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Short communication

Comparison of high-performance capillary electrophoresis and liquid chromatography on analysis of zinc 5-aminosalicylate dihydrate and related materials

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Abstract

Separation and determination of zinc 5-aminosalicylate dihydrate (ZASA) and related materials by high-performance capillary electrophoresis (HPCE) and liquid chromatography (HPLC) have been studied systematically. The R.S.D. of peak time and area and detection limits of materials were lower in HPLC compared to HPCE. HPCE and HPLC were used for determining ZASA and related materials in crude and treated ZASA using acid and heat, and similar results with both techniques showed the principal impurities in the crude ZASA were 5-aminosalicylic acid (ASA) and salicylic acid (SA), the compounds from ZASA decomposing under conditions of acid and heat were ASA, *p*-aminophenol and 5-benzeneazosalicylic acid. © 1998 Elsevier Science B.V.

Keywords: Zinc 5-aminosalicylate dihydrate; 5-Aminosalicylic acid; Salicylic acid; *p*-Aminophenol; 5-Benzeneazosalicylic acid

1. Introduction

Zinc 5-aminosalicylate (1:2) dihydrate ($C_{14}H_{12}N_2O_6Zn{\cdot}2H_2O$, ZASA), a new compound, is a more effective drug than 5-aminosalicylic acid (ASA) for the treatment of Crohn's disease and ulcerative colitis [1,2], in accordance with the results (unpublished) found at Henan Medical University. ZASA may include ASA and salicylic acid (SA), and decompose to *p*-aminophenol (AP) and 5-benzeneazosalicylic acid (BSA) under the influence of light, temperature and acid. Some papers have been published on the high-performance liquid chromatographic determination of ASA in plasma and urine [1] and in tablets and suppositories [2]. However, no report on the analysis of ZASA and ASA by high-performance capillary electrophoresis (HPCE) has been seen. In this paper, a simple, rapid and reproducible HPCE method for analyzing ZASA and related materials was developed and the results were

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compared to those found using high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Reagents

ZASA (99%) and BSA (99%) were made in our laboratory and tested by nuclear magnetic resonance (NMR), Fourier-transform infra-red (FTIR), EA and mass spectrometry (MS). AP, SA, ASA and other reagents were from Beijing Chemical Factory (Beijing, China). ZASA (2 mg/ml), ASA (2 mg/ml), SA (1 mg/ml), BSA (1 mg/ml) and AP (1 mg/ml) stock solutions were prepared using 50% methanol.

2.2. HPLC

An LC-6A HPLC system with a CLC-ODS column (150×6.0 mm I.D., 7 μm; Shimadzu, Tokyo, Japan) was used for the separation of ZASA and related materials. Samples were introduced by an injector with a 20-μl loop, and eluted with an appropriate mobile phase at a flow-rate of 1 ml/min. The temperature of the column was maintained at 20±0.2°C and the detection wavelength was set at 280 nm.

2.3. HPCE

HPCE separations were carried out by using a 1229 HPCE analyser (Beijing Institute of New Technology and Application, Beijing, China) with a fixed wavelength UV detector, which was set at 280 nm. A 65.5-cm long (57.3 cm to the detector), 50 μm bare fused-silica capillary (Yongnian Optical Factory, Yongnian, Hebei Province, China) that was filled with buffer was used for the separation. The pH values of the buffers were adjusted with concentrated H_3PO_4 and measured using a No. 5944 pH meter (Cole-Parmer, Chicago, IL, USA). The HPCE system was operated in the conventional mode with the injection occurring at the anode by applying 20 kV for 20 s and the temperature was maintained at 22±0.5°C throughout. The separation voltage was 20±0.3 kV. The capillary was rinsed with 0.1 mol/l NaOH and double-distilled water for 5 min after each run and was then re-equilibrated with running buffer for 10 min.

3. Results and discussion

3.1. Optimal HPCE buffer

The effects of the concentration of borax and β-cyclodextrin (β-CD), the proportions of ethanol (EtOH) and the pH values of the buffer on the behaviour of ZASA and related materials during HPCE were investigated.

When the borax concentration was increased from 20 to 60 mmol/l, the capillary current was increased from 18 to 60 μA and the peak shape became asymmetric, due to the temperature gradient caused by joule heating. The effect of β-CD on the migration time of these materials is shown in Fig. 1. Over the range of 5–15 mmol/l β-CD, the increase in migration times resulted primarily from the increase in viscosity and the decrease in the electroosmotic flow mobility. Fig. 2 shows the effect of EtOH on the migration times of these materials. It can be seen

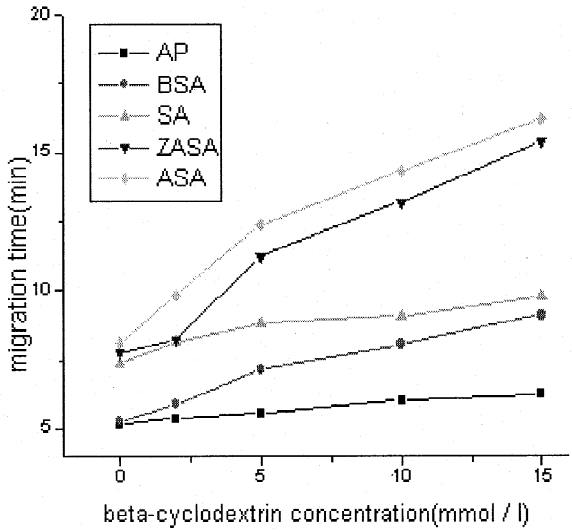

Fig. 1. Effect of β-cyclodextrin concentration on the migration time of ZASA and related materials. The detection wavelength was 280 nm. A 57.3-cm effective length×50 μm I.D. bare fused-silica capillary was used. The running buffer was 40 mmol/l borax–β-cyclodextrin (0–15 mmol/l)–10% ethanol–H_3PO_4 (pH 7.0) and the separation voltage was 20 kV.

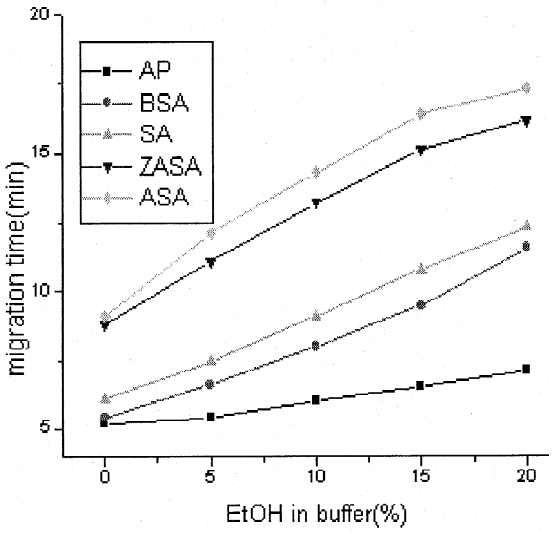

Fig. 2. Effect of the organic modifier, ethanol, on the migration time of ZASA and related materials. The running buffer was 40 mmol/l borax–10 mmol/l β-cyclodextrin–ethanol (0–20%)–H_3PO_4 (pH 7.0) and the other conditions were the same as in Fig. 1.

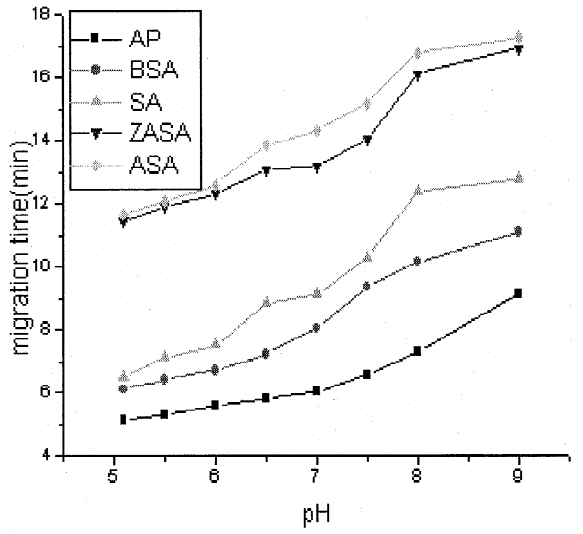

Fig. 3. Effect of pH on the migration time of ZASA and related materials. The running buffer was 40 mmol/l borax–10 mmol/l β-cyclodextrin–10% ethanol–H_3PO_4 (pH 5–9). Other conditions were the same as in Fig. 1.

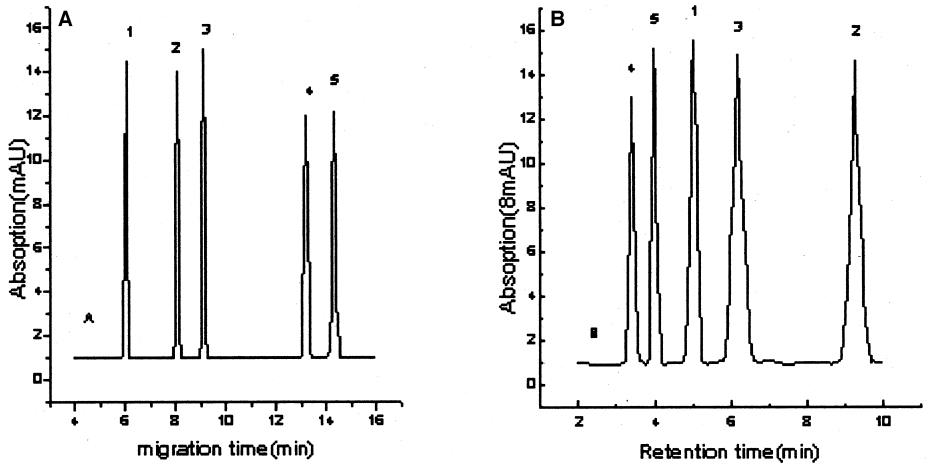

Fig. 4. Typical separation of ZASA, ASA, SA, AP and BSA by (A) HPCE and (B) HPLC. (A) The running buffer was 40 mmol/l borax–10 mmol/l β-cyclodextrin–10% ethanol–H_3PO_4 (pH 7.0) and the other conditions were the same as in Fig. 1. (B) The detection wavelength was 280 nm. A CLC-ODS column (150×6.0 mm I.D., 7 μm) was used and the temperature was maintained at 20°C. The mobile phase was 1% acetic acid–methanol (4:6, v/v) and the flow-rate was 1.0 ml/min. Peaks: 1, AP (20 μg/ml); 2, BSA (20 μg/ml); 3, SA (50 μg/ml); 4, ZASA (50 μg/ml); 5, ASA (50 μg/ml).

Table 1
Linear equations, detection limits and recoveries of ZASA and related materials

Method	Material	Concentration range (μg/ml)	Linear equation ($C=a+b\times A$)				Detection limit (μg/ml)	Recovery (%)
			a	*b*	*r*	*n*		
HPLC	ZASA	1.0–100	0.41±0.011	0.0163±0.00006	0.9986	7	0.73	100.8±1.21
	ASA	1.0–100	0.37±0.009	0.0142±0.00005	0.9966	6	0.55	103.3±1.32
	SA	1.0–100	0.21±0.006	0.0123±0.00008	0.9945	6	0.46	98.4±2.12
	BSA	0.8–50	0.17±0.008	0.0092±0.00007	0.9987	7	0.38	99.2±1.67
	AP	0.4–40	0.11±0.004	0.0108±0.00003	0.9996	6	0.33	100.9±1.07
HPCE	ZASA	10–150	2.57±0.083	0.167±0.0013	0.9972	6	5.90	96.4±2.89
	ASA	10–150	2.37±0.052	0.154±0.0010	0.9986	7	5.41	98.2±2.31
	SA	10–150	2.01±0.028	0.143±0.0011	0.9965	5	4.86	103.3±1.43
	BSA	5–100	1.72±0.031	0.098±0.0007	0.9997	5	3.72	100.9±1.76
	AP	5–100	1.64±0.019	0.110±0.0012	0.9976	5	3.94	99.1±2.95

in Fig. 2 that the migration time increased as the percentage of EtOH increased, in the range of 5–20% EtOH. The increase in viscosity and the decrease in the electroosmotic mobility resulted in an increase in the migration times. The peak shapes of ZASA and BSA were achieved because of reduced adsorption and the increased solubility of the analyte. The effect of pH on the migration times of these materials is shown in Fig. 3. As the pH was decreased from 9.0 to 5.0, the negative charge on the inner wall of the capillary was suppressed, so the peak shape was improved due to reduced adsorption of the analyte to the inner wall of the capillary. However, the migration time was decreased as a result of the increasing electrophoretic mobility of the analyte, even though the electroosmotic flow decreased.

Taking the migration time, peak symmetry and the current in the capillary into consideration, 40 mmol/l borax–10 mmol/l β-CD–10% EtOH–H_3PO_4 (pH 7.0) was selected as the running buffer for the HPCE separation of ZASA and related materials with a capillary current of 40 μA, and an asymmetry factor of near 1.0 was achieved. Fig. 4A shows a typical electropherogram of ZASA, ASA, SA, AP and BSA, which were completely separated within 16 min.

3.2. The optimal mobile phase for HPLC

The behaviour of the five materials on a CLC-ODS HPLC column, eluted with different mobile phases, was investigated. Taking the analytical time, resolution and peak symmetry into consideration, 1% acetic acid–methanol (4:6, v/v) was selected as the

Table 2
R.S.D. of peak time and area of ZASA and related materials

Method	Material	Concentration (μg/ml)	Peak area (μV·s)	R.S.D. (%)		Peak time (min)	R.S.D. (%)	
				Within day	Between days		Within day	Between days
HPLC	ZASA	50.0	2989	1.5	2.3	3.40	1.4	3.8
	ASA	50.0	3541	2.1	2.5	3.98	1.6	3.2
	SA	50.0	4038	1.2	1.7	6.16	1.2	2.1
	BSA	20.0	2175	2.3	2.7	9.26	1.4	2.8
	AP	20.0	1852	1.8	2.1	5.02	1.8	3.1
HPCE	ZASA	100.0	580	2.2	3.5	13.18	4.5	6.4
	ASA	100.0	641	2.7	2.9	14.30	3.9	4.8
	SA	100.0	697	1.9	2.8	9.10	4.5	5.1
	BSA	50.0	470	3.2	4.0	8.05	3.4	4.9
	AP	50.0	438	2.8	3.7	6.02	2.8	6.1

Table 3
Results of crude and treated ZASA (100 μg/ml) by HPLC and HPCE (n=5)

	ZASA		ASA		Concentration detected (μg/ml)					
					SA		AP		BSA	
	by HPLC	HPCE	HPLC	HPCE	HPLC	HPCE	HPLC	HPCE	HPLC	HPCE
Crude:										
No. 960612	91.88±1.21	92.21±2.52	4.32±0.12	5.82±0.18	2.64±0.13	2.38±0.27	–	–	–	–
No. 960815	92.02±1.25	91.67±2.43	4.38±0.13	4.11±0.32	3.72±0.11	4.03±0.31	–	–	–	–
No. 960919	97.34±0.67	97.09±1.42	2.12±0.11	2.41±0.19	–	–	–	–	–	–
No. 961013	98.81±0.41	98.13±1.39	0.72±0.05	1.02±0.08	–	–	–	–	–	–
Standard ZASA (99%) treated (10 min):										
22°C	98.21±1.16	98.37±1.48	–	–	–	–	–	–	–	–
50°C	89.38±1.24	90.43±1.56	4.87±0.12	6.10±0.47	–	–	–	–	–	–
80°C	56.12±1.21	58.52±1.48	38.22±1.25	36.16±1.13	–	–	4.08±0.29	4.53±0.42	–	–
100°C	9.21±0.42	10.25±0.72	82.65±2.76	81.74±4.27	–	–	4.11±0.32	4.20±0.39	2.21±0.18	2.72±0.22
0.5% H_2SO_4	62.38±1.87	64.76±2.18	30.82±1.26	28.31±2.98	–	–	–	–	–	–
1.0% H_2SO_4	19.76±1.21	20.12±1.97	11.68±0.69	12.23±1.05	–	–	–	–	–	–
5.0% H_2SO_4	–	–	–	–	–	–	–	–	–	–

mobile phase for the separation of these materials. Fig. 4B shows a typical chromatogram of standards that were baseline separated within 10 min.

3.3. Comparison of analytical data of ZASA, ASA, SA, AP and BSA by HPCE and HPLC

Using HPCE and HPLC, the linear equations, detection limits (S/N=3), recoveries (30 μg/ml, n=5) and reproducibilities (samples were tested ten times per day for seven days) of peak time and area of the five materials were determined and the results are presented Tables 1 and 2, respectively.

It can be seen from Tables 1 and 2 that the detection limits and R.S.D.s of peak time and area obtained for these materials were lower for HPLC than for HPCE. The poor values of peak time R.S.D.s may be caused by inadequately controlled temperature and separation voltage. There was no significant difference in recovery between the HPCE and HPLC methods.

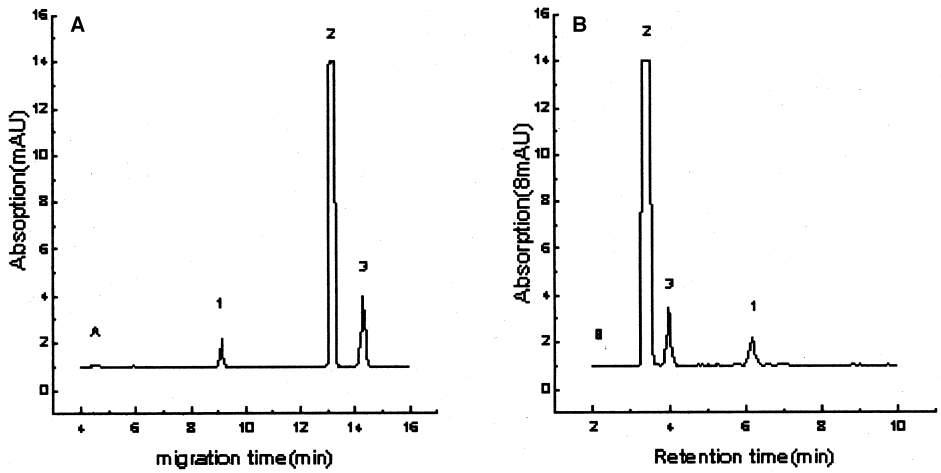

Fig. 5. Separation of crude ZASA (No. 960815) by (A) HPCE and (B) HPLC. The conditions were the same as in Fig. 4. Peaks: 1, SA; 2, ZASA; 3, ASA.

3.4. HPCE and HPLC determination of ZASA, ASA, SA, AP and BSA in crude and treated ZASA

Some crude and treated ZASA under conditions of heat and acid were determined by HPLC and HPCE. The results are listed in Table 3 and an extracted result is shown in Fig. 5.

Table 3 shows that the results determined by HPLC were similar to those obtained by HPCE. The results also illustrated that the principal impurities in crude ZASA were ASA and SA, and the decomposition compounds of ZASA under conditions of acid and heat were ASA, AP and BSA.

4. Conclusion

HPCE and HPLC were developed to determine ZASA and related materials under crude and treated conditions. The study showed that the accuracy of both methods was similar. HPLC had a lower detection limit, R.S.D. of peak time and area and analytical time than did HPCE. Both methods can be used for determining ZASA and its principal impurities. In addition, the methods are applicable to the determination of ZASA in plasma and urine for pharmacokinetic studies with some modification.

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