Capillary electrophoresis with capacitively coupled contactless conductivity detection: Recent applications in food control

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Abstract

Food quality control has become much more important during the last decade and demanded the development of robust, efficient, sensitive, and cost-effective analytical techniques. Capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C⁴D) is a powerful separation method based on the different migration time of components in the background electrolyte solution, and it is suitable for all charged ions. There is a large number of compound groups in food that can be determined by the CE-C⁴D method, such as amino acids, biogenic amines, fatty acids, food additives, sweeteners, and nutrients. This paper provides an overview of recent applications of the CE-C⁴D in food analysis over the last decade. General conclusions and future prospects of applicability of the CE-C⁴D method in food analysis are presented.

Keywords: Capillary electrophoresis, capacitively coupled contactless conductivity detection, food control.

1. INTRODUCTION

Capillary electrophoresis (CE) is a separation technique based on the different migration time rates of components (which are usually charged ions) in the background electrolyte solution (BGE), under an external applied electrical field E generated by a high voltage V. It is much simpler than chromatography for ion separations but still have many advantageous features such as high separation efficiency, short analysis time, low power requirements, less consumption of chemicals, and easy to install, operate, and maintain [1]. CE can be classified according to the mechanism of separation: capillary zoneelectrophoresis - CZE; isoelectric focusing - IFF; isotachophoresis - ITP; capillary gel electrophoresis - CGE; and micellar electrophoresis capillary chromatography - MECC. The development applications of CE method cover a wide range of target analytes, including antibiotics [2], vitamins [3], sweeteners [4-5], food additives [6-7], etc. Along with the new generations of microchip electrophoresis, many groups of specific substances on a much more complex matrix could be quantified by CE. The popular application areas of CE methods are pharmaceutical and biomedical, and food analysis [7-11].

Capacitively coupled contactless conductivity detection (C⁴D) has appeared to be convenient for all charged ions and when coupled with the capillary electrophoresis method, its response is proportional to the different mobility of the analyte and the co-ion of the BGE [12]. In general, any charged species which can be separated in electrophoresis can also be detected by a conductivity detector. C⁴D detection has shown its suitability with CE methods because it creates detection signals based on the same properties as CE separation, which has offered many benefits [1]. For example, UV absorption does not work well with most inorganic ions and organic ions lacking a strong chromophore. As a result, C⁴D detection can be established as the method of choice in the determination of inorganic species by capillary electrophoresis and a useful alternative to other detectors in the determination of non-UV-absorbing as well as UV-absorbing organic ions [13].

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Recently, quality control and safety in food production have received great attention, demanding the development of robust, efficient, sensitive, and cost-effective analytical methodologies. While other popular methods for food analysis such as gas chromatography (GC), high-performance liquid chromatography (HPLC) combined with optical (i.e., photodiode array PDA) or mass spectrometry (MS) detectors required high costs for sample analyses and instrumental maintenance, CE method seems to be much cheaper. Several reviews have been published focusing on the application of CE to determine some specific group of compounds [12, 14-15]. CE-C⁴D has been proved to be a versatile and robust tool, providing fast, efficient and automated separations with sample volumes and low consumption of solvents. Some groups of compounds in food can be determined by CE-C⁴D method, including amino acids, biogenic amines, fatty acids, food additives, sweeteners, nutrients, etc. The sample preparation for CE-C⁴D method is relatively simple. In many cases, liquid samples were only filtered and/or diluted before injection. Solid samples were homogenized, extracted by ultrasonication, filtered, and analyzed.

In this review, we focus on recent developments and applications of capillary electrophoresis method with capacitively coupled contactless conductivity detection (CE-C⁴D) in food analysis, covering the period of the last ten years, from 2011 up to now.

2. APPLICATIONS OF CE-C⁴D METHOD IN FOOD CONTROL

2.1. Nutrients

2.1.1. Biogenic amines and amino acids

In food industries, amino acids (AAs) play an important role in determining nutritional values, and they create a distinctive flavor for food. In addition, AAs analysis is also applicable for the determination of protein compositions. Biogenic amines (BAs), low-molecular-weight organic bases, are the products of metabolism of AAs by microbial activity. Some old foods, spoiled foods usually contain certain amounts of BAs, which affect the freshness of food. Most BAs are substances that have potential toxicity, therefore, it is essential to control the BA concentrations to ensure the quality of food.

Significant attention has been given to the analysis of biogenic amines in foods. A CZE-C⁴D method was described by Li et. al. (2014) for the simultaneous determination of eight typical biogenic amines: spermine, spermidine, histamine, putrescine, cadaver, tyramine, β -phenylethylamine, and tryptamine [16]. Several buffer compositions, such as citrate, acetate, and acetic acid were investigated and acetic acid (concentration of 500 mM, pH 2.5) + 150 mM 18-crown-6 used as background electrolyte could achieve the best separation and response. The limits of detection (LOD) and limits of quantification (LOQ) ranged from 44.3 - 149.0 ng/mL and 148.0 - 497.0 ng/mL, respectively. The proposed method was successfully applied for the analysis in real samples including tap water and Chinese hard liquors, and indicated its high potential for the direct determination of non-UV-absorbing BAs without derivatization procedures.

In the analysis of amino acids by CE, Duong et al. (2020) developed an in-house-made duel-channeled portable CE-C⁴D instrument for the determination of 10-hydroxy-2-decanoic acid (10-HDA) and free amino acids in royal jelly supplements [17]. In the first channel, 10-HDA was analyzed using the background electrolyte composed of 20 mM tris(hydroxymethyl)aminomethane (TRIS)/Ace (pH 8.5) under a voltage of -17 kV applied, while in the second channel, free amino acids were analyzed with BGE of 2 M lactic acid under -16 kV separation voltage. The electropherograms for a standard solution containing 10-HDA and free amino acid, as well as those for the sample RJ9 (with and without spiking) were shown in Figure 1 and Figure 2. The limits of detection and the limit of quantification varied from 0.039 - 0.090 mg/g and from 0.130 - 0.300 mg/g, respectively. The results obtained from analyzing various royal jelly-based dietary supplements were cross-checked with the reference method HPLC-PDA to verify the reliability of CE-C⁴D method, which showed a very good agreement. The homemade CE instrument using splitting mode has displayed several advantage features, including fast analysis time, low operation cost and little consumption of reagents for the determination of free amino acids in food supplements.

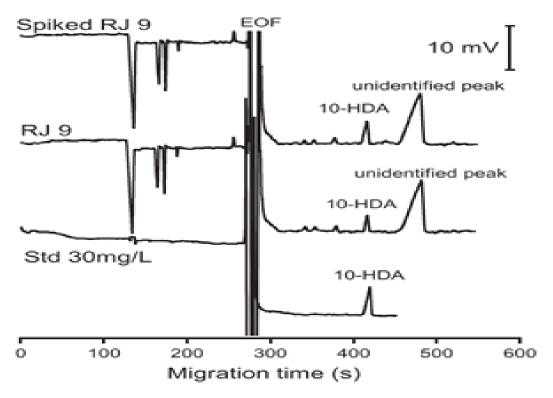


Figure 1. Electropherograms of standard solution, RJ9 samples (with and without spiking) containing 10-HDA [17]

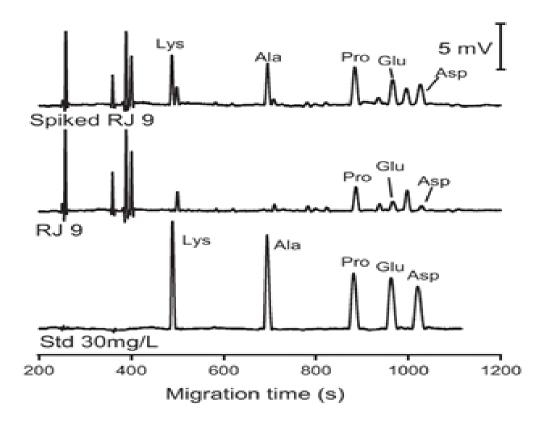


Figure 2. Electropherograms of standard solution, *RJ9* samples (with and without spiking) containing free amino acids [17]

2.1.2. Fatty acids

Fatty acids (FAs) are the building blocks of the fat in our bodies and in the food we eat. An excess of free fatty acids accumulated in skeletal muscles is associated with insulin resistance and overall cellular dysfunction [18]. Therefore, consumptions of fats and their effects on human health have become an active topic of research so that fatty acids also received much attention because of their health impacts.

An efficient CE-C⁴D method for the rapid analytical separation and simultaneous determination of 11 fatty acids in margarine was published by Wong et al. (2013) [19]. A buffer solution containing methyl- β -cyclodextrin (M β CD, ~ 6 mM) and heptakis-(2,3,6-tri-O-methyl)- β -cyclodextrin (TM β CD, 8 mM) dissolved in a mixture of Na₂HPO₄/KH₂PO₄ (5 mM, pH 7.4) : ACN : MeOH : *n*-octanol (3 : 4 : 2.5 : 0.5, v/v/v/v) was used as background electrolyte. The limit of detections, with a signal-to-noise ratio of three for nine fatty acids (0.9 - 1.9 µg/mL), were lower than some previously reported methods [20-21]. The validated method was successfully applied for determining the contents of nine fatty acids in commercial margarine samples, and some samples were randomly analyzed by the gas chromatography method with a flame ionization detector (GC-FID) to prove the validity of the proposed method.

The suitability of CE-C⁴D was shown to successfully separate and determine free fatty acids (FFAs) in edible oil by Wu *et al.* (2014) [22]. Buffer solution containing 39 mM TRIS, 3 mM pelargonic acid, 30 mM Brij 35, 35 % acetonitrile, 15 % 2-propanol, 2.5 % 1-octanol, and 300 μ M polyamidoamine (PAMAM) (pH 8.53) was chosen as the optimum conditions of background electrolyte compositions. The detection limits for most FFAs ranged from 0.46 - 3.28 μ M, equivalent to the mass concentrations of 0.12 - 0.57 μ g/mL. The developed method was applied for the determination of free fatty acids in plant oil samples. For comparison, free fatty acid contents in the oil samples were also analyzed by GC-MS, and the results from two methods agreed well. CE-C⁴D has been demonstrated to be fast, cost-effective, and easier to operate than the traditional chromatography techniques, with a high potential working in the food, nutrition, and oleochemical industries. *2.1.3. Other nutrients*

In addition to some groups of compounds presented in the above sections, other groups of substances such as micronutrients (inorganic ions), vitamins, and small-sized organic ions also received much attention because of their existence in many food categories such as drinking water, canned food, etc.

Inorganic ions as metal ions present a small amount to provide mineral elements for the human body. A simple CE-C⁴D method was developed by Lemos et al. (2014) for simultaneous determination of potassium, sodium, calcium, and magnesium in virgin olive oils [27]. To obtain the best signal, with asymmetry sharp peaks and good peak resolutions, a BGE was composed of 20 mM HIS and 22 mM lactic acid (pH 4.7). Limit of quantification of this method ranged from 0.029 to 0.044 mg/kg. To analyze the contents of metal ions in virgin olive oil, five different commercial samples were experienced a simple liquid-liquid extraction procedure: added 1 mL 10-fold diluted BGE, then homogenized under optimum sonification condition (15 minutes) before analyzing. The proposed method was proved to have acceptable precision and accuracy. In other group work, Nguyen et al. (2018) described a CE-C⁴D determination of K⁺, Ca²⁺, and Mg²⁺ mineral cations in rice, using BGE composed of 12 mM HIS/ acetic acid (pH 4.2) added 2 mM 18-crown-6 [28]. Limits of detection ranged from 0.8 to 1.5 ppm for three analytes. The obtained analytical results in rice samples showed good agreement with the reference methods (titration methods followed in TCVN), which contributed to a new application of CE-C⁴D in the quality control of rice aurine (2-amino ethanesulfonic acid) and choline are nutrients frequently added in infant formulas, energy drinks, and dietary supplements, which help to maintain physical comfort, stimulate thought processes, improve performance and awareness [29]. Nguyen et al. (2019) have developed a dual-channeled CE-C⁴D method for rapid determination of choline and taurine in energy drinks, and dietary supplements. In the first channel, taurine was analyzed using the BGE composed of 150 mM TRIS/lactic acid (pH 8.96) while choline was simultaneou ly determined in the second channel using BGE containing 150 mM TRIS/acetic acid (pH 9.50). The detection limits of choline and taurine were 0.27 and 0.45 mg/L, repetitively. A good agreement between the results obtained with CE-C4D and those with the standard confirmation methods (HPLC-DAD for taurine and LC-MS/MS for choline) was achieved.

The described method could help promote the use of CE-C⁴D as a straightforward and inexpensive solution for screening determination of various food additives in different food matrices.

2.2. Food additives

2.2.1. Organic acids and their salts

Some organic acids and their salts, which are important chemical preservatives, are usually added in pharmaceutical products, cosmetic, and food industries to delay nutritional losses of products and prevent the formation of microbial toxins. A great number of researches in food analysis focused on acid additives have been published.

Wei *et al.* (2011) presented a sensitive method of online preconcentration, namely field-enhanced sample injection (FESI), followed by CE-C⁴D for the determination of sorbic and benzoic acids in soy sauce [6]. The compositions of background electrolyte were 20 mM acetic acid/8 mM TRIS (pH 4.8) added 0.2 mM cetrimonium bromide (CTAB) as EOF modifier. To reduce the complex matrix interference of soy sauce samples, a suitable sample clean-up procedure was also investigated for real sample pretreatment. Under the optimized conditions, sorbic acid and benzoic acid were separated within 10 minutes, and the detection limits were 0.05 and 0.08 μ M, respectively. The quantitative results were further evaluated by the HPLC method, which showed that the data obtained from the proposed method were reliable.According to the authors, that was the first study using FESI-CE-C⁴D technique for the simultaneous determination of sorbic acid and benzoic acid in a very complex matrix such as soy sauce samples. The FESI-CE-C⁴D technique revealed its advantage in terms of simplicity, cost-effectiveness, high sensitivity, and excellent repeatability.

An in-house-made portable CE-C⁴D instrument with manual or semi-automated has recently emerged as a powerful and versatile separation techniques for the analysis of some acid additives in many food samples in Vietnam was described by Le *et al.* (2017) [7]. Citric acid is used as synthetic acidulant while citrate and tartrate play the role of antioxidants. A BGE composed of 50 mM Histidine (HIS)/30 mM MES added 25 μ M CTAB (pH 6.0) was employed for the determination of oxalate, tartrate, and citrate and LODs for these three acids were 0.22, 0.37, and 0.92 ppm, respectively. Other low-molecular-mass organic acids (i.e. formic acid, acetic acid, propionic acid, and butyric acid) can also be added to food for antimicrobial purpose. Thus, CE-C⁴D was developed for organic acids (C1-C4) analysis with a buffer solution containing 30 mM HIS/ 40 mM 2-(N-morpholino) ethanesulfonic acid (MES) (pH 5.8). The LODs for C1-C4 carboxylates were 0.05, 0.12, 0.15, and 0.17 ppm, respectively. The electropherograms obtained for two standard solutions with i) citrate, tartrate, oxalate (class 1) and ii) formate, acetate, propionate, butyrate (class 2) ware demonstrated in Figure 3.

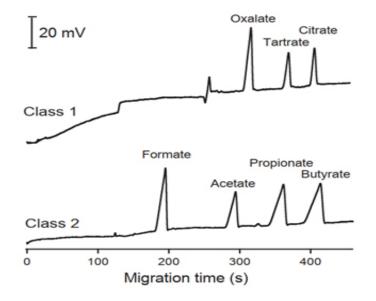


Figure 3. Electropherograms obtained for two standard solutions with i) citrate, tartrate, oxalate (class 1) and ii) formate, acetate, propionate, butyrate (class 2) [7]

Determinations were carried out in a wide range of samples, including beer, instant noodles, wine, coffee, vinegar, and tea. The results were cross-checked with those from HPLC-PDA, with relative errors for the two pairs of data smaller than 13 %. CE-C⁴D was found to be a simple and inexpensive solution for the analysis of various food additives in different food matrices.

2.2.2. Artificial sweeteners

Artificial sweeteners are widely used as a low-calorie alternative to natural sugar in food and beverages. However, the excess intake of sweeteners may negatively affect the health of consumers, so the usage management of artificial sweeteners in food is essential.

Bergamo *et al.* (2011) proposed a capillary electrophoresis with C⁴D detection for the simultaneous determination of aspartame, cyclamate, saccharin, and acesulfame-K in soft drinks and tabletop sweetener formulations [4]. Completed separation of analytes could be attained in about six minutes under the optimum BGE composition of 100 mM TRIS/10 mM HIS (pH 9.2). The limits of detection were from 1.4 to 4.2 mg/L and the limits of quantification were from 4.7 to 14.1 mg/L, which were considered better than CE with the photometric detection method [23]. In addition, CE-C⁴D was also developed for analyzing these four sweeteners in beverages, jelly, and black bean sweet soup by Le *et al.* (2017) [7]. The electropherogram for a standard solution containing aspartame, cyclamate, saccharin, and acesulfame-K is shown in Figure 4. Acesulfame-K was found most frequently in many kinds of drinks in Vietnam, with the highest concentration of 600 µg/g found in a Lipton ice tea sample. The data was cross-checked with the confirmation method (HPLC-PDA), showing a very good agreement between the results from two methods. CE-C⁴D method has been demonstrated to be a simple, rapid, and accurate analytical technique for the determination of some artificial sweeteners in soft drinks and tabletop sweetener formulations, and have a high potential in analysis of other matrices, such as juices, teas, etc.

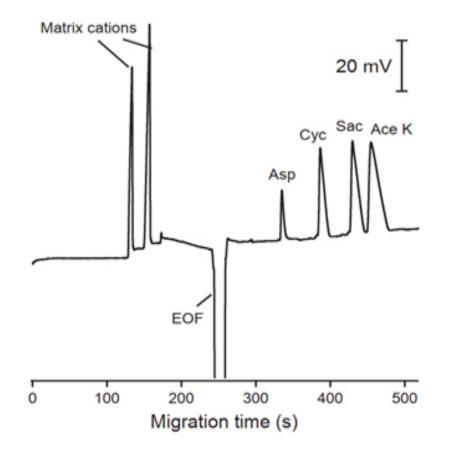


Figure 4. Electropherogram for a standard solution containing aspartame (100 ppm), cyclamate, saccharin, and acesulfame-K (50 ppm) [7]

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Coupling with a simple and easy-operating preconcentration technique such as solid-phase extraction (SPE) technique, a CE-C⁴D method was described by Xia *et al.* (2019) for the simultaneous determination of three sulfanilamide artificial sweeteners (acesulfame-K, sodium saccharin, sodium cyclamate) in foodstuffs [5]. A buffer solution (pH 9.74) containing 20 mM TRIS, 20 mM Na₂B₄O₇, and 50 μ M CTAB was used as background electrolyte. SPE clean-up procedure with porous aromatic framework-6 (PAF-6) as sorbent was optimized for extracting and enriching three analytes in samples. The detection limits achieved were 0.09, 0.12, and 0.22 μ M and the quantification limits were 0.32, 0.37, and 0.75 μ M for acesulfame-K, sodium saccharin, and sodium cyclamate, respectively. The proposed method was proved to be rapid, simple, cheap, and sensitive for the determination of artificial sweeteners in beverage samples and candied fruits when using capillary electrophoresis with SPE sample treatment.

3. CONCLUSIONS AND PERSPECTIVES

This review has described some applications of capillary electrophoresis - capacitively coupled contactless conductivity detection (CE-C⁴D) in food analysis. Summary of the applications in the world and in Vietnam presented in this article are shown in Table 1.

Some of the polyol compounds (sugar alcohols) that are found in food and beverages, also received much concern in terms of nutritional information and quality control. Coelho *et al.* (2016) demonstrated a CE-C⁴D method to separate erythritol, maltitol, xylitol, and sorbitol [24]. A background electrolyte composed of 25 mM sodium borate and adjusted the pH to 8.5 (using boric acid solution) was chosen to provide the best peak resolutions. LODs of four sweeteners were about 2.7 - 4.8 μ g/g and LOQs were 9.0 - 15.9 μ g/g, which were better than those obtained by other works reported in the literature using derivatization of the polyols [25] or indirect UV detection [26]. The presented method was successfully applied for the determination of these polyols in sugar-free chocolate sold in the commercial market.

References	Analyte	BGE composition	Detection limit		
Biogenic amines and amino acids					
Li et. al. (2014)	Biogenic amines: spermine, spermidine, histamine, putrescine, cadaver, tyramine, β - phenylethylamine, tryptamine	150 mM 18-crown-6/500 mM HAc (pH 2.5)	44.3 - 149.0 ng/mL		
Duong et. al. (2020)	10-HDA Free AAs: Lys, Ala, Pro, Glu, Asp	20 mM TRIS/Ace (pH 8.5) 2 M lactic acid	0.039 mg/g 0.039 - 0.090 mg/g		
Fatty acids Wong et. al. (2013)	FAs: lauric, myristic, trideca- noic, palmitic pentadecanoic, stearic,oleic, elaidic, linoleic, arachidic acids	6 mM M β CD, 8 mM TM β CD, Na ₂ HPO ₄ /KH ₂ PO ₄ (5 mM, pH 7.4) : ACN : MeOH : n-octanol (3 : 4 : 2.5 : 0.5, v/v/v/v)	0.9 - 1.9 μg/mL		
Wu et. al. (2014)	FAs: hexanoic, octanoic, capric, lauric, myristic, pal- mitic, linoleic, oleic, elaidic, stearic acids	39 mM TRIS, 3 mM pelargonic acid, 30 mM Brij 35, 35% acetonitrile, 15% 2-propanol, 2.5% 1-octanol, 300 μM PAMAM (pH 8.53)	0.46 - 3.28 μM		

Table 1. Application of CE-C⁴D in the analysis of food samples

Dinh Le Minh, Hoang Quoc Anh, Pham Thi Mai Huong, Nguyen Thi Anh Huong	Dinh Le Minh, Hoang	Quoc Anh, Pham	Thi Mai Huong,	Nguyen Thi Anh Huong
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References	Analyte	BGE composition	Detection limit		
Food additives					
Wei et. al. (2011)	Sorbic acid, benzoic acid	20 mM HAc/8 mM TRIS, 0.2 mM CTAB (pH 4.8)	5.6 - 9.8 µg/L		
Le et. al. (2017)	Oxalate, tartrate, citrate	50 mM HIS/30 mM MES, 25 μM CTAB (pH 6.0)	0.22 - 0.92 ppm		
Le et. al. (2017)	Formate, acetate, propionate, butyrate	30 mM HIS/40 mM MES (pH 5.8)	0.05 - 0.17 ppm		
Artificial sweeteners					
Bergano et. al. (2011)	Aspartame, cyclamate, saccharin, acesulfame-K	100 mM TRIS/10 mM HIS (pH 9.2)	1.4 - 4.2 mg/L		
Coelho et. al. (2016)	Erythritol, maltitol, xylitol, sorbitol	25 mM sodium borate/boric acid (pH 8.5)	2.7 - 3.7 μg/g		
Le et. al. (2017)	Aspartame, cyclamate, saccharin, acesulfame-K	100 mM TRIS/10 mM HIS (pH 9.2)	0.7 - 5.0 mg/L		
Xia et. al. (2019)	Acesulfame-K, sodium saccharin, cyclamate	20 mM TRIS, 20 mM Na ₂ B ₄ O ₇ , 50 μM CTAB (pH 9.74)	0.09 - 0.22 μM		
Other nutrients			LOD: NA ^a		
Lemos et. al. (2014)	Trace elements: Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺	20 mM HIS/22 mM lactic acid (pH 4.7)	LOQ: 0.029 - 0.044 mg/kg		
Nguyen et. al. (2018)	Minerals: K ⁺ , Ca ²⁺ , Mg ²⁺	12 mM HIS/Ace, 2 mM 18-crown-6 (pH 4.2)	0.8 - 1.5 ppm		
Nguyen et. al. (2019)	Choline Taurine	150 mM TRIS/Ace (pH 9.5) 150 mM TRIS/lactic acid (pH 8.96)	0.27 mg/L 0.45 mg/L		

^{*a}NA: Not available*</sup>

In general, the CE-C⁴D methods have been used to determine the contents of amino acids and biogenic amines, fatty acids, organics acids, artificial sweeteners, and other nutrients. A number of advantageous features of capillary electrophoresis include the simplicity, fastness, low-cost operation, and reduced consumption of reagents. With these outstanding features, the CE-C⁴D method has been successfully applied for on-site measurement of food additives. The CE-C⁴D devices have been installed and operated in both central and local laboratories in developing countries. In addition, C⁴D detection has been proved to be a suitable detector for all charged ions and an alternative for non-UV-absorbing compounds. The sensitivity is one of the major challenges of CE-C⁴D method for the analysis of complex matrices. Recently, there has been a number of sample pretreatment techniques, e.g. on-line preconcentration FESI, and solid-phase extraction (with the innovation of absorbent materials) that could be coupled with CE-C⁴D. We expect that the CE-C⁴D method along with the development of sample preparation techniques will contribute substantially to original research and real tasks in the fields of food safety and management.

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Phương pháp điện di mao quản sử dụng detector độ dẫn không tiếp xúc: Một số ứng dụng trong kiểm nghiệm thực phẩm

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Tóm tắt

Trong những năm gần đây, kiểm nghiệm thực phẩm đóng một vai trò quan trọng và đòi hỏi sự phát triển của các kỹ thuật phân tích nhanh, hiệu quả, cho độ nhạy cao và tiết kiệm chi phí. Điện di mao quản sử dụng detector độ dẫn không tiếp xúc kết nối kiểu tụ điện (CE-C⁴D) là một phương pháp phân tách hiệu quả, dựa trên tốc độ di chuyển khác nhau của các ion trong dung dịch điện ly dưới tác dụng của điện trường sinh ra khi áp điện thế cao vào hai đầu mao quản. Rất nhiều nhóm chất trong thực phẩm có thể được xác định bằng phương pháp CE-C⁴D, như acid amin, amino sinh học, acid béo, phụ gia thực phẩm, chất tạo ngọt, chất khoáng, ... Bài tổng quan này trình bày một số ứng dụng gần đây của phương pháp CE-C⁴D trong phân tích thực phẩm chung và triển vọng trong tương lai về khả năng ứng dụng của phương pháp CE-C⁴D trong phán tích thực phẩm cũng được trình bày.

Từ khóa: Điện di mao quản, detector độ dẫn không tiếp xúc, kiểm nghiệm thực phẩm.