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Frontiers of Separation Analysis

Development of a useful single-reference HPLC method for therapeutic drug monitoring of phenytoin and carbamazepine in human plasma

Yuko Sakaguchi[1] · Ryo Arima[1] · Runa Maeda[1] · Takuji Obayashi[2] · Akihide Masuda[2] · Mari Funakoshi[2] · Yumi Tsuchiya[2] · Nobuhiro Ichikawa[1] · Koichi Inoue[1]

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Abstract
A single reference high-performance liquid chromatographic (SR-HPLC) method was developed and validated for the therapeutic drug monitoring (TDM) of phenytoin (PHT) and carbamazepine (CBZ) in plasma from patients. The analytical parameters evaluated were linearity, limit of quantification (LOQ), selectivity, accuracy, and stability according to the US Food and Drug Administration (FDA) guideline. The developed method shows good linearity ($r2 > 0.999$; LOQ—50 µg/mL), and LOQ values were 1.56 µg/mL for PHT and 0.40 µg/mL for CBZ at 254 nm. For the development of SR-HPLC method, we evaluated to improve the detection wavelength, stirred retention time, and stability for SR, and selected 5-(p-methylphenyl)-5-phenylhydantoin for PHT (relative molar sensitivity, RMS = 0.848) and 10-methoxyiminostilbene for CBZ (RMS = 0.263). The established differential definite quantities of PHT and CBZ in plasma samples were similar using the RMS and absolute calibration methods based on RSD < 5.10%. A preliminary application was performed using chemiluminescent immunoassay and SR-HPLC method, in which the detectable values of the correlation coefficient and the slope of the intercept were PHT: 0.964 and 0.992647, and CBZ: 0.969 and 1.072089, respectively. Based on these results, we propose that the SR-HPLC method with RMS would be offered to the useful and accurate TDM of various medicines in plasma/serum samples.

Keywords Phenytoin · Carbamazepine · Therapeutic drug monitoring (TDM) · Single reference HPLC

Introduction

Therapeutic drug monitoring (TDM) has been broadly defined as the clinical analysis of chemical factors influencing drug prescription plans. In other words, the TDM procedure refers to the individualization of drug dosage based on plasma/serum monitoring concentration level. In addition, by combining the recognition of pharmaceutics, pharmacokinetics, and pharmacodynamics, the TDM procedure contributes to assessing the efficacy and safety of personal drug treatments. For clinical pharmacists in the pharmaceutical department, an accurate, useful, and routine TDM procedure is needed for the pharmacokinetic principles to assess these interpretations and decisions regarding the administration schedule. When drug concentration is closely related to therapeutic and side effects, this is an index of administration design based on the TDM procedure. In contrast, the analytical technique for TDM has been used in immunoassays, but it is accepted that frequent kits have a common weakness with non-specific interference from similar structures, metabolite-interference, and/or unknown biological matrix effects. Thus, it is necessary to develop chromatographic techniques for specific antiepileptic drugs, antibiotics, and anticancer drugs. In the concrete, a high-performance liquid chromatography (HPLC) method was reviewed for efficacy technique for the application of TDM procedures [1].

✉ Yuko Sakaguchi
yuko-s@fc.ritsumei.ac.jp

[1] Laboratory of Clinical and Analytical Chemistry, College of Pharmaceutical Sciences, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577, Japan

[2] Department of Pharmacy, Japanese Red Cross Kyoto Daiichi Hospital, 15-749 Honmachi, Higashiyama-Ku, Kyoto, Kyoto 605-0981, Japan

Frequently, HPLC methods have been applied for the selective and useful quantitation of plasma/serum drugs, particularly antiepileptic phenytoin (PHT) and carbamazepine (CBZ) [2–4]. PHT and CBZ are the main antiepileptic drugs used for TDM screening in clinical trials. Thus, HPLC methods have been developed to determine PHT and CBZ in plasma/ serum samples from patients [5–10]. Moreover, it is insufficient with these immunoassays in biological samples that previous reports showed biased data compared with HPLC methods for these matrixes and/or metabolites [11–13]. Recently, HPLC coupled with mass spectrometry (HPLC–MS) and tandem mass spectrometry (HPLC–MS/MS) methods has been used for simultaneous TDM of PHT and CBZ in plasma/serum samples [14–16]. However, based on these HPLC–MS and HPLC–MS/MS methods in the TDM procedure, it is inadmissible to use routine practices in clinical trials and pharmaceutical departments for tangled, high-priced, and maintenance instruments (specifically, MS detectors). Thus, the HPLC (coupled with UV detector) technique is suitable for useful, inexpensive, easy-to-use, and routine TDM procedures for PHT and CBZ in plasma/serum samples. However, these previous HPLC methods showed that the comprehensive traceability and certified referenced quantitative performance cannot be used to apply an accuracy control TDM procedure for PHT and CBZ in plasma/serum samples based on these individual standard curves [5–10]. In this first clinical study, we propose a single-reference (SR-) HPLC method for the TDM of PHT and CBZ in plasma samples from patients based on certified referenced quantitation without respective calibration curves using authentic standards.

In 2018–2022, we examined an HPLC method based on relative molar sensitivity (RMS), which involved an SR quantitative assay for determining natural chemicals in various food-related products [17–20]. This concept is that an SR standard is used as an internal standard for quantitative nuclear magnetic resonance (qNMR) and chromatographic methods (ex. HPLC) to estimate the RMS value of targeted analyte in various samples. The RMS values can be calculated from the molar ratios between the qNMR and HPLC response ratios and be lightly affected with each instrument and mobile phase. The concentrations of targeted analyte are determined using static RMS of analyte to SR standard (knowing concentration levels) added in the sample solution without reliable standard and calibration curve. Thus, the concentrations of analytes in sample solution could be calculated using the following equations [17–19]. Based on the Beer–Lambert law, the peak response (absorbance, R) is represented by the absorption coefficient (ε), the concentration (C), and the distance of the medium (ℓ). These factors are summarized for analyte from the SR standard:

$$R = \varepsilon \times C \times \ell .$$

In this case, ℓ is the same for the HPLC instrument. Based on the calculation of each calibration curve, including the origin, the ratio of the absorption coefficients (ε of analyte/ ε of SR) is shown in the following equation:

$$\begin{aligned} \varepsilon \text{ of analyte}/\varepsilon \text{ of } SR &= (R \text{ of analyte}/R \text{ of } SR) \\ &\quad \times (C \text{ of } SR/C \text{ of analyte}) \\ &= RMS. \end{aligned}$$

Thus, based on the RMS value, the concentrations of analyte can be easily derived using the existing and/or calculated SR concentration and peak ratio on a single running HPLC chromatogram. It performed an SR-HPLC method of analytes based on a certified standard such as 1,4-bis(trimethylsilyl) benzene-d_4 (1,4-BTMSB-d_4) for quantitative ^{1}H-qNMR and an SR standard related to RMS. In that, SR standards with similar chemical structures to analytes were typically employed as internal standards for qNMR experiments and HPLC methods to estimate the RMS values of each analyte with the slope of preliminary calibrations. On the other hand, the SR standard should have a similar absorption maximum wavelength to the targeted analyte, have stable physical properties, have sufficient chromatographic separation from the targeted analyte, have high-purity, be inexpensive, and be easily accessible for their use in HPLC assays. In our previous study, we designed SR standards based on the same molecular framework for the determination of analytes in various products by HPLC assay using RMS [18, 20]. However, it is possible to obtain the useful, comfortable, and low-cost similar chemical structures regent for good idea about selection criteria of SR. In this study, we can obtain the similar chemical structures regents of PHT and CBZ that showed the similar absorption maximum wavelengths of PHT and CBZ were observed.

In the first clinical study for the TDM procedure, the quantitative values of PHT and CBZ in plasma samples were determined based on the RMS of the analytes employing a selected SR standard, which was added to the sample solutions before the SR-HPLC method. We propose a useful, inexpensive, easy-to-use, and routine TDM procedure that involves the SR-HPLC method. Furthermore, in the application, the validity of the SR-HPLC method is assayed by comparing it with the quantitative values of immunological measurement methods commonly used in clinical settings.

Materials and methods

Chemicals and reagents

The PHT and CBZ standards were purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan). As SR reagents, 5-(*p*-methylphenyl)-5-phenylhydantoin (SR1), which is structurally similar to PHT, and 10-methoxyiminostilbene (SR2), which is structurally similar to CBZ, were selected. SR1 was purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and SR2 was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Their chemical structures are shown in Fig. 1. Methanol, acetonitrile, formic acid (FA, HPLC grade, 99%), trifluoroacetic acid (TFA, special grade, 98%), and other solvents were purchased from Fujifilm Wako Pure Chemical Industries Ltd. (Osaka, Japan). Purified water was obtained using a PURELAB Flex 5 system (ELGA Co., Inc., London, U.K.). Stock solutions of PHT, CBZ, SR1, and SR2 were prepared using methanol. The concentrated solutions were diluted as required by adding methanol/water (60/40, *v/v*).

HPLC instrument and condition

A TSKGEL ODS-100 V column (4.6 × 150 mm, 5.0 µm; Tosoh Co., Tokyo, Japan) was used to separate analytes in this method. The mobile phase in HPLC separation was composed of 0.1% FA in water (solvent A)/0.1% FA in methanol (solvent B). The isocratic conditions were as follows: 60% solvent B for 20 min. The system was operated at a flow rate of 1.0 mL/min. The column temperature was set to 40 °C, and the volume of the injected sample was 10 µL. The analytes were monitored using UV/visible absorbance in the 200–500 nm range (monitoring wavelength: 254 nm). The HPLC system comprised Hitachi High-Tech Science Chromaster (Hitachi Ltd., Tokyo, Japan).

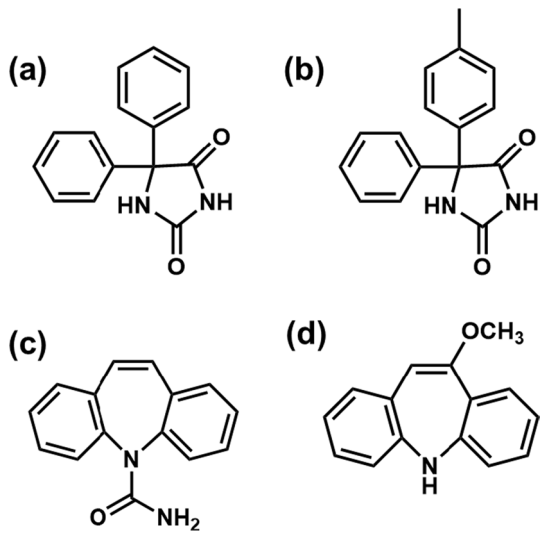

Fig. 1 Chemical structure of phenytoin (PHT) (**a**), 5-(*p*-methylphenyl)-5-phenylhydantoin (SR1) for PHT (**b**), carbamazepine (CBZ) (**c**) and 10-methoxyiminostilbene (SR2) for CBZ (**d**)

Calculation of RMS for PHT and CBZ

After qNMR (Supplementary Information), sample solutions were removed from the NMR tube and diluted with methanol/water (60/40, *v/v*) for the HPLC analysis. Using these solutions, we generated preliminary absolute calibration curves prepared at various concentrations (Fig. S1) widths for PHT, CBZ, SR1, and SR2, respectively [17]. The peak areas when the analyte concentrations were 0 µg/mL were set as the point of origin. The HPLC method was performed three times for each sample, and the average of the values obtained by that method was calculated. The RMS values were calculated from the ratio of the slopes of the absolute calibration curves. The following equation was used to determine the RMS values:

$$\text{RMS value of PHT} = \text{Slope of PHT}/\text{Slope of SR1}$$

$$\text{RMS value of CBZ} = \text{Slope of CBZ}/\text{Slope of SR2}.$$

Sample preparation of plasma

To validate the SR-HPLC method for accurate TDM procedure, 200 µL of plasma sample was prepared with 200 µL of acetonitrile containing 0.1% TFA. After stirring processing for 30 s, the centrifugation was performed at 9100 × g for 10 min at 4 °C using a CF15RN centrifuge (Hitachi, Tokyo, Japan). The supernatant was evaporated to dryness, and the mixed SR solution was adjusted to 10 µg/mL in a solvent of methanol/water (60/40, *v/v*) and redissolved in 200 µL for the HPLC analysis.

Analytical validation for HPLC method

Based on the US Food and Drug Administration (FDA) guideline [21], we investigated selectivity, accuracy, precision, and stability. For validation, the L-suitrol product (control plasma based on ISO15189) was obtained from Nissui Co., Tokyo, Japan. PHT and CBZ levels were quantified using six-point internal calibration with a quality control (QC) sample. For validation, we used the absolute calibration of PHT and CBZ; the acceptance criteria for these calibration curves had a correlation coefficient (r^2) of ≥ 0.999

(Fig. S1). To determine the intra-day accuracy, replicate ($n=6$) analyses of the plasma samples were performed on the same day. The inter-day accuracy was determined twice daily for 3 days ($n=6$). The process sample stability was evaluated using stable samples (5, 10, and 20 µg/mL) after 24 h in an autosampler at 4 °C. The bench-top stability was evaluated for 6 h at room temperature. Freeze–thaw stability was determined by comparing the freeze–thaw QC that had been frozen and thawed three times at −80 °C with the stable QC samples. Long-term stability was evaluated by analyzing stable QC samples stored at −80 °C for 30 days.

Application for clinical study

This clinical application was approved by the Japanese Red Cross Kyoto Daiichi Hospital Ethics Committee (Number: 1300, Date: 26/5/2021) and Ritsumeikan University Medical Research Ethics Review Board for Humans (Number: BKC-Human-medicine-2021–036, date: 25/6/2021) in accordance with the World Medical Association Declaration of Helsinki. These clinical samples included 25 patients from the Japanese Red Cross Kyoto Daiichi Hospital, Japan. The patient group consisted of individuals in oral CBZ treatment who received 100 ($n=3$), 200 ($n=7$), 300 ($n=1$), 400 ($n=2$), 600 ($n=4$), or 1000 ($n=1$) mg/day. The patient group consisted of individuals who received 90 ($n=1$), 200 ($n=2$), 300 ($n=1$), and 480 ($n=1$) mg/day oral PHT treatment and 375 ($n=1$) and 600 ($n=1$) mg/day infusion PHT treatment. The clinical parameters of the participants are presented in Table S1. In the clinical site, these drug concentrations in the plasma samples were measured by conventional evaluation (chemiluminescent immunoassay, CLIA reagent: ARCHITECT®, device: ARCHITECT® i2000SR). In this clinical application to evaluate these concentrations in residual plasma samples using developed SR-HPLC method, the correlative values were expressed using the Passing and Bablok regression analysis with Spearman's correlation coefficient that these p values less than 0.05 ($p<0.05$) were considered statistically significant [22]. In addition, the MedCalc® statistical software was used for this statistical evaluation (version 20.111, MedCalc Software Ltd, Ostend, Belgium).

Results

HPLC validation for the determination of PHT, CBZ, and SR in QC samples

Previous studies based on HPLC coupled with a UV detector for the simultaneous determination of PHT and CBZ in human plasma/serum samples showed that the C18 column and mobile phase consisted of acetonitrile/acetone/methanol/phosphate buffer, acetonitrile/methanol/phosphate buffer, or water/acetonitrile [9, 23, 24]. On the other hand, the recent trend of used organic solvents showed that the Japanese Pharmacopoeia is regulated using available residual solvents such as methanol and safety solutions for remaining in drug substances, excipients, and products [25]. Thus, in this study, we recommend a TDM procedure with an HPLC method using a C18 column and methanol/water. The optimal separation and retention of PHT, CBZ, and SRs could be achieved using this condition. Figure 2 shows the HPLC chromatograms of PHT, CBZ, SR1, and SR2 obtained using TSKGEL ODS-100 V column and the mobile phase composed of 0.1% FA in water/0.1% FA in methanol. PHT was detected at the retention time of 4.6 min, CBZ at 5.4 min, SR1 at 6.4 min, and SR2 at 14.3 min, confirming that the four targeted chemicals could be analyzed without interfering with each other. In addition, all peaks were detected within 15 min, and the measurements were achieved within a short time. Then, the absolute calibrations of PHT and CBZ from the LOQ to 50 µg/mL were verified ($r^2 \geq 0.999$, Fig. S1). In addition, the LOQ values of PHT and CBZ showed 1.56 and 0.40 µg/mL, respectively. Next, the analytical validation for the sample preparation is examined and shown in Table 1. The FDA guidelines require that the deviation in accuracy and accuracy be $\leq \pm 20\%$. The recovery and stability rates of PHT and CBZ at QC were within permissible standard values under all conditions and could be measured with good reproducibility. Based on this developed HPLC method, we concluded that the useful, inexpensive, easy-to-use and routine TDM procedure of PHT and CBZ was assessed, as well as the selectivity, accuracy, precision and

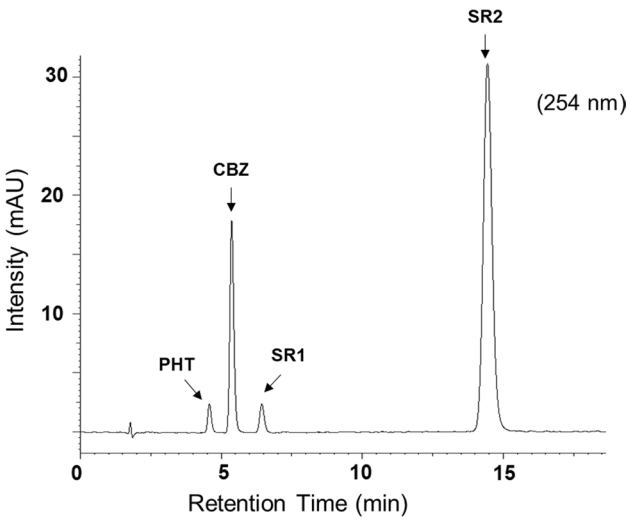

Fig. 2 SR-HPLC chromatogram of PHT, CBZ, SR1, and SR2 standard solution (concentration levels of 10 µg/mL). PHT was detected at a retention time of 4.6 min, CBZ at 5.4 min, SR1 at 6.4 min, and SR2 at 14.3 min based on 254 nm

Table 1 Analytical validation of PHT and CBZ in QC samples

Validation test	Analytes	Spiked levels (μg/mL)	Detected concentrations (μg/mL)	Accuracy (%)	Precision (%)
Intra-day	PHT	5	4.52	90.4	4.06
		10	8.62	86.2	4.42
		20	17.62	88.1	2.93
	CBZ	5	4.64	92.8	2.28
		10	9.10	91.0	2.40
		20	18.58	92.9	2.96
Inter-day	PHT	5	4.52	90.4	4.03
		10	9.26	92.6	3.23
		20	17.68	88.4	1.43
	CBZ	5	4.78	95.6	3.72
		10	9.36	93.6	1.30
		20	17.67	88.4	0.91
Process/wet extract	PHT	5	4.80	96.0	3.71
		10	9.52	95.2	2.38
		20	18.71	93.6	0.90
	CBZ	5	4.72	94.4	3.76
		10	9.35	93.5	1.46
		20	18.49	92.5	0.29
Bench-top	PHT	5	4.51	90.2	1.77
		10	8.86	88.6	1.30
		20	17.61	88.1	1.01
	CBZ	5	4.40	88.0	1.06
		10	8.90	89.0	0.36
		20	17.77	88.9	0.53
Freeze and thaw	PHT	5	4.59	91.8	2.29
		10	8.91	89.1	7.38
		20	19.60	98.0	1.70
	CBZ	5	4.57	91.4	0.99
		10	9.86	98.6	1.51
		20	19.33	96.7	2.42
Long-term	PHT	5	4.54	90.8	2.66
		10	9.31	93.1	1.25
		20	18.26	91.3	0.58
	CBZ	5	4.71	94.2	0.69
		10	9.24	92.4	0.63
		20	18.15	90.8	0.25

($n = 6$)

stability of the QC samples (spiked levels of 5, 10 and 20 μg/mL, respectively).

Calculation of RMS values of PHT and CBZ for SR-HPLC method

To develop the SR-HPLC method, a reference compound is needed to investigate the simultaneous separation of PHT and CBZ based on previous conditions. In this study, we selected SRs with very similar chemical structures that could be obtained from a common reagent company (Fig. 1). The

Table 2 RMS values of PHT and CBZ for SR-HPLC method

Analytes	Range (μg/mL)	RMS values
PHT	LOQ-20	0.859
	15–50	0.837
	LOQ-50	0.839
	Mean (RSD, %)	0.848 (1.41%)
CBZ	LOQ-20	0.256
	15–50	0.269
	LOQ-50	0.257
	Mean (RSD, %)	0.263 (2.76%)

Table 3 Compared data from RMS and absolute calibration methods for accuracy of PHT and CBZ in QC samples

Analytes	Spiked levels (μg/mL)	RMS method		Absolute calibration method	
		Quantitative value	RSD (%)	Quantitative value	RSD (%)
PHT	5	4.35	2.9	4.36	3.2
	10	9.27	1.43	9.27	2.5
	20	18.99	3.04	19.97	1.49
CBZ	5	4.05	1.06	4.45	2.52
	10	8.38	1.1	9.23	1.74
	20	20.49	0.42	18.88	1.49

purities of PHT, CBZ, SR1, and SR2 were evaluated using qNMR, and the purities were high such as PHT (99.99%), CBZ (99.04%), SR1 (99.53%), and SR2 (98.78%). The reproducible values of all standards were relative standard deviation (RSD) < 1.0% ($n = 3$). Using the adjusted solutions of PHT, CBZ, SR1, and SR2, the averaged RMS values (PHT = 0.848 based on SR1 and CBZ = 0.263 based on SR2 for 3 d) were calculated based on the calibration slopes of six ranges, that is, 0 (origin)–20, 15–50 and 0–50 μg/mL using previous conditions at 254 nm (Table 2). To examine the accuracy and applicability, the RMS and the absolute calibration methods were compared with QC samples at three concentrations (5, 10, and 20 μg/mL). The results are presented in Table 3. The established differential definite quantities of PHT and CBZ were similar with that of RMS and the absolute calibration methods based on RSD < 5.10%. In the developed and validated SR-HPLC method, the calibration range levels of PHT and CBZ were derived using the peak ratio with RMS values on a single run.

Clinical application for TDM of PHT and CBZ in plasma samples

An exploratory comparative study was performed on 7 patients with PHT and 18 with CBZ administration. At the clinical site, a common practice with CLIA was performed according to the TDM procedure. CLIA showed that PHT and CBZ levels ranged from 2.2 to 18.8 μg/mL and 2.7 to 10.1 μg/mL, respectively. On the other hand, the SR-HPLC method indicated that PHT and CBZ levels ranged from 1.4 to 16.7 μg/mL and 2.7 to 10.1 μg/mL, respectively. Figure 3 shows the different observed chromatograms of PHT and CBZ in plasma from the patients. The Passing and Bablok regression analysis and the Bland–Altman plot are shown in Figs. 4 and 5. The values of the correlation coefficient and slope of the intercept were PHT = 0.964 and 0.992647 and CBZ = 0.969 and 1.072089, respectively.

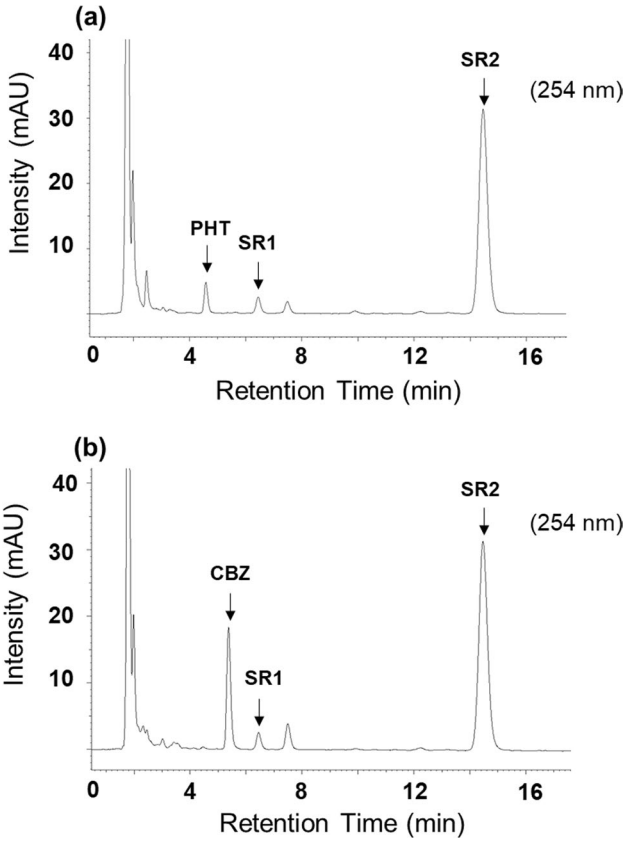

Fig. 3 SR-HPLC chromatograms of plasma samples from patients receiving 300 mg/day PHT treatment (**a**), CLIA: 18.8 μg/mL, SR-LC assay: 16.74 μg/mL, and receiving 600 mg/day CBZ treatment (**b**), CLIA: 10.1 μg/mL, SR-LC assay: 10.1 μg/mL

Discussion

Typical previous study showed that the simultaneous quantification of PHT and CBZ in human serum using simple HPLC method [9]. These validations indicated that LOQ and accuracy (absolute recovery) values were 0.2 μg/mL, and range from 95.65 to 103.33% [9]. On the other hand, our HPLC method showed that the LOQ and accuracy values were 1.56 (PHT)/0.40 (CBZ) μg/mL and range from 86.2 to 98.6%. These both methods were suitable for TDM procedure and can also be used for pharmacokinetic studies conducted in humans. In addition, using the SR-HPLC method for first clinical application, our study made simultaneous quantification of PHT and CBZ possible in less time and more affordable regarding to TDM procedure. The SR-HPLC method have been used in the food industry but has not been clinically applied. Our study has shown the potential for the clinical application of the SR-HPLC method and concept. Several studies have reported the simultaneous quantitation of PHT and CBZ using HPLC techniques [5–10]. However, in cases of simultaneous quantitation using HPLC techniques, it is necessary to purchase standard

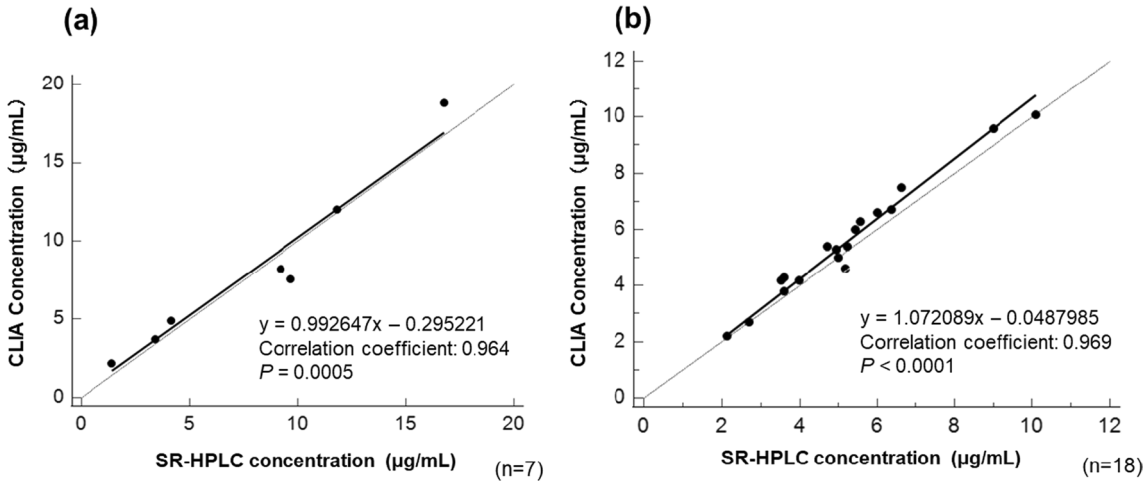

Fig. 4 Passing and Bablok regression analysis results for a comparative study between SR-HPLC and CLIA concentrations of PHT ($n=7$) (**a**) and CBZ (n = 18) (**b**)

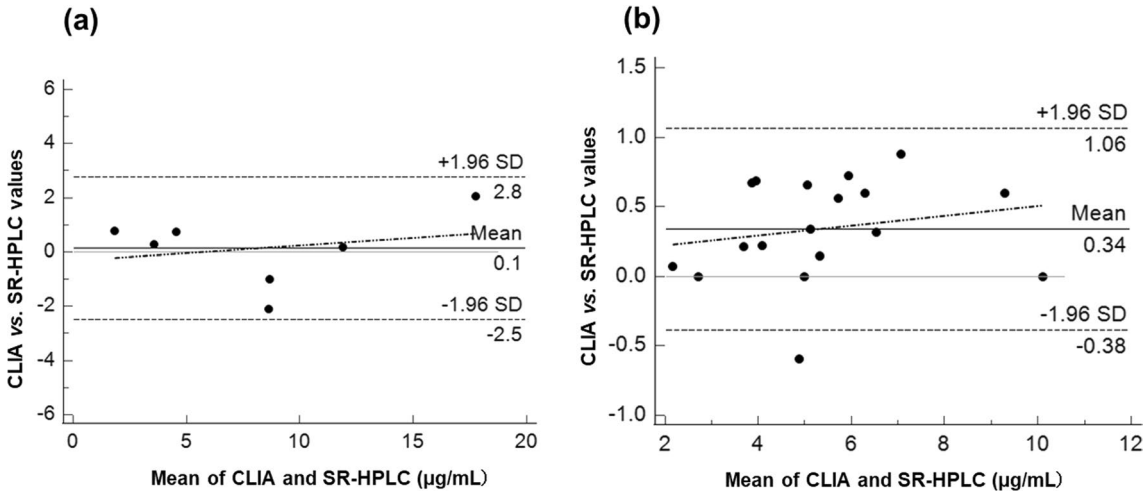

Fig. 5 Bland–Altman plots for compared study between SR-HPLC and CLIA concentrations of PHT ($n=7$) (**a**) and CBZ ($n=18$) (**b**)

products of each drug and prepare a calibration curve for quantitation. However, these HPLC methods are costly and require time to create a calibration curve. Thus, the SR-HPLC method based on RMS values was developed for the clinical application of useful and accurate TDM procedure.

We also compared the concentrations of PHT and CBZ with the frequently used CLIA evaluation. The results showed that the SR-HPLC method can accurately quantify PHT and CBZ in plasma samples from patients. It has been reported that blood drug concentration measurement by immunoassay, which is currently frequently used, shows cross-reactivity with analogs because of its principle. In particular, cross-reactivity with hydroxyzine and cetirizine, antihistamines, and carbamazepine-10, 11-epoxide (CBZ-E), an active metabolite of CBZ, has been reported [26, 27]. Among them, there are some reports that the crossing property of CBZ-E is approximately 90% [26–28]. The crossing of CBZ-E depends on the reagent, and the CLIA carbamazepine used in this study has been reported to have low crossing of CBZ-E [29]. In this study, there was no significant difference between the quantitative values obtained using the SR-HPLC method and CLIA evaluation. However, from the results of the Bland–Altman plot, the difference between the mean values of the quantitative values (CLIA *vs.* SR-HPLC) was PHT: 0.14 and CBZ: 0.34. Quantitative values of CLIA were higher for CBZ than for PHT. The difference between the quantitative values of these SR-LC and CLIA values were not considered a major clinical problem. However, although the average value of CBZ-E/CBZ was 0.23

[30], the correlation coefficient tended to decrease when it exceeded 0.4 [29]. In cases of high CBZ-E concentrations, attention should be paid to the measured CLIA values. Considering the cross-reactivity of CBZ-E, it was suggested that CBZ quantification based on chromatographic techniques is necessary.

The SR-HPLC method could be applied to drugs other than PHT and CBZ and is considered a breakthrough method for measuring blood concentrations of other drugs. Using this developed method, simultaneous quantitation of various drugs in plasma/serum samples would be possible in the future for TDM procedures.

Conclusions

Using this developed SR-HPLC method in clinical study, simultaneous quantitation of PHT and CBZ in plasma samples with ease has now become possible, at a low cost, and in a short time without using a calibration curve. In addition, it is possible that this quantification method can be applied to other drugs and metabolites for clinical stage. The proposed SR-HPLC method is expected to significantly contribute to the TDM field because of the simple and highly accurate quantitation of drugs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s44211-023-00266-z.

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Data availability On reasonable request, derived data supporting the findings of this study are available from the corresponding author after approval from the Ethical Committee of the Ritsumeikan University.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

References

1. S.H.Y. Wong, J. Pharm. Biomed. Anal. (1989). https://doi.org/10.1016/0731-7085(89)80041-X
2. A.H. Kumps, J. Pharm. Biomed. Anal. (1982). https://doi.org/10.1007/BF00313405
3. H.M. Neels, A.C. Sierens, K. Naelaerts, S.L. Scharpé, G.M. Hatfield, W.E. Lambert, Clin. Chem. Lab. Med. (2004). https://doi.org/10.1515/CCLM.2004.245
4. P.A. Datar, J. Pharm. Anal. (2015). https://doi.org/10.1016/j.jpha.2015.02.005
5. D.C. Turnell, S.C. Trevor, J.D. Cooper, Ann. Clin. Biochem. (1983). https://doi.org/10.1177/000456328302000106
6. H. Sayo, H. Hatsumura, M. Hosokawa, J. Chromatogr. (1988). https://doi.org/10.1016/s0378-4347(00)81978-7
7. G.K. Szabo, R.J. Pylilo, R.J. Perchalski, T.R. Browne, J. Chromatogr. (1990). https://doi.org/10.1016/s0021-9673(01)88952-4
8. B. Rambeck, T.W. May, M.U. Jürgens, V. Blankenhorn, U. Jürges, E. Korn-Merker, A. Sälke-Kellermann, Ther. Drug Monit. (1994). https://doi.org/10.1097/00007691-199412000-00013
9. K.M. Patil, S.L. Bodhankar, J. Pharm. Biomed. Anal. (2005). https://doi.org/10.1016/j.jpba.2005.02.045
10. E. Greiner-Sosanko, D.R. Lower, M.A. Virji, M.D. Krasowski, Biomed. Chromatogr. (2007). https://doi.org/10.1002/bmc.753
11. M.J. Tutor-Crespo, J. Hermida, J.C. Tutor, J. Clin. Lab. Anal. (2007). https://doi.org/10.1002/jcla.20115
12. J. Hermida, M.D. Bóveda, F.J. Vadillo, J.C. Tutor, Clin. Biochem. (2002). https://doi.org/10.1016/s0009-9120(02)00299-0
13. Ó. Guerrero Garduño, D.F. González-Esquivel, C. Escalante-Membrillo, Á. Fernández, I.S. Rojas-Tomé, H. Jung Cook, N. Castro, Biomed. Chromatogr. (2016) https://doi.org/10.1002/bmc.3631
14. T. Liu, R.R. Kotha, J.W. Jones, J.E. Polli, M.A. Kane, J. Pharm. Biomed. Anal. (2019). https://doi.org/10.1016/j.jpba.2019.112816
15. S.T. Hassib, H.M.A. Hashem, M.A. Mahrouse, E.A. Mostafa, Biomed. Chromatogr. (2018). https://doi.org/10.1002/bmc.4253
16. L. Yin, T. Wang, M. Shi, Y. Zhang, X. Zhao, Y. Yang, J. Gu, J. Sep. Sci. (2016). https://doi.org/10.1002/jssc.201501067
17. M. Takahashi, Y. Nishizaki, N. Sugimoto, K. Sato, K. Inoue, J. Chromatogr. A (2018). https://doi.org/10.1016/j.chroma.2018.04.029
18. M. Takahashi, Y. Nishizaki, K. Morimoto, N. Sugimoto, K. Sato, K. Inoue, Sep. Sci. Plus 1(2018) https://doi.org/10.1002/sscp.201800081
19. M. Takahashi, Y. Nishizaki, N. Masumoto, N. Sugimoto, K. Sato, K. Inoue, J. Sci. Food Agric. (2021). https://doi.org/10.1002/jsfa.11013
20. M. Takahashi, K. Morimoto, Y. Nishizaki, N. Masumoto, N. Sugimoto, K. Sato, K. Inoue, Chem. Pharm. Bull. (2022). https://doi.org/10.1248/cpb.c21-00621
21. Guidance for Industry, Bionanalytical Method Validation, US Department of Health and Human Services/Food and Drug Administration Centre for Drug Evaluation and Research (CDER)/Centre for Veterinary Medicine (CVM), 2018, May.
22. H. Passing, Bablok. J. Clin. Chem. Clin. Biochem. (1983). https://doi.org/10.1515/cclm.1983.21.11.709
23. R.H. Queiroz, C. Bertucci, W.R. Malfará, S.A. Dreossi, A.R. Chaves, D.A. Valério, M.E. Queiroz, J. Pharm. Biomed. Anal. (2008). https://doi.org/10.1016/j.jpba.2008.03.020
24. M.M. Bhatti, G.D. Hanson, L. Schultz, J. Pharm. Biomed. Anal. (1998). https://doi.org/10.1016/s0731-7085(97)00265-3
25. The Japanese Pharmacopoeia, Eighteenth Edition, 2.46 Residual Solvents, June 7 (2021) 58–64. https://www.mhlw.go.jp/content/11120000/000943877.pdf
26. A. Dasgupta, M.A. Reyes, B.G. Davis, A.M. Marlow, M. Johnson, J. Clin. Lab. Anal. (2010). https://doi.org/10.1002/jcla.20400
27. G.A. McMillin, J.M. Juenke, G. Tso, A. Dasgupta, Am. J. Clin. Pathol. (2010). https://doi.org/10.1309/AJCPFAHVB26VVVTE
28. G.A. McMillin, J.M. Juenke, M.J. Johnson, A. Dasgupta, J. Clin. Lab. Anal. (2011). https://doi.org/10.1002/jcla.20460
29. R. Takahashi, K. Imai, S. Sugai, T. Yoshida, M. Nakamura, S. Hamano, F. Iwasaki, Iryo Yakugaku. (2012). https://doi.org/10.5649/jjphcs.38.741
30. D.A. Svinarov, C.E. Pippenger, Ther. Drug Monit. (1996). https://doi.org/10.1097/00007691-199612000-00006