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Quantification of pilsicainide in serum by capillary electrophoresis

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Abstract

A new method for determining pilsicainide concentration in serum by rapid and selective capillary electrophoresis has been developed and validated. For pretreatment, serum was made alkaline and then extracted with diethyl ether. Procainamide was used as an internal standard. Sodium dihydrogenphosphate buffer (pH 2.29; 0.1 M) was used as a running buffer. A fused-silica capillary tube was loaded with a voltage of 25 kV and detection was performed at UV 200 nm. Good linearity (0–2.5 μg/ml) was obtained with the minimum limit of detection being 0.04 μg/ml serum (signal-to-noise ratio, 3:1). The R.S.D. of within-run reproducibility was 0.798–2.32%, that of between-run reproducibility was 4.74–5.12% and the recovery rate was 61–63%. Disopyramide, another anti-arrhythmic drug, was close to pilsicainide in terms of migration time. This method was applied to determination of pilsicainide in serum samples.

Keywords: Pilsicainide; Serum pilsicainide concentration; Capillary electrophoresis; Liquid–liquid extraction; Procainamide

1. Introduction

Pilsicainide (Fig. 1) is an anti-arrhythmic drug which falls into the class Ic of Vaughan's [1] anti-arrhythmic classification. It exhibits an anti-arrhythmic effect by inhibiting the Na^+ channel of cardiac muscle and, therefore, shows excellent efficacy against ventricular and supraventricular tacharrhythmias. Mexiletine, another anti-arrhythmic drug, is metabolized primarily in the liver and excreted through the kidney [2], while pilsicainide remains mostly unmetabolized, 90% of which is excreted through the kidney as the unchanged drug [3]. Its protein binding ratio is about 35% [4] and its half-life is about 4–5 h. Its therapeutic concentration in blood is 0.2–0.9 μg/ml [3]. Since pilsicainide is excreted through the kidney type its half-life is prolonged in patients with decreased renal function [5]. Its administration interval and dosage need to be adjusted according to the renal function status of the patient. Like other anti-arrhythmic drugs, pilsicainide has serious side effects such as ventricular fibrillation and ventricular tachycardia and, monitoring of its concentration in blood is necessary [6,7].

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High-performance liquid chromatography (HPLC) is the main method of determining concentration in blood of anti-arrhythmic drugs (quinidine, disopyramide, lidocaine, mexiletine, procainamide, *N*-acetylprocainamide) that are traditionally more widely used than others [8–11]. However, the determination by capillary electrophoresis (CE) has also been reported recently [12–14]. In such reports, anti-arrhythmic drugs, whose concentration in blood is relatively low, are pretreated (either by extraction with an organic solvent or by solid phase extraction) before use as samples for determination. In determining the concentration of pilsicainide in blood, the range around 0.1 μg/ml should be measured precisely. For this reason, serum samples need to be purified in a more specific manner and enriched to a higher concentration during the pretreatment. As a result, final sample volume becomes low. We paid attention to the fact that, compared with HPLC, CE offered higher theoretical plate number, only required samples and reagents of extremely low volumes, and, therefore, enabled specific analysis in a highly economical manner. CE's measurement sensitivity is often reported to be unsuitable to measuring drugs of low concentration in biological fluid by UV detection [15]. However, because pilsicainide showed strong UV absorption, as well as high ionization in the capillary tube, analysis could be performed with sensitivity that could be applied to clinical use. The purposes of this research was to develop a new determination method for pilsicainide concentration in serum by CE and to incorporate the method into a clinical laboratory for drug determination. We will herein report on the method and the results of its evaluation.

2. Experimental

2.1. Materials

Pilsicainide hydrochloride was provided by Suntory Ltd. (Tokyo, Japan). Procainamide hydrochloride used as an internal standard (IS) and all other drugs used for measurement interference evaluation were purchased from Sigma (St. Louis, MO, USA). Sodium dihydrogenphosphate, sodium hydroxide (0.1 N), diethylether and phosphoric acid (0.1 N) were purchased from Wako Pure Chemicals (Osaka, Japan).

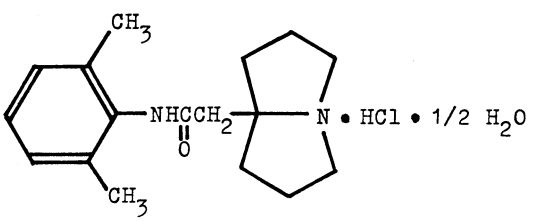
2.2. Preparation of standard solutions

Pilsicainide stock standard solution was adjusted with distilled water (1.0 mg/ml) and diluted with distilled water in an appropriate manner to be used as a working standard solution. Serum standards were prepared by adding the working standard solution to blank serum. Procainamide to be used as IS was dissolved in distilled water (1.0 mg/ml solution), and diluted with distilled water to be used as a working standard solution.

2.3. Sample preparation

Serum of a patient orally administered with a pilsicainide hydrochloride (Sunrythm®) preparation (Suntory, Tokyo, Japan) was used for the determination.

Patient serum (0.5 ml) and serum standard (0.5 ml) was added to 0.05 ml of the IS (10 μg/ml) solution and then treated with 0.5 ml of sodium hydroxide (0.1 N). These solutions were added to 5.0 ml of diethylether, shaken and mixed with the Recipro Shaker (Taitec Tokyo, Japan) for 10 min, and centrifuged at 1500 g for 5 min in a refrigerated centrifuge (Model RS-18GL, Tomy Seiko, Tokyo, Japan). Subsequently, 4.0 ml each from the diethylether layer was collected, poured into a 10 ml conical glass test tube and evaporated to



N-(2,6-dimethylphenyl)-8-pyrrolizidinylacetamide hydrochloride hemihydrate

Fig. 1. Structure of pilsicainide.

dryness under reduced pressure at 40 °C using a Centrifugal concentrator (VC-36, Taitec, Tokyo, Japan). The residues were re-dissolved in 50 μl of distilled water to be used as CE samples.

2.4. Electrophoretic instrumentation and running conditions

A P/ACE™ system MDQ51 equipped with a photo-diode array detector was used as the CE system and an untreated fused-silica capillary tube (e CAP™, effective length 500 mm × 75 μm I.D.) was used as the capillary tube, both of which were made by Beckman Coulter (Fullerton, CA, USA). Sodium dihydrogenphosphate buffer (pH 2.29; 0.1 M) was used as the electrophoresis buffer and its pH was adjusted with phosphoric acid (0.1 N). The electrophoresis buffer was passed through a 0.45 μm filter (Millipore, Bedford, MA, USA) and then, degassed ultrasonically for 5 min before actual use. Analysis was conducted in the following manner: The capillary tube was rinsed first with sodium hydroxide (0.1 N) for 3 min and then with distilled water for 2 min, and was conditioned with the electrophoresis buffer for 5 min (20 psi). Sampling was performed for 16 s (0.5 psi). Electrophoresis was conducted with a loading voltage of 25.0 KV, normal polarity and detection was performed at 200 nm. During these procedures, the temperature in the capillary tube was maintained at 25 °C and that in the sample vials at 15 °C.

2.5. Validation of the assay

2.5.1. Linearity

Serum standards of 0.2, 0.4, 1.0, 2.0 and 2.5 μg/ml were prepared by adding the pilsicainide working standard solutions of various concentrations to blank serum. These standards were pretreated (Section 2.3) and then, measured by CE.

2.5.2. Precision

In order to obtain within-run reproducibility, serum standards were prepared by adding the pilsicainide working standard solutions at four different concentrations to blank serum, which were measured in a consecutive and repetitive manner so that relative standard deviation (R.S.D.) could be calculated for each concentration. In order to obtain between-run reproducibility, serum standards at two different concentrations were prepared in the same manner. Measurement was performed twice a day for each concentration in 7 consecutive days, while samples were stored frozen (− 20 °C) and thawed at room temperature before each measurement.

2.5.3. Recovery

Samples were prepared by adding the pilsicainide working solutions of various concentrations to blank serum by 5% or less of the amount of the blank serum, and they were measured by CE. At the same time, the pilsicainide standard solutions of various concentrations were measured by CE without any preceding process, so that the recovery rate could be calculated (absolute recovery rate).

2.5.4. Sensitivity

Standard serum samples containing pilsicainide of various concentrations were prepared by diluting 1.0 μg/ml serum standard with normal pooled serum. These samples were measured by CE and the concentration with which signal-to-noise ratio was detected as 3:1 was determined to be the minimum limit of detection and quantitation.

2.5.5. Selectivity

Interference with the determination from endogenous substances in serum was evaluated by measuring blank sera. Interference from exogenous substances was evaluated by comparing, on the electropherogram, the migration time of pilsicainide with that of other anti-arrhythmic drugs, i.e. quinidine, disopyramide, propafenone, mexiletine, flecainide, lidocaine, propranolol, procainamide and *N*-acetylprocainamide.

3. Results and discussion

As pH of the electrophoresis buffer declined, the migration times of pilsicainide and IS became shorter and their sensitivities increased. From this, pH 2.29 is deemed to be the most suitable for

this method. To further increase the sensitivity, setting of sampling time is an important factor. Generally, the longer sampling time is, the more sensitivity increases. With this method, up to 20 s of sampling is possible and stable analysis was achieved with 16 s of sampling for this particular determination. Deproteinization and extraction (extraction with organic solvent and solid phase extraction) are generally methods for serum pretreatment [16,17]. Evenson et al. [12] used solid phase extraction as the pretreatment method for determination of procainamide, *N*-acetylprocainamide and disopyramide. With this method, however, serum samples needed to be enriched to a higher concentration because the concentration of pilsicainide in the serum samples was low. Thus, extraction with organic solvent was adopted, which also enabled easier operation. Since the pK_a of pilsicainide is 10.1–10.3, serum samples were treated with alkali and then extracted with diethylether. In relation to the choice of the solution for re-dissolving samples with which the CE system was charged, the electrophoresis buffer, distilled water and 10% ethanol were examined and distilled water was found to be the most suitable. As to what should be used as IS, Examination showed both procainamide (relative migration time [RMT] compared with pilsicainide, 0.790) and propericyazine (RMT = 1.11) were options suitable for use as IS. In order to perform fast analysis, procainamide was chosen. Fig. 2 shows electropherograms of measurement examples of a serum blank, serum standard, and serum of a patient administered with pilsicainide. It indicates that specific analysis of pilsicainide and IS was successfully performed without interference from endogenous substances in serum. The minimum limit of detection and quantitation obtained from evaluation using serum samples were, respectively, 0.04 and 0.10 μg/ml at a signal-to-noise ratio of 3:1. Some reports on the determination of other anti-arrhythmic drugs by HPLC show that the minimum limit of detection was 0.2–0.5 μg/ml [9,10]. Although measured drugs were different, this method exceeded these reports in sensitivity. Linearity (0–2.5 μg/ml) was good ($r = 0.999$). Evaluation results of within-run reproducibility and between-run reproducibility are shown in Table 1. A recovery rate test showed recovery rates of 62.5, 62.0 and 61.0% when 0.4, 1.00 and 2.00 μg/ml of pilsicainide was added, respectively. Accuracy of the reproducibilities was almost the same as reported for other anti-arrhythmic drugs determined by HPLC [8,9], while it was better than the one reported for a determination using CE [13]. Habler et al. [10] measured lidocaine in serum and its metabolites after pretreatment by solid phase extraction and reported that the recovery rate was 44–76%. This figure, like the recovery rate obtained by this method (determination of pilsi-

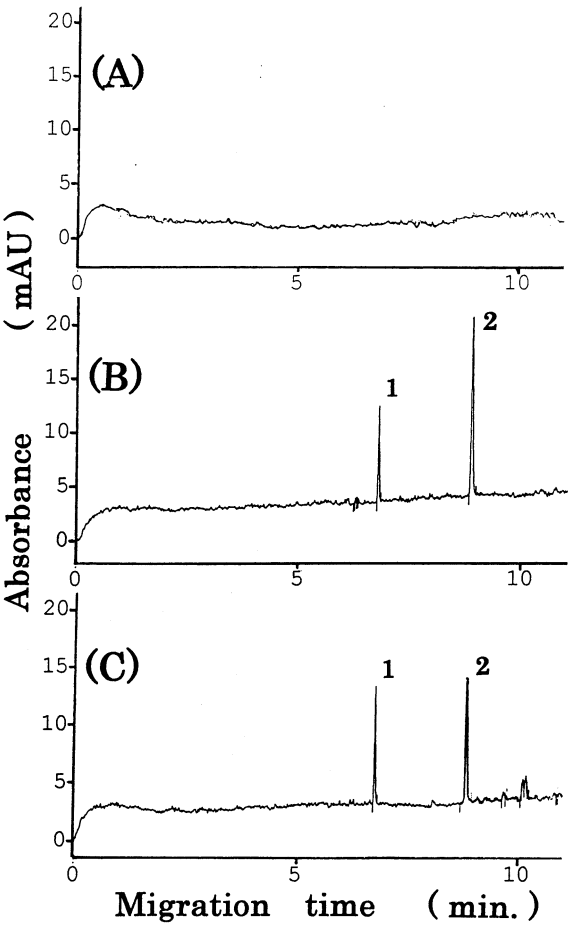


Fig. 2. Typical electropherograms of a blank serum (A), a serum standard spiked with pilsicainide (1.0 μg/ml) (B), and a serum sample from a patient on pilsicainide (0.57 μg/ml) (C). (1) Internal standard; (2) pilsicainide.

Table 1
Precision of the present method

Mean value (μg/ml)	S.D. (μg/ml)	R.S.D. (%)	Mean migration time (min) pilsicainide/IS	S.D. (min) pilsicainide/IS	R.S.D. (%) pilsicainide/IS
Within-run reproducibility ($n = 6$)					
0.25	0.0058	2.320	9.72/7.47	0.1140/0.0522	1.170/0.699
0.62	0.0120	1.940	9.97/7.62	0.0302/0.0170	0.303/0.223
1.22	0.0107	0.877	9.90/7.58	0.0615/0.0254	0.621/0.335
2.33	0.0186	0.798	9.94/7.62	0.0292/0.0403	0.294/0.529
Between-run reproducibility (7 *days*)					
0.41	0.021	5.12	9.21/6.98	0.447/0.258	4.85/3.70
0.78	0.037	4.74	9.21/6.98	0.433/0.252	4.70/3.61

cainide), was lower than around 100% that was shown in other reports [8,9,13]. These reports do not indicate how recovery rate was calculated, while it was calculated as an absolute recovery rate in this method. Generally speaking, a recovery rate of this level should be regarded as satisfactory for extraction with organic solvent [18]. Elution behaviors of other anti-arrhythmic drugs under determination conditions of this method were evaluated and RMT of these drugs relative to that of pilsicainide were calculated. The results were: quinidine, 0.729; mexiletine, 0.884; lidocaine, 0.975; disopyramide, 1.01; propranolol, 1.04; *N*-acetylprocainamide, 1.08; propafenone, 1.10; flecainide, 1.14. Migration time of disopyramide was close to that of pilsicainide. Therefore, perfect separation of these two drugs was difficult to achieve.

4. Conclusion

This method has high sensitivity, is easy to perform and is economical. Diethylether used for pretreatment could be evaporated under reduced pressure quickly, which in turn enabled the whole operation to be finished in a short period of time. In the assay validation, linearity, precision, sensitivity, recovery, selectivity were quite satisfactory. Thus, this method can be used in research and clinical laboratories.

References

[1] W.E.M. Vaughan, J. Clin. Pharmacol. 24 (1984) 129–147.
[2] V. Harserbartth, J.E. Doevendas, M. Wolf, Clin. Pharmacol. Ther. 29 (1981) 729–736.
[3] M. Yokota, T. Miyahara, N. Enomoto, H. Inagaki, N. Gotoh, H. Hayashi, et al., Ther. Res. 10 (1989) 2135–2147.
[4] T. Shiga, M. Hashiguchi, A. Urae, H. Kasanuki, T. Rikihisa, Clin. Pharmacol. Ther. 67 (2000) 222–228.
[5] T. Takabatake, H. Ohta, Y. Yamamoto, Y. Ishida, H. Hara, Y. Shioki, et al., Basic Pharmacol. Ther. 17 (1989) 3195–3205.
[6] J. Morganroth, J.L. Anderson, G.D. Gentzkow, J. Am. Coll. Cardiol. 8 (1986) 607–615.
[7] The cardiac arrhythmia suppression trial (CAST) investigators, New Engl. J. Med. 321 (1989) 406–412.
[8] C.M. Lai, B.L. Kamath, Z.M. Look, A. Yacobi, J. Pharm. Sci. 69 (1980) 982–984.
[9] R.R. Bridges, T.A. Jennison, J. Analyt. Toxicol. 7 (1983) 65–68.
[10] M.K. Halbert, R.P. Baldwin, J. Chromatogr. B 306 (1984) 269–277.
[11] W. Mastropaolo, D.R. Holmes, M.J. Osborn, J. Rooke, T.P. Moyer, Clin. Chem. 30 (1984) 319–322.
[12] M.A. Evenson, J.E. Wiktorowicz, Clin. Chem. 38 (1992) 1847–1852.
[13] P. Lukkari, H. Siren, M. Pantsar, M.L. Riekkola, J. Chromatogr. 632 (1993) 143–148.
[14] Z.K. Shihabi, J. Chromatogr. A 817 (1998) 25–30.
[15] G. Hempel, Electrophoresis 21 (2000) 691–698.
[16] Z.K. Shihabi, in: J.R. Petersen, A.A. Mohammad (Eds.), Clinical and Forensic Applications of Capillary Electrophoresis Part 17, Humana Press, New Jersey, 2001, pp. 355–384.
[17] D.K. Lloyd, J. Chromatogr. A 735 (1996) 29–42.
[18] R. Majors, G. Slack, in: L.R. Snyder, J.J. Kirkland, J.L. Glajch (Eds.), Practical HPLC Method Development Part 4, Wiley, New York, 1997, pp. 100–173.