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Analytical Methods

Determination of capsaicin and dihydrocapsaicin in *Capsicum anuum* and related products by capillary electrophoresis with a mixed surfactant system

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ABSTRACT

An easy, rapid, sensitive, and cheap capillary electrophoresis (CE) method based a mixed surfactant system formed by sodium dodecyl sulphate (SDS) and polyoxyethylene sorbitan monolaurate (Tween 20) as modifier in the buffer was reported. Quantitative analysis of capsaicin and dihydrocapsaicin in *Capsicum anuum*, pepper sauce and porous capsicum plaster was demonstrated. After conducting a series of optimisations, baseline separation was obtained for the analytes within 5 min under the optimum conditions (15 mM sodium tetraborate–0.05% (v/v) Tween 20–2.2 mM SDS buffer (pH 10.1), 20 kV voltage, 214 nm UV detection). The method resulted in excellent linearity, with r^2 of regression equation of 0.9994 and 0.9996 for capsaicin and dihydrocapsaicin, respectively. Recoveries were in the range 90–107% and 92–109% for capsaicin and dihydrocapsaicin, respectively.

1. Introduction

Capsicum anuum are savory food additives that are widely utilised in many parts of the world, and are highly valued for their attributes of colour, pungency, and aroma. It is used for preparing spicy sauces and also in Mexican and Asian cuisines. Both hot pepper and chillies are produced in increasing amounts in Europe and India. International demand for strongly coloured hot pepper fruit arouses hope for an increase in the preference for spiciness in food products. Capsaicinoids are the compounds responsible for the hot, spicy flavour presented by many varieties of peppers. Among the many natural capsaicinoids found in hot chilli peppers, two compounds are predominant: capsaicin (trans-8-ethyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide) (Kosuge & Furuta, 1970). Interest in capsaicin is based on its toxicity (likely carcinogenic action) (Archer & Jones, 2002), its effects on the nervous system and its nutritional and therapeutical benefits at low doses (Gamse, Wax, Zigmond, & Leeman, 1981). So that it is imperative to establish a sensitive, selective and practicable technique for fast detection of capsaicinoids because of the increasing demand by consumers for spicy foods, and the increasing use in pharmaceuticals (Kaale, Van Schepdael, Roets, & Hoogmartens, 2002).

Previous chromatographic methods have been reported for analytical separation, quantitation and identification of naturally occurring capsaicinoids by gas chromatography (Cisneros-Pineda et al., 2007; Hawer, Ha, Hwang, & Nam, 1994), reversed-phase high-performance liquid chromatography (HPLC) (Barbero, Liazid, Palma, & Barroso, 2008) and micellar electrokinetic chromatography (MEKC) (Laskaridou-Monnerville, 1999). The most recent methods for the determination of capsaicinoids have used HPLC coupled to more selective techniques such as mass spectrometry (Garces-Claver, Arnedo-Andres, Abadia, Gil-Ortega, & Alvarez-Fernandez, 2006; Kozukue et al., 2005; Schweiggert, Carle, & Schieber, 2006; Thompson, Phinney, Welch, & White, 2005). However, all these methods, though attaining sometimes low detection limits, are very laborious, long-lasting, expensive or the requirement of tedious pretreatment. Nowadays, rapid and low cost of analysis is increasingly being demanded in many areas including food and pharmaceutical analysis. The aim of our study was to find an easy, rapid, sensitive, and cheap method for the determination of capsaicin and dihydrocapsaicin.

It is well known that capillary electrophoresis (CE) has become an important liquid separation tool as a complimentary technique to liquid chromatography (LC). CE has the advantages of high-resolution capability and small sample volume. To date, various surfactants have been available as additive in CE, such as anionic surfactants SDS (Baher, Fatemi, Konoz, & Golmohammadi, 2007; Liu, Chen, & Hu, 2007a) and nonionic surfactant Tween 20 (Esaka, Sawamura, Murakami, & Uno, 2006; Liu, Chen, & Hu, 2007b). However, mixed surfactants systems, which are composed of two or three surfactants, are not commonly used. In our study, it has been found that they have unique selectivity. It may be because the fact that synergism is sought when anionic and nonionic surfactants are mixed (Esaka, Tanaka, Uno, & Goto, 1997).

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In this report, a new CE method, rapid and reproducible, based on a mixed surfactant system formed by SDS and Tween 20 is adapted for quantitative analysis of capsaicin and dihydrocapsaicin in *C. anuum*, pepper sauce and porous capsicum plaster. The capsicum plaster, which contains natural herbal product extracts, is used for long-lasting relief of muscular fatigue, lumbago, back pain, stiff shoulders, and arthritis. Various parameters such as the concentration of SDS and Tween 20 mixed surfactant, concentration of sodium tetraborate, buffer pH value, and applied voltage were studied. Besides, the separation efficiency, the linearity, the detection limit, and the repeatability of the separation methods were investigated.

2. Experimental

2.1. Chemicals and materials

Capsaicin and dihydrocapsaicin were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, China. For their chemical structures, see Fig. 1. The standards were used as received. *C. anuum* from different provinces (see Table 1) were purchased in local stores. Wild hill pepper sauce was purchased from Hainan Dezhong Food Co. Ltd. (Qionghai, China). Hongfantian pepper sauce was purchased from Zhuzhou Hongfantian Co. Ltd. (Zhuzhou, China). Porous capsicum plaster was purchased from Shaanxi Lübei health products Co. Ltd. (Xi'an, China). Sodium tetraborate was purchased from Tianjin Yongda chemical reagents and development centres (Tianjin, China). Ethanol was purchased from Tianjin Jingu Industry Co. (Tianjin, China). SDS and Tween 20 were purchased from Guangdong Guanghua Chemical Factory Co. Ltd. (Guangzhou, China). All chemicals were of analytical reagent grade and were used as received. All solution and buffer were made in distilled water.

2.2. Apparatus

A CL1030 high-performance capillary electrophoresis (HPCE) apparatus (Beijing Cailu Instrumental Co. Beijing, China) was used. The HPCE was equipped with a power supply (up to constant voltage 30 kV). Uncoated silica separation capillaries of 50 cm (42 cm effective length) × 50 μm I.D. × 375 μm O.D. (Yongnian Optical Fiber Factory, Hebei, China) were used throughout the study. UV detection was carried out at 214 nm. The data acquisition was carried out with a HW-2000 Chromatography Workstation (Shanghai Qianpu Software Company, Shanghai, China). Samples were introduced into the capillary by hydrodynamic injection, where the sample vial was raised by 15.5 cm for 8 s. A PH-3C acidity metre (Shanghai Hongyi Instrument Co. Ltd., Shanghai, China) was used for the pH measurement.

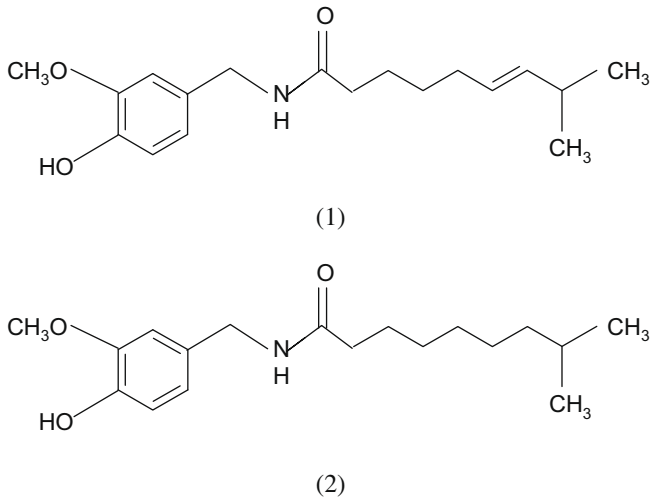

Fig. 1. Structures of (1) capsaicin and (2) dihydrocapsaicin.

Table 1
Contents of the analytes in real samples and RSD ($n = 4$). Conditions: 15 mM sodium tetraborate–0.05% (v/v) Tween 20–2.2 mM SDS buffer (pH 10.1), 20 kV voltage, 214 nm UV detection.

Sample no.[a]	Capsaicin (mg/g)	Dihydrocapsaicin (mg/g)
1	0.86 (1.4)[b]	0.59 (3.3)
2	0.73 (2.2)	0.47 (3.5)
3	0.42 (3.6)	0.49 (2.8)
4	1.18 (1.5)	1.08 (1.6)
5	1.41 (0.82)	1.03 (4.2)
6	2.40 (4.2)	1.45 (3.1)
7	0.84 (0.68)	0.81 (1.1)
8	0.047 (2.0)	0.050 (2.8)
9	0.075 (5.0)	0.089 (2.8)
10	0.070 (2.1)	0.046 (1.9)

[a] Fruits of *Capsicum anuum* in different Provinces, China, Hunan (1), Sichuan (2), Guizhou (3), Hubei (4), Yunnan (5), Jiangxi (6), Henan (7), and Wild hill pepper sauce (8), Hongfantian pepper sauce (9), Porous capsicum plaster (10).
[b] The data in parentheses refer to the RSD (%).

2.3. Preparation of the reagents

The electrophoretic buffer was freshly prepared and typically consisted of 15 mM sodium tetraborate–2.2 mM SDS–0.05% (v/v) Tween 20 (pH 10.10). The buffer was prepared daily from stock solution of 100 mM sodium tetraborate, 100 mM SDS and 10% (v/v) Tween 20, and then adjusted to the desired pH using either 1 M NaOH or 1 M HCl. The pH values were determined by a PH-3C pH meter. Standard stock solutions (1000 μg/mL) of capsaicin and dihydrocapsaicin were dissolved in 50% (v/v) aqueous ethanol degassed in an ultrasonic bath and filtered through a 0.45 μm membrane filter. The solutions at various concentrations were prepared by appropriate dilution of the stock solution with 50% (v/v) aqueous ethanol solution.

The *C. anuum* fruits were oven-dried at 60 °C for 12 h, and then the dried fruits was added to a FastPrep instrument (model DFT100, Linda machine Co. Ltd., Wenling, China), in which it was ground for 60 s. Porous capsicum plaster was cut into small pieces prior to the following steps. Fine powder of 1.00 g, pieces and sauce of the difference samples were respectively weighed and extracted with 5 mL 50% (v/v) aqueous ethanol for 60 min in an ultrasonic bath. Next, extracted solutions were filtered through 0.45 μm syringe filters until the filtrate was limpid and transparent. The filtrate was directly introduced into capillary for determination. All solutions were filtered through 0.45 μm syringe filters before use.

3. Results and discussion

3.1. Method development

Capsaicin and dihydrocapsaicin were hydrophobic compounds. Initially, MEKC was tried out with buffer of sodium tetraborate–SDS (the concentration of SDS was higher than the CMC of

8.1 mM). The main drawback of using lower concentration SDS was peak tailing, which could be due to the relatively strong ion pair interaction between micelle and analyte. To improve peak shape, SDS concentration was increased, which resulted in longer migration time. At 30 mmol SDS, capsaicin and dihydrocapsaicin could be separated completely with symmetrical peak shape, however, dihydrocapsaicin was poor separated from other unknown compounds in the herbal medicines. Attempts to improve the peak shape by modifying the buffer with organic solvents without increasing migration time, such as acetonitrile and methanol, were unsuccessful, because although the peak shape improved, the selectivity was not improved in the real samples. Therefore, further attempts were made to obtain rapid separation and symmetrical peak shape by adding Tween 20 to the electrolyte. Interestingly, both selectivity and peak efficiency (peak shape) improved when Tween 20 was added to the SDS solution without increasing migration time, and SDS concentration is lower than the CMC of 8.1 mM. Under the optimal condition, capsaicin and dihydrocapsaicin were separated completely with unknown compounds in the herbal medicines. The peak sequence of the two compounds in CE was capsaicin and dihydrocapsaicin.

3.2. Effect of the buffer pH

The influence of the buffer pH in the range of 9.1–10.1 on the separation of capsaicin and dihydrocapsaicin in a 15 mM sodium tetraborate–2.0 mM SDS–0.05% (v/v) Tween 20 buffer was investigated. The results indicated that with the increasing pH value, from 9.1 to 10.1, the migration time and resolution between the analytes change a little, but the peak area and peak height increased all through. Additionally, the peak shape of the analytes was obviously improved at the same time. On considering the resolution, migration time, peak area and peak shape of the analytes, the optimised buffer pH was selected at 10.1.

3.3. Effect of sodium tetraborate buffer concentration

Buffer concentration had an obvious influence on the separation because it can influence the electroosmotic flow (EOF) and the viscosity of the electrolyte. The effect of varying the sodium tetraborate buffer concentration from 5 to 30 mM at pH 10.1 was investigated. As expected, the migration times of the analytes increased with the increase of buffer concentration. This is as a result of the decreased EOF since this effect is directly related to the decrease of the zeta potential at the capillary wall-solution interface. The resolution between capsaicin and dihydrocapsaicin increased with increasing the concentration of sodium tetraborate until it reached a maximum value at 20 mM, but decreased with further increasing the concentration. The peak area and peak height increased all through. Additionally, good peak shape of the analytes was obtained at 15 mM, whilst peak splitting and tailing were obtained at 30 mM sodium tetraborate. With concurrent consideration of resolution and migration time, peak area and peak shape, 15 mM sodium tetraborate was therefore preferred for further studies.

3.4. Effect of Tween 20

Although capsaicin and dihydrocapsaicin were completely separated under the above optimal conditions, the peak shape of the analytes was poor and peak tailing was serious without Tween 20. As we know, peak tailing phenomena was resulted from conductivity differences between the sample and surrounding buffer. It may be because that the electric field of the sample zone was higher than buffer zone. Therefore, further attempts were made to improve the separation by adding the organic modifier to the electrolyte. Tween 20, a nonionic surfactant, was applied in this paper. We investigated the effect of Tween 20 contents changed from 0% to 0.09% (v/v) on the separation behaviour of capsaicin and dihydrocapsaicin using 15 mM sodium tetraborate–2.0 mM SDS (pH 10.1). The results indicated that the migration time, peak area and resolution of the analytes decreased with the increasing of Tween 20 concentration. The addition of Tween 20 provides a significant improving in the peak shape all through. Fig. 2 shows separations of the analytes in the absence (A) and the presence of 0.05% (v/v) Tween 20 (B). With concurrent considerations peak area, peak shape and resolution, 0.05% (v/v) Tween 20 was chosen as an optimal surfactant concentration.

3.5. Effect of SDS

Influence of the SDS on the resolution and migration time of capsaicin and dihydrocapsaicin was studied in a range from 0 to 5 mM using 15 mM sodium tetraborate–0.05% (v/v) Tween 20 at pH 10.1 and 20 kV applied voltage. Based on the structure and hydrophobicity of the compounds the separation was affected by the SDS concentration differently (Fig. 3). When the concentration of SDS was increased from 0 to 5 mM whose concentration is lower than the CMC of 8.1 mM, there was an increase in the migration time from 3.2 to 4.9 min and 3.3 to 6.1 min for capsaicin and dihydrocapsaicin, respectively. The resolution increased with increasing SDS concentration. SDS (2 mM) can achieve baseline separation. Considering the resolution, migration time, 2 mM SDS was therefore preferred for further studies. However, it was unfavourable to the analytes' separation in real samples. The results in this work indicated that the peak sequence of dihydrocapsaicin and unknown peaks was changed when the concentration of SDS was increased from 2.0 to 2.5 mM (Fig. 3). Capsaicin and dihydrocapsaicin in real samples could be completely separated without interference of unknown peak at 2.2 mM SDS. Accordingly, the SDS concentration was fixed at 2.2 mM for the following experiments.

3.6. Final optimisation

Final conditions were as follows: 15 mM sodium tetraborate–0.05% (v/v) Tween 20–2.2 mM SDS buffer (pH 10.1), 20 kV voltage, 214 nm UV detection.

3.7. Method validation

Under the optimum conditions, calibration graphs were obtained by injecting standard solutions at seven different concentrations. Each point on the calibration graph corresponded to the

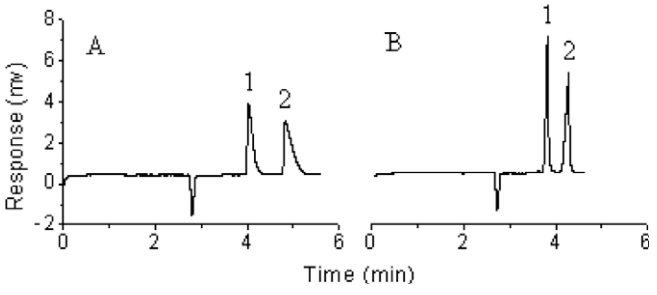

Fig. 2. Separations of capsaicin and dihydrocapsaicin in the absence (A) and the presence (B) of 0.05% (v/v) Tween 20 in electrolyte solution. Conditions: 50 μm I.D. × 375 μm O.D. × 50 cm length (41 cm effective length), uncoated; buffer, 15 mM sodium tetraborate–2 mM SDS (pH 10.1); voltage, 20 kV; detection wavelength, 214 nm; sample: 100 μg/mL capsaicin and dihydrocapsaicin; sample solution: 50% (v/v) aqueous ethanol; peaks: 1, capsaicin; 2, dihydrocapsaicin.

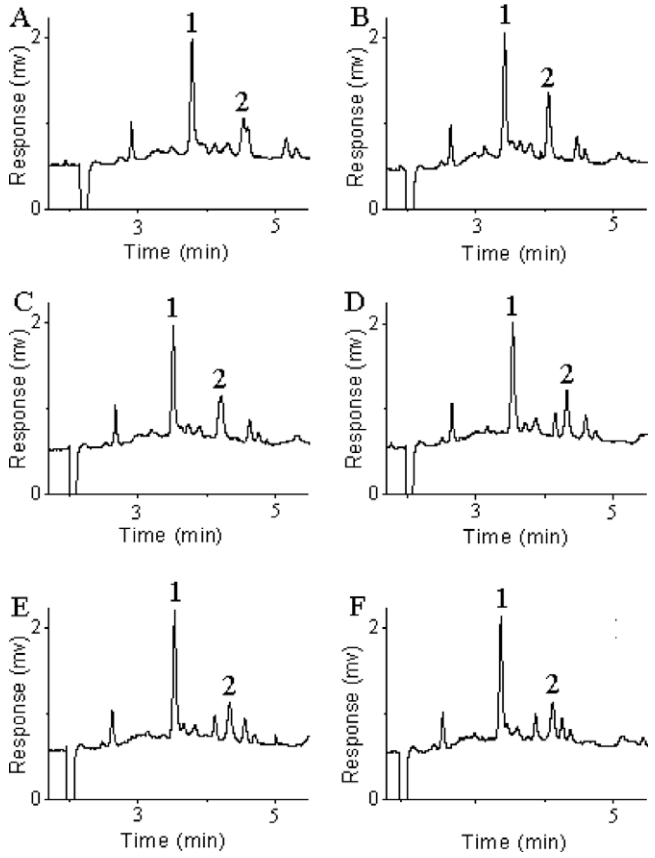

Fig. 3. Influence of SDS concentrations on the separation of the peaks. (A) 1.9 mM, (B) 2.0 mM, (C) 2.1 mM, (D) 2.2 mM, (E) 2.3 mM, and (F) 2.4 mM. Other conditions as in Fig. 2.

mean value obtained from three independent peak area measurements. The calibration curves exhibited excellent linear behaviour over the concentration range of 1–400 μg/mL with correlation coefficient (r) 0.9997–0.9998. The detection limit of capsaicin and dihydrocapsaicin were 0.66 and 0.73 μg/mL, respectively. The peak areas were employed for quantification. The repeatability of the method was determined with a standard mixture solution at the level of 200 μg/mL for the analytes. The electropherograms obtained were shown in Fig. 4. The repeatability was expressed as the relative standard deviations (RSDs) (n = 4) of the migration times and peak areas obtained for four injections. The RSDs (n = 4) of the migration times and peak areas were 0.09–0.1, 1.8–2.1% (intra-day), and 0.73–0.78, 1.6–2.3% (inter-day), respectively.

3.8. Real samples

Quantification of the two alkaloids present in different Provinces of hot chilli peppers cultivated in China and products related to *C. anuum* were implemented by the present method under the optimum conditions. The peaks were identified by the standard addition methods. The accuracy of the methods and the potential matrix effects were established by analysing spiked samples. The typical electropherograms of the samples were shown in Fig. 5. The contents of the two alkaloids found in the sample solutions mentioned above together with their RSDs were given in Table 1. As can be seen, the RSDs (n = 3) for the samples were slightly higher than those for the standards. The recoveries of the method were determined with the standard addition method for capsaicin and dihydrocapsaicin in the extracts of *C. anuum* and porous capsicum plaster with the result of 90–107% for capsaicin, 92–109% for dihydrocapsaicin, respectively.

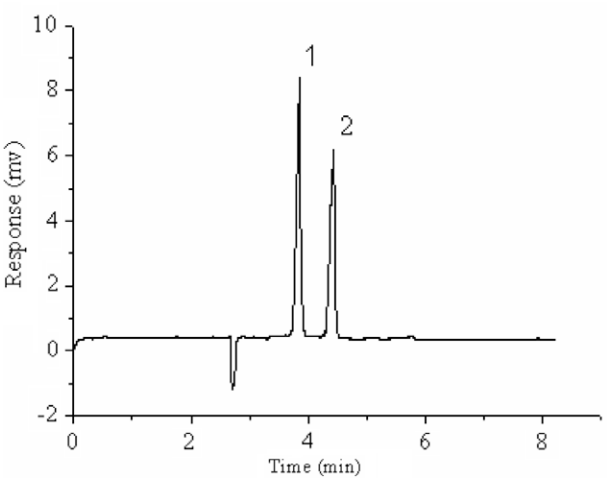

Fig. 4. Electropherogram of a standard solution of capsaicin and dihydrocapsaicin. Running buffer: 15 mM sodium tetraborate–2.2 mM SDS–0.05% Tween 20 (pH 10.1). Other conditions as in Fig. 2.

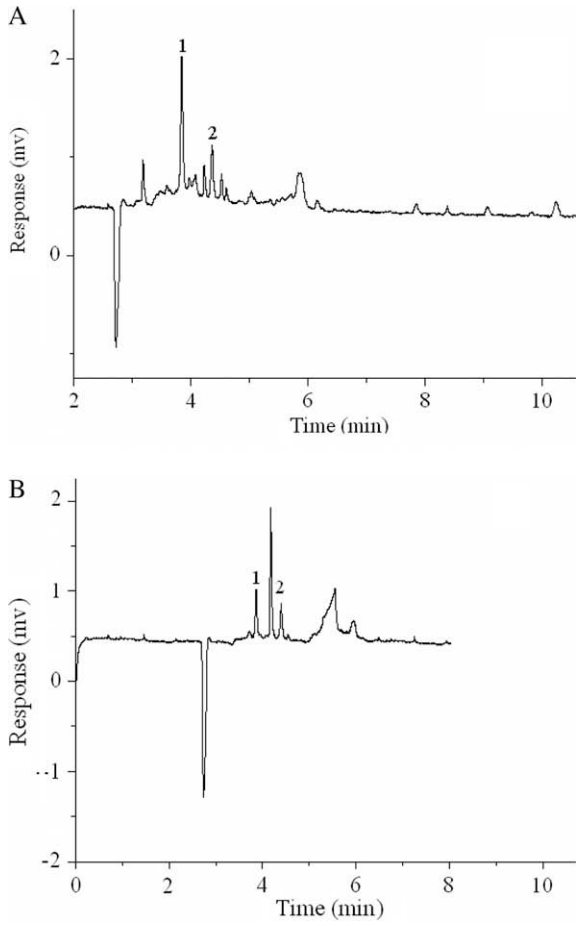

Fig. 5. Electropherograms of the real samples. (A) *Capsicum anuum* (Sichuan Province), (B) porous capsicum plaster, other peaks, unknown. Other conditions as in Fig. 4.

4. Conclusions

In this study, a CE method based a mixed surfactant system formed by SDS and Tween 20 as modifier in the buffer was reported and its application was discussed. The effective separation

of capsaicin and dihydrocapsaicin in *C. anuum*, pepper sauce and porous capsicum plaster were achieved by CE. The separation of capsaicin and dihydrocapsaicin could be achieved within 5 min. The developed method, for their rapidity, simple and the high selectivity, was very suitable for the quality control of plants and products containing capsaicin and dihydrocapsaicin.

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References

Archer, V. E., & Jones, D. W. (2002). Capsaicin pepper, cancer and ethnicity. *Medical Hypotheses, 59*(4), 450–457.

Baher, E., Fatemi, M. H., Konoz, F., & Golmohammadi, H. (2007). Prediction of retention factors in micellar electrokinetic chromatography from theoretically derived molecular descriptors. *Microchimica Acta, 158*, 117–122.

Barbero, G. F., Liazid, A., Palma, M., & Barroso, C. G. (2008). Fast determination of capsaicinoids from peppers by high-performance liquid chromatography using a reversed phase monolithic column. *Food Chemistry, 107*, 1276–1282.

Cisneros-Pineda, O., Torres-Tapia, L. W., Gutierrez-Pacheco, L. C., Contreras-Martin, F., Gonzalez-Estrada, T., & Peraza-Sanchez, S. R. (2007). Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico. *Food Chemistry, 104*, 1755–1760.

Esaka, Y., Sawamura, M., Murakami, H., & Uno, B. (2006). Surfactant gradient methods using mixed systems of cetyltrimethylammonium chloride and nonionic surfactants possessing polyoxyethylene chains for electrokinetic separation of benzoate anions as model analytes. *Analytical Chemistry, 78*, 8142–8149.

Esaka, Y., Tanaka, K., Uno, B., & Goto, M. (1997). Sodium dodecyl sulfate–Tween 20 mixed micellar electrokinetic chromatography for separation of hydrophobic cations: Application to adrenaline and its precursors. *Analytical Chemistry, 69*, 1332–1338.

Gamse, R., Wax, A., Zigmond, R. E., & Leeman, S. E. (1981). Immunoreactive substance P in sympathetic ganglia: Distribution and sensitivity towards capsaicin. *Neuroscience, 6*(3), 437–441.

Garces-Claver, A., Arnedo-Andres, M. S., Abadia, J., Gil-Ortega, R., & Alvarez-Fernandez, A. (2006). Determination of capsaicin and dihydrocapsaicin in Capsicum fruits by liquid chromatography-electrospray/time-of-flight mass spectrometry. *Journal of Agricultural Food and Chemistry, 54*, 9303–9311.

Hawer, W. S., Ha, J., Hwang, J., & Nam, Y. (1994). Effective separation and quantitative analysis of major heat principles in red pepper by capillary gas chromatography. *Food Chemistry, 49*, 99–103.

Kaale, E., Van Schepdael, A., Roets, E., & Hoogmartens, J. (2002). Determination of capsaicinoids in topical cream by liquid–liquid extraction and liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 30*, 1331–1337.

Kosuge, S., & Furuta, M. (1970). Studies on the pungent principle of Capsicum. Part XIV: Chemical constitution of the pungent principle. *Agricultural and Biological Chemistry, 34*, 248–256.

Kozukue, N., Han, J. S., Kozukue, E., Lee, S. J., Kim, J. A., Lee, K. R., et al. (2005). Analysis of eight capsaicinoids in peppers and pepper-containing foods by high-performance liquid chromatography and liquid chromatography–mass spectrometry. *Journal of Agricultural Food and Chemistry, 53*, 9172–9181.

Laskaridou-Monnerville, A. (1999). Determination of capsaicin and dihydrocapsaicin by micellar electrokinetic capillary chromatography and its application to various species of Capsicum, Solanaceae. *Journal of Chromatography A, 838*, 293–302.

Liu, L. H., Chen, X. G., & Hu, Z. D. (2007a). Separation and determination of alpinetin and cardamonin in Alpinia katsumadai Hayata by flow injection–micellar electrokinetic chromatography. *Talanta, 71*, 155–159.

Liu, L. H., Chen, X. G., & Hu, Z. D. (2007b). On-line concentration by head-column field amplified sample injection for the analysis of berberine, palmatine and jatrorrhizine in herbal medicine by flow injection–micellar electrokinetic chromatography. *Microchimica Acta, 159*, 125–131.

Schweiggert, U., Carle, R., & Schieber, A. (2006). Characterization of major and minor capsaicinoids and related compounds in chili pods (*Capsicum frutescens* L.) by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Analytica Chimica Acta, 557*, 236–244.

Thompson, R. Q., Phinney, K. W., Welch, M. J., & White, V. E. (2005). Quantitative determination of capsaicinoids by liquid chromatography–electrospray mass spectrometry. *Analytical and Bioanalytical Chemistry, 381*, 1441–1451.