

Fluorescence labeled capillary electrophoresis fingerprint analysis of sulfonamides residues in tea garden soil and tea

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Abstract. A fluorescence labeled capillary electrophoresis fingerprint method for the analysis of sulfonamides residues in tea garden soil and tea was established by using o-phthalaldehyde (OPA) as pre-column derivatization reagent. The effects of background electrolyte concentration, pH, column temperature and voltage on the separation conditions were investigated. The optimum separation conditions were as follows: Glycine sodium hydroxide slow concentration: 20 mmol/L; pH: 9.0; Column temperature: 20 °C; Separation voltage: 17 kv, pressure: 50 mbar, injection time: 8 s. Under the established optimal conditions, 13 sulfonamide derivatives could be separated efficiently within 9 min, and the linear range is 0.35~100µg/kg, the detection limit (signal-to-noise ratio is 3) is in the range of 0.12-0.25 µg/kg, the quantitative limit (signal-to-noise ratio is 10) is in the range of 0.35-0.70µg/kg.

Keywords; Derivatization; Phthalaldehyde; Sulfa; Soil; Tea; Capillary electrophoresis

1. Introduction

Tea is an important cash crop in the world. Organic fertilizer fermented by animal feces is usually applied during tea planting. The sulfonamide drugs used to treatment of animal bacterial diseases were mainly excreted with the animal feces and urine [1]. Therefore, the use of organic fertilizer will lead to the increase of sulfonamide content in soil environment [2, 3] and finally enriched by tea and the human body through the food chain [4], which causing adverse reactions such as human allergic reaction, hemolytic anemia and cancer [5, 6]. There are many methods for the detection of multi-component residues of sulfonamides, including microbial detection method [7], enzyme-linked immunosorbent assay [8], gas chromatography-mass spectrometry [9], high performance liquid chromatography tandem mass spectrometry [10] and capillary electrophoresis [11]. However, the microbial detection method has the disadvantages of poor stability and low sensitivity, which cannot meet the requirements of the detection of trace residues [12]. Enzyme linked immunosorbent assay is mainly used in qualitative tests, and may produce false positive or false negative results. Gas chromatography-mass spectrometry has high specificity and sensitivity, but the high polarity and low volatility of sulfonamides limited the application of gas chromatography. High performance liquid chromatography-tandem mass spectrometry [13] has a high sensitivity for the detection of sulfonamides, with a detection limit of about 0.5 µg/kg. It is the most accurate method for the detection of

sulfonamides residues, but expensive equipment and difficult operation limit the application of this method.

Capillary electrophoresis is a new liquid phase analysis technology with the characteristics of fast analysis, high separation efficiency and environmental friendliness [14, 15]. The operating system is mainly water and inorganic salt solution, and the sample consumption is in the microliter level. However, there are few studies on the detection of sulfonamides residues in tea and its soil environment, and there are no reports on the detection of sulfonamides residues by pre-column derivatization capillary electrophoresis.

In this study, o-phthalaldehyde (OPA) was used as the pre column derivatization reagent to establish the fluorescence labeled capillary electrophoresis fingerprint analysis method of sulfonamides in tea and tea garden soil. The detection limit nearly reached the detection limit level of high performance liquid chromatography-mass spectrometry, and the detection time was short. The concentration, pH, operating temperature, voltage and other conditions of the buffer solution were optimized, and the sulfonamides standard solution and the actual samples of tea garden soil and tea were analyzed and determined.

2. Material and method

2.1 Instruments and chemicals

The Capillary electrophoresis instrument was purchased from Beckman (USA). Capillary with a dimension of 50 µm I.D. (365 µm O.D.) and a total length of 58.5 cm

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(effective length of 50 cm) was purchased from Xinnuo Optical (China). 2475 fluorescence detector was purchased from Waters (USA). 5810 high speed centrifuge was purchased from Eppendorf (Germany). Sulfaxazole (SMO), sulfamethylpyrimidine (SMR), sulfaisoxazole (SFZ), sulfaclopyridazine (SCP), sulfadimethylpyrimidine (SIM), sulfamethoxy pyridazine (SMP), sulfamethoxypyrimidine (SMM), sulfamethoxypyrimidine (SMD), Sulfapyridine (SPD), sulfadiazine (SD), sulfathiazole (ST), sulfadimethylpyrimidine (SDM), sulfamethothiadiazole (SMT), O-phthalaldehyde (OPA) (purity $\geq 99.0\%$), were purchased from Sigma (USA). Acetonitrile, dichloromethane, chloroform, methanol, n-hexane, acetone, phosphoric acid, acetic acid, boric acid, glycine, sodium hydroxide (analytical-reagent grade) were purchased from Beijing Chemical Factory (China).

2.2 Sample collection

Soil samples with organic fertilizer, including chicken manure soil, pig manure soil, cow manure soil, and 3 control soil samples without organic fertilizer were collected from tea gardens in Meitan County, Guizhou Province, China, and named as S1, S2, S3, Sc1, Sc2 and Sc3, respectively. The organic fertilizer used in this study has been stored and fermented for more than 1 year and has been applied to the soil for more than half a year. All soil samples were collected 3-5 cm below the surface. Tea leaves were collected from tea trees where the soil was collected and named as T1, T2, T3, Tc1, Tc2 and Tc3, respectively.

2.3 Standard solution preparation and derivatization

Individual standard stock solution (10 mg L⁻¹): 10 mg of each sulfonamide was accurately weighed, dissolved in 1000 mL of methanol under ultrasound, and stored in a brown bottle at 4 °C. Mixed standard solution (100 µg/L): 1 mL of each individual standard stock solution was mixed and diluted to 100 mL with methanol in brown volumetric flask. Phthalaldehyde (OPA) solution (5 mg/L): accurately weigh 5.0 mg of OPA reagent and dissolve it in 1000 mL of methanol solution. Britton-Robinson (B-R) buffer solution: A certain volume of 2 mol/L NaOH was added to 0.4 mol/L triacid mixture (phosphoric acid, acetic acid, boric acid), and adjusted the pH to 1.5.

The derivatization is based on the Tang et al and Yu et al methods with some modifications [16, 17], briefly 100 µL of the mixed standard solution was added in a colorimetric tube, then 100 µL of B-R buffer solution and 600 µL of OPA solution were added, the mixture was reacted in a water bath at 80 °C for 20 min and cooled to room temperature. The corresponding low-concentration mixed standard derivative was diluted from it.

2.4 Sample pretreatment and derivatization

The extraction method of sulfonamides in soil samples was as follows: soil samples were dried naturally and ground. Then 3.0 g of soil powder was dipped in 30 mL

of 1.0 % formic acid-methanol (7 : 3, V/V) and followed by ultrasonic extraction for 30 min. After centrifugation for 30min at 3000 r/min, the supernatant was collected and filtered through 0.45 µm syringe filter.

The extraction method of sulfonamides in tea samples was as follows: The freeze-dried tea sample was ground and 0.5 g of tea powder was added with 25ml methanol and followed by ultrasonic extraction for 30 min. Sample was centrifuged at 3000 rpm for 10 min. The supernatant was transferred into a test tube. The obtained pellet was extracted again with 25 mL methanol. The supernatants were combined and evaporated under a gentle stream of nitrogen. Finally, the dry residue was dissolved in 5 mL of 0.1 mol/L hydrochloric acid solution and filtered through 0.45 µm syringe filter. The soil and tea sulfonamides extract samples were derivatized according to the above method.

2.5 Instrument operation

Detection conditions: fluorescence detector wave length $\lambda_{ex}/\lambda_{em}$: 295 nm/ 420 nm, pressure: 50 mbar, injection time: 8 s. The buffer solution was ultrasonically degassed for 5 min before the experiment. Before each sample injection, the capillary column was rinsed with 0.1 mol/L NaOH solution, ultrapure water, and buffer for 5 min. The flushing time was extended to 10 min when the buffer was changed. The influences of pH, buffer solution concentration, operating temperature and voltage on the separation effect were detected.

3. Results and discussion

3.1 Optimization of separation conditions

3.1.1 The influence of glycine sodium hydroxide concentration on separation

Buffer system consists of buffer reagent and pH regulator. The buffer concentration affects the electroosmotic flow and the electrophoresis behaviour of the sample, which determines the separation efficiency, selectivity, and analysis time of capillary electrophoresis [18]. Therefore, the effect of the glycine sodium hydroxide concentration on the separation parameters in the range of 16-24 mmol/L was detected. Seven sulfonamide derivatives examples were detected under the condition of buffer pH 9.0. As shown in Figure 1 (A and B), the separation degree increased with the increase of buffer concentration. However, when the concentration was increased to 20 mmol/L, the increasing trend of separation degree slowed down with the increase of concentration, and the separation time was obviously delayed. Considering the separation efficiency and separation time, 20 mmol/L was selected as the buffer concentration.

3.1.2 Effect of pH on separation

The pH of buffer system is also an important factor to determine the separation effect [19]. The pH of the buffer

solution determines the effective mobility of the sample and controls the size and direction of electroosmotic flow. In the present study, the effect of pH on the separation was tested in the range of 8.8-9.2 under the condition of buffer solution was fixed at 20 mmol/L. The effect of pH on the separation effect is particularly prominent in this experiment, as shown in Figure 1 (C, D). When the pH is lower than or equal to 8.9, 7 sulfonamide derivatives cannot achieve baseline separation, especially in the separation of SMO and SD; When the pH increased to 9.0, the baseline separation of 7 sulfonamide derivatives was achieved within 8.7 minutes; When the pH is higher than 9.0, the separation time is obviously delayed and the peak shape is poor; Therefore, pH =9.0 was selected as the separation condition.

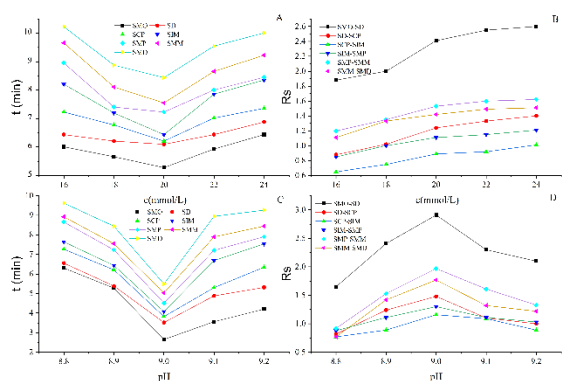


Fig.1. Effect of buffer concentration and pH on separation time and separation degree. (A) Buffer concentration on separation time; (B) Buffer concentration on separation degree; (C) pH on separation time; (D) pH on separation degree .

3.1.3 Influence of voltage and temperature on separation

The separation voltage determines the electric field intensity, and affects the migration rate of the samples [20]. Although a higher separation voltage will increase the migration rate and decrease the analysis time, higher voltage results in increased Joule heat, decreased baseline stability and sensitivity of electrophoresis, which ultimately reduced separation efficiency. In this study, the separation degree between the components became worse when the separation voltage exceeds 17 kV (data not shown), and 17 kV was selected as the separation voltage. Temperature affects the reproducibility and efficiency of separation [21]. With the increase of temperature, the viscosity of buffer decreases, the migration speed increases, and the analysis time decreases. However, with the increase of temperature, the joule heating effect increases and the column effect decreases, leading to the decrease of separation efficiency. In this study, the increase of temperature between 10°C and 30°C has a positive effect on the separation of components. However, in order to prevent the large joule heat generated by high temperature, 20 °C was selected as the separation temperature.

According to the above experimental results, the optimum capillary electrophoresis conditions were set as follows, glycine sodium hydroxide buffer solution concentration:

20 mmol/L; pH: 9.0; Column temperature: 20 °C; Separation voltage: 17 kV; Pressure: 50 mbar, injection time: 8 s.

3.2 Method establishment and verification

According to the above determination methods, 13 sulfonamide derivatives could achieve rapid baseline separation within 9 min, and the separation effect is good (Fig. 2).

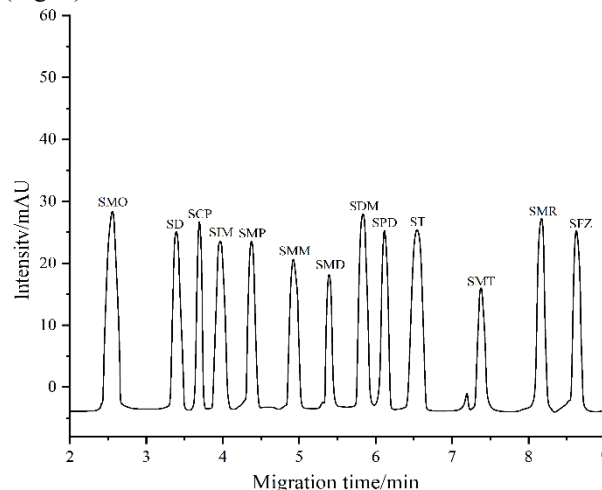


Fig. 2. Capillary electrophoresis spectrogram of standard samples (20 µg / kg).

Separation conditions: Glycine sodium hydroxide buffer solution concentration: 20 mmol / L; pH: 9.0; column temperature: 20 °C; separation voltage: 17 kV; pressure: 50 mbar; injection time: 8 s

A series of concentration samples of 13 kinds of sulfonamide derivatives were prepared and detected, and the linear regression was carried out according to the peak area, and the linear regression equation and correlation coefficient were obtained. All of the 13 sulfonamide derivatives had good linear relationship in the range of 0.35-100 µg/kg. The sulfonamide standards were added to the blank sample to check the sensitivity. The detection limit of the method was set as the mass concentration corresponding to 3 times signal-to-noise ratio ($S/N \geq 3$), and the quantitative limit of the method was set as the mass concentration corresponding to 10 times signal-to-noise ratio ($S/N \geq 10$). The investigation experiment of migration time and peak area reproducibility is realized by repeating the sample injection analysis of mixed sulfonamide standard solution for 5 times under the above optimization conditions, and the relative standard deviation of migration time is between 0.68% - 1.22%, The relative standard deviation of peak area is between 0.70% - 1.38%. It could be seen in Table 1 that the method has good linear relationship and high sensitivity.

Table 1. Linear equation, correlation coefficient, linear range, limit of detection, limit of quantitation, relative standard deviation of migration time and relative standard deviation of peak area of sulfonamide derivatives (n=5)

SA S	Linear regression equation	Correlation coefficient	Linear range	L O D	L O Q	Migration time RSD (%)	Peak area RSD (%)
S M O	Y=1.56+7.88x	0.9932	0.7-100	0.25	0.70	0.86	1.02
SD	Y=9.66+8.44x	0.9866	0.7-100	0.25	0.70	0.99	1.13
SC P	Y=11.23+1.27x	0.9912	0.3-100	0.12	0.35	1.01	0.98
SI M	Y=9.66+3.21x	0.9854	0.7-100	0.25	0.70	1.22	0.87
S M P	Y=8.38+10.96x	0.9910	0.7-100	0.25	0.70	0.87	0.70
S M M	Y=3.65+8.10x	0.9892	0.7-100	0.25	0.70	0.98	1.10
S M D	Y=10.22+7.62x	0.9991	0.7-100	0.25	0.70	1.12	1.38
SD M	Y=8.55+4.21x	0.9990	0.7-100	0.25	0.70	1.07	0.84
SP D	Y=8.91+7.88x	0.9991	0.3-100	0.12	0.35	0.85	0.82
ST	Y=6.21+10.68x	0.9993	0.3-100	0.12	0.35	0.68	0.78
S M T	Y=7.62+4.54x	0.9989	0.3-100	0.12	0.35	1.03	1.02
S M R	Y=8.55+3.20x	0.9971	0.3-100	0.12	0.35	1.11	1.13
SF Z	Y=10.11+5.99x	0.9923	0.3-100	0.12	0.35	1.07	0.99

The recovery and precision of the method were tested by adding standard sulfonamides solution to blank tea sample, and 6 parallels were detected for each concentration. As shown in Table 2, the average recoveries ranged from 94.0% ~ 103.8% with the relative standard deviations of 1.07% ~ 2.64% within the concentration of 10-40 µg/kg. The results indicated that the method was sufficiently stable for the simultaneous determination of sulfonamide derivatives.

Table 2. Recoveries and relative standard deviations of 13 Sulfonamides in tea (n = 6).

SA S	Add ed (µg/kg)	Recov ery (%)	RS D (%)	SA S	Add ed (µg/kg)	Recov ery (%)	RS D (%)
	10.0	98.4	1.33		10.0	97.2	1.47
SM O	20.0	102.3	1.55	SD M	20.0	103.3	1.56
	40.0	96.3	1.42		40.0	94.6	2.56
	10.0	95.0	2.64		10.0	94.9	1.36
SD	20.0	95.6	1.11	SP D	20.0	94.8	1.20
	40.0	103.0	1.85		40.0	95.2	1.36
	10.0	94.1	1.45		10.0	96.3	1.33
SC P	20.0	95.3	1.46	ST	20.0	99.8	1.61
	40.0	98.6	1.51		40.0	95.6	1.23
	10.0	95.1	1.16		10.0	94.8	1.09
SI M	20.0	94.5	2.01	SM T	20.0	95.8	2.36
	40.0	97.7	1.44		40.0	102.3	1.12
	10.0	94.6	2.29		10.0	95.4	1.48
SM P	20.0	97.2	1.57	SM R	20.0	95.8	1.22
	40.0	101.8	1.41		40.0	97.3	2.55
	10.0	94.0	1.21		10.0	95.1	1.07
SM M	20.0	99.3	1.23	SF Z	20.0	95.2	1.33
	40.0	96.5	1.32		40.0	103.8	1.21
	10.0	96.5	1.46				
SM D	20.0	96.1	1.36				
	40.0	95.8	2.31				

3.3 Method application

This method was applied to detect sulfonamides in the soil without and without organic fertilizer in a tea garden in Meitan County, Guizhou Province and the tea picked in the corresponding soil environment. Among them, 5 and 3 kinds of sulfonamide drugs were detected in the two soil samples with chicken manure and pig manure organic fertilizer, respectively. None sulfonamide drug was detected in the soil with cow manure and the soil without organic fertilizer. Three kinds and two kinds of

sulfonamide drug residues were detected in the tea samples produced by applying chicken manure and pig manure organic fertilizer, respectively, and no sulfonamides drug residues were detected in the tea produced by other soils.

Table 3. Detection of sulfonamides in soils and teas ($\mu\text{g} / \text{kg}$).

S	S	S	S	S	S	S	T	T	T	T	T	T
A	1	2	3	c	c	c	1	2	3	c	c	c
S	1											
S	1											
M	2.	N	N	N	N	N	N	N	N	N	N	N
O	2	D	D	D	D	D	D	D	D	D	D	D
	2											
S	1	1					5	4				
D	3.	3.	N	N	N	N	.	.	N	N	N	N
	6	4	D	D	D	D	6	8	D	D	D	D
	9	7					3	3				
S	N	N	N	N	N	N	N	N	N	N	N	N
C	D	D	D	D	D	D	D	D	D	D	D	D
P	D	D	D	D	D	D	D	D	D	D	D	D
SI	N	N	N	N	N	N	N	N	N	N	N	N
M	D	D	D	D	D	D	D	D	D	D	D	D
	1	1					4	6				
S	8.	7.	N	N	N	N	.	.	N	N	N	N
M	4	4	D	D	D	D	3	7	D	D	D	D
P	5	1					3	5				
S	N	N	N	N	N	N	N	N	N	N	N	N
M	D	D	D	D	D	D	D	D	D	D	D	D
M	D	D	D	D	D	D	D	D	D	D	D	D
S	N	N	N	N	N	N	N	N	N	N	N	N
M	D	D	D	D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D	D	D	D	D
	1											
S	7.	N	N	N	N	N	N	N	N	N	N	N
D	3	D	D	D	D	D	D	D	D	D	D	D
M	9											
S	N	N	N	N	N	N	N	N	N	N	N	N
P	D	D	D	D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D	D	D	D	D
S	N	N	N	N	N	N	N	N	N	N	N	N
T	D	D	D	D	D	D	D	D	D	D	D	D
S	N	N	N	N	N	N	N	N	N	N	N	N
M	D	D	D	D	D	D	D	D	D	D	D	D
T	D	D	D	D	D	D	D	D	D	D	D	D
	1	1					4					
S	3.	7.	N	N	N	N	.	N	N	N	N	N
M	8	5	D	D	D	D	5	D	D	D	D	D
R	4	5					0					
S	N	N	N	N	N	N	N	N	N	N	N	N
F	D	D	D	D	D	D	D	D	D	D	D	D
Z	D	D	D	D	D	D	D	D	D	D	D	D

(Note: nd means the content is lower than the detection limit)

4. Conclusion

A fluorescence labeled capillary electrophoresis fingerprint method for the analysis of sulfonamide residues in tea garden soil and tea was established by

using o-phthalaldehyde (OPA) as pre column derivatization reagent. The buffer solution concentration, pH, operating temperature, voltage and other conditions were optimized. At the optimal separation conditions, 13 sulfonamide derivatives could achieve rapid baseline separation within 9 min, and the method has good linear relationship and high sensitivity. The actual samples of soil and tea were determined under the optimal conditions. Three soil samples applied with organic fertilizers and three control soil samples, as well as corresponding tea samples, were tested in this method, and no sulfonamide residue was detected in unfertilized soil and corresponding tea leaves. However, 5 residues were detected in soil samples applied with organic fertilizers. The organic fertilizers were stored and fermented for more than 1 year before use, these results indicated that sulfonamides sulfa drugs degrade slowly in organic fertilizers and will enter the soil. More significantly, tea produced from soil treated with organic fertilizer also contained low levels of sulfa residues. These results indicated that sulfonamides could be absorbed and accumulated by tea and finally enter the human body through the food chain. The detection limit of this method nearly reaches the detection level of high performance liquid chromatography-mass spectrometry. At the same time, it eliminates the disadvantages of liquid chromatography-mass spectrometry, such as using a large number of organic solvents and complex gradient elution operation.

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