 mM SDS, 10% tetrahydrofurane, pH 1.82	UV at 264 nm with sweeping	[65]
Berberine, jatrorrhizine, palmatine	*Coptidis chinensis*	CEC: strong cation-exchange monolithic silica column. Mobile phase: 12.5 mM phosphate pH 7.4-acetonitrile (40:60, v/v)	UV at 263 nm	[67]
Sanguinarine, chelerythrine	*Macleaya cordata*, *Chelidonium majus*	NACE:40 mM ammonium acetate, acetonitrile–methanol (1:1), acetic acid pH* 5.6	LIF (native fluorescence)	[60]
Chelerythrine, nitidine	*Zanthoxylum nitidum*	NACE: 100 mM sodium acetate (pH 5.0) in methanol:water (4:1, v/v)	UV at 228 nm	[55]
Coptisine, palmatine, *N*-methyllaudanine, allocryptopine, protopine, corycavidine, glaucine, corydine, bulbocapnine, corydaline, corypalmine, tetrahydropalmatine, canadine, thalictrivacine	*Corydalis* species	NACE: 50 mM ammonium acetate, 1M acetic acid and 10% methanol in acetonitrile	ESI-MS	[62]
Protopine, cryptopine, corydamine, sinactine, (+)-adlumine, (−)-α-hydrastine, (+)-corlumine, (−)-fumarophycine, (−)-*O*-methylfumarophycine, (+)-parfumine	*Fumaria officinalis* and phytopreparations	NACE: 60 mM ammonium acetate in acetonitrile-methanol (9:1) and 2.2 M acetic acid	ESI-MS	[61]
Fangchinoline, tetrandrine	Radix *Stephania tetrandrae* and phytopreparations	NACE: 50 mM ammonium acetate, 0.5% acetic acid in acetonitrile-methanol (50:50, v/v)	UV at 214 nm	[57]

Table 1 (*Continued*).

Alkaloids	Sample	Separation conditions	Detection	Ref
Fangchinoline, tetrandrine	Radix *Stephania tetrandrae*	Flow injection MEKC: 15 mM acetic acid–15 mM sodium acetate–3% Tween 20–5% methanol at pH 5.5	UV at 254 nm	[66]
Pyrrolizidine alkaloids				
Senkirkine, senecionine, retrorsine, seneciphylline	*Kuan donghua* and *Qian liguang*	MEKC: 20 mM borate pH 9.1, 30 mM SDS and 20% methanol	UV at 220 nm	[70]
Sencionine, seneciphylline	*Gynura segetum*	MEKC: 120 mM Tris–35 mM lauric acid (pH not reported) and 20% methanol	UV at 220 nm	[71]
Senkirkine, senecionine, retrorsine, seneciphylline	*Kuan donghua*	MEKC: 20 mM borate pH 9.1, 30 mM SDS and 20% methanol; analysis using dynamic pH junction sweeping technique	UV at 220 nm	[69]
Indole alkaloids				
Strychnine, brucine	*S. nux-vomica*	NACE: 30 mM ammonium acetate, 1.0% acetic acid and 15% acetonitrile in methanol	UV at 214 nm	[73]
	Shen jin huo luo wan	NACE: 25 mM Tris-boric acid (pH 4.0) and 40% methanol in acetonitrile	UV at 214 nm	[74]
		MEKC: 50 mM phosphoric acid (pH 2.0), 100 mM SDS and 20% acetonitrile analysis performed using sweeping technique	UV at 203 nm	[75]
Mesembrine, mesembrenone, Δ^7 mesembrenone, mesembranol, epimesembranol	*Sceletium tortuosum* tablets	CZE: 50 mM phosphate pH 1.5	UV at 228 nm	[77]
Harmaline, tetrahydroharmine, harmine, harmane, harmol norharmane	*Psychotria viridis* l	CZE: 200 mM formic acid and 7 mM ammonia in water with 10% acetonitrile	LIF (ex 266 nm) and CE-MS	[78]
Yohimbine	*Pausinystalia yohimbe*	NACE: 30 mM ammonium acetate, 0.5% acetic acid in methanol	UV at 220 nm	[76]
Methylxanthine alkaloids				
Caffeine, theophylline, theobromine	guarana	CZE: 20 mM tetraborate pH 9.2	UV at 212 nm	[80]
Caffeine, theophylline, theobromine	Beverages (coca cola, tea drinks) roasted coffee, tea leaves	CZE: 15 mM tetraborate (pH 9.2) analysis performed using a nano-valve as sample injector	UV at 274 nm	[81]
Theobromine, caffeine	Green tea	CD-MEKC: 25 mM borate-phosphate, pH 2.5, 90 mM SDS and 25 mM HP-βCD	UV at 200 nm	[86]
Thebromine, caffeine	*Theobroma cacao*	CD-MEKC: 50 mM Britton-Robinson buffer at pH 2.5, 90 mM SDS and 12 mM HP-βCD	UV at 220 nm	[85]
Theobromine, caffeine	Tea leaves at different fermentation degree	MEKC: 10 mM phosphate, 4 mM borate (pH 7.0), 45 mM SDS and 0.5% ethanol	UV-DAD at different wavelengths in the range 200–280 nm	[84]
Caffeine	Decaffeinated coffee	MEKC: 10 mM carbonate (pH 11.0), 50 mM SDS	UV at 206 nm	[83]
Caffeine, theobromine	Beverages (yerba mate, coffee, cocoa, tea)	MEKC: 90 mM borate buffer (pH 8.5), 50 mM SDS	UV at 200 nm	[82]
Caffeine, theophylline	Beverages	CEC: organic–inorganic hybrid silica monolith C18-sulfonic groups using a mobile phase containing 25 mM phosphate–borate pH 8–acetonitrile (40:60, v/v)	UV at 254 nm	[87]
Morphinane alkaloids				
Morphine, codeine, thebaine, papaverine, narcotine	Opium	CZE: 100 mM sodium acetate–acetic acid (pH3.1) and 70% methanol	UV at 224 nm	[88]

Table 1 (*Continued*).

Alkaloids	Sample	Separation conditions	Detection	Ref
Morphine, codeine, oripavine, thebaine	Industrial liquors (from extraction processes)	CD-CZE: 100 mM Tris–phosphate (pH 2.8) + 30 mM HP-βCD	UV at 214 nm	[90]
Morphine, codeine, thebaine	*Papaver* species	CD-CZE: phosphate buffer 100 mM (pH 3.0) + 5 mM αCD	UV at 214 nm	[91]
Morphine, codeine, thebaine, narcotine	Dried poppy samples	IL-CZE: 25 mM borate–80 mM 1E-3MI-TFB, pH 9.18	ECL	[92]
Papaverine, narceine, noscapine, thebaine, reticuline, oripavine, codeine, morphine	Poppy straw samples	MEKC: 50 mM phosphate, 80 mM SDS and 25% methanol	UV at 200 nm	[89]
Morphine, thebaine, codeine, papaverine, narcotine	*Pericarpium papaveris*	CEC: hydrophilic/cation-exchange monolith;mobile phase: 5 mM phosphate pH 4.0–90% acetonitrile (v/v).	UV at 224 nm	[95]
Sinomenine: 7,8-didheydro-4-hydroxyl-3,7-dimthoxy-17-methylmorphinan-6-one	*Sinomenium acutum*	CZE: 50 mM phosphate pH 5.0	ECL	[93]
	Sinomenium acutum	NACE: 80 mM ammonium acetate, 2.0% acetic acid, 20% acetonitrile in methanol	UV at 262 nm	[94]
Miscellaneous alkaloids				
Galanthamine	*Bulbus Lycoridis Radiatae*	CZE: 18 mM phosphate at pH 9.0	ECL	[99]
Galanthamine, haemanthamine	*Narcissus* species	NACE: 90 mM ammonium acetate, 0.5% acetic acid, 25% acetonitrile in methanol	UV at 280 nm	[98]
Verticine, verticinone	*Fritillariae* species	IL-CZE: 8 mM phosphate – 40 mM 1B-3MI-TFB	ECL	[101]
Camptothecin, 9-methoxycamptothecin	*Nothapodytes foetida*	MEKC: 10 mM borate pH 8.6, 90 mM SDS and 20% dimethyl sulfoxide	UV at 369 nm	[96]
Pyrido[1,2-a]azepines: stemofoline, oxystemokerrin, didehydrostemofoline, stemocurtisinol	*Stemona curtisii*, *S. Morakot*, *S. collinsae*, *S. tuberose*	NACE-MS: 50 mM ammonium acetate, 1 M acetic acid, 10% methanol in acetonitrile	ESI-MS	[102]

BES: *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; 1B-3MI-TFB: 1-butyl-3-methylimidazolium tetrafluoroborate-based ionic liquid; 1E-3MI-TFB: 1-ethyl-3-methylimidazolium tetrafluoroborate-based ionic liquid; αCD: α-cyclodextrin; βCD: β-cyclodextrin; DM-βCD: Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; EC: electrochemical detection; ECL: electrochemiluminescence detection; FASS: field amplified sample stacking; HP-βCD: Hydroxypropyl- β-cyclodextrin; SRM: standard reference material.

In addition, organic solvents are widely used in CE analysis of alkaloids because non-aqueous media help in increasing the solubility of hydrophobic compounds; further they can improve selectivity. Based on their general structures, alkaloids are divided into several subgroups and the most important of them are reported in Fig. 1. In Table 1 the CE approaches applied in analysis of alkaloids in a number of herbal drugs, plant materials and phytopharmaceuticals, are summarized.

2.1. Tropane alkaloids

Solanaceae contain mainly tropane alkaloids such as atropine, anisodamine and scopolamine; these plants are extensively used both in traditional medicine and as sources for the extraction of the pharmacologically important (parasympatolytic and anti-cholinergic) alkaloids. The analytical challenge is represented by the weak UV absorption and the sensitivity of direct UV detection may not meet the requirement of quality control of herbal drugs and related extracts. Indirect UV detection was applied in analysis of nortropane alkaloids calystegines A_3 and B_3 in potatoes (*Solanaceae*) using histidine as the UV marker (detection at 300 nm); the detection limit (LOD) of 3 μg/mL was achieved [18].

Electrochemiluminescence (ECL) is frequently combined to CE separation, as a useful and sensitive detection mode. ECL is based on the chemiluminescence emission of reactive species generated by electrochemical reactions. The complex tris(2,2′-bipyridyl)ruthenium, $Ru(bpy)_3^{2+}$, is often selected as an ECL label and it fills a reservoir of the detection cell; the complex undergoes oxidation on the electrode surface at the voltage of +1.3 V to gen-

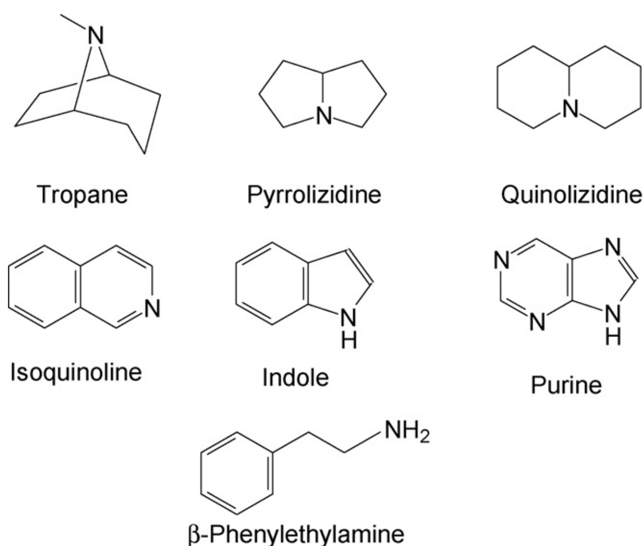


Fig. 1. Backbone structure of the principal alkaloids.

erate the reactive $Ru(bpy)_3^{3+}$ [19]. In particular, in CE analysis of alkaloids, the alkyl amine groups react with the complex to form the excited state $[Ru(bpy)_3^{2+}]^*$ which will decay to the ground state emitting at 610 nm. Gao et al. proposed the application of CE-ECL to the determination of atropine and scopolamine in *Flos daturae*. The detection system was based on a three-electrode configuration (a Pt disk working electrode, an Ag/AgCl reference electrode and a Pt wire as a counter electrode). The ECL detection reservoir contained a solution of 5 mM $Ru(bpy)_3^{2+}$ and 50 mM phosphate buffer. The CE separation of the two alkaloids was obtained under aqueous alkaline conditions (pH 8.48); by means of this method the detection limits for atropine and scopolamine were found in the order of 0.3–13 ng/mL [20]. Using the same detection mode, anisodamine beside scopolamine and atropine was analysed in *Flos daturae* using β-cyclodextrin (βCD) as additive to the aqueous BGE [21] or under NACE conditions [22].

CE combined to electrospray (ESI)-mass spectrometry (CE-ESI-MS) was applied in analysis of tropane alkaloids in *Atropa belladonna* leaf extracts [23], steam-bark of *Schizantus grahamii* [24] and hairy root cultures of *Cannabis sativa* [25]; detection limit of about 0.32 μg/mL was achieved.

2.2. Phenylethylamine alkaloids

Medicinal herbs containing phenylethylamine alkaloids (i.e. *Citrus* species and *Ephedra sinica*) are widespread used for their effects on human metabolism in particular by stimulating lipolysis and thus promoting the fat mass reduction in obese people. Specifically, ephedra extracts such as Ephedra Herba (Ma Huang) contain alkaloids such as: (1R,2S)-(−)-ephedrine, (1S,2S)-(+)-pseudoephedrine, (1R,2S)-(−)-norephedrine, (1S,2S)-(+)-norpseudoephedrine, (1R,2S)-(−)-*N*-methylephedrine, and (1S,2S)-(+)-*N*-methylpseudoephedrine [26]. The possible adverse effects associated to the use of these products prompted the U.S. Food and Drug Administration (FDA) to ban ephedra containing dietary supplements depending on the dosage [27]. In addition, the National Institute of Standards and Technology (NIST) issued standard reference materials with certified values for ephedra alkaloids, synephrine and caffeine [28]. As a consequence several analytical methods have been developed with the aim to provide reliable quantitation of ephedrine and pseudoephedrine in ephedra extracts and herbs [29]. Details on the CE methods in analysis of ephedra alkaloids in real samples are reported in Table 2. In order to obtain adequate selectivity in separation of these alkaloids, Li and co-workers developed a CZE method under acidic conditions (tris-phosphate pH 4.5) in the presence of Cu(II)-L-lysine complex as a ligand-exchange modifier. The hydroxyl group and nitrogen atom of the alkaloids, can yield a complex with Cu(II); in addition the π-electrons of the aromatic ring can interact with the protonated L-lysine. Under these conditions high selectivity for ephedrine and pseudoephedrine was achieved. The authors provided spectroscopic data confirming the hypothesized mechanism and applied the validated method to quantitation of ephedra alkaloids in *Ephedra* Herba samples (Ma Huang) extracted using ethanol. Recovery values ranged within 95.0–104.0% (RSD % < 2.54) and the sensitivity (LOD 5.0 μg/mL) was adequate for application to real samples [30].

Higher sensitivity was obtained by combining MEKC separation and Laser-Induced Fluorescence detection (LIF). The applied achiral MEKC method was based on SDS as the surfactant and acetonitrile (ACN) as organic modifier. Ephedrine and pseudoephedrine were derivatized using 5-(4,6-dichloro-s-triazin-2-ylamino)fluorescein (DTAF). The derivatization reaction was carried out under aqueous conditions (pH 9.5 in carbonate/bicarbonate buffer, 0.05 M) in 30 min at 45 °C using a molar ratio (derivatization reagent to analyte) of 20:1. The method was applied to real samples of ephedra herb and its preparations (tablets and capsules); adequate recovery (89.6–106.8%) and very high sensitivity (LOD lower than 4×10^{-4} ng/mL) were obtained [31]. Zhou et al. used 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) as labelling reagent for in-capillary derivatization of ephedrine and pseudoephedrine. Briefly, aqueous samples were injected hydrodynamically into the CE capillary at the constant temperature of 25 °C; successively, sequential injections of derivatization buffer plug (tetraborate 20 mM, pH 8.6) and reagent solution plug (NBD-F 5 mM in ACN) were performed. After mixing time of 15 s, a stand-by time of 1 min was found to be the optimum to allow the derivatization reaction to take place. The electrophoretic separation was then carried out under MEKC conditions and the LIF detection was performed using excitation wavelength at 488 nm and emission at 520 nm. The method was applied to samples such as powdered ephedra herb, Keke capsules and tablets (Hei Pian and Bai Pian) extracted with water in ultrasonic bath [32]. Similar sensitivity was reported by Dong et al., using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-Cl) as derivatization reagent and LIF detection. A conventional off-line derivatization procedure was applied using a non-aqueous media composed of methanol:acetonitrile 80:20 (v/v) containing ammonium acetate 40 mM; the reaction time was 40 min at 55 °C. In order to have a full compatibility of the derivatization sample mixture with the separation buffer, NACE conditions (ammonium acetate 80 mM–acetic acid 3% in methanol) were applied. Herbal preparations (Gejie Dingchuan capsules Feibao Sanxiao tablets and Hei and Bai Pian tablets) used in TCM (Traditional Chinese Medicine) were subjected to extraction with methanol for the analysis of ephedrine and pseudoephedrine [33].

Biosynthetic pathways involved in plant kingdom drive often to enantiomerically pure compounds. Furthermore, although several factors affect the chiral composition of the plant metabolites, detecting a distortion of the enantiomeric ratio can be considered as an acceptable hint of the natural origin of the compounds [1]. Chiral analysis is one of the main fields of the chemical quality control of pharmaceuticals in which CE plays a pivotal role [34,35]. In characterization of *Ephedra sinica* and its medicinal phytopreparations, chiral analysis provides a useful tool because only (1R,2S)-(−)-ephedrine, (1S,2S)-(+)-pseudoephedrine enantiomers are found in nature. Simple CZE analysis of ephedrine and pseudoephedrine in herbal drugs (tablets) was carried out in a 25 mM triethanolamine phosphate buffer (pH 2.5) in condition of reversed EOF. Highly sulphated β-cyclodextrin was supplemented as the chiral additive to obtain the required enantioselectivity and to allow the separation of ephedrine and pseudoephedrine enantiomers from other herbal drug constituents [36]. A combination of three neutral cyclodextrins, namely βCD, hydroxypropyl-β-cyclodextrin (HP-βCD) and Heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-βCD) under acidic conditions was used as a chiral system to achieve the simultaneous enantioresolution of octopamine and synephrine enantiomers in the presence of tyramine and *N*-methyl tyramine in *Citrus* species dietary supplements. In particular the chiral analysis was applied to evaluate the thermal racemisation of l-synephrine. Although information on the kinetics of the conversion was not provided, the study demonstrated that the extraction procedure applied to the real samples did not lead to any chiral artefacts being the racemisation of synephrine effective at relatively high temperature (i.e. 100 °C) [37].

Phinney and co-workers developed three CE-UV methods that allowed the enantioseparation of ephedrine and pseudoephedrine using neutral HP-βCD, DM-βCD and charged sulfated βCD as chiral selectors. The combination of negatively charged sulfated βCD (2.8%) and DM-βCD (1.2%) under acidic conditions (pH 2.5) pro-

Table 2
Details on the CE analysis of ephedra alkaloids.

Sample	CE method	Sample preparation	Alkaloid content[a]	LOD (μg/mL)	Ref.
Ephedra dietary supplements	Chiral CZE-UV	Ultrasonication (water-HCl)	(−)-E: 2% (+)-PE: 0.5%	0.6	[36]
Ephedra fruits	Chiral CZE-UV	Ultrasonication (methanol)	(−)-E: 2.3–3.3% (+)-PE: 1.6–2.5% (−)-ME: 0.98%	n.r.	[39]
SRM *Ephedra sinica*	Chiral CZE-UV	Ultrasonication (methanol)	(−)-E: 0.03–7.6% (+)-PE: 0.005–0.92%	n.r.	[38]
SRM *Ephedra sinica*	Chiral MEKC-ESI-MS	Ultrasonication (methanol)	(−)-E: 0.018–1.29% (+)-PE: 0.0012–0.393% (−)-Nor-E: 0.0003–0.039% (+)-Nor-PE: 0.00037–0.073% (−)-ME: 0.00093–0.121% (+)-MPE: 0.000015–0.0061%	0.0625–0.125	[41]
Ephedra herb Keke capsule Hei Pan tablets Bai Pan tablets	MEKC-LIF Derivatization with DTAF[b]	Ultrasonication (water)	E: 0.56–0.05% PE: 0.016–2.94%	1.41×10^{-7}	[31]
Ephedra herb Keke capsules Hei Pan tablets Bai Pan tablets	MEKC-LIF in-capillary derivatization with NBD-F[c]	Ultrasonication (water)	E: 0.589–0.03% PE: 0.014 – 3.0%	$1.6–4.8 \times 10^{-3}$	[32]
Ephedra herba (Ma Huang)	Ligand-exchange CE	Reflux ethanol	E: 0.60–0.84% PE: 0.19–0.22%	5.0	[30]

n.r.: not reported.

[a] Symbols: (−)-E, (−)-ephedrine; (+)-PE: (+)-pseudoephedrine; (−)-ME: (−)-methylephedrine; (−)-Nor-E: (−)-norephedrine; (+)-Nor-PE: (+)-norpseudoephedrine; (+)-MPE: (+)-N-methylpseudoephedrine.

[b] DTAF, 5-(4,6-dichloro-*s*-triazin-2-ylamino)fluorescein.

[c] NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole.

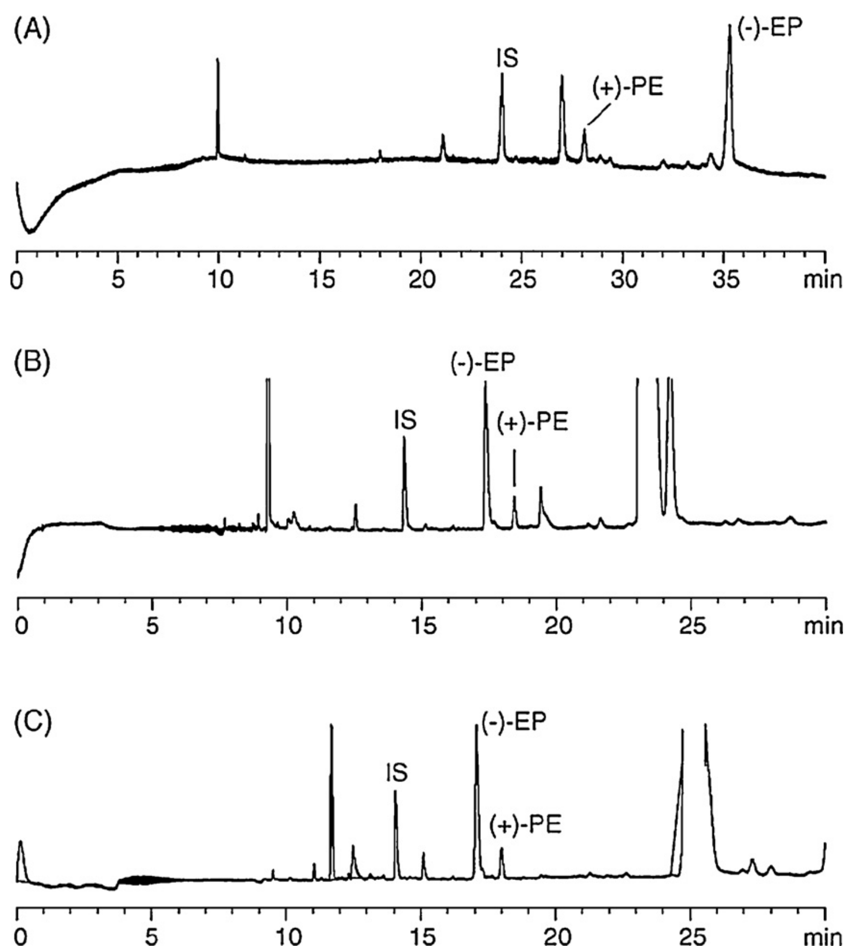
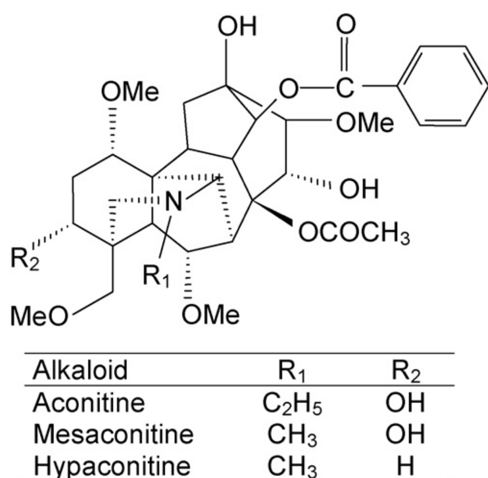


Fig. 2. Analysis of standard reference material (by NIST) using three different chiral CE methods. The method conditions and symbols are reported in Tables 1 and 2 (see Ref. [38]). Modified from [38].



Alkaloid	R_1	R_2
Aconitine	C_2H_5	OH
Mesaconitine	CH_3	OH
Hypaconitine	CH_3	H

Fig. 3. Structure of aconitine, mesaconitine and hypaconitine.

vided the best separation of the two couples of enantiomers. Standard reference materials (SRMs) containing ephedra in development at NIST, were analysed by each of three CE methods. The obtained electropherograms are reported in Fig. 2; in the SRM samples, only the naturally occurring enantiomers (−)-ephedrine and (+)-pseudoephedrine were found, however the method proved to be suitable in detecting product adulteration by its potential in identification of specific stereoisomers [38]. The neutral HP-βCD was also found to be a useful chiral selector in the presence of tetrabutylammonium chloride as additive, for the simultaneous enantioseparation of ephedrine, pseudoephedrine, *N*-methylephedrine and norephedrine enantiomers in *Ephedra sinica* extracts [39].

The comprehensive characterization of ephedra extracts was performed by chiral MEKC-ESI-MS, which provided the simultaneous enantioseparation of all ephedra alkaloids. Polysodium *N*-undecenoxy-carbonyl-L-leucinate was used as polymeric chiral surfactant because it was found to be compatible with ESI-MS detection. In the optimised conditions the enantioseparation of four pairs of ephedrine enantiomers was achieved and the ESI-MS detection allowed high sensitivity (LOD ranging within 0.0625–0.125 μg/mL) [40,41].

2.3. Aconitine alkaloids

Aconitum plants (*Ranuncolaceae*) are widely distributed across Asia and North America and two species of them, namely *A. carmichaeli* Dexb. and *A. kusnezoffii* are listed in the Chinese Pharmacopoeia. Aconitine and the congeners mesaconitine and hypaconitine (Fig. 3) are the main diester-diterpene alkaloids of aconitum plants and although they show high toxicity they can also be used at low doses because of pharmacological effects such as anti-inflammatory and anti-pain [42]. Owing to the biological activity (and toxicity), CE analytical methods for the quality control of aconitum alkaloids in herbal drugs have been considered.

CZE was applied in analysis of aconitine, hypaconitine and mesaconitine in extracts from aconitum plants using alkaline buffer such as phosphate at pH 8.5–9.5 in aqueous or mixed aqueous–organic media [43], and in the presence of the ionic liquid 1-butyl-3-methylimidazolium (1B-3MI-TFB) [44]. UV detection at 235 or 254 nm allowed the sensitivity required in analysis of real samples, however improved LOD (10^{-8} M, thus in the order of ng/mL) was achieved by means of ECL detection using tris(2,2′-bipyridil)ruthenium(II), $Ru(bpy)_3{}^{2+}$, 5 mM in phosphate buffer (50 mM) [45]. Song et al. focused on the poor stability of the native aconitum alkaloids, which undergo to hydrolysis of the ester moiety. Monester-diterpenes, namely benzoylaconine, benzoylmesaconine and benzoylhypaconine, can occur in processed aconitum herbal drugs and, interestingly, these artefacts show less toxicity compared to the parent compounds. Using a BGE composed of 200 mM Tris buffer containing 150 mM perchloric acid and dioxane 40% at pH 7.8, the simultaneous separation of three diester-diterpene alkaloids (the native aconitine, hypaconitine and mesaconitine) and the related degradation products (benzoylaconine, benzoylmesaconine and benzoylhypaconine) was accomplished in about 25 min (Fig. 4). The obtained detection limit was lower than 45 ng/mL. The analysis of crude and processed herbal preparations clearly showed that the manufacture process affected the content of aconitum alkaloids with a significant decrease of the native diester-diterpenes [46].

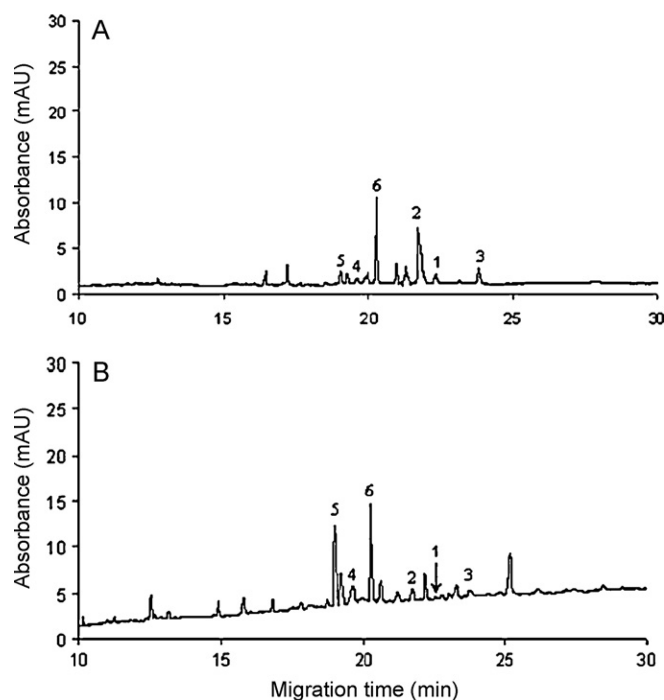


Fig. 4. Typical electropherograms of crude aconite roots (A) and processed roots (B) from *A. carmichaeli*. Conditions. Fused-silica capillary (39.6 cm effective length, i.d. 50 μm); BGE 200 mM Tris buffer containing 150 mM perchloric acid and dioxane 40% at pH 7.8. Detection at 214 nm; electrokinetic injection at 10 kV for 10 s; Voltage 25 kV; temperature 25 °C. Peaks: aconitine (1); mesaconitine (2); hypaconitine (3); benzoylaconine (4); benzoylmesaconine (5); benzoylhypaconine (6). Modified from [46].

2.4. Quinolizidine alkaloids

Radix sophorae flavescentis (*Sophora flavescens*) is frequently used in TCM for treating acute hepatitis and jaundice; it was found that quinolizidine alkaloids were the main constituents of this herbal drug. Furthermore, these compounds exhibit adverse effects and the quality control of the quinolizidine-containing herbal drugs needs for the accurate quantitation of the alkaloids content. The p*K*a values determined by CZE of quinolizidine alkaloids such as matrine, sophoridine, sophocarpine, lehmannine, sophoranol, oxymatrine, oxysophocarpine, were found in the range 5.90–7.72 [47]. Their analysis was performed by CE-UV using aqueous running buffers both in alkaline (borate and phosphate pH 8.5 [48]) and acidic conditions (phosphate pH 2.5 [49]). The sample preparation involved the extraction of plant materials using chloroform. Similarly for other alkaloid types, quinolizidines, owing to the presence of tertiary amine function, react with tris(2,2′-bipyridyl)ruthenium

(II) to produce ECL response. This approach was applied to the highly selective and sensitive analysis of *S. flavescens* extracts. Unexpectedly, ECL response was also observed for oxymatrine (quinolizidine *N*-oxide), which should not be determined because of the lack of tertiary amino function. Under these detection conditions the LOD values were 1000-fold compared to those achieved by CE-UV at 200 nm [50]. NACE was applied in analysis of matrine and oxymatrine in *S. flavescens* extracts by UV detection [51]; hyphenation with ESI-MS was reported in analysis of quinolizidine alkaloids in *Lupinus* species. The BGE was modified by addition of a small percentage of water (10%) in order to obtain the separation of the potentially toxic alkaloids sparteine, lupanine, angustifoline and 13α-hydroxylupanine [52]. MEEKC using sodium cholate as surfactant at pH 6.5 (borate–phosphate electrolyte), 1-butanol (1.2%) as the co-surfactant and ethylacetate (0.6%) as the oil, was found to be useful in analysis of the quinolizidine alkaloids (including cytisine) of *S. flavescens*. Interestingly, Mg^{2+} was supplemented at low concentration (4 mM) as an EOF modifier; it was found to improve the separation selectivity. The developed MEEKC system allowed the application of field amplified injection technique; the reported limit of detection was 0.1 ng/mL [53].

2.5. Isoquinoline alkaloids

Bis-benzylisoquinoline alkaloids have attracted attention for the important pharmacological effects; in particular protoberberines are a structural class of organic cations (quaternary ammonium alkaloids) mainly distributed in Ranuncolaceae (e.g., *Rhizoma coptidis*), Berberidaceae (e.g. *Cortex berberdis*), Papaveraceae (e.g. *Herba chelidoni*) and Rutaceae (e.g. *Cortex phellodendri*). The most considered protoberberine alkaloids are berberine, palmatine and jatrorrhizine. Protoberberine alkaloids display a great variety of biological and pharmacological activities, including inhibition of DNA synthesis, protein biosynthesis, inhibition of membrane permeability, and the uncoupling of oxidative phosphorylation. These processes likely contribute to the allelochemical and toxic effects observed against bacteria, fungi, other plants, insects, and vertebrates [54].

NACE has shown to be a useful technique in analysis of these compounds in herbal drugs and preparations. The high selectivity of a mixed organic-aqueous running buffer (methanol:water, 4:1 v/v, containing sodium acetate 100 mM, pH* 5.0) was applied in fingerprint analysis of the Chinese herbal medicine *Zanthoxylum nitidum* containing the protoberberine alkaloids chelerythrine and the isomer nitidine [55]. Quantitation of berberine, palmatine and jatrorrhizine, in complex samples such as *Rhizoma coptidis*, *Cortex berberdis*, *Herba chelidoni*, *Cortex phellodendri* and *Caulis mahoniae* herbal drugs, was performed using a NACE buffer composed of 50 mM ammonium acetate, 0.5% acetic acid and 10% ACN in methanol. UV detection at 214 nm allowed LOD of about 0.3 μg/mL [56]. Under similar NACE conditions, bisbenylisoquinoline alkaloids, namely fangchinoline and tetrandrine were determined in Radix *Stephaniae tetrandrae* and its medicinal preparations; high detection sensitivity (0.3 ng/mL) was obtained by injecting a short plug of ethanol before the injection of the sample plug, in order to achieve a field amplified stacking effect [57].

Mixed organic-aqueous BGE system (ammonium acetate at pH 3.4 with acetic acid in water–methanol, 1:5 v/v) was applied in separation of berberine and hydrastine in root powder of *Hydrastis canadensis*; detection was performed at 225 nm [58]. Significant improvement of sensitivity (at ng/mL level) was achieved by profit of the native fluorescence of the compounds (emission at 520 nm) under laser excitation at 488 nm [59,60]. Hyphenation of NACE with electrospray ion trap mass detector was applied to the analysis of isoquinoline alkaloids in *Fumaria officinalis*. A mixture ACN–methanol (9:1, v/v) containing 60 mM ammonium acetate and 2.2 M acetic acid was used as the running buffer. Under these conditions the migration order was found to follow the p*K*a of the compounds. Furthermore the ESI (positive ionization mode) and MS/MS experiments, provided typical fragmentation useful to the unambiguous identification of 10 alkaloids. The NACE-MS method was applied in quantitative analysis of the major isoquinoline alkaloids in *F. officinalis* herba and its phytotherapeutic tablets containing protopine and cryptopine [61]. The same Authors applied NACE-MS conditions to analyze a set of 79 samples belonging to *Corydalis* species; 39 analytical peaks were characterized by the MS spectra and a number of them were unambiguously identified as isoquinoline alkaloids. Principal component analysis (PCA) performed using peak areas as the data basis, allowed to select 8 compounds (among them allocryptopine, protopine, corycavidine, bulbocapnine and terahydropalmatine) as putative biomarkers for discrimination of the *Corydalis* species. The proposed method provided also the sensitive quantitation of palmatine, protopine, bulbocapnine, corydaline, tetrahydropalmatine (LOD values in the range 0.7–8.3 μg/mL) [62]. A mixed organic-aqueous buffer was also used in CE coupled to ESI-TOF (Time of Flight) mass spectrometry in analysis of *Rhizoma coptidis*. A number of alkaloids were identified: berberine, palmatine, jatrorrhizine, columbamine, epiberberine, coptisine, tetradehydroscoulerine and tetrahydrocheilanthifolinium. In addition, the CE-MS method was validated for linearity, sensitivity and precision on selected alkaloids namely berberine, palmatine and jatrorrhizine. The analysis was carried out on real samples represented by dried roots of *Rhizoma coptidis*; the sample preparation was performed by accelerated solvent extraction using ethanol (80% in diluted HCl). The CE-MS method proved to be suitable also in routine analysis [63].

MEKC analysis of benzylisoquinoline alkaloids is applied mainly to provide separation of complex mixtures in plant samples including bioactive neutral components. Using sodium deoxycholate 50 mM as surfactant, the MEKC separation of coptisine, berberine, and palmatine was achieved simultaneously to that of neutral bioactive compounds such as flavonoids (wogonin, baicalenin, baicalin) and anthraquinones (sennoside A and B, emodin, rhein, physcion, chrysophanol and aloe-emodin). In Fig. 5 the electropherograms of a standard mixture of the considered analytes and a real sample (TCM preparation San-huang-xie-xin-tang extract), are reported. The method showed excellent selectivity and was applied to the analysis of *Coptidis Rhizoma*, *Scutellariae Radix*, *Rhei Rhizoma* (rhubarb) and phytopreparations containing the three herb mentioned above. The sample preparation was carried out by exhaustive extraction using methanol–water (70:30, v/v) in ultrasonic bath. The extracts were concentrated under vacuum and dissolved in acetonitrile–water (70:30, v/v). The method was validated for linearity, precision, accuracy (recovery values were in the range 98.12–107.58%, RSD % inter-assay < 5.21, $n = 9$) and the detection limits in analysis of protoberberine alkaloids were < 0.65 μg/mL [64]. Higher sensitivity (in the range of ng/mL) was obtained in the determination of berberine, coptisine and palmatine by MEKC using sweeping technique [65]. MEKC separation of tetrandrine and fangchinoline was obtained using the neutral surfactant Tween 20, which was found to enhance the solubility of cationic hydrophobic compounds. The separation system was applied in flow injection capillary electrophoresis for the analysis of *Stephaniae tetrandrae* capsules [66].

Very fast separation of the major protoberberine alkaloids in extracts of *Coptis chinensis* Franch could be obtained by CEC using both a monolithic silica column and a monolithic silica column bearing sulfonic acid groups for strong cation-exchange interac-

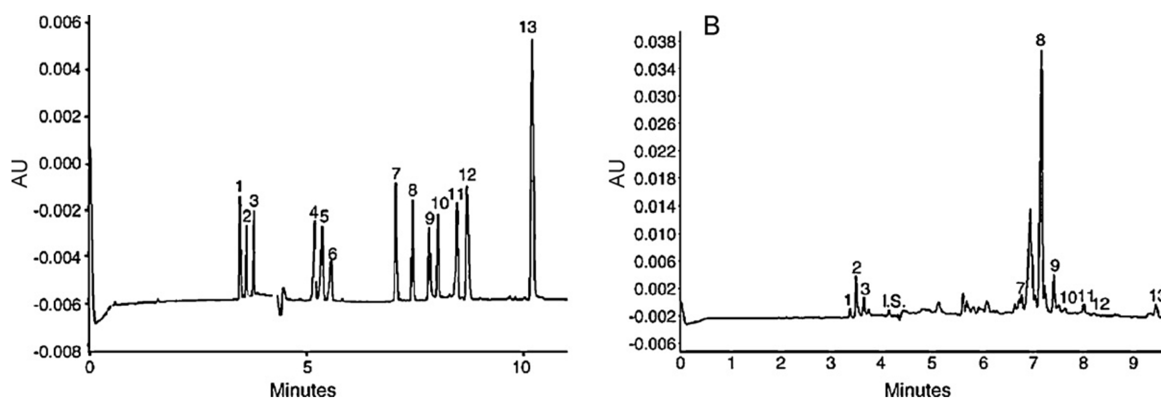


Fig. 5. (A) Electropherogram of standard mixture of coptisine (1), berberine (2) and palmatine (3) in the presence of neutral compounds: aloe-emodine (4), chrysophanol (5), physcion (6), wogonin (7) baicalin (8), baicalein (9), sennoside B (10), sennoside A (11), emodin (12), rhein (13). IS: tetrandrine (the internal standard). (B) Electropherogram of a real sample (San-huang-xie-xin-tang extract). Conditions: 3 mM sodium tetraborate–10 mM sodium dihydrogen phosphate (pH 7.3), 50 mM sodium deoxycholate, 30% acetonitrile. Fused silica capillary (49 cm effective length, i.d. 50 μm); detection at 270 nm. Modified from [64].

tions (MS-SCX). The mobile phase used in both the applications was composed of phosphate buffer pH 7.4 in the presence of 60% ACN as organic modifier. The two methods (conventional CEC monolithic column and MS-SCX column) showed similar separation selectivity (separation profile and migration/retention time) [67].

2.6. Pyrrolizidine alkaloids

Pyrrolizidine alkaloids are found in a variety of plant species growing wideworld such as *Gynura segetum* that belongs to the Compositae family and *Senecio* and *Tussilago* genera. Some of these plants and phytopreparations are officially used in TCM with the vernacular name of Qian liguang and Kuan donghua for the treatment of pharyngeal infection and chronic bronchitis, asthma and influenza, respectively. However, beside the pharmacological effect, due to the presence of pyrrolizidine alkaloids, these TCM remedies are also focus of attention of health control Authorities (e.g. German Federal Health Bureau) for their potential genotoxicity and carcinogenicity [68].

Analytical separation methods involving HPLC and CE have been proposed for the quantitation of these toxic pyrrolizidines. The most important pyrrolizidine alkaloids senecionine, seneciphylline, retrorsine and senkirkine, contain the 4-azabicyclo[3.3.0]octane system with senecionine and seneciphylline differing only for the presence in the latter of the C_{13}-C_{23} double bond. Owing to the same p*K*a values (5.44) [69], these strictly related compounds can be separated only by MEKC. SDS [70] and lauric acid [71] as surfactants were used in MEKC analysis of Qian liguang and Kuan donghua and *Gynura segetum* plant material, respectively. Using both the MEKC systems the addition of methanol (20%) to the BGE was necessary to achieve the required selectivity. Yu and Li introduced a dynamic pH junction–sweeping technique to improve the sensitivity of the SDS based MEKC method; in particular, the pH of the sample matrix extract was adjusted to acidic value and injected as a large volume into the capillary filled with the SDS separation solution (pH 9.1). The positively charged pyrrolizidines migrated into the sample plug towards the boundary with the separation solution. Due to the high pH value of the MEKC BGE the cationic pyrrolizidine are converted to the neutral free base forms and are focused by partitioning into SDS micelles. The achieved sensitivity was 90-fold higher than that obtained using conventional injection [69].

2.7. Indole alkaloids

Indole alkaloids constitute a wide class of natural products most of them pharmacologically important and characterized by very different activities.

Strychnos nux-vomica is used in TCM as anti-inflammatory and analgesic, however the major indole alkaloids, strychnine and brucine, are well-known for their toxic effects consisting in significant rise of blood pressure and induction of violent convulsions [72]. The quantitation of these compounds in plant materials as well as in phytopreparations is thus of importance, the therapeutic index being very low.

NACE was useful in reducing the peak tailing observed in aqueous media and caused by interaction of the cationic hydrophobic alkaloids with the Si–OH groups of the inner capillary wall. Ammonium acetate was the selected electrolyte in the presence of acetic acid dissolved in ACN–methanol mixture. Fast separation of strychnine and brucine was obtained and the method was applied to the analysis of *Strychnos nux-vomica* plant materials and Yaotontning (TCM) capsules, after extraction using ammonium hydroxide in methanol [73]. Alternatively a BGE composed of Tris-borate at pH* 4.0 in methanol–ACN (60:40, v/v), was used [74]. Although the applied electrophoretic conditions of the two described methods were very similar, the limit of detections for strychnine was 0.02 μg/mL using ammonium acetate as supporting electrolyte [73] and 0.578 μg/mL using Tris-boric acid [74]; the sharper peak shapes observed using ammonium acetate could explain the better UV response.

MEKC was used in combination with sweeping concentration technique. In particular, using acidic running buffer (50 mM phosphoric acid at pH 2.0) containing SDS 100 mM, a strong suppression of the EOF was obtained. The acidified sample matrix was injected as a large sample volume and the hydrophobic cationic alkaloids could be partitioned into the SDS micelles. The achieved sensitivity (LODs were 0.05 and 0.07 μg/mL for strychnine and brucine, respectively using UV detection at 203 nm) was 100-fold higher compared to that obtained using conventional injection [75].

The monoterpenoid indole alkaloid yohimbine is the typical bioactive compound of *Pausinystala yohimbe* whose barks are used in tropical West Africa as an aphrodisiac. Methanol extracts of *P. yohimbe* were directly injected in an optimized NACE-UV method based on ammonium acetate–acetic acid as the BGE in methanol. The samples were simultaneously analysed in NACE-UV and in gas chromatography–mass spectrometry (GC–MS), used as a refer-

ence method. As expected, GC–MS demonstrated to be superior in terms of efficiency and opportunities in identification of analytes; however the results obtained in quantitation of yohimbine in real samples using NACE and GC–MS were not significantly different. In particular the analysed samples showed to contain about 1.1–1.2% of the active alkaloid [76].

Hydroindole alkaloids such as mesembrine and congeners (mesembrenone, Δ^7 mesembrenone, mesembranol and its stereoisomer epimesembranol) have been isolated from *Sceletium* species used for the psychoactive effects. A simple CZE method based on phosphate buffer at pH 1.5 was successfully applied to the separation of the alkaloids of *Sceletium* phytopreparations. The quantitation was performed only on mesembrine by UV detection at 228 nm; the LOD was 1.5 μg/mL and the commercially available tablets were found to contain mesembrine at 0.03% level [77].

Psychotria viridis, a plant used by the Shamans from South America as psychotropic and sacramental drug, contains β-carboline alkaloids that can be included among indole alkaloids. Huhn et al. developed a mixed aqueous-organic CE method for the analysis of β-carbolines in extracts of *P. viridis*. A dual detection system, LIF and ESI-MS, was developed; in particular, the use of optical fibers allowed the LIF detection to be set close to ESI source making easy the comparison of the LIF and MS electropherograms. Using 200 mM formic acid and 7 mM ammonia in water with 10% ACN, the baseline separation of norharmane, harmane and tetrahydroharmane was accomplished. In addition, the method provided the simultaneous separation of harmine, harmaline and harmol. It was observed that a solution of concentration 40 nM, provided a MS signal around the detection limit. LIF detection was applied by profit of the native fluorescence of β-carbolines (λ_{ex} = 266 nm); with the exception of tetrahydroharmane, which possesses a low fluorescence quantum yield, the sensitivity of LIF detection was found to be about 5-fold higher than ESI-MS [78].

2.8. Methylxanthine alkaloids

Methylxanthine alkaloids (purine backbone) caffeine, theophylline and theobromine are present in a variety of plants and plant products used for preparation of beverages (coffee, tea, guarana). Because of their stimulant effects on cardiovascular and nervous system, methylxanthine-containing plants are sometimes used as phytopharmaceuticals and dietary supplements. Depending on the country, caffeine level in beverage could be limited; i.e. the U.S. FDA set the limit of 0.02% [79], however the so-called energy drinks when classified as “supplements” may contain more. Further, natural caffeine levels are exempt from these regulations, thus coffee based drinks may have more caffeine than 0.02% without being classified as a supplement. Methylxanthines content is also used as an index of the quality of coffee, tea and cocoa.

Analytical methods for the analysis of methylxanthines in plants and derived products have been developed in order to provide useful tools in the quality control of such a wide variety of preparations.

CZE in alkaline conditions (tetraborate, pH 9.2) was applied to the analysis of guarana (*Paullinia cupana*) phytopreparations; the dissociation of acidic hydrogen of theobromine (H-1) and that of N-H of the imidazol-moiety of theophylline, made these compounds migrating as anions whereas caffeine occurred with the EOF [80]. Under similar conditions this effect was not observed by Li et al., that however focused their study on the development of a sample injector for high precision analysis of methylxanthines containing drinks, tea leaves and roasted coffee extracts [81].

MEKC was developed for methylxanthines analysis in drinks (*yerba mate*, coffee, cocoa, tea, decaffeinated coffee, etc.) using SDS as surfactant under alkaline conditions (tetraborate, pH 8.5 [82] or carbonate pH 11.0 [83]). However alkaline buffers provided poor peak shapes and detrimental peak splitting due to the keto-enol tautomerism of methylxanthines [82]. The levels of caffeine found in different beverages showed high variations depending on the type; i.e. some of the analysed decaffeinated coffee samples were found to contain levels of caffeine above the limit set by authorities (Brazilian legislation set the limit of 0.1%) [82,83]. MEKC was found to be useful also in simultaneous separation and analysis of methylxanthines and catechins in tea and *Theobroma cacao* samples. In particular the use of cyclodextrins in cyclodextrin-modified MEKC (CD-MEKC) mode offered the opportunity for high selectivity tuning. The poor stability of catechins at pH > 8 led to choose neutral [84] or acidic running buffers [85,86]; under these conditions also the peak splitting of methylxanthine due to keto-enolate tautomerism was circumvented and fast analysis of methylxanthines, catechins, procyanidins, phenolic acids, vitamin C and theflavin was obtained [84–86].

CEC using hybrid silica monolith columns allowed a very fast analysis of methylxanthines in tea beverages; in analysed samples, theophylline was not detected whereas the caffeine content was found to be more than 10-fold lower compared to the tea infusions [87].

2.9. Morphinane alkaloids

Morphinane alkaloids (opium alkaloids) such as morphine, codeine, thebaine, papaverine and narcotine belong to isoquinoline derivatives and show a broad range of pharmacological activities; their major application is in analgesia, sedation and cough depression. They also exert a number of side effects as respiratory depression. In addition morphinane alkaloids are strongly addictive and illicitly used as narcotic drugs; for this reason are under strict international control. Opium, the exudates from *Papaver somniferum*, contains more than 30 alkaloids and is the raw material for extraction; also the dried heads of *P. somniferum*, so-called poppy straw, is used as a source of morphine and thebaine. Analytical methods are of importance for the determination of these compounds in plant materials because the content of specific morphinane alkaloids could be used to differentiate the origin of opium that is a very important issue to help the law enforcement agencies in checking the illicit production of narcotic drugs. CZE of morphinane alkaloids (100 mM sodium acetate–acetic acid, pH 3.1), was applied by Sashidhar and co-workers to the determination of thebaine, codeine, morphine, papaverine and narcotine in 124 opium samples from different regions of India. The alkaloids profile was found to be dependent on sample type. Application of discriminant analysis made it possible to cluster the analysed samples on the basis of their geographical origin [88]. A chemometric approach to the differentiation of opium and poppy straw samples was also successfully applied by Reid and co-workers. The analysis of the samples was carried out by MEKC using 50 mM phosphate buffer (pH 2.5) and 80 mM SDS in the presence of 25% methanol. Under these conditions a sweeping concentration technique was applied to improve sensitivity; the obtained LOD values were in the range 0.05–0.14 μg/mL (UV detection at 200 nm) [89].

CD-modified CZE was applied to improve the separation selectivity in the analysis of morphinane alkaloids in different *Papaverum* species and in the liquors (rich extracts, spent extracts rich solvent liquors) produced during the industrial manufacture processes of alkaloids extraction. Fast separation of the morphinane alkaloids was achieved by using acidic running buffer (pH 2.8–3.0) supplemented with HP-βCD (30 mM) or α-cyclodextrin (αCD) (5 mM) [90,91]. The UV detection allowed for adequate sensitivity (LOD values were in the order of 0.1 μg/mL). However a significant improvement of sensitivity was obtained by ECL detection using tris(2,2′-bipyridyl)ruthenium(II) in the detection cell. In this application the separation buffer was based on 25 mM borate alkaline solution supplemented with the ionic liquid 1-ethyl-3-

methylimidazolium tetrafluoroborate (1E-3MI-TFB). Borate buffer was useful in improving the separation selectivity by complexation of borate anions with the hydroxyl groups of alkaloids. On the other hand, the ionic liquid, because of the higher conductivity compared to that of phosphate buffer present in the detection cell, provided a field amplified effect, resulting in improved sensitivity (LOD of 1×10^{-6} to 1×10^{-9} M) [92]. ECL detection was applied in CZE analysis of sinomenine (7,8-didehydro-4-hydroxyl-3,7-dimethoxy-17-methylmorphinan-6-one), the morphinane alkaloid found in *Sinomenium acutum*. The achieved detection limit was reported to be 2×10^{-3} μg/mL [93], thus about 1000-fold lower than that obtained using a conventional NACE-UV method [94].

CEC was applied to the determination of narcotine, papaverine, thebaine, codeine, morphine in *Pericarpum papaveris* using a monolith consisting of hydrophilic/cation-exchange mixed stationary phase. The cation-exchanger function was based on sulfonic groups which also afforded for the EOF, whereas the diol groups effected as hydrophilic interaction sites. It was shown that high concentration of organic modifier (90% ACN) promoted hydrophilic interaction of the analytes with the stationary phase improving separation [95].

2.10. Miscellaneous alkaloids

Camptothecines are valuable quinoline alkaloids present in *Camptotheca acuminata*, *Nothapodytes foetida*, *Ophiorrhiza pumila* and *Tabernaemontana heyneana*. The increasing interest towards these compounds is due to the demonstrated activity in the treatment of colorectal and ovarian cancer. Camptothecin is currently obtained from natural sources both for the clinical use and for the downstream synthesis of the derivatives. The main issue in developing analytical methods for the determination of camptothecin in plant materials is the poor water solubility. Extraction of active alkaloids from plant material was optimized using dimethyl sulfoxide; subsequently a CE method was developed for the separation of camptothecin and 9-methoxycamptothecin. In order to achieve compatibility of the CE separation medium with the extraction solvent, a MEKC running buffer containing 90 mM SDS and 20% dimethyl sulfoxide in 10 mM borate buffer was used. Interestingly, borate (pH > 8) as supporting electrolyte was found to be effective in increasing the solubility of camptothecin and derivatives by promoting the equilibrium of the lactone forms to the carboxylate as a consequence of the complexation with borate ions. Owing to the characteristic UV spectrum, the selective detection in real samples was performed at the wavelength of 369 nm and the LOD could be assessed as about 2 μg/mL thus adequate to this application (3.76 mg/g of camptothecin in *N. foetida* plant materials) [96].

Amaryllidaceae alkaloids are an important class of isoquinoline derivatives; among them galanthamine, that is found in *Galanthus* and *Narcissus* species, has been approved for the pharmacological treatment of Alzheimer's disease [97]. GC–MS analysis is considered the method of choice for the characterization of amaryllidaceae alkaloids; by means of this approach a number of specific compounds have been identified in *Narcissus* species (i.e. lycoramine, narwedine, haemanthamine, tazettine). NACE-UV was applied as a confirmatory and orthogonal method in analysis of *Narcissus pseudonarcissus* and *Narcissus jonquilla*. NACE was performed using 90 mM ammonium acetate and acetic acid (0.5%) in methanol–ACN mixture (75/25, v/v). The organic running buffer was compatible with the real samples obtained by extraction of plant material (crushed bulbs) using methanol. The amount of galanthamine and haemanthamine in the analysed *Narcissus* extracts was found to be 2.2 and 0.32 mg/g, respectively. The analytical performances of NACE-UV in terms of sensitivity and accuracy were similar to those of the GC–MS reference method [98]. The use of ECL detection after CZE separation (18 mM aqueous phosphate buffer, pH 9.0) was applied to the analysis of galanthamine in *Bulbus Lycoridis Radiatae*, widely used in TCM. The ECL detection based on tris(2,2′-bipyridyl)ruthenium(II) allowed LOD of 0.25 ng/mL with a significant gain compared to the UV detection; however the level of galanthamine found in the *Bulbus Lycoridis Radiatae* phytopreparations was 0.13 mg/g thus affordable also by UV detection [99].

Steroidal alkaloids such as verticine and verticinone are characterized by cholestane carbon skeleton (isosteroid alkaloids) with a hexacyclic benzo[7,8]fluoreno[2,1-b]quinolizine nucleus. These compounds have been isolated from plants belonging to Liliaceae family i.e. *Bulbus fritillariae* used as a traditional herb remedy in Japanese, Turkish, Pakistani and south-east Asian folk medicines [100]. The *B. fritillariae*-based phytopreparations are used as antitussive, anti-asthmatics and expectorants. Extracts from different *Fritillariae* species were obtained with chloroform in the presence of ammonia to afford the free base of the alkaloids. The organic residues was dissolved in methanol and analysed by CZE using ECL detection. In particular the running buffer was constituted of 8 mM phosphate pH 8.0 in the presence of 40 mM 1B-3MI-TFB ionic liquid. Under these conditions a very low LOD (about 50 pg/mL) in analysis of verticine and verticinone was achieved. The analysis of real samples showed that verticine was found in any of the analysed extracts (*Fritillariae hupehensis*, *F. waluajewii*, *F. cirrhosa*, *F. thunbergii*, *F. ussuriensis*, *F. pallidiflora* and *F. przewalski*) at amounts ranging within 5.40–12.35 mg/L, whereas verticinone was found at lower levels (5.66–8.07 mg/L) only in *F. hupehensis*, *F. cirrhosa*, *F. thunbergii* and *F. ussuriensis* [101].

Stemona, from the family of Stemonaceae, is known in traditional medicine of south-east Asia, China and Japan because its phytopreparations (primary the roots) are used to treat bronchitis, pertussis and tubercolosis. Interestingly a number of alkaloids, structurally defined as pyrido[1,2-a]azepines, have been identified in this plant species and are considered to be responsible for the mentioned pharmacological activity. NACE coupled with ESI-MS detection has been developed to characterize the highest number of alkaloids in *S. curtisii*, *S. collinsae*, *S. tuberosa*. In particular by means of MS^n experiments at least 40 and 50 alkaloids or charged *N*-bearing compounds, were detected. The method was successfully applied to the quantitation of stemofoline, oxystemokerrin, didehydrostemofoline and stemocurtisinol [102].

3. Polyphenols

Epidemiological studies have shown that natural phenolic compounds play an important role against biomarkers for cancer, cardiovascular diseases and other degenerative diseases [103–105]. Several thousand molecules with polyphenol structure have been identified in higher plants and edible plants; these compounds may be classified as phenolic acids, flavonoids, stilbenes and lignane. In natural sources, polyphenols are contained as complex mixtures and their composition is highly variable in amount and quality; as a consequence the analytical profile (quali- and quantitative) is very important in standardization of herbal drugs containing polyphenols as well as in monitoring the food changes such as oxidation. Detailed reviews dealing with advanced separation methods [106–108], including electromigration techniques [106,109–112] for analysis of polyphenols, are available in the recent literature.

The most important aspects involved in capillary electrophoretic analysis of phenolic compounds in herbal drugs and medicinal plants, will be dealt below by following the chemical classification based on the constitutive phenolic skeleton: phenols (phenolic acids and coumarins); quinones and naphtoquinones; flavonoids (flavones, isoflavones, flavanones, flavonols, flavanols, anthocyanins). General structures of flavonoids are reported in

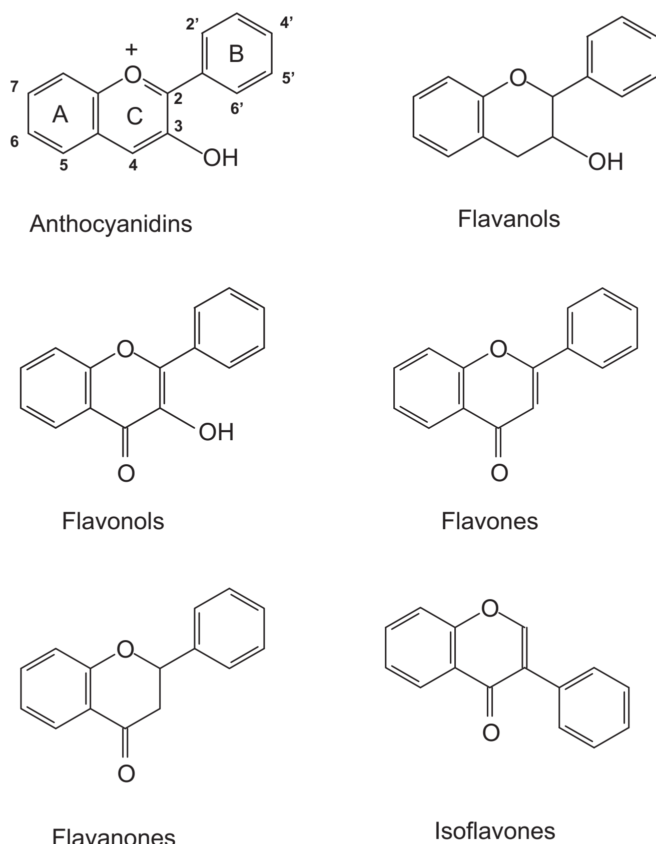


Fig. 6. Structure of the main flavonoids.

Fig. 6. These compounds share a common structure consisting of two aromatic rings (A and B) that are bound together by three carbon atoms that form an oxygenated heterocycle (C). Their classification is based on both the substitution of C ring and the position of B ring; they are often hydroxylated in positions 3, 5, 7, 3′, 4′ and /or 5′. In plants, flavonoids are often present as O– or C– glycosides.

3.1. Phenolic acids

Phenolic acids are secondary plant metabolites having hydroxycinnamic or hydroxybenzoic structures. Hydrobenzoic acids content in plants and herbal drugs is generally very low, whereas hydroxycinnamic acids are very common (i.e. *p*-coumaric, caffeic, ferulic acid). These compounds are rarely found in the free form; the bound forms are glycosilated derivatives or esters [103,104]. The determination of phenolic acids profile by separation techniques can be used as a fingerprint and for the standardization of herbal drugs, however, often the analytical methods are developed to generally include the determination of phenolic compounds together with other flavonoids [106–112]. In the following section some CE methods specifically developed for the analysis of simple phenolic compounds in herbal drugs are discussed.

CZE using direct UV detection is the method of choice in the analysis of these compounds because of their ionic character and strong UV absorption; tetraborate buffer (native pH of 9.2) was found to be the most used BGE. The separation and quantitation of cinnamic, chlorogenic, syringic, ferulic, benzoic, *p*-coumaric, vanillic, caffeic and protocatechuic acid in extracts of *Bromus inermis* was obtained in about 14 min using a BGE constituted of 20 mM tetraborate in the presence of 5% methanol. The sample was obtained by Soxhlet extraction of the plant material using 2 M HCl and subsequent Solid Phase Extraction (SPE) procedure (reversed-phase sorbent) before injection. The amounts of phenolic acids in real samples ranged within 2.8–4.3 μg/g of the dried plant material with recovery varying from 96 to 101% and RSD < 6% (n = 3). The sensitivity of the method (LOD < 0.12 μg/mL with UV detection at 254 nm) was suitable in the analysis of this specific plant which was selected as a model sample containing a broad spectrum of phenolic compounds in detectable concentrations [113]. Stacking techniques and hyphenation with isotachophoresis (ITP) were applied for the improvement of sensitivity. Šafra et al. determined cinnamic, chlorogenic, coumaric, ferulic, caffeic, gentisic, syringic and vanillic acid in extracts of *Herba epilobi* using 50 mM sodium tetraborate at pH 9.0 containing 2% of αCD as the running buffer. A large injection plug (representing 70% of the capillary volume) of the sample solution using low conductivity matrix was introduced; a negative voltage was then applied to remove electroosmotically the sample matrix while the negatively charged analytes were stacked at the boundary between the sample zone and the BGE. Under these conditions the limit of detection at 200 nm was less than 0.025 μg/mL [114]. The same research group developed a combination of ITP (pre-separation) and CZE (analytical separation) for the analysis of caffeic, rosmarinic, *p*-coumaric, chlorogenic, ferulic acid and quercitrin in extracts of *Melissae herba*. Boric acid 50 mM at pH 8.2 was used as terminating electrolyte and Tris–HCl 10 mM pH 7.2 and 0.2% hydroxyethylcellulose was the selected leading electrolyte. The optimum BGE for the CZE separation was composed of 25 mM 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid, 50 mM Tris, 0.2% hydroxyethylcellulose, 40 mM boric acid and 20% methanol. Under these conditions the analysis time was quite long and the separation of *p*-coumaric acid and chlorogenic acid was not complete, however the sensitivity was adequate for the analysis of real samples (i.e. 0.018 μg/mL for caffeic acid) [115]. MEEKC was also applied in analysis of phenolic acids when the samples to be analysed contained additional neutral compounds. Oil in water O/W microemulsion composed of 0.6% (w/v) cyclohexane, 3.0% (w/v) SDS, 6.0% (w/v) 1-butanol, 3% (w/v) acetonitrile and 87.4% (v/v) sodium tetraborate allowed the complete separation of 13 compounds, including the phenolic acids danshensu, salvianolic acid B and C, lithospermic acid and the neutral terpenoids dihydrotanshinone I, cryptotanshinone, methylene tanshiquinone and tanshinone IIA. The method was useful in characterization of extracts from Radix et Rhizoma Salviae Miltiorrhizae [116].

3.2. Coumarins

Coumarins are shikimate–derived, benzo-α-pyrone derivatives; although their distribution in plant kingdom is limited to some plant families (Apiaceae, Rutaceae, Asteraceae and Leguminosae) the natural coumarins are currently object of several pharmacological studies. These compounds have demonstrated anti-inflammatory and analgesic activity and chemopreventive effects when administered in the diet [17].

CZE analysis of the coumarins naringin, sophoricoside, esculine, genisteine, isofraxidine and esculetin was performed by indirect laser-induced fluorescence detection using fluoresceine as the background fluorophore. The method optimization was performed by taking into account that high concentration of background electrolyte can reduce sensitivity; a 5 mM borate (pH 9.4) buffer in the presence of methanol 20% (v/v) was found to be effective in the separation (less than 5 min) of the six coumarins in standard solution. However in analysis of real samples (extracts of *Fructus sophorae japonicae* and *Herb sarcandrae*) the analytes were partially overlapped to co-existing components of the matrix. This problem was overcome by application of the second-order derivative electropherograms without loss of sensitivity. The method was validated for linearity, reproducibility and sensitivity (LOD < 4.0 μM). Rela-

tively high levels (>85 mg/g, recovery of 102%) of sophoricoside, an inhibitor of interleukins, were found in *Fructus sophorae japonicae* [117].

CEC and pCEC (pressurized CEC) have been widely applied in analysis of coumarins in plant extracts. Chen et al. used a CEC capillary (i.d. 100 μm) packed with ODS-18 of 3 μm silica particles, in the presence of 10% of bare silica particles in order to provide a suitable EOF. Conventional frits were prepared by silica sintering. The separation of xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, byakangelicin was obtained under pCEC conditions using a mixture of methanol–ACN–phosphate buffer (pH 4.8, 15 mM) as the mobile phase under the application of voltage (1 kV) and the pressure of 13.8 MPa. This system allowed the separation of coumarins in alcoholic extracts of dried root of *Angelica dahuricae*. In this application, the efficiency obtained by pCEC resulted higher than that achieved by capillary HPLC and HPLC; the sensitivity (LOD < 1 μg/mL, UV detection at 216 nm) was adequate for applications to real samples (the amount of the coumarins was in the range 0.201–1.230 mg/g) [118]. A further advance in pCEC application was achieved by using a ODS packed column with monolithic outlet frit obtained by the reactive monomer (glycidyl methacrylate) in the presence of a crosslinker and a thermal initiator of polymerization. Bergapten, imperatorin, osthole and other coumarins could be separated in short analysis time using a mobile phase composed of a binary mixture of ammonium acetate (10 mM, pH 4.0)–ACN 50:50 (v/v). The considered coumarins were assumed as phytomarkers in standardization of *Fructus cnidii*, one of the most popular traditional Chinese medicinal herbs. In Fig. 7 the pCEC separations of coumarins in standard solutions and real samples, are reported [119]. Using a similar mobile phase, *Fructus cnidii* extracts were analysed by CEC in a methacrylate ester-based monolithic column. The main advantages of this stationary phase are the fine control of porous property and the high stability even under extreme pH conditions. Separation of isopimpinelline, bergapten, imperatorin and osthole was achieved within 5 min. The method was validated for linearity, repeatability (intraday peak area RSD % < 3.81, $n = 6$) and column-to-column reproducibility (peak area RSD % < 6.62, $n = 3$), sensitivity (LOD of 0.1–0.5 μg/mL) and recovery (in the range 94–99% with RSD % < 3.0). The method was applied to the analysis of real samples from different geographical origins; osthole was found the most represented coumarin (maximum content 23.47%) in the analysed extracts [120].

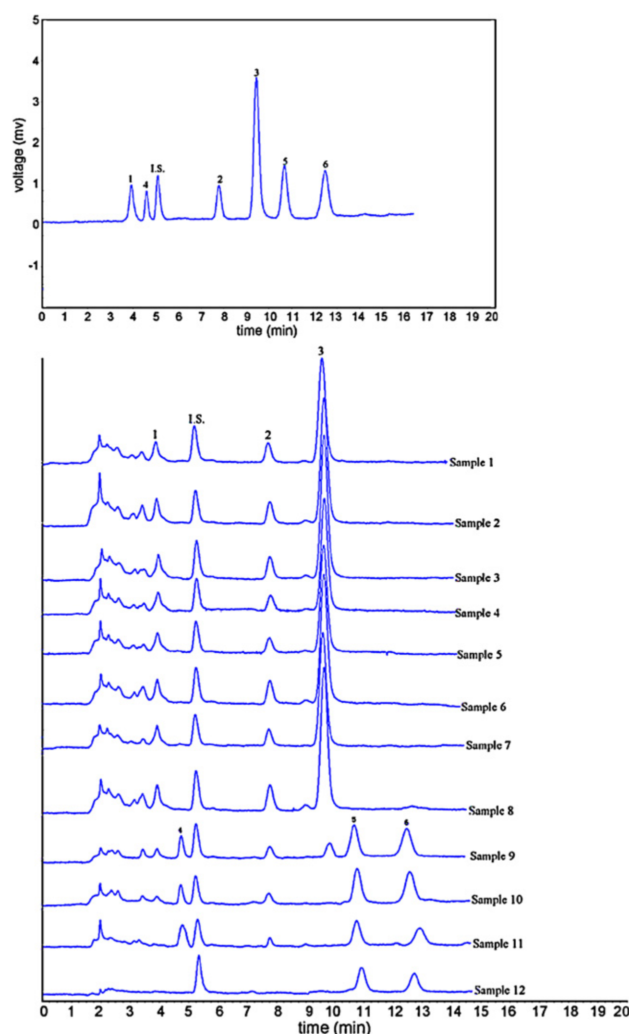


Fig. 7. Pressurized capillary electrochromatograms of (A) six standard coumarins and (B) coumarins from Fructus cnidii extracts from different regions of China (samples 1–12). Column: 100 μm i.d., C_{18}, 3 μm particles packed column with monolithic outlet frit. pCEC conditions: 10 mM ammonium acetate buffer (pH 4.0)/ACN (50/50, v/v); injection: 40 nL; applied voltage: −6 kV; backpressure: 10.3 Mpa; flow rate: 0.05 mL/min; detection wavelength: 320 nm. Peaks: (1) bergapten, (2) imperatorin, (3) osthole, (4) 2′-acetylangelicin, (5) oroselone, and (6) O-acetylcolumbianetin; (IS) diazepam (the internal standard). Modified from Ref. [119].

3.3. Naphtoquinones and anthraquinones

Naphthoquinones are bioactive phenolic compounds derived almost exclusively from the shikimic acid pathway. The pharmacological significance of this group of compounds is limited to few examples; interestingly, eleuthoside B, isoeleutherin, eleutherin, eleutherol and eleutherinoside A were found as the main bioactive components of the extracts of *Eleutherine americana* (Iridaceae), used to treat coronary diseases and as diuretic and purgative. Ganzera et al. compared MEKC and CEC in the analysis of plant material subjected to simple extraction with methanol. CEC was carried out using a polymeric methacrylate-based monolithic column with strong cationic properties. However the mechanism of separation did not involve the ionic exchange because of the lack of negative charge of the analytes. Ammonium formate solution in a mixture water–ACN was used as the mobile phase and the retention of the analytes was driven by reversed-phase interactions; under these conditions the positively charged functions of monolith material only generated the EOF. The separation was complete in about 10 min and the retention order was similar to that obtained using a reference RP-HPLC method; exception was observed for inversion of elution order of eleuthoside B and eleutherinoside A. A completely different migration pattern was shown by MEKC separation in which 50 mM sodium cholate was used as the surfactant in 25 mM borate solution in the presence of tetrahydrofurane (THF) as organic modifier. Furthermore, MEKC did not allow for the separation of the critical couple of naphtoquinones isoeleutherine/eleutherine. The comparison of the validation parameters of the two methods (CEC and MEKC) revealed that both fulfill the required criteria; on the other hand the HPLC reference method resulted to be more sensitive (10 fold) than the electrodriven methods but significantly more time expensive (analysis time twice longer) [121].

Anthraquinones are the major constituents of Rhubarb, a well-known Chinese herbal medicines used also in the west as a laxative medicine. An intensive literature has been available regarding different electrodriven techniques in the analysis of anthraquinones in extracts from Rhubarb. The structural features of anthraquinones bearing phenols moieties make these weak acids (p*K*a in the range 9.14–10.19) dissociated under alkaline conditions [122]; in particular at pH 10.1 (borate–phosphate buffer) physcion, chrysophanol, aloe-emodin, emodin showed to be well separated in conventional CZE using ACN (42%, v/v) as organic modifier. The CZE conditions were used in flow injection-CE mode allowing for

the continuous automated sampling without physical movement of the CE capillary. The method was applied to the analysis of Rhubarb and different herbal drugs and preparations. However, rhein, one of the most characteristic anthraquinones in Rhubarb, was not considered in this study [122]. Actually, when similar CZE conditions involving 50 mM borate (pH 8.2) in the presence of isopropyl alcohol and 25% ACN, as the running buffer, were applied to the analysis of powdered Rhubarb, rhein showed a migration time of about 40 min [123]. A significant reduction of analysis time (analysis time < 12 min) was obtained using phosphate buffer (35 mM, pH 10) containing βCD 20 mM as additive. Urea (2 M) was introduced into the running buffer to increase the solubility of the cyclodextrin. The method was applied to the analysis of rhubarb plant material and *Polygonum cuspidatum* subjected to extraction in ultrasonic bath using ethanol as the solvent [124]. A major number of compounds was found in Rhubarb powder after Soxhlet extraction using methanol for 10 h. In particular, emodin, chrysophanol, aloe-emodin, emodin-1-β-D-glucoside, emodin-8-β-D-glucoside, chrysophanol-1-β-D-glucoside, chrysophanol-8-β-D-glucoside, rehin-8-β-D-glucoside, and sennoside A and B were detected using a BGE constituted of borate 30 mM (pH 10) containing αCD (5 mM). Under these conditions the separation took about 30 min; however as observed in other CZE conditions, rhein showed an impressively long migration time (about 60 min) and was thus neglected in routine analysis. An orthogonal complementary method was developed by RP-HPLC; it provided the analysis of the 11 considered compounds, including rhein, in about 20 min, however the separation of rehin-8-β-D-glucoside, and sennoside A and B was not complete. The validation parameters of CE and HPLC resulted to be comparable except for sensitivity, which was found to be 10-fold higher using HPLC [125]. Shorter analysis time in the determination of physcion, chrysophanol, aloe-emodin, emodin and rhein was achieved by MEKC approach using sodium deoxycholate as the surfactant. Borate buffer (pH 9.0, 20 mM) in the presence of 15% ACN was used as the micellar BGE allowing for the complete separation of the anthraquinones (including rhein) in less than 14 min. The method was applied to the analysis of *Cassia obtusifolia*; under these conditions the migration order of the analytes resulted to be different from that obtained using CZE, making MEKC a convenient separation system, suitable in the analysis of antraquinones from different sources [126]. Significant advantages in terms of analysis time was achieved by pCEC on monolithic stationary phase obtained by in situ polymerization of iso-butyl methacrylate, ethylene dimethacrylate and methacrylic acid in the presence of 1-propanol and 1,4-butanediol (70/30, v/v) as porogenic solvents. The separation of five anthraquinones from Rhubarb extracts was achieved in about 5 min providing a different separation profile in comparison to CZE and MEKC [127].

3.4. Flavonoids

Flavonoids are undoubtedly one of the most important classes of bioactive polyphenols [103–105]. Their basic structure is 2-phenyl chromane and they are biosynthetically derived from a combination of shikimic acid and acetate pathways. They are divided in different subgroups (Fig. 6) and they either occur as aglycones or as O- or C-glycosides. Flavonoids are ubiquitous in plant kingdom and are also known as components (natural pigments) in fruits and vegetables.

3.4.1. CZE of flavonoids in borate buffer

Capillary zone electrophoresis can be conveniently applied in analysis of flavonoids; borate is used as the electrolyte of choice because of its ability in complexation of polyphenols aglycones and/or saccharides. In particular, borate ions form charged and mobile five-membered-ring complexes (with 1,2-diols) and six-membered ring complexes (with 1,3-diols) thus increasing the selectivity of separation [128]. A typical ion-dipole or ion-induced dipole interaction between flavonoids and borate was also confirmed by means of CE using as model compounds luetolin-7-O-glucoside, isorhamnetin, apigenin, luteolin, quercetin. Additionally, the association constants and the change in Gibbs free energy of the interaction were estimated. Applications to real samples of *Lamiophlomis rotata*, a Chinese folk medicine used to promot blood circulation, were carried out using a 30 mM borate pH 9.0 buffer in the presence of ACN (8%, v/v). Luteolin-7-O-glucoside was found to be the major active component [129]. Sodium tetraborate 50 mM, pH 8.2 was used as the BGE in separation of rutin, vitexin-2′-O-rhamnoside, hyperoside and chlorogenic acid in extracts from *Crategus monogyna* Jacq. (hawthorn), known in TCM as a cardiotonic, vascodilatoric and hypotensive supplement [130]. Very similar conditions were also applied in the analysis of icariin, epimedin A, B and C in Herba Epimedii, a Chinese herbal medicine prepared from the dried aerial parts of *Epimedium brevicornum* and other *Epimedium* species [131]; in the determination of the uncommon flavonoid glycosides 12-hydroxy-desmethoxyyangonin, 12-β-D-glucopyranoside-desmethoxyyangonin and luteolin 3′-(6-E-*p*-coumaroyl-β-D-glucopyranoside) in real samples of *Scorzonera austriaca* [132]; in the determination of 7-O-α-L-rhamnopyranosyl-kaempferol-3-O-β-D-glucopyranoside and 7-O-α-L-rhamnopyranosyl-kaempferol-3-O-α-L-rhamnopyranoside in the traditional Chinese herb *Cynanchun Chinese* [133]; in the determination of apigenin, apigenin-7-O-glucoside, luteolin, luteolin-7-glucoside and rutin in *Achillea millefolium* [134]; in the analysis of quercetin-3-O-robinobioside, hyperin, isoquercetin and myricetin in the flowers of *Abelmoschus manihot* (L.) [135]. Furthermore, analysis of catechin, epicatechin besides phenolic acids, namely vanillic, rosmarinic, caffeic and gallic acid in Salvia extracts was accomplished in less than 10 min in a BGE composed of borate 40 mM pH 9.2 using UV detection at 280 nm [136].

3.4.2. CZE of flavonoids in phosphate or mixed phosphate-borate buffer

Mixed phosphate-borate buffer was found to be suitable in the separation of nine flavonoids potentially present in extracts of *Anaphalis margaritacea*, an herbal drug used in northwest and southwest of China for cough and respiratory problems. In particular, a 25 mM borate and 10 mM phosphate BGE (pH 9.5) allowed for the separation of 5,7-dihydroxy-3,6,8-trimethoxyflavone, 5,7-dihydroxy-3,8-dimethoxyflavone, 5,7-dihydroxy-3-methoxyflavone, kaempferol-3-O-β-D-glucopyranoside, tiliroside, 3-methylquercetin, spiraein, apigenin, and quercetin [137]. Borate–phosphate buffer was also exploited in analysis of flavonoids aglycons and glycosides in *Flos Lonicerae*, a traditional Chinese medicine prepared from the dried flower buds of several species of the genus Lonicera [138].

Suntornsuk and Anurukvorakun used sodium dihydrogen phosphate-disodium hydrogen phosphate buffer at pH 8.0 in separation of flavonoids (kaempferol, rutin, quercetin, myricetin and apigenin) from Thai plants such as *Centella asiatica*, *Rosa hybridis* and *Chromolaena odorata*. The addition of organic solvents ACN (10%, v/v) and methanol (6%, v/v) was necessary to obtain separation of critical compounds, namely kaempferol, quercetin and myricetin [139].

3.4.3. CZE of flavonoids using additives to BGE

Beside the use of mixed borate–phosphate buffer as well as that of organic solvents, the selectivity tuning in CZE of flavonoids was performed by addition of complexing agents to the BGE. Neutral cyclodextrins, in particular 2-hydroxypropyl-γ-cyclodextrin was an effective chiral selector in enantioseparation of bioactive

enantiomers of the flavanone eriodictyol in *Balanophora involucrata* Hook. f. [140]. Jáč et al. proposed a novel electrophoretic buffer containing tungstate as complex-forming reagent for the analysis of flavonoids. In the presence of tungstate, the solutes bearing vicinal hydroxy groups can form negatively charged complexes with W(VI) as the central ion. A running buffer composed of 50 mM *N*-(2-hydroxyethyl)piperazine-2′-(2-ethansulfonic acid) at pH 7.4, supplemented with tungstate 2.2 mM and 25% (v/v) of methanol, was effective in the separation of apigenin, luteolin, hyperoside, quercetin, rutin and phenolic acids in *Hypericum perforatum* extracts [141].

Also ionic liquids were explored as additive to borate running buffer in separation of flavonoids. Ionic liquids are ionic substances with melting points at or close to room temperature and their use as additive to improve the CE separation is increasing. Yanes and co-workers used 1-alkyl-3-methylimidazolium-based ionic liquid as additive to aqueous running electrolyte. The imidazolium cations cover the inner capillary wall generating an anodic EOF and they are also supposed to be able in establishing hydrophobic, hydrogen bonding, ion-dipole/ion-induced interactions with the analytes. In particular, the association of polyphenols with imidazolium ions was hypothesized to be occurring through π–π interactions [142]. Two independent studies involving application of ionic liquid as additives to borate running buffer in separation of flavonoids have been recently reported. In both the studies 1-butyl-3-methylimidazolium tetrafluoroborate was found to be suitable in separation of flavonoids, namely kaempferol, quercetin, isorhamnetin in *Hippophae rhamnoides* extracts and phytopharmaceutical preparations (tablets) [143]. The separation of arteanoflavone, eupatilin hispidulin and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone was obtained only in the presence of βCD as a further additive [144].

The majority of the flavonoids are freely soluble in water; few exceptions are represented by puerarin, daidzein and wogonin, the bioactive components of *Puerariae radix* and *Scutellariae radix*. Hu and co-workers applied advantageously NACE (mixture of ACN–methanol) to improve the solubility of these analytes [145]. Addition of sodium cholate to NACE buffer was necessary to achieve the separation of fraxin, esculin and esculetin [146], eupatilin, arteanoflavone, 5,7,4′-trihydroxy-6,3′5′-trimethoxyflavone [147] in different medicinal plants extracts.

3.4.4. Electrochemical detection in CZE analysis of flavonoids

The applications described above were performed by means of UV detection which was conveniently applied both in aqueous and non-aqueous buffers. The UV spectra of flavonoids exhibit two major absorption bands in the region of 240–400 nm; the band at 300–380 nm is associated with the absorption of the cinnamoyl system, whereas the band at 240–280 nm is associated with the absorption of the benzoyl system [148]. The detection limits reported in the cited literature varied within 0.01–6 μg/mL in general depending on the flavonoid type, the selected wavelength, inner capillary section and running buffer nature and pH. Owing to the relatively high content of bioactive flavonoids in medicinal plant extracts, the detection sensitivity is not a challenging concern; e.g. the total flavonoids extract in *Epimedium* is reported to range within 39.0–172.1 mg/g depending on the considered species [131]; rutin is found at a level of about 9.2 mg/g in *Hypericum perforatum* leaves [141] and esculin levels in *Cortex fraxini* ranged within 6.779–27.89 mg/g [146].

Electrochemical detection (ED) is a useful alternative detection approach, widely applied in the determination of flavonoids upon CE separation. Since polyphenols can be electrochemically oxidized at a relatively moderate potential, the CE-ED detection can be very selective also when real samples are analysed after a minimum pretreatment. In fact, matrix constituents such as proteins, carbohydrates and lipids, normally do not engender signal interference. The design of the CE-ED detector is based on the end-column approach in which the working electrode (a carbon-disk electrode with 300–500 μm diameter) is simply placed at the outlet of the separation capillary and the detection is carried out in the buffer reservoir that contains the ground electrode of the CE instrument. The detection cell contains, together with the working electrode, a platinum auxiliary electrode and a reference electrode (such as saturated calomel electrode, SCE, or Ag/AgCl). In CE-ED, the potential applied to the working electrode affects the sensitivity and the stability of the system. The investigation of the best detection conditions involves the evaluation of the peak current of the analyte when increasing potential is applied. The hydrodynamic voltammograms (peak current versus potential) obtained for most of the flavonoids, show that a progressive increase of the response is achieved when the potential is increased. However, when the applied potential is higher than +950 mV (e.g. vs SCE), both the baseline noise and the background current increase, resulting in unstable baseline. In most of the published applications the potential of +950 mV was used. Several examples of CE-ED determinations of flavonoids in plant extracts are available in the literature; the assay by means of this approach is rapid and low cost and provides satisfying sensitivity. In Table 3 recent examples of CZE analysis of flavonoids by CE-ED are reported. The detection limits reported for most of the bioactive polyphenols ranged within 0.02–6 μg/mL, thus comparable to those obtained using UV detection.

Borate running buffer resulted to be suitable in CZE separation of flavonoids with ED detection; in general tetraborate in the concentration range 50–100 mM (pH 8.45–9.0) was used [149–155]. As already mentioned, the separation selectivity is improved in the presence of relatively high concentrations of borate; however in CE-ED applications, the use of concentration values higher than 100 mM resulted in baseline noise and sensitivity loss. Addition of phosphate to borate buffer was found to be beneficial in the CZE separation of flavonoids using ED detection; $Na_2B_4O_7$-NaH_2PO_4 showed not only good resolution but also allowed for high detection sensitivity. The optimum pH values ranged within 7.5–8.8 and under these conditions the analysis of flavonoids was performed in a variety of herbal drugs [156–160]. Addition of low levels of cyclodextrins (in particular 3 mM βCD) to borate–phosphate buffer (pH 8.8) was effective in improving the separation of catechin and epicatechin beside flavonoids such as apigenin, luteolin, kaempferol, quercetin. The additive did not affect the EC detection and the method was applied in the analysis of chrysanthemum extracts [161].

Improved sensitivity in CE-ED of flavonoids was achieved by means of a carbon nanotube/poly(ethylene-co-vinyl acetate) composite working electrode that showed to significantly reduce the overpotential. Under conventional separation conditions using a 50 mM borate buffer (pH 9.2), esculine and esculetin were separated in about 8 min and detected by CE-ED using the modified electrode by applying a potential of +0.8 V (*versus* Ag/AgCl reference electrode). The method showed an improved sensitivity (in the order of 30-fold) compared to the CE-ED method employing conventional carbon working electrodes and it was applied in analysis of extracts of *Cortex fraxinii* [162]. Beside the improvement of sensitivity, modified working electrodes have been developed with the aim to enhance the range of detectable compounds. He and co-workers applied a copper–disc working electrode to analyze bioactive components of oolong tea infusions. Copper can be oxidized into its higher transitional state Cu(II) and Cu(III), which complexed with the polyhydroxyl compounds and resulted in electrocatalysis oxidation. By means of this system, upon CE separation using alkaline buffers, sugars and amino acids produced good current response. A phosphate-borate (30 mM–40 mM, pH 8.5) BGE

Table 3
CE-ED determination of flavonoids in herbal drugs.

Sample	Flavonoids	Buffer/electrode	Ref.
Ilex purpurea Hassk	Kaempferol, quercetin, phenolic acids	Borate/c.-d.	[149]
Ricinus communis	Rutin, quercetin	Borate/c.-d.	[150]
Frucus aurantii	Naringin, naringenin, hesperidin	Borate/c.-d.	[151]
Ipomea batatas	Rutin, quercetin	Borate/c.-d.	[152]
Sophora japonica L.	Genistin, genistein, quercetin, kaempferol, rutin	Borate/c.-d.	[153]
Cynomorium songaricum	Epicatechin, catechin, luteolin, quercetin, naringenin, rutin, phloridzin	Borate/c.-d.	[154]
Perilla frutescens L.	Catechin, apigenin, luteolin	Borate/c.-d.	[155]
Agrimonia pilosa Ledeb	Catechin, quercetin, rutin, quercitrin, hyperoside	Borate-phosphate/c.-d.	[156]
Portulaca oleracea L.	Kaempferol, apigenin, myricetin, quercetin, luteolin	Borate-phosphate/c.-d	[157]
Lonicera confusa DC.	Luteolin, phenolic acids	Borate-phosphate/c.-d	[158]
Houttuynia cordata Thub. *Saururus chinensis* (Lour) Bail	Quercetin, hyperoside, rutin, quercitrin	Borate-phosphate/c.-d	[159]
Leonurus heterophyllus Sweet	Kaempferol, quercetin, hyperoside rutin, quercitrin	Borate-phosphate/c.-d	[160]
Chrysanthenum	Catechin, epicatechin, apigenin, luteolin, kaempferol quercetin	Borate-phosphate + 3 mM βCD/c.-d.	[161]
Cortex fraxinii	Esculine, esculetin	Borate/carbon nanotube	[162]
Oolong tea infusions	L-theanine, L-glutamine, sucrose, glucose, fructose, ascorbic acid, epigallocatechin gallate	Borate-phosphate/copper	[163]

c.-d.: carbon–disk electrode.

allowed the simultaneous separation and analysis of L-theanine, L-glutamine, sucrose, glucose, fructose, ascorbic acid and epigallocatechin gallate [163].

3.4.5. EKC in analysis of flavonoids

MEKC is widely applied in analysis of polyphenols mainly because of its high resolution power and for the wide opportunity of selectivity tuning. Examples of applications of MEKC and MEEKC in analysis of herbal drugs and plant extracts are given in Table 4.

The CZE analysis of extracts from *Scutellaria baicalensis* Georgi, one of the most used herbal medicine in China and other East Asian countries, was unsuccessful because of the interference of the matrix components and due to the neutral character of some constituents of the raw material. A MEKC system was optimized by central composite design to provide the separation of baicalin, baicalein and wogonin in real samples from different origin. Baicalin, reported to be an anti-inflammatory, anti-HIV and anti-tumor compound, was found the most represented flavone in *Scutellaria baicalensis* (amount within 24.74–143.56 mg/g); the level of baicalein ranged within 1.53–15.12 mg/g and that of wogonin within 0.36–4.14 mg/g. The high specificity of the method allowed for a comparative analysis of fingerprint of samples from different origin. In particular, the relative area values of eight copossesing peaks in each of the obtained electropherograms, were used to construct an eight-dimensional vector to characterize the chemical pattern of the fingerprint. By means of this comparative analysis it was found that the samples from the same origin showed high similarity [164].

Catechins represent one of the most studied classes of bioactive flavonoids; they are mainly found in green tea, which has been included among the herbal drugs. Actually, a variety of studies including epidemiological and nutritional investigations have shown the possible relationships between tea consumption and incidence of cancer and cardiovascular diseases [165,166]. Analysis of catechins content in green tea, tea beverages and tea extract dietary supplements is thus very important and MEKC has been applied as method of choice.

A simple MEKC method allowed for the separation of a number of catechins, namely (+)-catechin ((+)-C), (−)-epigallocatechin ((−)-EGC), (−)-epigallocatechin gallate ((−)-EGCG), (−)-epicatechin ((−)-EC) and (−)-epicatechin gallate, ((−)-ECG) and the methylxanthine caffeine and it was applied in analysis of green tea extract dietary supplements [167]. Under similar conditions the same set of green tea catechins were analysed in a polydimethylsiloxane (PDMS) microchip platform using pulsed amperometric detection [168]. MEKC was also off-line coupled to LC separation in bidimensional analysis of complex mixtures of phenolic acids and flavones in green tea extracts. The samples were analysed by liquid chromatography on a PEG column using ammonium acetate–ACN under gradient elution (first dimension of separation). The eluted fractions were collected and successively analysed by MEKC, which showed full compatibility with the fractions collected from the first chromatographic dimension. Green tea samples were analysed under the comprehensive 2D method; besides the typical green tea catechins, flavones such as naringin, biochanin A, hesperetin were detected [169].

Catechins undergo rapid oxidation under alkaline conditions, thus acidic conditions should be preferred not only in sample preparation but also during separation [170]. The lack of charge in catechins molecules at low pH values makes MEKC mandatory in their analysis. The use of acidic running buffers (pH 2.5) results in the strong suppression of the EOF and the negatively charged SDS micelles show anodic migration. Using this approach, defined as reversed–flow MEKC (RF-MEKC), the analytes showing stronger interaction with the micelle are the faster migrating towards the anodic detection end. Addition of cyclodextrins to the MEKC system

Table 4
MEKC and MEEKC in analysis of flavonoids.

Sample	Flavonoids	Buffer	Ref.
Scutellaria baicalensis Georgi	Baicalin, baicalein, wogonin	15 mM SDS, 15 mM borate–40 mM phosphate; 15% ACN, 7.5% 2-propanol	[164]
Green tea dietary supplements	Green tea catechins	50 mM SDS, 5 mM borate–60 mM phosphate at pH 7.0	[167,168]
Green tea samples	Green tea catechins, flavones such as naringin, biochanin A, hesperetin	10 g/L SDS, 25 mM borate (pH 9.05) + 1.85 g/L heptakis(6-*O*-sulfo)-β-cyclodextrin	[169]
Theobroma cacao beans, chocolate	(−)-Epicatechin, caffeine, theobromine and procyanidin B1 and B2	90 mM SDS, 50 mM Britton-Robinson buffer (pH 2.5) + 12 mM HP-βCD	[85]
Green tea samples	Green tea catechins	90 mM SDS, 25 mM borate-phosphate pH 2.5, 25 mM HP-βCD	[86]
Green tea beverages	Green tea catechins	50 mM SDS, 50 mM phosphate pH 2.0, 75 mM NaCl.	[173]
Azadirachta indica	Astragalin, nicotiflorin quercetin, isoquercetin, rutin	50 mM SDS, 20 mM phosphate buffer (pH 2.5), 15% ACN and 5% THF	[174]
Alpinia katsumadai Hayata	Cardamonin, alpinetin	RMEKC: SDS (20.9%, w/v), water (13.0%, v/v), 1-butanol (18%, v/v), heptane (1.5%, v/v), ACN (8.0%, v/v)	[175]
tea leaves, tea beverages, grapes, apples,red wines	Green tea catechin, syringic acid, *p*-cumaric acid, vanillic acid, caffeic acid, gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid	MEEKC: 2.89% w/v SDS, 1.36% w/v heptane, 7.66% w/v cyclohexanol, 2% w/v ACN, phosphate buffer pH 2.0	[176]
silymarin capsule, *Saururus chinensis*	Puerarin, catechin, epicatechin, liquiritin, rutin, baicalein, hyperoside, quercitrin, naringin, naringenin, luteolin, silymarin, alpinetin, wogonin	MEEKC: 0.9% w/v SDS, 0.9% w/v sodium cholate, 0.9% w/v butan-1-ol, 0.6% w/v ethyl acetate, 98.2% v/v of 10 mM $Na_2B_4O_7$–20 mM H_3BO_3 buffer (pH 7.5)	[177]
Radix Astragali, Radix et Rhizoma Salvia Miltiorrhizae	Calycosin-7-O-b-D-glucoside, calycosin, formononetin, rosmarinic acid, danshensu, caffeic acid, salvianolic acid B, lithospermic acid, protocatechuic acid	MEEKC: 0.6% w/v heptane, 3.0% w/v SDS, 6.0% w/v 1-butanol, 5mM sodium tetraborate buffer (pH 9.0) + variable amounts of SC-SWNTs	[179]

ACN:, Acetonitrile; THF: tetrahydrofurane; SC-SWNTs:, single walled carbon nanotubes.

gives rise to the inclusion-complexation equilibria of the analytes into the cyclodextrin cavity, which occurs simultaneously to the partitioning into the SDS micelle. Moreover also the inclusion into the macrocyclic cavity of the SDS monomer has to be considered as an additional event affecting the separation ability of the mixed system [171,172].

Using a 50 mM Britton-Robinson buffer at pH 2.5, with SDS at 90 mM in the presence of 12 mM HP-βCD, the separation of (−)-EC, caffeine, theobromine and procyanidin B1 and B2 was obtained; furthermore these conditions showed to be enantioselective for catechin enantiomers. The analysis of *Theobroma cacao* beans extracts and chocolate revealed that (−)-C was found only in chocolate whereas *Theobroma cacao* beans exclusively contained (+)-C. Being (−)-C a non-native molecule, its presence in manufactured products (chocolate) was ascribed to the epimerization of the native (−)-EC occurring under thermal manufacturing treatment. Thus, (−)-C was assumed as a chiral marker of thermal treatment of natural products containing catechins [85]. Under similar RF-MEKC modified by cyclodextrins, the simultaneous separation of all the green tea catechins and methylxanthines (theophylline, caffeine and theobromine) was achieved in less than 8 min. Beside the chiral resolution of (±)-C, enantioseparation was also obtained for (±)-gallocatechin and in analogy with the occurrence of the non-native distomer (−)-C, also (−)-gallocatechin was considered as an additional non-native marker of thermal degradation (generated by epimerization of (−)-epigallocatechin). The CD-MEKC analysis of green tea from different origin showed that in a number of samples thermal epimerization occurred likely as the result of fixing (steam treatment to inactivate oxidase enzymes or roasting process [166]) [86].

RF-MEKC was also applied to the development of on-line preconcentration technique for improved sensitivity. The method was based on the large sample volume injection of flavonoids dissolved in alkaline diluted borate buffer, whereas the CE capillary was filled with acidic and highly conductive BGE. When the flavonoids associated with borate ions migrated into the acidic buffer, they became neutral because of the conversion of borate ions to boric acid. The flavonoids in their neutral form could be concentrated at the boundary with BGE and were moved to the anodic detection end carried by SDS solution under acidic pH. The method was applied to the analysis of ECG, gallocatechin gallate (GCG) and EGC in tea beverages; the achieved LOD (UV detection at 214 nm) values were within 1.4–17.5 nM [173].

RF-MEKC in the presence of organic solvents was applied in analysis of flavonoids in Neem (*Azadirachta indica*) samples; the method allowed the separation of seventeen polyphenols in less than 12 min. The real samples showed to contain some specific and unusual flavonoids such as astragalin and nicotiflorin beside the more common quercetin, isoquercetin and rutin. The study was also addressed to the investigation on the relationships between

migration behavior and flavonoid structure. The evaluations of solvent effects on the solute mobility confirmed that the flavonoids solubilize preferentially in the interfacial region of the SDS micelle rather than in their core [174].

Other electrokinetic methods applied in separation of flavonoids include the use of reverse micelle electrokinetic chromatography (RMEKC) and MEEKC. Reverse micelle are formed i.e. using SDS as the surfactant in non-polar solvents (such as 1-butanol or n-butyl chloride) in the presence of relatively low water percentages. Under these conditions the core of the micelle is constituted by the polar groups, whereas the SDS hydrocarbon chains form the outer layer. A RMEKC method was optimized for the separation and quantitation of cardamonin and alpinetin in *Alpinia katsumadai* Hayata and phytopreparations [175]. MEEKC was found to be a more versatile technique than RMEKC in analysis of flavonoids and it was applied in conventional mode and in reversed-flow conditions (using acidic running buffer i.e. phosphate buffer pH 2.0). Reversed–flow MEEKC was obtained using acidic BGE (phosphate buffer, 86.1%, v/v) containing heptane (1.36%, w/v) as the oil phase, SDS (2.89%, w/v) as the surfactant and cyclohexanol as co-surfactant (7.66%, w/v). Addition of 2% (w/v) ACN was necessary to obtain the separation of EGCG, EC, EGC, C, caffeic acid and gallic acid [176]. Although the microemulsion composition can be considered quite complex it showed to be compatible with electrochemical detection of flavonoids [177].

Recently, carbon nanotubes, that are graphene cylinders with different diameters and chirality, have been used as separation tools in μHPLC stationary phase and monolithic CEC materials [178] as well as additives to pseudostationary phases i.e. in MEEKC. Efficient and appropriate dispersion of these materials can be obtained by means of the technique defined as surfactant-coated single walled carbon nanotubes (SC-SWNTs).

In particular the addition of SWNTs to a conventional O/W MEEKC allowed the baseline resolution of the critical couple of phenolic acids namely salvianolic and lithospermic acid simultaneously to the separation of calycosin-7-O-β-D-glucoside, calycosin, formononetin, rosmarinic acid, danshensu and caffeic acid in Radix Astragali and Radix et Rhizoma *Salvia Miltiorrhizae* [179].

CEC has shown to be a suitable technique in analysis of flavonoids and it has been applied to the separation of complex mixtures of very similar compounds by profit of the high efficiency and selectivity provided by the hybrid separation mechanism (CZE and partition). Bonn and co-workers used a home-made reversed phase CEC column packed with 3 μm particle (silica-C_{18}) for the separation of flavonoids in standard solution; (−)-EC, quercetin, myricetin, hesperetin and naringenin were selected as model compounds [180]. Commercially available CEC column Hypersil C_{18} (3 μm, 100 μm i.d./25 cm length) was the selected stationary phase in separation and analysis of the flavonoids liquiritin, isoliquiritin, ononin, liquiritigenin and isoliquiritigenin in *Glicyrrhiza* species [181] and analysis of hexandraside E, kaempferol-3-O-rhamnoside, hexandraside F, icariin, epimedin A, B, and C in *Epimedium* species (herba Epimedii) [182].

The mobile phase was a binary mixture constituted of phosphate and ACN; the pH of the mobile phase had a strong influence on the separation and in these applications pH 3 (analysis of *Glycirrhiza* extracts) and pH 4 (analysis of *Epimedium*) were found as the optimum. The phosphate concentration was relatively low (maximum concentration 20 mM) and ACN was used at 65–70% (v/v). The CEC runs were performed at 30 °C and 25 kV. The methods were validated for linearity, precision, accuracy and sensitivity. The limit of detections were below 9 μg/mL, thus the methods could be applied to the analysis of real samples. The plant material, constituted by dried roots and rhizomes of *Glycyrrhiza* species [181] and herba Epimedii [182], were prepared by pressurized liquid extraction (PLE) using as the solvent a mixture of ethanol/water (70/30, v/v) at the temperature of 100–120 °C in static mode conditions for 5 min. In both the methods the recovery values for the considered flavonoids ranged within 95.2–103.8 (RSD% <3.7).

Horváth and co-workers applied CEC using a 75 μm i.d. capillary packed with Spherisorb ODS (5 μm diameter particle and 80 Å mean pore) in analysis of phenolic compounds in extracts of *Chamomilla recutita*. A low pH mobile phase (pH 2.8 phosphate buffer) was used to suppress the ionization of the solute thus avoiding electrophoretic behavior. Flavonoids (apigenin, apigenin-7-O-glucoside, luteoline, luteoline-7-O-glucoside, quercetin, rutin, naringenin), coumarins (umbelliferone, herniari) and phenylpropanoids (caffeic acid and chlorogenic acid) were selected as model compounds. Because of the low EOF velocity the separation of 10 out of the 11 compounds took about 28 min. In order to increase the EOF velocity, a commercially available SCX-C_{18} column was tested. This column was packed with a "mixed mode" stationary phase containing strong cation exchange sites that remain ionized also at low pH; simultaneously the aliphatic chains provide site for hydrophobic interactions. The negatively charged particle surface provide a good EOF. The elution order was the same as for the ODS but analysis time was reduced about 4-fold; the separation profile of a real samples is reported in Fig. 8 [183].

4. Carbohydrates

Carbohydrates are the first products plant obtained by photosynthesis; they are classified as sugars (mono- and oligosaccharides) and polysaccharides. In addition to their use as bulking agents in pharmaceutical industries, some of these compounds have been recognized as immuno-modulatory, anticoagulants, hypoglycaemics and antivirals [17]. CE analysis of mono- and polysaccharides from natural sources has been applied in standardization of herbal drugs (i.e. Moutan Cortex) as well as for the characterization of polysaccharides with potential bioactivity.

The analytical challenge in determination of saccharides is mainly related to the lack of significant UV absorption precluding the direct UV detection; CE-ED is a very convenient approach to overcome the problem because of the good sensitivity provided and simple operation procedure. In carbohydrate analysis, the copper working-electrode has been employed at a constant potential in strongly alkaline media. This approach was applied to the standardization of Moutan Cortex constituted by root of *Paeonia suffruticosa*. The bioactive components are paeoniflorin, a monoterpene glucoside, and paeonoside; in addition, the primary metabolites sucrose, glucose and fructose were considered as important phytomarkers that can indicate the quality of this herbal drug. A 300 μm diameter copper disc electrode at the working potential of +0.60 V (versus SCE) was used as ED detector. The separation of paeoniflorin, sucrose, glucose and fructose was obtained in fused-silica capillary, using a 75 mM NaOH solution as the BGE. The method showed to be of practical application for quantitation and fingerprint studies [184].

Analysis of polysaccharides can be carried out after hydrolysis using trifluoroacetic acid at 100 °C for 6 h, to generate the related monosaccharides. In general the polysaccharides can be extracted with water starting from the defatted plant materials; the combined extracts are concentrated and the polysaccharides are precipitated with ethanol 95%. After dissolution in water, a purification by dialysis is performed and the retentate is concentrated and deproteinated.

The characterization of the bioactive polysaccharides from Danggui, represented by the roots of *Angelica sinensis* (Umbelliferae), was achieved by means of CE-ED analysis of the monosaccharides (fucose, galactose, glucose, arabinose, ramnose and xylose) released after hydrolysis [185].

Pre-column off-line derivatization of saccharides is a useful alternative to the CE-ED; 1-phenyl-3-methyl-5-pyrazolone (PMP)

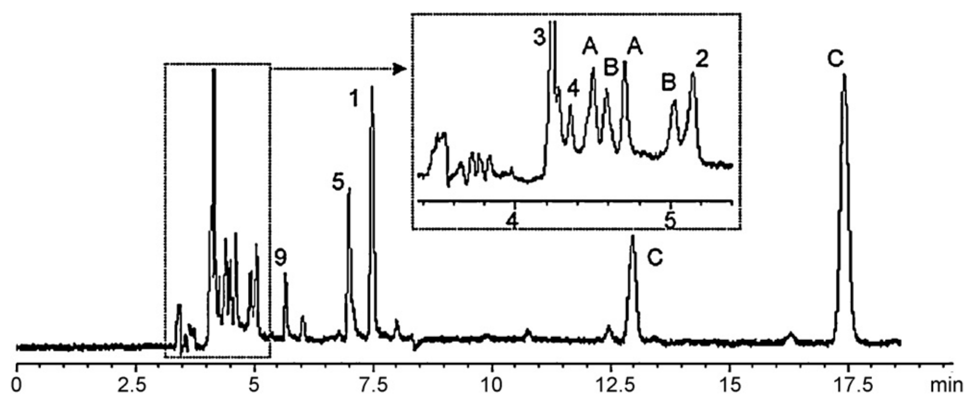


Fig. 8. Capillary electrochromatograms of chamomile's phenolic compounds in a real sample. Column: 50 μm i.d., SCX/C_{18}, 3 μm particles (33.5 cm total/25 cm effective length). CEC conditions: 50 mM phosphate buffer (pH 2.8)/ACN (50/50, v/v); injection: 12 bar/0.4 min; applied voltage: +25 kV; temperature: 25 °C; detection wavelength 337 nm. Peaks: herniarin (1), umbelliferone (2), caffeic acid (3), chlorogenic acid (4), apigenin (5), quercetin (9). B and C identified by UV DAD as phenylpropanoid derivatives. Modified from ref. [183].

is one of the popular labels that react with reducing carbohydrates under mild conditions. In addition, the derivatization reaction does not produce desialylation and isomerization and the resulting PMP derivatives can yield strong UV absorbance at 250 nm.

Some polysaccharides from plants have been regarded as important biological response modifiers; i.e. polysaccharide from *Gynostemma pentaphyllum* herb tea has shown immunostimulating activity and it was proved as an inhibitor of the proliferation of human colon carcinoma HT-29 and SW-116 cells. Similarly, polysaccharides were demonstrated to be the main bioactive constituents of *Codonopsis pilusula*, well known as Dangshen in China for treatment of dyspepsia and poor appetite. Interest is also raised by the polysaccharides from the fruits of pumpkin *Cucurbita moschata* Duchesne ex Poirethas, which showed ability in reducing the blood glucose levels and improves tolerance of glucose. After hydrolysis of the polysaccharides, the released monosaccharides were labelled using PMP. The CE analysis was then carried out in highly concentrated borate buffers (200–400 mM pH 11.0). The baseline separation of several PMP-derivatized monosaccharides (xylose, arabinose, glucose, rhamnose, fucose, galactose, mannose, glucuronic acid, galacturonic acid) can be achieved in about 40 min providing reliable electrophoretic profiles useful in the characterization of the polysaccharides [186–188].

5. Lipids

Lipids are classified as fatty acids, glycerolipids, glycerophospolipids, sphingolipids and sterol lipids [17]. Traditional methods for the analysis of lipids are GC and HPLC; Otieno and Mwongela reviewed the CE-based methods for the determination of lipids, describing advantages and disadvantages of the different modes of CE compared to the well-established chromatographic techniques [189].

Some lipids are represented in the plant kingdom and possess interesting biological activity. As an example, γ-linoleic acid, which is contained in vegetable oils from Onagraceae, Saxifragaceae and Boraginaceae, is involved in platelet aggregation and inflammatory processes. However, very few examples have been reported regarding the CE analysis of lipids as bioactive phytomarkers in medicinal plants and herbal drugs.

An interesting example has been given by Bannore et al., about the determination of some fatty acids in peanut seeds used for the edible oil production. The oleic acid content in peanut seeds is an important marker for their classification, being involved in reducing the blood level of LDL cholesterols. Furthermore the high oleic acid content positively affects the product flavor and the shelf life. A suitable CE system was developed by taking into account some critical points in analysis of fatty acids: (i) poor UV absorption, (ii) poor water solubility and (iii) structural similarity of the considered fatty acids (namely, stearic, palmitic, nonadecanoic, oleic, linoleic and linolenic acid). NACE was thus selected as a method of choice in combination with indirect UV detection. The use of relatively small percentages of water in NACE buffers improved the separation; in particular, 20% of water was used in the presence of NMF (*N*-methylformamide) and dioxane; Tris electrolyte (40 mM) was used in the presence of adenosine 5′-monophosphate (2.5 mM) as the UV marker (detection at 254 nm). Under these initial conditions, palmitic and linolenic acid co-migrated; their separation was achieved by addition of αCD (7 mM). The extraction of lipids from peanut seeds was carried out by following a procedure involving saponification; the validation data were excellent regarding the recovery (about 97%). Data on sensitivity were not provided, however the method was able in quantifying level of linoleic acids below 5% [190]. NACE was applied also in analysis of bioactive phospholipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in plant seeds (soybean, sunflower, peanut, apricot kernel, filber and walnut). The CE separation was achieved in a medium composed of ACN/2-propanol (3:2, v/v) in the presence of acetic acid (0.3%) and 60 mM of ammonium acetate. The detection was performed at 200 nm and the proposed method was compared with a MEKC method based on the use of sodium cholate as surfactant. The two methods showed similar validation parameters, i.e. reproducibility and sensitivity (LOD < 0.025 mg/mL) [191]. A CD-MEKC method was developed and validated for the analysis of anacardic acids in *Anacardium occidentale* nut shell liquid (the cashew). Anacardic acids are 6-alkylsalycilic acids (alkyl moiety is represented by 15 carbon chain and can be included among lipid compounds); they are mainly present in *Ginko biloba* extracts and show potent antibacterial activity against Gram-positive bacteria. These compounds were extracted from nut shell liquid, as free acids, using ethyl acetate and were subsequently purified by silica gel column chromatography. The sample mixture was dissolved in aqueous methanol (30%) containing urea in order to increase the solubility of the compounds. Two different MEKC approaches were developed to achieve the complete separation of three anacardic acids labelled as An15:3 (three double bonds), An15:2 (two double bonds) and An15:0. The first MEKC method was based on conventional conditions using SDS in 40 mM borate buffer (pH 9.2) in the presence of ACN (24%). Since the separation was not satisfactory, a reversed-flow MEKC was preferred; in particular the EOF could be strongly suppressed by using a polydimethylacrylammide-coated capillary, furthermore

cyclodextrins were supplemented to the BGE to modulate the separation selectivity. In particular two CDs, namely βCD (10 mM) and heptakis-6-sulfo-βCD (1 mM) were added to the MEKC system composed of 20 mM SDS in phosphate pH 6.5 (10 mM) containing ACN 20%. An independent ESI-MS method was also applied; using MS, two additional anacardic acids isomers were identified in real samples. The results from MS and MEKC allowed for the complete characterization of nut shell liquid from *A. occidentale* [192]. A dual CD system was also applied in CE separation of ergostane and lanostane compounds from the fruits of *Antrodia camphorata* (syn. *Taiwanofungus camphorates*) used in TCM to enhance immune system and liver function. The active compounds were extracted by Soxhlet ($CHCl_3$) and silica gel column chromatography (n-hexane and ethyl acetate) followed by thin layer chromatography (TLC) for a further fractionation. Dimethyl formamide (DMF) was introduced into the BGE to support the solubility of the hydrophobic analytes during separation. The optimum conditions for separation involved high levels of CDs; precisely, 20 mM of methyl-βCD and 30 mM of sulfobutylether-βCD were added to 20 mM borate buffer pH 9.3 and 5% DMF. The separation of seven compounds (methyl antcinate B, dehydroeburicoic acid, antcin B, dehydroesulfurenic acid, zhankuic acid C, antcin A and antcin C) was achieved in less than 40 min. Because of the favourable UV absorption due to the conjugated system, the detection limit at 243 nm was 1 μM [193].

6. Terpenes

6.1. Monoterpenes

Monoterpenes are commonly found in essential oils and have attracted interest because of antibacterials, anti-fungal and anthelmintic activity [17]. The method of choice for their analysis is GC, which is applied using flame ionisation detector (GC-FID) for quantitative analysis (mainly based on normalization to 100%); GC–MS is useful in structural characterization and identification [194]. As an alternative to the well-established GC approach, electromigration techniques could be potentially applied, however only limited examples of CE analysis of monoterpenes are reported in the recent literature. Interestingly a CEC application using a C_{18} stationary phase and UV detection was developed for the separation and quantitation of monoterpens in methanol extracts and essential oil (steam distillation) of pepper. Twelve compounds were separated and analysed within about 25 min. 3-Caryophillene, 3-carene and limonene were the most representative compounds in essential oils from white and black pepper [195].

6.2. Diterpenes

Diterpenes constitute a wide group of C_{20} compounds biosynthesized from 2E,6E,10E-geranylgeranyl pyrophosphate. Some of the components of this class were used in therapeutic treatments. Thi Phuong et al. provided an interesting example of application of simple CZE analysis of bioactive diterpenoid isomers (continentalic, acanthoic and kaurenoic acids) from *Acanthopanax* species. Extracts from this plant showed potent biological activity (mainly anti-inflammatory, anti-fibrosis and hepatoprotective effects). The CE separation of diterpenes was obtained by using sulfobutyl ether βCD as additive to 50 mM (pH 8.5) borate buffer. The method, after full validation, was applied to extracts from different *Acanthopanax* species, allowing for their classification according to the diterpenoids content [196].

CE-ESI-MS was applied in analysis of polar diterpenes in rosemary extracts obtained by subcritical water extraction. This environmentally friendly extraction mode provides extracts rich in polar compounds because of the use of water as the extraction solvent. However by application of RP-HPLC the loss in relatively highly polar constituents of the extract was observed (mainly rosmarinic acid). Differently, the use of CE-ESI-MS, although less sensitive than HPLC–MS, allowed for the determination of high polar compounds that elute not retained in LC approach [197].

The bioactive diterpenoids (miltirone, dihydrotanshinone I, tanshinone IIA, methylene tanshiqunone and cryptotanshinone) from Radix and Rhizoma *Salviae miltiorrhizae*, one of the most widely used TCM, were separated by MEEKC and non-aqueous MEKC [116,198]. A comparison of the analytical performances of O/W MEEKC and W/O MEEKC showed the superiority of the conventional O/W MEEKC based on SDS as surfactant, 1-butanol as co-surfactant and cyclohexane as the oil. The separation was carried out using borate buffer as the background electrolyte and a small percentage of acetonitrile was found to be essential for the separation of the five diterpenoids. The W/O MEEKC system showed lower resolving power and different separation profile compared to the O/W MEEKC. Interestingly, it can be concluded that microemulsion inversion can be used to obtain orthogonal separation profiles. MEEKC (conventional O/W microemulsion) was reported to be useful in analysis of diterpene lactones andrographolide and dehydroandrographolide in *Andrographis paniculata* using heptane or ethyl acetate as the oil component [199,200].

Two diterpenoids, namely horminone and 7-O-acetylhorminone were isolated from aerial parts of *Salvia chionantha* and *Salvia kronenburgiii* using acetone; after fractionation on silica gel columns with different eluents, the two compounds were finally separated on TLC and characterized by ^{1}H -NMR, EI-MS, UV and IR. The isolated compounds were used as standard references for the MEKC quantitation in root extracts of Salvia species, recognized as an important medicinal plant used in the treatment of many disorders [201,202].

6.3. Triterpenes

Triterpenes are C_{30} compounds structurally related to steroids; among them, glycyrrhetic acid (GAc), the aglycon of glycirrhizin (GL) is one of the most studied. It is one of the bioactive components in Radix Glycyrrhizae, or commonly licorice, the underground material from species of the genus *Glycyrrhiza* L. Licorice is extensively used as an herbal drug in Western and Eastern medicine for a number of diseases [203]. A simple CZE method based on 70 mM borate buffer (pH 9.22) was developed and optimised to allow the separation of GL and GAc from the other components of the *Glycirrhiza* plants extracts such as liquiritin, glabridin and licochalcone. The method was applied in the analysis of extracts from different *Glycyrrhiza* species (65 samples) from Europe and China; PCA analysis of peak area of the detected components, allowed their differentiation. The CZE method showed an excellent selectivity allowing also for fingerprint analysis [204]. The CE separation of two stereoisomers, namely 18α- and 18β-glycyrrhetic acid was achieved using a pH 10 carbonate buffer (25 mM) in the presence of βCD (0.4%) and diethylene glycol (10%) [205]. The sensitivity of the method using UV-DAD (LOD of 1–2.5 μg/mL) was sufficient for the intended applications, however the combination of isotachophoresis with CZE allowed a significant improvement of sensitivity (LOD of 8 ng/mL). This method was applied in the analysis of liqueurs, sweets and food supplements [206].

The CE separation of ursolic and oleanoic acid was obtained by non-aqueous buffer in analysis of extracts of *Rabdosia japonica* and *Piper kadsura* [207]; however the most applied approach to the analysis of these isomeric acids and their congeners is based on the use of CDs in aqueous or organic-aqueous media. The separation of ursolic, oleanoic and betulinic acid in *Prunella vulgaris* L. was obtained in tetraborate buffer (pH 9.4) in the presence of methanol and βCD 2 mM [208]. Similarly, oleanoic, ursolic, maslinic, 2α-

hydroxyursolic, arjunic, 2α,3α,19α-trihydroxy-12-oleanen-28-oic and euscaphic acid were resolved using tetraborate 200 mM and 12.5% methanol with addition of 15 mM of βCD. The method was validated and applied in analysis of *Rubus chingi*, however because of sample matrix interferences the complete analysis of these compounds in real samples was not achieved [209]. Yang et al. applied CD-CZE (borate buffer supplemented with 8 mM of βCD) in analysis of oleanoic and ursolic acid in extracts of *Pterocephalus hookeri*, a Chinese herbal medicin used in treatment of flu, inflammation and rheumatism [210]. Under similar CD-CZE conditions, in the presence of mono-3-phenylcarbamoyl-βCD, Lin et al. analysed saikosaponins a, c and d in extracts of *Bupleurum* Chinese DC [211].

MEKC was also applied to the separation of oleanoic and ursolic acid in dry fruits of *Cornus officinalis* used in TCM for the treatment of hepatobiliary and renal disorders. The use of conventional MEKC SDS-based was not successful, thus HP-βCD was supplemented to improve the separation. Experimental design was applied to the optimisation of the separation conditions and the best results were obtained using tetraborate 40 mM, SDS 25 mM and HP-βCD 15 mM. The content of oleanoic and ursolic acid in the real samples of cornel was in the order of 0.1 and 0.3 mg/g [212]. In general, UV detection (i.e. 210 nm) was applied in the analysis of these compounds providing adequate sensitivity (reported LODs 2.65–26 μg/mL) [207–212].

Further examples of the usefulness of cyclodextrins as additives to achieve separation of triterpenoids in herbal drugs and medicinal plant extracts are related to the analysis of *Antrodia camphorata* a Ganoderma-like fungus used to manage various diseases such as abdominal pain, diarrhea, hypertension. Zhankuic acid and antcin A, B and C, ergostane and lanostane compounds related to ganoderic acids [213] were separated by CZE modified by a dual CD system (methyl-βCD and sulfobutylether-βCD) in tetraborate buffer (pH 9.3). Under the optimised conditions the separation of chiral antcin C was achieved [214].

Dubber and Kanfer proposed a CD-MEKC method for the analysis of terpene trilactones (ginkgolides A, B, C, J, M) and the sesquiterpene bilobalide, in *Ginko biloba* preparations, one of the top selling herbal drugs in the world. Beside separation of the terpene trilactones, the MEKC method had to be selective also for flavanols glycosides. Optimum separation was obtained under acidic conditions, where EOF is strongly suppressed (25 mM phosphoric acid and 40 mM SDS supplemented with 12 mM of βCD). Under the optimized RF-MEKC the method was able to provide quantitation of quercetin and rutin and simultaneously allowed the fingerprinting of terpenes [215]. The CE fingerprint of *Ginkgo biloba* extracts by CE was also carried out under conventional MEKC (alkaline borate buffer as the BGE) [216].

6.4. Sesquiterpenes

A simple CZE analysis of 4 sesquiterpenes eremophilenolides from methanol extracts of *Ligulariopsis shichuana* was developed using tetraborate pH 10.0 running buffer. Sensitivity was not reported, however the good UV absorption of the analytes and their relatively high content in plant extract allowed for reliable determination [217].

The analysis of ginsenosides, the main effective components of the two ginseng genuses, i.e. *Panax ginseng* and *Panax quinquefolium* is challenging because of the weak UV absorption; furthermore, ten strictly related compounds had to be separated in the presence of matrix components. Reversed flow MEKC using phosphoric acid (10 mM) as supporting electrolyte in the presence of 20% ACN and 15% 2-propanol as organic solvents and SDS (140 mM) as the surfactant, was found to be able in providing the complete separation of the analytes in about 32 min. Under these separation conditions the samples dissolved in micelle solution were injected by electrokinetic mode with an improvement of sensitivity which was proved to be around 10-fold compared to that obtained using conventional hydrodynamic mode [218].

7. Conclusion

The opportunity for application of different separation principles have made CE a very versatile techniques and one of the most suitable tools in analysis and quality control of herbal drugs and medicinal plants.

The optimization of experimental parameters affecting separation, such as: (i) nature, concentration and pH values of background electrolyte in CZE; (ii) choice of surfactants type and concentration in MEKC and MEEKC; (iii) use of organic solvents, (iv) use of additives which can form inclusion complexes such as CDs and (v) opportunity to combine hybrid separation mechanisms (chromatographic and electrophoretic in CEC), represent valuables features for the development of selective and fast analytical methods for complex natural products. Furthermore the possibility of coupling CE with LIF and ECL detectors beside the conventional UV detector, greatly enhances the sensitivity of the electromigration techniques making them well suited in the standardization of phytopharmaceuticals. More advanced detection approach, as CE-ESI-MS and CE-ESI-TOF-MS are mainly addressed to the phytochemical characterization of new potentially bioactive plant metabolites.

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