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Detection of multiple anions by thin-layer chromatography

Noriyuki Kato[a,*], Masataka Sakayanagi[a], Takahiro Nakayama[a], Hideo Nishimura[a], Akira Ogamo[b]

[a]*Scientific Criminal Investigation Laboratory, Kanagawa Prefectural Police Headquarters, 155-1 Yamashita-cho, Naka-ku, Yokohama 231-0023, Japan*

[b]*School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*

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Abstract

A novel detection method for 21 different anions by thin-layer chromatography is presented. Anions on the target plate form salts with amine in a developing solvent and are visualized after staining with citric acid–acetic anhydride reagent as white spots contrasting against a pale red-pink background. This method has particularly high sensitivity for anions of chlorate, sulfate, phosphate, chromate and dichromate (0.02–0.05 μg). The method is demonstrated to efficiently detect toxic arsenite in curry sauce as an example application. The proposed method offers highly efficient indirect detection for a wide range of anions, and serves as a purification procedure for the preparation of anionic sample solutions for other analytical methods.

Keywords: Mobile phase composition; Forensic analysis; Inorganic anions

1. Introduction

Anions are present in many substances, and in many cases are useful markers of substances used in crime. A notable case in Japan, involving poisoning by the arsenite component of white-ant insecticide, has been studied extensively, and the existence of a simple method of rapidly identifying anion markers of such poisons would be of immense benefit to such forensic investigations. Capillary electrophoresis (CE) and ion chromatography (IC) are the primary methods employed in forensic science to analyze many anions in one analysis [1–4]. These methods can give good results, however the instruments and reagents are very expensive and sample pretreatment is often complex and dominates the sensitivity of detection.

Thin-layer chromatography (TLC) has received relatively little attention compared with instrumental analysis, even though the technique is a simple and basic analytical procedure and is used for widely applicable molecule separation. Furthermore, TLC requires very little pretreatment and the costs of essential materials such as TLC plate, developing solvents and color reagents are relatively inexpensive compared with those of instrumental analysis. Therefore, a TLC method is a strong candidate for the initial screening stage in forensic science. The present authors investigated a TLC method that would allow the analysis of a wide range of anions in one analysis. Although the use of TLC has been reported

*Corresponding author. Tel.: +81-45-662-0395; fax: +81-45-662-0395.

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for the detection of one or a few ions [5–13], the technique has yet to be adapted to the analysis of a wide spectrum of analyte ions. In addition, these previous methods involved the use of expensive and/or special materials and reagents, and complex procedures and pretreatment of the sample and/or TLC plate.

The present authors have already reported a TLC method for the detection of tertiary amines in forensic applications as a red-purple material by staining with citric acid–acetic anhydride reagent (CAR) [14]. In the application of this method, we observed white spots corresponding to the reaction products (salt) formed by ammonium ions in the developing solvent and chloride ions in the tertiary amine hydrochloride on the plate. This phenomenon of white marking is the process exploited for the indirect anion detection method proposed here. Such a method requires only inexpensive materials and reagents, and the procedure is relatively simple. In this study we refined the technique, and examined its utility with respect to the detection of 24 different anion species, both inorganic and organic.

2. Experimental

2.1. Materials and reagents

Twenty-four species of anions were tested, as listed in Table 1. Aqueous solutions of 1 and 10 mg/mL were prepared from reagent-grade chemicals

Table 1
R_F values of 24 kinds of anions and solvent systems using TLC

No.	Sample	Solvent system[a]					
		DS1(S)[b]	DS2(S)	DS3(S)	DS4(S)	DS5(S)	DS6(R)
AN-1	Potassium perchlorate	0.81	0.96	0.59	0.93	0.75	0.80
AN-2	Sodium chlorate	0.78	0.91	0.35	0.90	0.69	0.51
AN-3	Sodium hypochlorite	0.78; 0.69	0.89; 0.80	0.32; 0.05	0.89; 0.85	0.68; 0.58	0.52; 0.20
AN-4	Potassium nitrate	0.77	0.90	0.28*t*	0.87	0.66	0.50
AN-5	Sodium nitrite	(0.72)[c]	(0.85)	ND	(0.86)	ND	0.59
AN-6	Ammonium sulfate	0.22*t*[d]	0.59	0.00	0.51*t*	0.11	0.00
AN-7	Sodium thiosulfate	0.63; 0.29	0.86; 0.71	0.25; 0.06	0.76	0.17	0.25
AN-8	Ammonium phosphate	0.06	0.52*t*′	0.00	0.32	0.03	0.00
AN-9	Sodium chloride	0.70	0.83	0.06*t*	0.81	0.59	0.21
AN-10	Potassium fluoride	ND	0.58*t*	ND	0.37	0.17*t*′	ND
AN-11	Potassium iodide	0.80	0.94	0.39	0.90	0.73	0.61
AN-12	Potassium bromide	0.76	0.90	0.18	0.87	0.65	0.39
AN-13	Ammonium acetate	ND	ND	ND	ND	ND	ND
AN-14	Potassium cyanide	ND	ND	ND	ND	ND	ND
AN-15	Ammonium thiocyanate	0.80	0.90; 0.02*t*	0.47; 0.00	0.90; 0.08*t*	0.72; 0.00	0.63
AN-16	Sodium arsenite	0.48	0.49	0.02	0.45	0.37	ND
AN-17	Potassium arsenate	0.10	0.56*t*	0.00	0.46*t*	0.05	0.00
AN-18	Sodium chromate	0.34*l*	0.86*t*	0.04*l*′	0.89*t*	0.20*l*	0.06*l*′
AN-19	Potassium dichromate	0.32*l*	0.86*t*	0.03*l*′	0.88*t*	0.19*l*	0.05*l*′
AN-20	Boric acid	0.33; 0.25	0.33*t*	0.06*t*	0.22	0.23	0.02
AN-21	Picric acid	0.86	0.97	0.72	0.95	0.79	0.72*t*
AN-22	L(+)-Tartaric acid	0.26	0.60	0.02	0.55; 0.05*t*	0.16	0.02
AN-23	Sodium carbonate	ND	ND	ND	ND	ND	ND
AN-24	Potassium ferricyanide	(0.44*t*′)	0.88	0.00	0.85	(0.22*t*′)	0.15*t*′

[a] The developing solvents used in this experiment were as follows: DS1, methanol–*n*-butanol–40% monomethylamine (3:1:1, v/v); DS2, methanol–*n*-butanol–40% *n*-butylamine (3:1:1, v/v); DS3, acetone–40% monomethylamine (10:1, v/v); DS4, methanol–*n*-propanol–water–40% *n*-buthylamine–10% trichloroacetic acid (50:30:15:8:1.5, v/v); DS5, methanol–*n*-butanol–40% monomethylamine (2:2:1, v/v); DS6, acetonitrile–40% monomethylamine (9:1, v/v).

[b] (S) and (R): the TLC plates used in this experiment were silica gel $60F_{254}$ and RP-18 F_{254}, respectively.

[c] Parentheses with the R_F value indicate spots not colored as white spot.

[d] *t*, tailing; *t*′, tailing from the spot to the origin; *l*, leading; *l*′, leading from origin; ND, not detected.

purchased from Kanto (Tokyo, Japan) and Wako (Osaka, Japan). For AN-3, a commercial 5.76% (w/w) solution was diluted to a concentration equivalent to the other sample solutions. All sample solutions were stored in a refrigerator until use.

Commercial TLC plates, silica gel $60F_{254}$ (silica gel), cellulose F (cellulose), RP-18 F_{254} (RP-18), polyamide 11F (polyamide), RP-8 $F_{254}S$ (RP-8) and CN $F_{254}S$ (CN) were purchased from Merck (Darmstadt, Germany).

A C_{18} extraction/clean-up column (100 mg) was obtained from Alltech (Deerfield, USA) and activated prior to use with methanol and purified water. A solid-phase column was used for the trial sample.

2.2. Preparation of CAR

The CAR used to detect the anion was prepared by dissolving 2.0 g citric acid in acetic anhydride, and making up to a volume of 100 mL with acetic anhydride. The solution was stored in a freezer until use.

2.3. Staining procedure

The staining procedure is shown schematically in Fig. 1. In step A, after TLC development, the dried plate was dipped in a suppressing solution containing 1.4 mL phosphoric acid in 200 mL acetone and then removed immediately. In step B, after drying, CAR was sprayed on the entire surface of the plate. Finally, in step C, the developed plate was covered and heated on a hotplate at 110 °C. Using this procedure, anions were revealed as distinct white material against a pink-red background within 3 min (Fig. 2) [14].

2.4. Solvent systems

Aqueous ammonium (28%), monomethylamine (40%) and *n*-butylamine (40%) solutions were used as the amine for development. The TLC solvent systems used in this experiment are modifications of those in other reports [5–13], and are listed in Table 1.

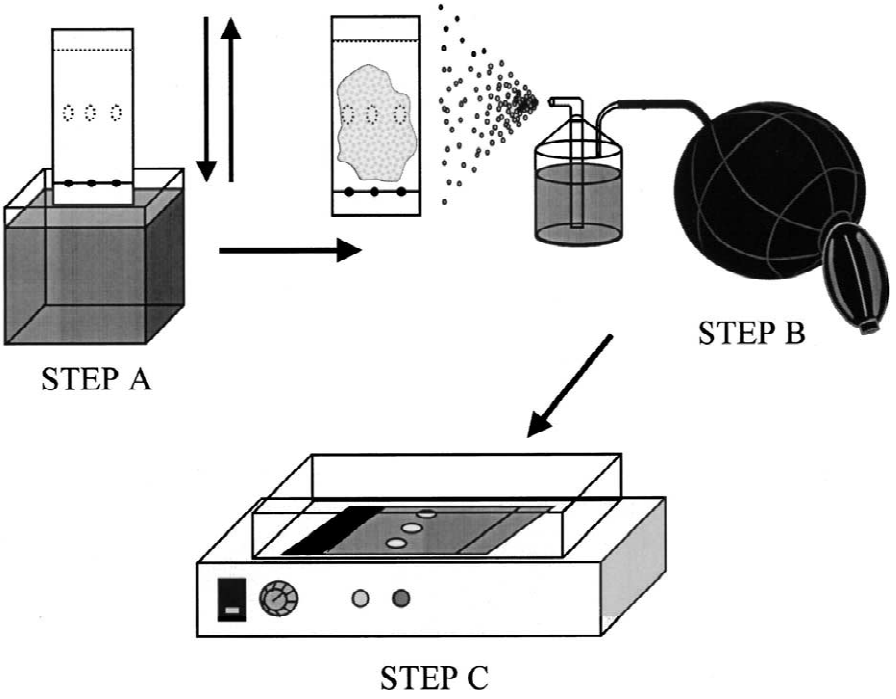

Fig. 1. Illustration of the coloration procedure after chromatographic development.

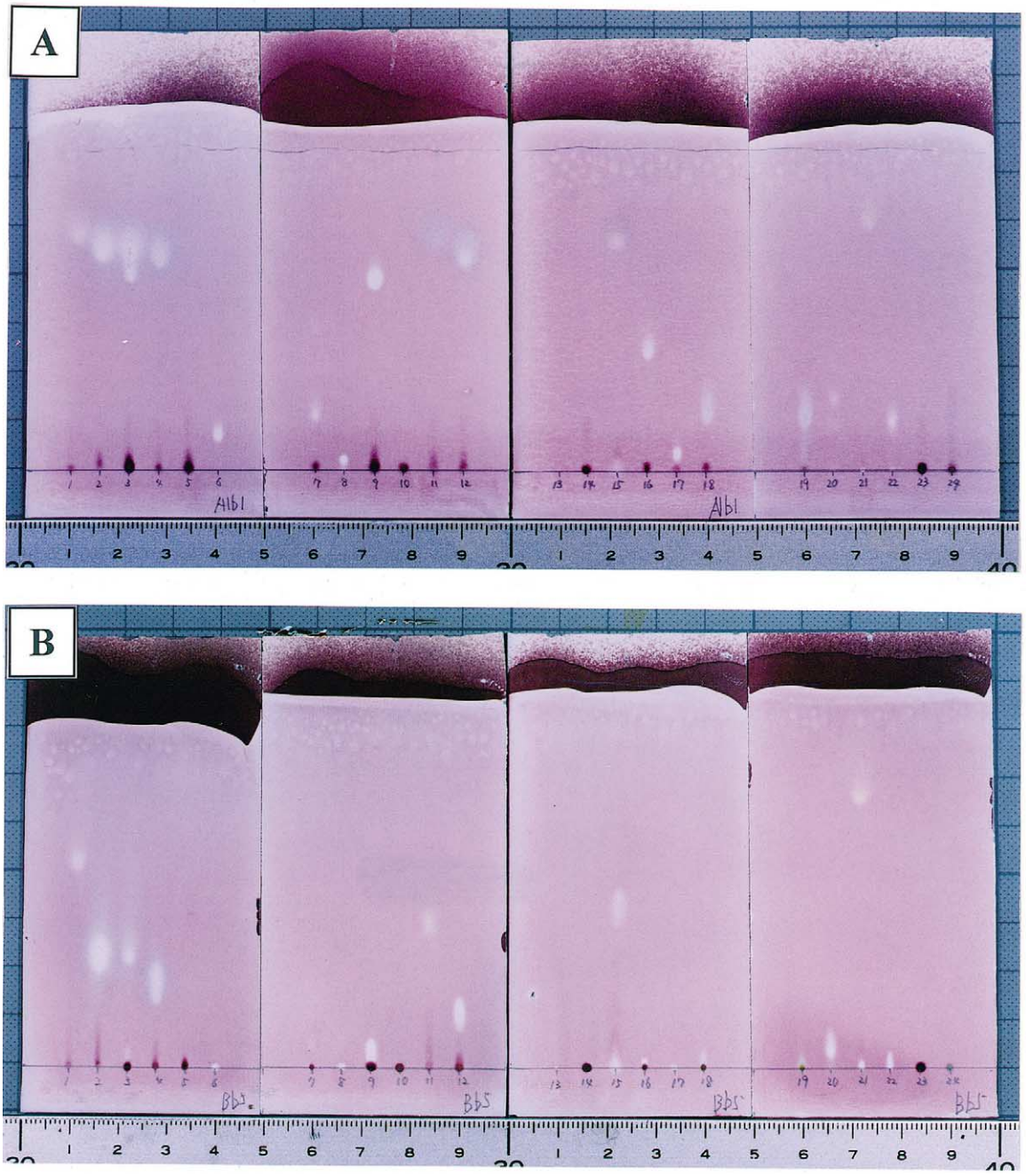

Fig. 2. Photographs of white spots corresponding to the salt formed by the amine and the anion. The solvent systems used were: (A) methanol–*n*-butanol–40% monomethylamine (2:2:1, v/v) and (B) acetone–40% monomethylamine (10:1, v/v) and the TLC plate used was a silica gel 60 F_{254}. From AN-1 to AN-8 and from AN-17 to AN-22, 10 μg was spotted on the TLC plate, and from AN-9 to AN-16 and from AN-23 to AN-24, 20 μg.

2.5. Trial sample

The effectiveness of the proposed method for practical qualitative application to a forensic sample (trial sample) was then examined. The trial sample was prepared by adding 0.5 g sodium arsenite (AN-16) to 100 g curry sauce, followed by shaking to dissolve the mixture. A 10 g amount of this well-mixed sample was used for analysis. The sample was then diluted with 20 mL of purified water, and centrifuged at 3000 rpm for 10 min. The supernatant solution was fractionated from this sample. This process was repeated three times and the liquid component combined. After centrifugation, the liquid component was diluted to 100 mL as the trial sample. A 1 mL aliquot of this sample was filtered through 0.5-μm filter paper, applied to a C_{18} solid-phase column, and eluted with purified water. The eluted solution was evaporated to dryness under reduced pressure using a vacuum pump, and the obtained residue was dissolved in 50 μL purified water. This solution was used as the trial sample for TLC.

3. Results and discussion

3.1. Optimum conditions for anion detection

The optimum TLC conditions for detecting these anions were examined using chlorate and chloride ion as anions and ammonia as amine, stained according to the procedure detailed above.

The optimum contact procedure for amine and anions before coloration was examined first. Three procedures of amine addition were tested: (1) adding the ammonia to the developing solvent, (2) spraying aqueous ammonia onto the plate after development, and (3) a combination of these two procedures. The anion could be identified as a white spot in the case of (1), but in cases (2) and (3) the white spot could not be confirmed.

The optimum kind and concentration of amine for salt formation with the anions was then examined. A previous report showed that ammonia and primary amines cannot be stained by CAR [14]. Monomethylamine, *n*-butylamine and ammonia were examined as developing agents for the formation of the white spots as counter-ions of the target anion. These amines were diluted with purified water to a specified concentration and mixed with 19 times their volume of isopropanol for use as the developing solvent. The concentration range of each amine was as follows: ammonia, 1–28% (v/v); monomethylamine 28–40%; *n*-butylamine 5–100%. These three amines in the developing solvent formed a white material that could be detected easily against the stained background. Monomethylamine was found to be the most suitable for salt formation, producing the most highly recognizable white spots and having the advantage that the unreacted amine could be vaporized easily. The optimum concentrations of each amine were 28% for ammonia, and 40% for monomethylamine and *n*-butylamine. It was considered that 40% primary amine in the developing solvent was appropriate for salt formation with anions. We selected 40% monomethylamine as the amine phase in the developing solvent for subsequent experiments, but, for the detection of fluoride ion, 40% *n*-butylamine was used because of its higher sensitivity for fluoride.

Finally, the optimum stationary phase for the TLC plate was examined using the above staining procedure, excluding step A. The coloration of the entire surface of the TLC plate was observed to ensure a degree of staining to allow the white anions to be resolved. The surface of the silica gel was colored too intensely to easily identify the white spots of the anions, and the cellulose remained unchanged. Plates made of RP-18, polyamide, RP-8, and CN were only slightly colored. Silica gel was selected as the most suitable stationary phase for the proposed method, including step A, because the other TLC plates did not take up the stain sufficiently and are not commonly used. The inclusion of a fluorescent component in the stationary phase was confirmed not to change the coloration of the silica gel plate. All of the fluorescent plates tested in this experiment might be stained to the same intensity as the plates without fluorescence.

3.2. Separation and detection of 24 different anions

Twenty-one of the 24 anions could be separated using six solvent systems, and were detectable by the

staining procedure shown in Fig. 2 and Table 1. The three undetectable anions, acetate, cyanide and carbonate, are considered to be decomposed and vaporized during the final heating step due to weak ionic bonds [15,16]. As cyanide ions readily form anionic metal complexes with ferric ions (ferricyanide), this toxic anion could be detected as a white-blue spot of ferricyanide using developing solvents (DS) 2, 3, 4 and 6 (Table 1). Hypochlorite ions decomposed into chlorate, and chloride ions were detected as two white spots, permitting indirect detection by TLC. Nitrite appeared as a red spot on the silica gel plate, but was detected satisfactorily as a white spot on the RP-18 plate using DS6. Although the 21 anions were largely observed as white spots, chromate, dichromate and ferricyanide ions, which contain heavy metals, appeared white-gray, white-gray and white-blue, respectively.

The detection limits for these 21 anions, ranging from 0.02 to 4 μg, are shown in Table 2. These values were determined by observation with the naked eye of three co-workers.

The results of the three co-workers were in fair agreement with each other. High sensitivity (<1 μg) was achieved for 16 target anions, and an even higher sensitivity (0.02–0.05 μg) was achieved for chlorate, sulfate, phosphate, chromate and dichromate. These five anions with higher sensitivity were strongly acidic ($pK_a<3$) in nature [15,17].

3.3. Detection of arsenous ion added to food

The toxic dose of arsenous acid for humans is reported to be between 5 and 50 mg, and the lethal dose is between 100 and 300 mg [18,19]. The addition of sodium arsenite to curry sauce in this experiment was carried out considering the Japanese poisoning case in 1998. The amount of arsenous acid added to 10 g of the curry sauce was at the toxic level. In Fig. 3(1A), the trial sample prepared above

Table 2
Detection limits of the 24 anions using TLC

No.	Sample	Detection limit (μg)	Solvent system[a] and plate type[b]
AN-1	Potassium perchlorate	0.4	DS1(S)
AN-2	Sodium chlorate	0.02	DS1(S)
AN-3	Sodium hypochlorite	0.02	DS1(S)
AN-4	Potassium nitrate	0.3	DS1(S)
AN-5	Sodium nitrite	4	DS6(R)
AN-6	Ammonium sulfate	0.03	DS1(S)
AN-7	Sodium thiosulfate	0.6	DS1(S)
AN-8	Ammonium phosphate	0.05	DS1(S)
AN-9	Sodium chloride	0.5	DS1(S)
AN-10	Potassium fluoride	3	DS4(S)
AN-11	Potassium iodide	0.2	DS6(R)
AN-12	Potassium bromide	2	DS1(S)
AN-13	Ammonium acetate	–	–
AN-14	Potassium cyanide	–	–
AN-15	Ammonium thiocyanate	4	DS1(S)
AN-16	Sodium arsenite	3	DS1(S)
AN-17	Potassium arsenate	0.3	DS1(S)
AN-18	Sodium chromate	0.03	DS1(S)
AN-19	Potassium dichromate	0.04	DS1(S)
AN-20	Boric acid	0.2	DS1(S)
AN-21	Picric acid	0.5	DS1(S)
AN-22	L(+)-Tartaric acid	0.5	DS1(S)
AN-23	Sodium carbonate	–	–
AN-24	Potassium ferricyanide	0.9	DS2(S)

[a] The solvent systems shown in Table 1 were used to examine the detection limit.
[b] TLC plate types were as follows: (S) silica gel 60 F_{254} and (R) RP-18F_{254}.

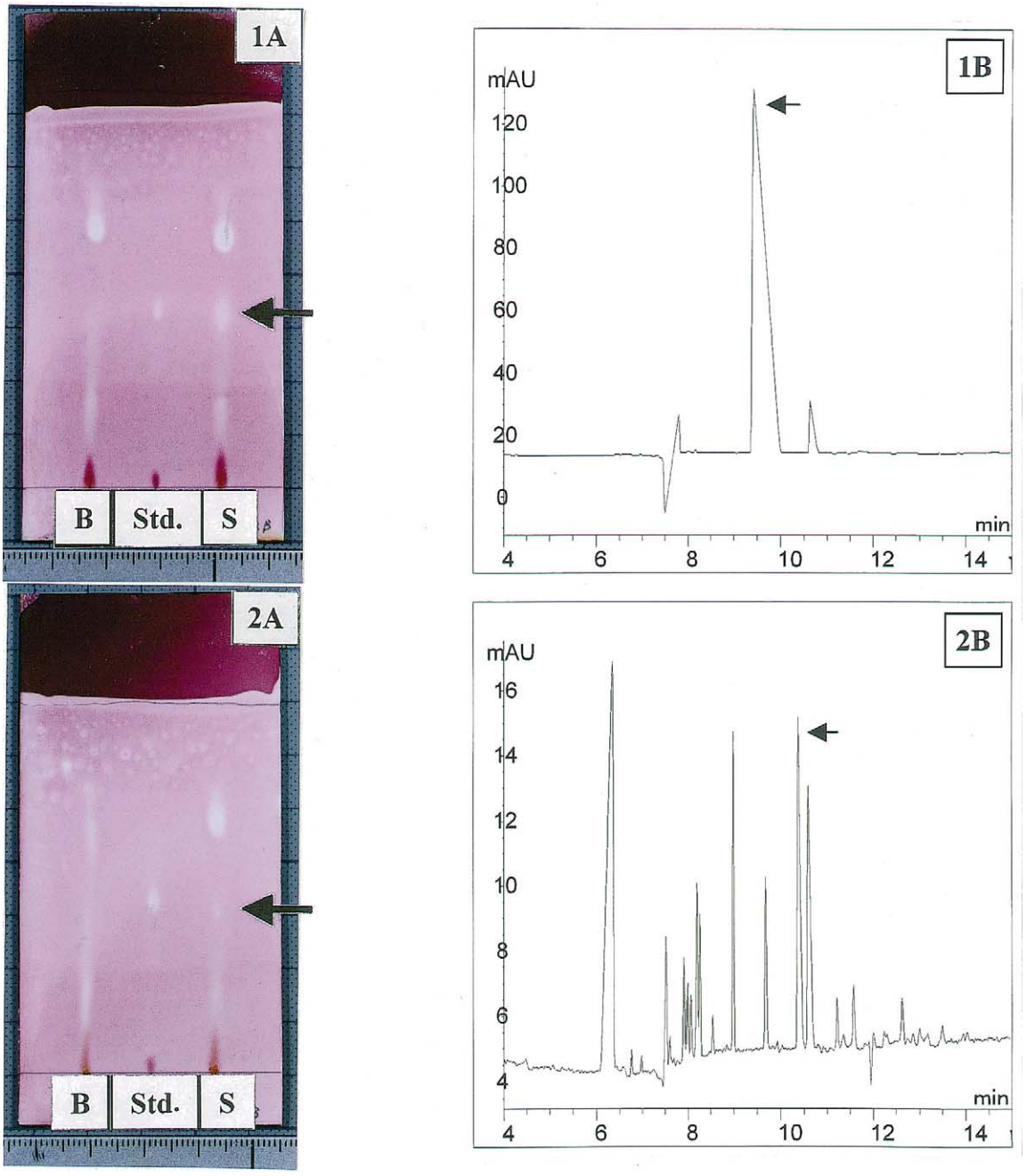

Fig. 3. Photographs of white spots with arsenite ion added to curry sauce: B, blank; Std., standard (20 μg); S, trial sample (40 μg). The arrow shows the spot of the arsenite component. DS1 shown in Table 1 was used as the developing solvent. (1A) Trial sample after treatment with a filter and a C_{18} column; (1B) electropherogram of the arsenite component extracted from the TLC plate of (1A) by CE; (2A) trial sample before filtration and passing through the C_{18} column; (2B) electropherogram of (2A) obtained by CE.

exhibits white spots corresponding to the arsenite–amine salt. After development, the arsenite component was extracted with purified water from stained silica gel, and the extract was analyzed by CE (G1600A; Agilent Technologies) after concentration to 100 μL under low pressure. Arsenite was confirmed as a single peak and did not appear to be affected by the other constituents of the curry sauce [Fig. 3(1B)]. The trial sample before filtration and passing through the C_{18} column was also examined by TLC and CE. In TLC, the white spot of the arsenite compound was obstructed by the other components of the curry sauce, and was difficult to confirm compared with Fig. 3(1A) [Fig. 3(2A)]. In the CE analysis of this sample, many constituent peaks originating from the curry sauce were confirmed in addition to arsenite ions [Fig. 3(2B)].

This result suggests that this method, namely TLC separation, detection, extraction, filtration and passage through a C_{18} column, is applicable to other analytical methods as a pretreatment procedure for CE or IC.

4. Conclusion

Twenty-one different anions were successfully detected using the proposed TLC method, and separated effectively using six different developing solvents. It is considered that other anions will also be detectable by identifying the most appropriate combination of amine, anion and TLC plate. Extending this method will be the subject of future investigations. The technique was demonstrated to provide good results as a detection method for various anions, and also to serve as an initial purification procedure for preparing ideal samples for other analytical methods.

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