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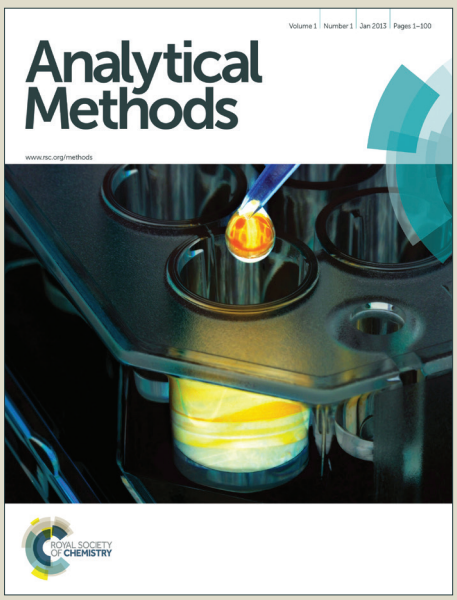

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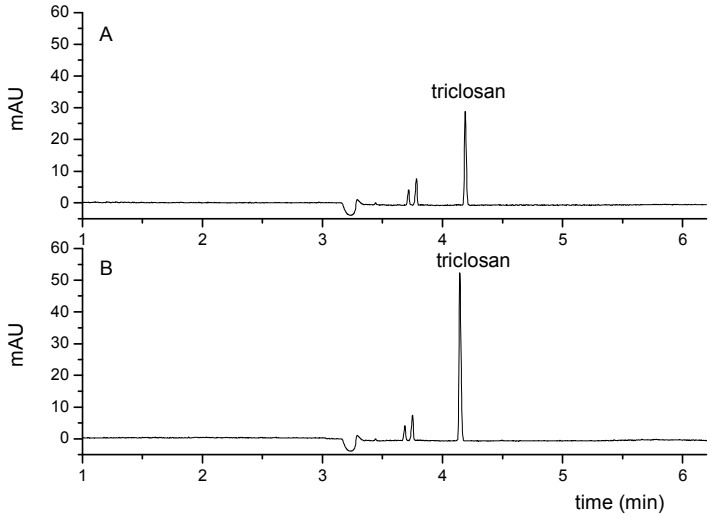

Application of a non-aqueous capillary electrophoresis method to the analysis of triclosan in personal care products

Application of a non-aqueous capillary electrophoresis method to the analysis of triclosan in personal care products

Hui Ma, Lijuan Wang, Huitao Liu*, Feng Luan,Yuan Gao*

College of Chemistry and Chemical Engineering, Yantai University, Yantai 264005, P. R. China.

* Corresponding author. Tel: +86-535-6902401; Fax: +86-535-6902401.
E-mail address: liuht-ytu@163.com, gaoyyt@163.com

ABSTRACT: A non-aqueous capillary electrophoresis (NACE) method was developed for analyzing triclosan in personal care products. Factors influencing NACE analysis were investigated. The non-aqueous running buffer was 60:40 (v/v) methanol-acetonitrile containing 1 mmol L^{-1} sodium tetraborate, with 9 s hydrodynamic injection, 25 kV applied voltage, and UV detection at 204 nm. The migration time of triclosan was about 4 min. Personal care product samples were extracted with methanol under ultrasonication for 20 min, with linear range of 0.25-50 μg mL^{-1} and detection limit at 0.075 μg mL^{-1}. The proposed NACE method obtained a low detection limit and showed satisfactory analytical performance.

Key Words: triclosan (TCS), non-aqueous capillary electrophoresis (NACE), personal care products

Introduction

Triclosan, (2,4,4′-trichloro-2′-hydroxy-diphenyl ether, TCS) is a broad-spectrum antimicrobial agent and bactericide. Because of its antimicrobial efficacy, it is widely used in personal health and skin care products, such as soaps, detergents, hand cleansers, cosmetics, toothpastes, etc.[1,2] However, TCS was accused for disrupting the endocrine system, for instance, thyroid hormone homeostasis and possibly the reproductive system.[3,4] The use of triclosan in personal care products is under close scrutiny.[5] Regulation (EC) clearly stipulates the total content of TCS should not exceed 0.3% (w/w) in cosmetic products.[6] And triclosan was listed as an priority substance in cosmetics by Norwegian Environment Agency.[7] Therefore, it is of great significance to develop simple and sensitive methods to determine triclosan.

Many methods have been reported for the determination of triclosan such as, HPLC,[8-11] GC, [12] GC-MS, [13] spectrophotometry,[14] electrochemical method,[15] and capillary zone electrophoresis (CZE) method[16-19] etc. Capillary electrophoresis (CE) as a powerful separation technique, offering high efficiency, sufficient selectivity and short analysis time, has greatly developed in these years. It is a versatile and efficient technique for the analysis of a large range of analytes in various sample matrices. Though CZE methods have been reported for the analysis of triclosan, [16-19] the sensitivity given by CZE can be improved further.

Non-aqueous capillary electrophoresis (NACE) was a branch of CE, it has many advantages for the analysis of compounds that are difficult to separate in aqueous buffers due to low solubility or lack of selectivity in aqueous buffers.[20] The

superiority of non-aqueous capillary electrophoresis is represented in excellent reviews. NACE is a field that continues to grow. Many areas of NACE including physicochemical properties of organic solvents, selectivity, theory and applications remain highly active areas of research.[21, 22]

In view of the hydrophobic property of triclosan, a non-aqueous capillary electrophoresis method was developed in this paper for the analysis of triclosan in personal care products. The developed NACE method showed superior analytical performance, including high sensitivity and fine selectivity. To the best of our knowledge, there is no literature reported using a NACE method for the determination of triclosan.

Experiments

Reagents, standards and samples

Triclosan (99.5%) was purchased from Dr.Ehrenstorfer GmbH (Germany); Acetonitrile and methanol were from Kemel Chemical Reagent Co., Ltd. (Tianjin, China). Sodium tetraborate was from Guangcheng Chemical Reagent Co., Ltd. (Tianjin, China). Personal care products were purchased from local supermarket and were stored at room temperature (25 °C). The organic solvents used in this experiment were of chromatographic grade. Purified water was used throughout.

Stock standard solution of triclosan (500 μg mL^{-1}) was prepared in methanol and stored under refrigeration (4 °C). Fresh calibration standard solutions were obtained by diluting stock solution with 50:50 (v/v) methanol-acetonitrile.

Instrumentation and conditions

All CE experiments were performed using a LUMEX CAPEL 105 Capillary Electrophoresis System (LUMEX Ltd., 19 Moskovsky Pr., St. Peterburg, 198005, Russia) equipped with a UV detector and a 1 - 25 kV high-voltage power supply. A fused-silica capillary (75 μm I.D.; effective length 50.5 cm; total length 60 cm; Yongnian Ruifeng Chromatographic Devices Limited Company) was used.

The new capillary was treated by flushing with purified water for 10 min, 0.5 mol L^{-1} NaOH for 40 min and purified water for 5 min. Capillary conditioning was done every morning by flushing with purified water for 3 min, 0.2 mol L^{-1} NaOH for 30 min, purified water for 3 min and with running buffer i.e. 60:40 (v/v) methanol-acetonitrile containing 1 mmol L^{-1} sodium tetraborate for 8 min. Between two injections, it was conditioned by running buffer for 5 min. This conditioning procedure led to a good reproducibility. All buffers and solutions were filtered through a 0.45 μm membrane filter.

Sample pretreatment

Personal care product samples (0.1000 g) were accurately weighed and treated with 8.0 mL methanol and sonicated for 20 min, following by centrifugation at 6000 rpm for 5 min. Then 2.0 mL of the supernatant was transferred into a 10 milliliter plastic capped tube and mixed with 2.0 mL acetonitrile. The solutions were filtered through a 0.45 μm membrane filter before analysis. The sample treatment method by methanol ultrasonic extraction is according to SN/T 1786-2006. [23]

Results and Discussion

Method development

To obtain fine NACE conditions for triclosan analysis, effects of detection wavelength, buffer composition, electrolyte concentration and injection solvent were taken into consideration.

Selection of wavelength. The determination was carried out with UV detector, therefore, evaluation of the best wavelength for triclosan was necessary to get high sensitivity. The analyte was dissolved in 50:50 (v/v) methanol-acetonitrile and running buffer, respectively, then UV absorption spectra from 200 to 400 nm were performed (Fig. 1). It can be observed that triclosan dissolved in 50:50 (v/v) methanol-acetonitrile and in running buffer has maximum absorption at 203 nm and 204 nm, respectively. Then the same triclosan standard solution was analyzed by CE with UV detection at 203 nm and 204 nm, respectively. The results indicated that the peak area of triclosan at 204 nm was about 10% larger than that at 203 nm. Thus, 204 nm was selected as the detection wavelength.

Electrolyte composition. Four different electrolytes including ammonium chloride, sodium tetraborate, sodium acetate and ammonium acetate, which are commonly used in non-aqueous capillary electrophoresis were tested. The electrolytes were dissolved in 50:50 (v/v) methanol-acetonitrile at a concentration of 10 mmol L^{-1} and were assayed by CE. No well separated peak can be seen in the electropherogram when ammonium chloride (Fig. 2, a) and ammonium acetate (Fig. 2, d) were used as electrolyte. A tiny and broad peak appeared when sodium acetate (Fig. 2, c) was used. When sodium tetraborate (Fig. 2, b) was employed as electrolyte, the peak shape of triclosan was good, showing it was the proper electrolyte. So, sodium tetraborate was

selected for further investigation.

Electrolyte concentration. Concentrations of sodium tetraborate in 50:50 (v/v) methanol-acetonitrile ranging from 0.5 to 10 mmol L^{-1} (0.5, 1, 2, 5, 10 mmol L^{-1}) were tested. Results are shown in Fig. 3. The electropherograms indicated that with the decreasing of electrolyte concentration from 10 to 0.5 mmol L^{-1}, the migration time of triclosan became shorter and the electric current became lower gradually. When the concentration decreased to 0.5 mmol L^{-1} (Fig. 3, a), although it gave shorter migration time, the reproducibility was not so good. And when real samples were analyzed at this concentration the analyte could not well separated from other components in the sample. Thus, 1 mmol L^{-1} (Fig. 3, b) sodium tetraborate was used in following analysis. The electric currents with different concentrations of sodium tetraborate (10, 5, 2, 1, 0.5 mmol L^{-1}) were 25.3, 14.8, 6.8, 3.6, 1.9 μA.

Organic solvent composition. Considering that organic solvent composition has a critical impact on migration time, sensitivity and peak shape,[21] different volume proportions of methanol and acetonitrile (v/v) varied from 40: 60, 50: 50, 60: 40 to 70: 30 were tested. Results are demonstrated in Fig. 4. We can see from Fig. 4 that when 60:40 (v/v) methanol-acetonitrile (Fig. 4, c) was used the peak shape and sensitivity of triclosan were the best with a short migration time of about 4 min. So 60:40 (v/v) methanol-acetonitrile was finally chosen for further testing.

Injection time, temperature, and applied Voltage. Appropriate injection time could help improving the sensitivity leading to a lower detection limit. Hydrodynamic injection times of 3s to 11s in steps of 2s at 30 mbar were investigated and the results

are given in Fig. 5. From 3s to 9s, peak area and height were visibly increased. When injection time was more than 9 s, peak height did not increase that much, yet the peak shape broadened. Considering the comprehensive aspects, injection time of 9 s was selected.

Temperature (in the range of 18-25 °C) and applied voltage (in the range of 18-25 kV) were tested. The results are shown in Fig. 6 and Fig. 7. We can see that at temperature of 25 °C and applied voltage of 25 kV short migration time and good analysis performance were obtained. Non-aqueous buffer has the advantage of low electric current and low Joule heat. Thus when the applied voltage was 25 kV, the corresponding electric current was just 3.6 μA with short migration time of about 4 min. Therefore, temperature of 25 °C and applied voltage of 25 kV was employed.

Injection solvent. In this paper, the solvents used in dissolving triclosan standard were investigated. We found that different injection solvents have vital effect on the sensitivity. Standard solutions at 5 μg mL^{-1} dissolved in methanol, acetonitrile, 50:50 (v/v) methanol-acetonitrile and running buffer were analyzed. Results are given in Fig. 8. It can be seen that when methanol was used as the solvent (Fig. 8, a), a leading peak appeared in electropherogram with the peak height about 14 mAU. When acetonitrile (Fig. 8, b) was used, the peak was symmetrical, and peak height was about 24 mAU. When 50:50 (v/v) methanol-acetonitrile (Fig. 8, c) was used, the peak is sharp and high, with the peak height of about 35 mAU, demonstrating a very good sensitivity. When running buffer (Fig. 8, d) was used as the solvent, the peak height was about 9 mAU and the peak was obviously broadened. There is no conductivity

difference between the injection solvent and the running buffer when using running buffer as the injection solvent, which can result in enrichment of the analyte. Perhaps that's why the peak was so broad. Therefore, 50:50 (v/v) methanol-acetonitrile was selected as the injection solvent.

From the results mentioned above, the NACE conditions are: 60:40 (v/v) methanol-acetonitrile containing 1 mmol L^{-1} sodium tetraborate, with 9 s hydrodynamic injection, 25 kV applied voltage, temperature of 25 °C and UV detection at 204 nm. The injection solvent was 50:50 (v/v) methanol-acetonitrile. Under these conditions, triclosan was determined within 5 min.

Validation of the method

Linearity and limit of detection (LOD). A five-point calibration curve was established between peak areas and the concentrations range of 0.25 - 50 μg mL^{-1} with correlation coefficient >0.9999. The linear equation was Y=7.59X-0.88. Each point of the calibration curve was the mean value from three independent injections (Fig. 9). The LOD based on signal to noise ratio (S/N) of 3 was 0.075 μg mL^{-1}.

Precision. Precision of the method was measured according to intra- and inter-day repeatability of the migration time and peak area.

The intra- and inter-day precision were obtained from five consecutive injections of 50:50 (v/v) acetonitrile - methanol extract of toothpaste carried out within the same day and on five different days. Intra-day RSD for migration time was 0.81% and for peak area was 1.56%. Inter-day RSD for migration time was 1.03% and for peak area was 2.17%.

Sample quantitative recoveries. Accuracy of the method was estimated by addition of triclosan standard to the samples. Two levels of triclosan standard were spiked in the samples before sample pretreatment. The amounts of triclosan were calculated from the regression equation of the calibration curve. Values obtained were listed in Table 1. Typical electropherograms of different samples and spiked samples are shown in Fig. 10.

Comparison with aqueous CE method

Compared with aqueous the CZE-UV method [17], the proposed NACE method has advantages of shorter analysis time and lower detection limit. The LOD of NACE was 0.075 μg mL^{-1} lower than that reported by the aqueous CE method [17] of 1.2 μg mL^{-1}.

Conclusion

A simple, rapid and sensitive NACE method has been developed. The comprehensive analysis performance is satisfactory. The results demonstrated that different injection solvents greatly affect the sensitivity of the NACE method. Compared with the aqueous CE method, the proposed NACE method provides shorter analysis time and lower detection limit.The method can be potentially applied to the routine analysis of triclosan in personal care products.

Acknowledgements

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Table 1 Determination results and recoveries of triclosan in samples

Sample	Contents ($mg\ g^{-1}$)	Recoveries				
		Original ($\mu g\ mL^{-1}$)	Added ($\mu g\ mL^{-1}$)	Recovery (%)	Average recovery (%)	RSD (%)
Toothpaste	0.67±0.01	4.48	2.0	93.2	94.2	1.57
			4.0	95.2		
Lotion	1.98±0.04	12.9	4.0	95.5	96.9	2.09
			8.0	98.3		
Facial cleanser	n.d.[a]	n.d.	2.0	95.4	97.7	3.29
			4.0	99.9		

[a] not detected

Figure captions

Fig. 1. UV spectra of triclosan. a: dissolved in running buffer and b: dissolved in 50:50 (v/v) methanol-acetonitrile.

Fig. 2. Electropherograms of triclosan with different electrolyte composition. a: ammonium chloride, b: sodium tetraborate, c: sodium acetate and d: ammonium acetate dissolved in 50:50 (v/v) methanol-acetonitrile with a concentration of 10 mmol L^{-1}. Other NACE conditions: 3 s hydrodynamic injection, 25 kV applied voltage, 25 ℃, UV detection at 204 nm, injection solvent: 50 to 50 (v/v) methanol-acetonitrile.

Fig. 3. Electropherograms of triclosan with different concentrations of sodium tetraborate. a: 0.5 mM; b: 1 mM; c: 2 mM; d: 5 mM; e: 10 mM.

Fig. 4. Electropherograms of triclosan with different compositions of organic solvent. The volume proportion of methanol and acetonitrile was a: 4 to 6, b: 5 to 5, c: 6 to 4, d: 7 to 3.

Fig. 5. Electropherograms of triclosan with different injection time. a: 3 s, b: 5 s, c: 7 s, d: 9 s and e: 11 s.

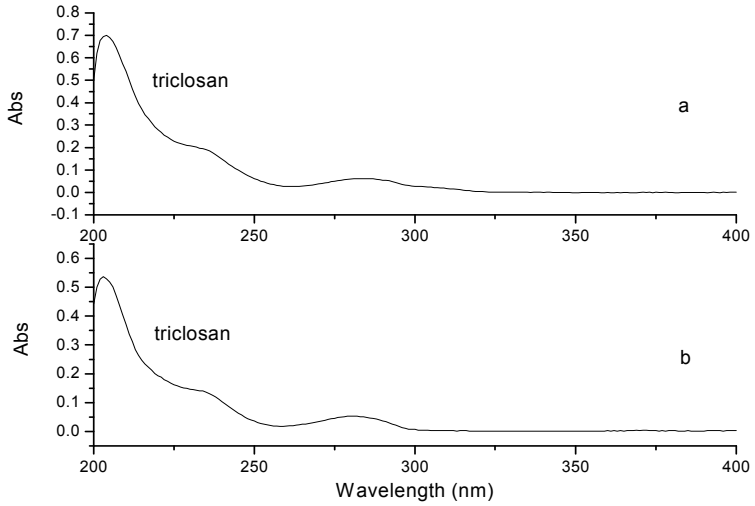
Fig. 6. Electropherograms of triclosan at different temperature. a: 18 ℃, b: 22 °C and c: 25 ℃.

Fig. 7. Electropherograms of triclosan with different applied voltage. a: 18 kV, b: 22 kV and c: 25 kV.

Fig. 8. Electropherograms of triclosan standards at 5 μg mL^{-1} dissolved in a: methanol, b: acetonitrile, c: 50:50(v/v) methanol-acetonitrile and d: running buffer.

Fig. 9. Calibration curve of triclosan. Error bars were represented by SD of peak area.

Fig. 10. Electropherograms of samples. a: toothpaste sample; b: toothpaste sample spiked with 4 μg mL^{-1} triclosan standard; c: lotion sample; d: lotion sample spiked with 4 μg mL^{-1} triclosan standard; e: facial cleanser sample; f: facial cleanser sample spiked with 4 μg mL^{-1} triclosan standard. NACE conditions: 60 to 40 (v/v) methanol-acetonitrile containing 1 mmol L^{-1} sodium tetraborate, 9 s hydrodynamic injection, 25 kV applied voltage, 25 °C, UV detection at 204 nm, injection solvent : 50 to 50 (v/v) methanol-acetonitrile.



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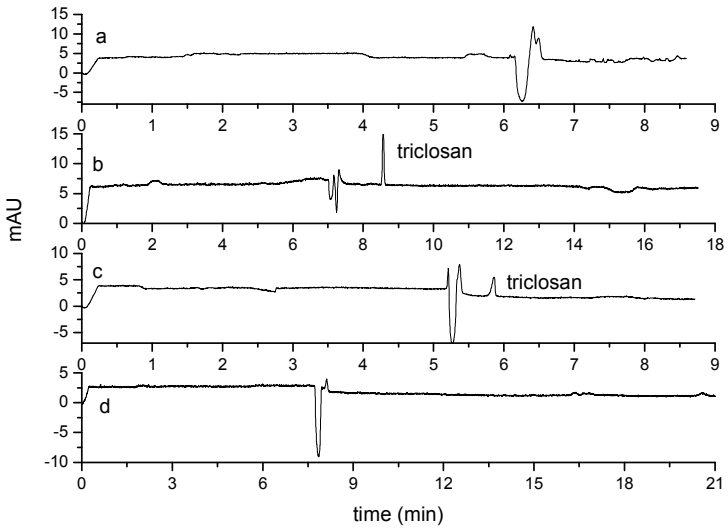

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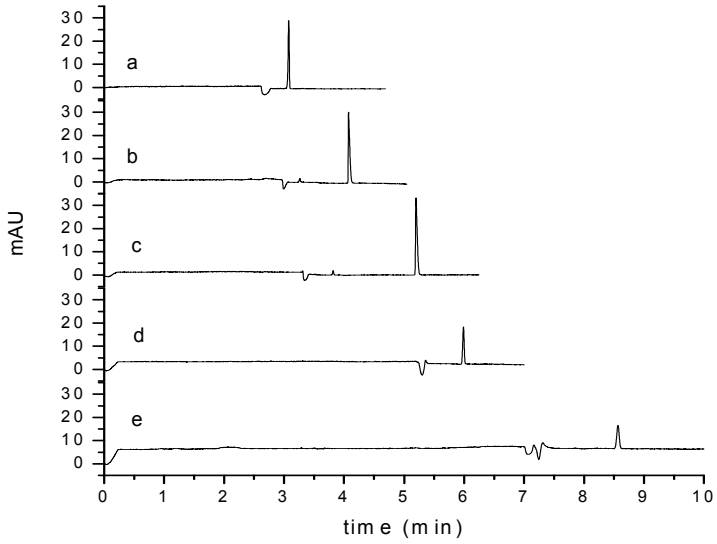

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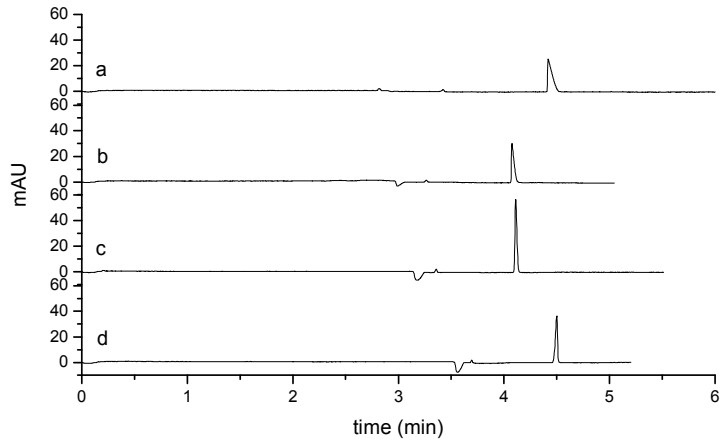

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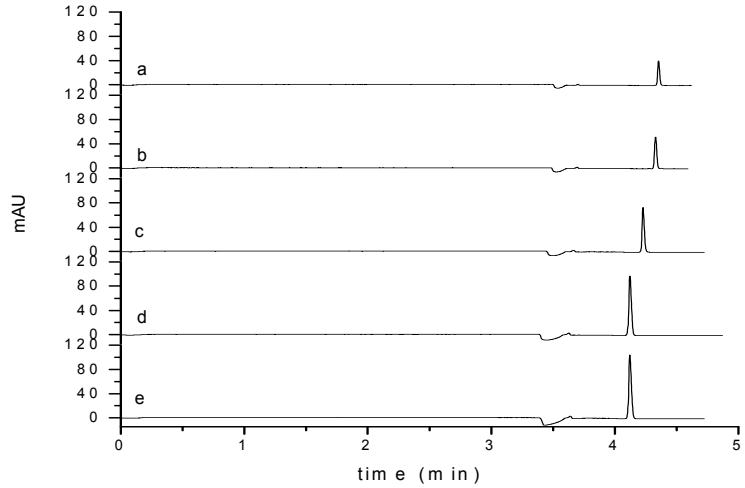

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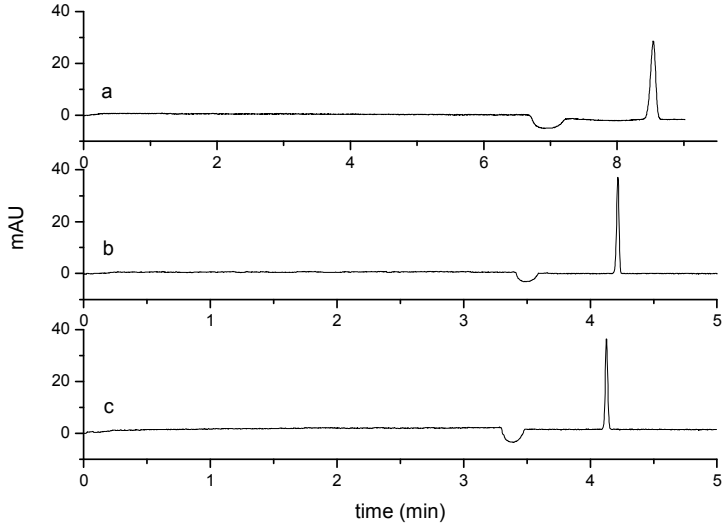

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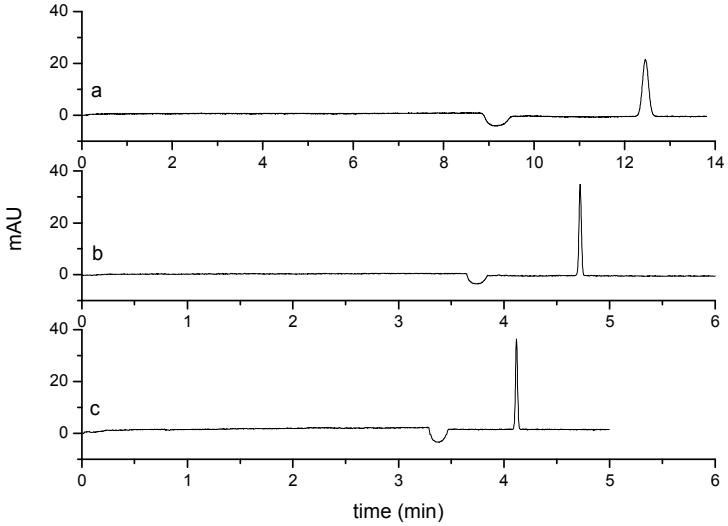


Fig. 7. Electropherograms of triclosan with different applied voltage. a: 18 kV, b: 22 kV and c: 25 kV.

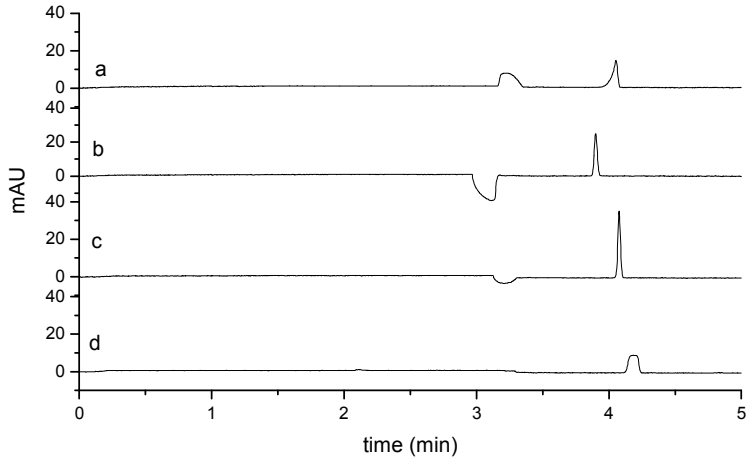
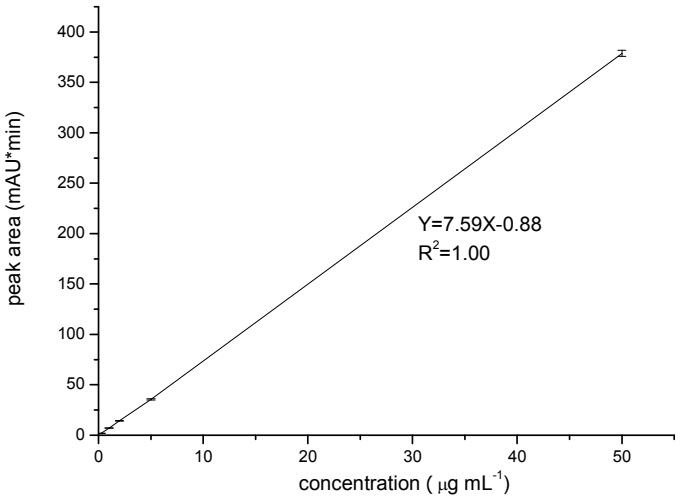


Fig. 8. Electropherograms of triclosan standards at 5 μg mL^{-1} dissolved in a: methanol, b: acetonitrile, c: 50:50(v/v) methanol-acetonitrile and d: running buffer.



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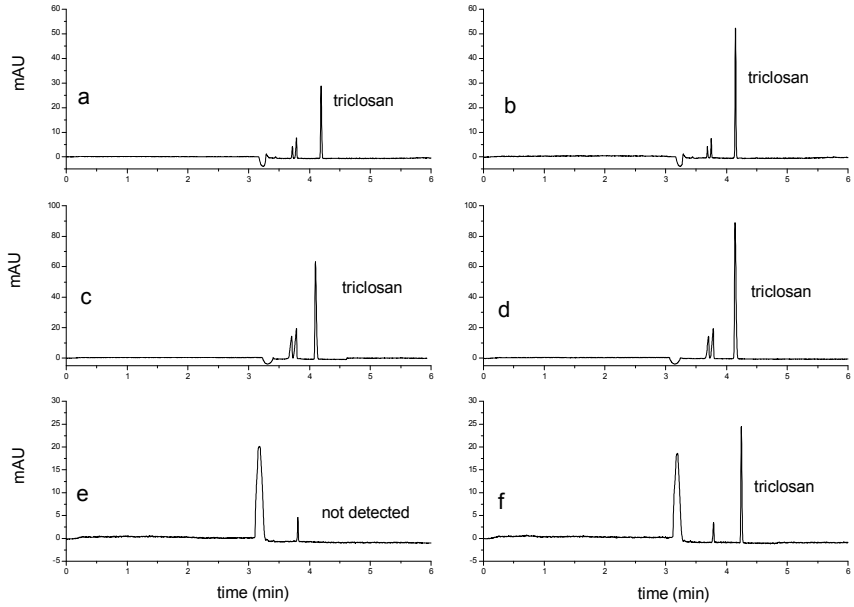

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