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Short communication

Quantitation of counter ion of a water-insoluble drug by nonaqueous capillary electrophoresis with indirect UV detection

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Abstract

This is a report on a capillary electrophoretic approach for the quantitation of the counter ions of drugs that are insoluble or slightly soluble in water. Using a nonaqueous run buffer, the sample solvents were not restricted, which is not the case with conventional aqueous run buffer systems. The separation selectivity could be manipulated using mixed solvents such as methanol and dimethylformamide as run buffers. Detection was carried out by indirect UV detection with phthalate. The linearity and the repeatability of the peak area of chloride was satisfactory. This approach was applied to the drug substance, azelastine hydrochloride, which is slightly soluble in water, and provided a value of chloride content consistent with the theoretical value.

Keywords: Buffer composition; Azelastine hydrochloride; Inorganic anions; Chloride

1. Introduction

The counter ions of drugs with ionic groups are of importance because the physicochemical properties, stability and bioavailability of the drugs depend on their salt forms. For quantitation of the counter ion, titration and ion chromatography are mainly used. However, the former method does not have sufficient selectivity for ions, i.e., chloride and bromide cannot be distinguished. Also in the latter method, the running cost is high. In addition, it is often difficult to apply both methods to water-insoluble compounds because the sample solvents are restricted.

Alternatively, capillary electrophoresis (CE) has been developed remarkably and applied to the separation of small ionic species [1–9]. Especially in the fields of pharmaceutical, environmental and food analyses, CE was effective for determining small ions [10–13]. In these cases, detection of ions without any chromophore was often carried out by indirect UV detection with additives such as chromate, phthalate and benzoate because the commercial CE equipment with UV detectors could be used without any modification. However, this approach could not be directly applied to quantify the counter ion of water-insoluble drugs because an additional step for the extraction of the ion from the sample matrix would be required.

Recently, some papers on the use of nonaqueous solvents such as methanol and dimethylformamide (DMF) as run buffers for CE have been published [14–16]. This approach has attractive features such as viability for compounds with lower solubility as well as its unique selectivity and high sensitivity [17].

In this paper, we describe an analytical method to

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quantify the counter ion of water-insoluble drugs using nonaqueous CE with indirect UV detection which was developed and was applied to the quantitation of the chloride content of azelastine hydrochloride.

2. Experimental

2.1. Capillary electrophoresis

All electrophoretic data were collected using a Beckman P/ACE 2100 system (Fullerton, CA, USA). Fused-silica capillaries were purchased from Beckman with both 50 or 75 μm I.D., 365 μm O.D. and 27 cm total length. The capillary temperature was maintained at 25°C by a liquid cooling system. Injection was performed by a pressure mode (4 s, 3447 Pa), and detection was performed at 254 nm. The applied voltage was −7 kV. Between injections, the capillary was rinsed with run buffer, which was 10 mmol/l potassium hydrogenphthalate (KHP), 0.5 mmol/l tetramethylammonium hydroxide (TMAH), and 2% (v/v) water in methanol–DMF (7:3). This solution was prepared by diluting a stock solution of KHP (500 mmol/l in water) and TMAH (50 mmol/l in methanol) with methanol and DMF. The obtained peak areas were converted to values normalized by the migration times.

2.2. Reagents

Sodium chloride, sodium bromide and potassium nitrate were obtained from Wako (Osaka, Japan). KHP was obtained from Toyama Yakuhin Kogyo (Toyama, Japan). Methanesulfonic acid was purchased from Aldrich (Milwaukee, WI, USA). The reagent-grade methanol, DMF, dimethylsulfoxide (DMSO), and TMAH were obtained from Wako. Water used in this study was purified by a Milli-Q system (Millipore, Milford, MA, USA).

2.3. Sample solutions

Each stock solution of chloride, bromide, nitrate and methanesulfonate (10 mg/ml) was prepared by dissolving them in water (in the cases of chloride, bromide and nitrate) or methanol (methanesulfonate).

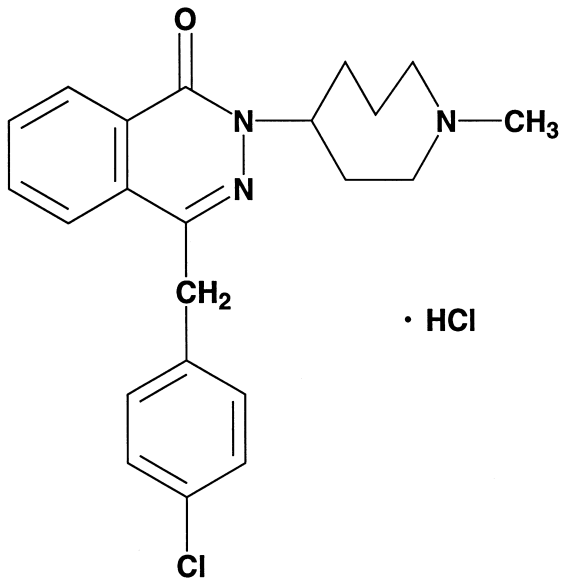

Fig. 1. Structure of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$).

These stock solutions were then mixed and diluted with methanol, DMF or DMSO (8 μg/ml each). This solution was used as a standard mixed solution for selectivity.

The chloride standard solutions for linearity and precision were prepared by diluting the stock solutions of chloride and nitrate with methanol. The concentration range of chloride for linearity was from 6 to 10 μg/ml. The test solution of azelastine hydrochloride was prepared by dissolving the compound in methanol. Azelastine hydrochloride, is slightly soluble in water and had been developed for some allergy diseases. As shown in Fig. 1, this compound has one hydrochloride group in salt form.

All solutions were filtered through a 0.45-μm GL 13A filter (GL Sciences, Tokyo, Japan).

3. Results and discussion

First, we investigated the possibility of the combination of an aqueous run buffer containing chromate and nonaqueous sample solvents such as methanol, DMF and DMSO for quantitation of chloride in water-insoluble drugs. However, in the cases of DMF and DMSO as sample solvents, the electrical current could not be maintained during analysis. In

addition, methanol as a sample solvent could not be employed in the case of the 75-μm I.D. capillary because of a sudden decrease in the electrical current. These phenomena might be caused by the discontinuity between the run buffer and the sample solvents in the capillary. Thus, the use of nonaqueous run buffers was investigated. In nonaqueous run buffers, it is well-known that the migration behavior of solutes differs from that in aqueous buffers because of the change in the hydration, ion-pairing, and the zeta potential of solutes [17]. In addition, a higher S/N value at lower electrical current would be expected, and various kinds of sample solvents would be applicable.

First, concerning the additive for indirect UV detection, some reagents were evaluated for detection of chloride by the similarity of their mobility to chloride in nonaqueous run buffer systems. Chromate, which is the most common additive for chloride in the aqueous run buffer systems, was not suitable for methanolic run buffer systems because of its instability in methanol [17]. Other additives such as phthalate [2,4,17,18], fumarate [4], *p*-toluenesulfonic acid, salicylate [2,4], pyromellitic acid [19], and 2,6-pyridinedicarboxylic acid [4,20], which were previously used in aqueous systems, were evaluated. As a result, their migration times, except for that of 2,6-pyridinedicarboxylic acid, were about 1.5 times that of chloride. The migration time of 2,6-pyridinedicarboxylic acid was about twice that of chloride. Among them, phthalate was used in this study, although further optimization is needed.

Next, the effect of nonaqueous solvents as the run buffers on the separation selectivity was investigated. In Fig. 2, the dependence of the migration times of chloride, bromide, nitrate and methanesulfonate (mesylate) on the mixing ratio of methanol with DMF is shown. In this case, the migration order of nitrate and chloride was reversed compared with that in the aqueous run buffer system [17].

Moreover, the migration times of all samples increased drastically as the fraction of DMF increased. Considering the analysis time and the separation between nitrate and bromide, the ratio of methanol–DMF (7:3) was selected. As for the buffer constituents, Salimi-Moosavi and Cassidy reported that the addition of *n*-butylamine improved the reproducibility of the migration times [17]. In this

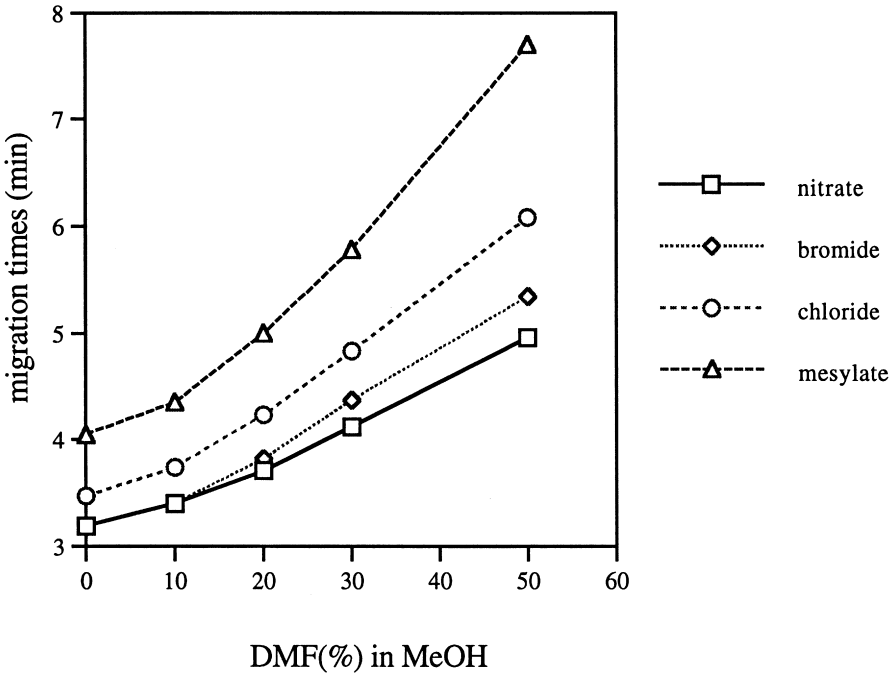

Fig. 2. Effect of DMF addition to methanol on the migration times of anions.

case, however, an unstable baseline was obtained due to the addition of *n*-butylamine, although the reason was not clear. Therefore, we employed TMAH instead of *n*-butylamine and could obtain reproducible results. The influence of TMAH in methanol for nonaqueous electrolyte on electroosmotic flow (EOF) was investigated. As a result, EOF was from anode to the cathode, as for aqueous electrolytes. The mobility was 0.73×10^{-4}(cm^2 s^{-1} V^{-1}).

The effect of the sample solvents was investigated. Fig. 3 shows a typical example of the sample solvents: DMF including 0.24% (v/v) water. Almost similar electropherograms were also obtained for the other sample solvents: methanol including 0.24% (v/v) water and DMSO including 0.24% (v/v) water (data not shown). As this is not the case with the aqueous buffer systems, the sample solvents were not limited in this methanol–DMF run buffer system.

For this system, the linearity was tested from 6 to 10 μg/ml, which corresponds to 75–125% of the target concentration (Table 1). Using nitrate as an internal standard (I.S.), the linearity of chloride was improved and the correlation coefficient was 0.9996.

Table 1
Repeatability and linearity of peak area and peak area ratio of chloride to internal standard[a]

	R.S.D. (%) (n=6)	r
Peak area	1.02	0.9814
Peak area ratio to I.S.[b]	1.65	0.9996
Migration time	0.75	—

[a]Sample for repeatability: 8 μg/ml of chloride and 10 μg/ml of I.S. [a]Sample for linearity: 6, 7, 8, 9 and 10 μg/ml of chloride including 10 μg/ml of I.S. Other conditions are given in Fig. 3. [b]Nitrate was used as an internal standard.

On the other hand, surprisingly, the repeatability of the peak area ratio was lower than that of the peak area, although both of these R.S.D. values were satisfactorily less than 2.0% (Table 1).

Azelastine hydrochloride, which is slightly soluble in water was tested, as an application to a drug substance. As a result, the chloride content of the drug substance was 8.59% by this method, using nitrate as the I.S., and this was in agreement with the theoretical value (8.47%).

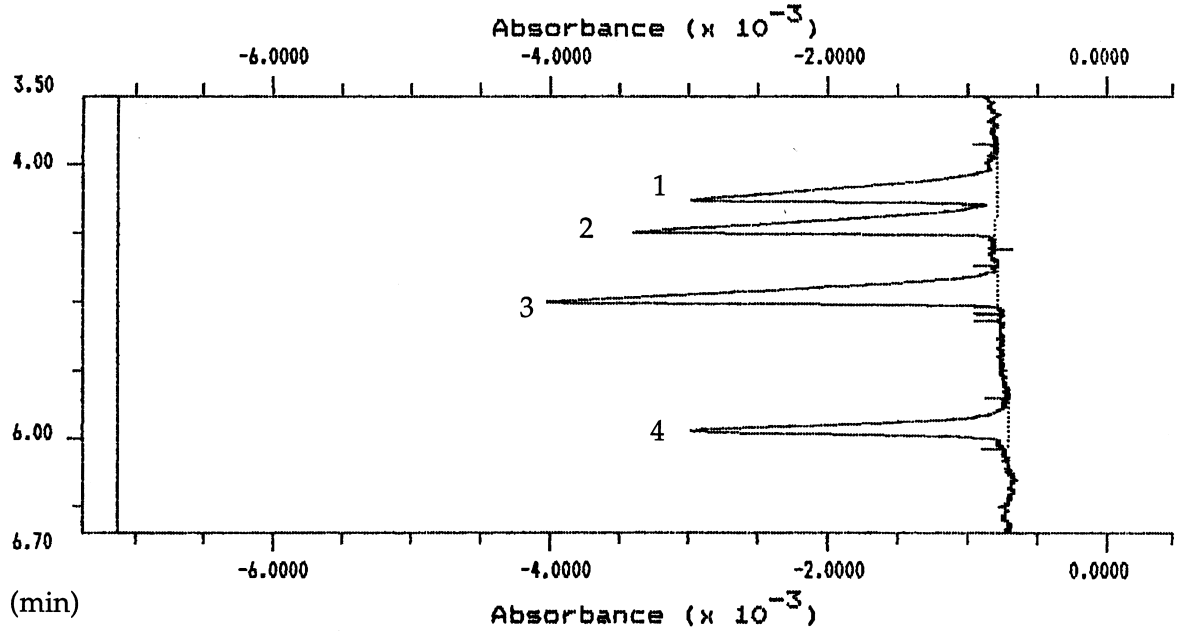

Fig. 3. Separation and detection of anions with phthalate. Conditions: run buffer, 10 mmol/l KHP, 0.5 mmol/l TMAH and 2% (v/v) water in methanol–DMF (7:3); detection, 254 nm; applied voltage, −7 kV; samples, nitrate (peak 1, 8 μg/ml), bromide (peak 2, 8 μg/ml), chloride (peak 3, 8 μg/ml), and mesylate (peak 4, 8 μg/ml); sample solvent, DMF including 0.24% (v/v) water.

4. Conclusions

In this study, CE using a nonaqueous run buffer system with a mixture of methanol and DMF was presented. Because this system permitted the injection of various kinds of such solvents, the counter ion of drugs that are insoluble or slightly soluble in water, such as chloride, could be determined using phthalate as an additive for indirect UV detection. The injection repeatability and the linearity in the target range were satisfactory using the peak area with or without I.S. This method provided a value of chloride content consistent with the theoretical value for azelastine hydrochloride, which is slightly soluble in water. This method would be applicable to other counter cations such as sodium and potassium, as well as other counter anions of drugs that are insoluble or slightly soluble in water with some modifications.

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