Brief Report

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Analytical method validation and feasibility of salivary pregabalin measurement in Japanese volunteers: A pilot study

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SUMMARY: This pilot study evaluated whether salivary pregabalin concentrations reflect plasma levels in Japanese volunteers to support non-invasive therapeutic drug monitoring (TDM). Four healthy adults received a single 75-mg orally disintegrating tablet; unstimulated saliva and venous blood were collected 1 hour post-dose using a standardized passive-drool protocol with pre-collection rinsing. Pregabalin was quantified by a high-performance liquid chromatography with fluorescence detection. The assay met bioanalytical performance criteria across both matrices (excellent linearity, recovery > 94.2%, precision ≤ 10%, stability within 5.2%). At 1 hour, median concentrations were 1.96 μg/mL (plasma) and 0.466 μg/mL (saliva). In paired analysis (n = 4), saliva and plasma showed a positive trend (r = 0.838, p = 0.298). Given the small sample size, these results are considered exploratory and demonstrate the feasibility of the analytical approach rather than providing definitive clinical evidence. Under standardized collection conditions, salivary pregabalin concentrations appear to yield clinically interpretable estimates of systemic exposure, warranting validation in larger, multi-time-point cohorts to establish actionable saliva-to-plasma conversion thresholds and evaluate clinical utility.

Keywords: Therapeutic drug monitoring, saliva, plasma, high-performance liquid chromatography, fluorescence detection, noninvasive sampling

1. Introduction

Pregabalin is a ligand of the α2δ subunit of voltage-gated calcium channels and attenuates excitatory neurotransmitter release. It is widely prescribed for neuropathic pain and fibromyalgia and is often considered among first-line options in chronic pain management (*1,2*). Its pharmacokinetic profile comprises rapid absorption (Time to maximum concentration: T_{max} of approximately 1 h), negligible plasma protein binding (< 1%), and predominantly renal elimination, with most of the dose excreted unchanged in urine (*1,2*).

Older adults frequently experience age-related declines in renal function, increasing the risk of accumulation and central nervous system adverse effects such as dizziness and somnolence, which contribute to falls, fractures, and functional decline; because exposure is strongly influenced by renal function, these risks merit particular attention (*1,2*). Although initiation at low doses and gradual titration are recommended, inter-individual variability limits dose-based safety assessments alone.

Therapeutic drug monitoring (TDM) may therefore improve safety. However, blood-based TDM is invasive and difficult to implement in home-care or community settings. Saliva has emerged as a non-invasive alternative matrix: good saliva–plasma correlations have been reported for several drugs, including lithium and phenytoin, and recent reviews have synthesized broader evidence supporting saliva as a TDM matrix when the unbound fraction is clinically relevant (*3-5*).

Given its small molecular size and extremely low protein binding, pregabalin is mechanistically expected to diffuse into saliva (*2*). Nonetheless, human evidence remains limited and derives largely from Jordanian patients (*6*); no data have been reported in Asian populations. Generating preliminary data in Japanese individuals is therefore an essential first step toward evaluating whether salivary pregabalin concentrations can be used for monitoring in broader clinical settings. As an initial investigation, this pilot study focuses on feasibility and analytical behavior. We quantified salivary and plasma pregabalin concentrations in healthy Japanese volunteers and examined their relationship to assess the analytical feasibility of salivary pregabalin monitoring as

a non-invasive approach.

2. Materials and Methods

2.1. Study design

This pilot study assessed whether salivary pregabalin concentrations can serve as a surrogate for plasma levels. To coincide with the expected T_{max}, saliva and plasma were collected 1 hour post-dose (*1*). The protocol was approved by the Ethics Committee of Tokyo Metropolitan Bokutoh Hospital (Approval No. 05-112); written informed consent was obtained from all participants. This study was conducted in accordance with the Declaration of Helsinki (revised in 2013).

2.2. Participants and dosing

Four healthy adults (21-41 years; mean 27.5 ± 9.1 years; 2 males, 2 females) received a single 75-mg dose of pregabalin as orally disintegrating tablets (ODT). To mitigate oral-cavity contamination, participants avoided food and beverages (except water) for ≥ 1 h before dosing, swallowed without chewing, and rinsed the mouth with water immediately after complete disintegration (*7,8*). Saliva sampling was performed 60 min post-dose; a pre-collection water rinse was repeated 10 minutes before sampling.

2.3. Reagents and chemicals

Pregabalin, gabapentin (internal standard, IS), and 4-chloro-7-nitrobenzofurazan (NBD-Cl) were from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). A high-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and distilled water were from Kanto Chemical Co., Inc. (Tokyo, Japan). Potassium dihydrogen phosphate (KH_2PO_4; FUJIFILM Wako, Osaka, Japan) was used in the mobile phase.

2.4. Analytical procedures

Pregabalin in plasma and saliva was quantified using a HPLC system (JASCO Corporation, Tokyo, Japan) consisting of a vacuum degasser, pump, gradient unit, and autosampler, with an FP-2020 spectrofluorometer (excitation/emission wavelengths [Ex/Em] of 470/530 nm). Separation was achieved on a C18 column (Capcell Pak C18 MG II, 250 × 4.6 mm, 5 µm; Osaka Soda Co., Ltd., Osaka, Japan) at 0.75 mL/min using 0.5% KH_2PO_4 (pH 4.5) and methanol. Samples (50 µL) were protein-precipitated, derivatized with NBD-Cl, and injected using gabapentin as the internal standard.

2.5. Sample preparation

Stock solutions of pregabalin and gabapentin (1 mg/mL each) were prepared in distilled water. To 50 µL of plasma or saliva, 10 µL of IS (0.2 µg gabapentin) and 140 µL of methanol were added. Following vortexing (60 s) and centrifugation (15,000× *g*, 10 min, 4°C), 100 µL of supernatant was mixed with 25 µL of borate buffer (0.25 mol/L, pH 10.5) and 100 µL of NBD-Cl (10 mg/mL). The mixture was reacted at 60°C for 15 min. A 20-µL aliquot was then injected.

2.6. Saliva collection

Unstimulated whole saliva was collected with participants seated and the head slightly tilted forward. After the standardized water rinse, saliva was collected by the passive drool method into polypropylene tubes for 3 min, targeting ≥ 1 mL.

2.7.1. Calibration and method validation

Calibration ranges were 0.1, 0.5, 1, 5, 10, and 20 µg/mL for plasma and 0.0125, 0.025, 0.050, 0.10, 0.50, and 1.0 µg/mL for saliva. Method validation followed FDA bioanalytical guidelines (*9*). Recovery and accuracy were determined at 0.1-20 µg/mL (plasma) and 0.0125-1.0 µg/mL (saliva). Precision was evaluated using five sets of control samples intra-day and on five different days inter-day at 0.1, 0.5, 1, 5, 10, and 20 µg/mL (plasma) and 0.0125, 0.025, 0.050, 0.10, 0.50, and 1.0 µg/mL (saliva).

2.7.2. Sample stability

Stability in plasma (0.1, 1.0, and 20 µg/mL) and saliva (0.0125, 0.10, and 1.0 µg/mL) was evaluated under bench-top (20°C, 6 h), processed sample (4°C, 24 h), long-term (−60°C, 4 weeks), and freeze-thaw (three cycles from −60°C) conditions ($n = 5$ for all conditions).

2.8. Statistical analysis

Pearson's correlation and Bland–Altman analyses were used to assess the plasma–saliva relationship, conducted with EZR (*10*) and JMP.

3. Results and Discussion

The assay demonstrated excellent linearity for plasma (0.1-20 µg/mL, $R^2 = 0.9994$) and saliva (0.0125-1.0 µg/mL, $R^2 = 0.9997$). Recovery exceeded 94.2%, with intra- and inter-day coefficient of variation (CV) s ≤ 10%. Stability tests under all conditions showed < 5.2% degradation, aligning with FDA bioanalytical guidance (*9*). While Idkaidek *et al*. (*6*) reported a TDM study in 44 Jordanian patients using capsules and proposed therapeutic ranges, the novelty of this study lies in its focus on the Japanese population using an ODT formulation and a standardized passive-drool collection protocol.

At 1 h after a single 75-mg oral dose, median concentrations were 1.96 µg/mL (CV 11.91%) in plasma and 0.466 µg/mL (CV 21.92%) in saliva (Table 1). Despite the risk of oral-cavity residue from the ODT formulation (*7,8*), the standardized passive-drool collection protocol was designed to mitigate potential contamination. The four paired observations showed a strong positive relationship (Pearson's $r = 0.838$) (Figure 1). However, this association did not reach statistical significance ($p = 0.298$), primarily due to the limited sample size ($n = 4$). These findings, along with the calculated saliva-to-plasma (S/P) ratios (median: 0.241; range: 0.185-0.275), suggest that salivary pregabalin concentrations may reflect systemic exposure, although confirmation in a larger cohort is required.

Bland–Altman analysis was performed for exploratory visualization (Figure 1). While all data points fell within the limits of agreement, these limits are highly unstable with $n = 4$ and should not be interpreted as a definitive demonstration of agreement. In Jordanian patients, trough and peak sampling at steady state showed good saliva–plasma correlations (0.71-0.83), and preliminary therapeutic ranges were proposed for both matrices, supporting feasibility while highlighting population characteristics, formulation (capsule vs ODT), and procedural differences as potential sources of variance (*6*).

Beyond pregabalin, strong saliva–serum relationships have been reported for lithium and for phenytoin; recent reviews underscore saliva's value as a non-invasive TDM matrix when the unbound fraction is clinically relevant (*3-5*). To enhance comparability across cohorts, future work should pre-specify formulation and sampling protocols (*e.g.*, capsule rather than ODT when feasible, standardized pre-rinse and timing).

From a clinical pharmacology perspective, pregabalin's pharmacokinetic profile renders exposure monitoring particularly relevant in individuals with impaired renal function. This profile also makes saliva—reflecting the diffusible, unbound fraction—mechanistically plausible as a surrogate matrix (*1,2*). However, older adults commonly exhibit reduced salivary flow, which may influence concentration measures; given our cohort's mean age (27 years), generalizability to elderly patients is limited (*11-13*). Future studies should incorporate multi-time-point paired sampling around and beyond T_{max}, capture salivary physiology (flow rate, pH) at collection and define clinically meaningful saliva–plasma thresholds for pregabalin TDM. Given the pilot nature of this investigation, the small sample size limits statistical power; accordingly, the observed

Table 1. Plasma and salivary pregabalin concentrations in healthy volunteers

Volunteer No	Plasma (µg/mL)	Salivary (µg/mL)	S/P ratio
1	1.94	0.359	0.185
2	2.23	0.551	0.247
3	1.66	0.389	0.234
4	1.98	0.544	0.275
Median	1.96	0.466	0.241
(Range)	(1.66-2.23)	(0.359-0.551)	(0.185-0.275)

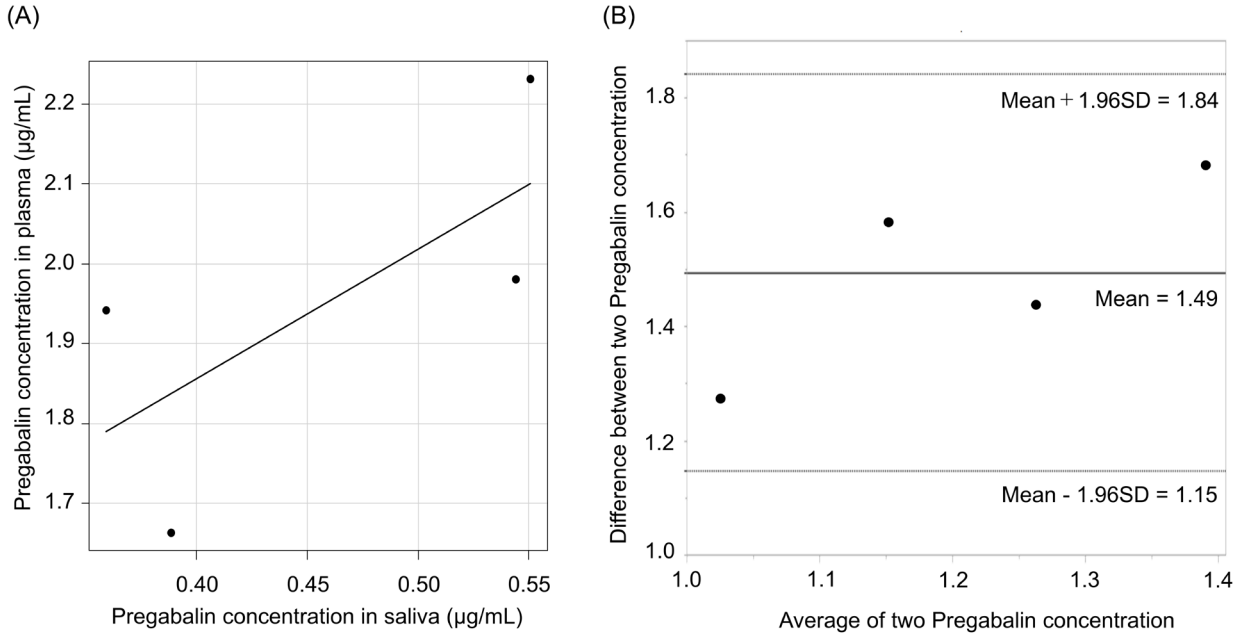

Figure 1. Correlation between plasma and salivary pregabalin concentrations. (**A**) Scatter plot showing the association between plasma and salivary pregabalin concentrations in healthy volunteers ($n = 4$), with Pearson's correlation coefficient indicating a positive relationship between the two matrices ($r = 0.838$). (**B**) Bland–Altman plot of paired plasma–saliva measurements ($n = 4$), showing the mean difference (1.49 µg/mL) and standard deviation (SD: 0.177 µg/mL), and the upper and lower limits of agreement (1.84 and 1.15 µg/mL), with all data points falling within the limits. This plot is intended for exploratory visualization of the differences between the two matrices.

correlation should be interpreted as exploratory rather than confirmatory. Nevertheless, the internal consistency of plasma concentrations across participants supports the feasibility of paired plasma–saliva assessment under a controlled protocol.

The lack of statistical significance ($p = 0.298$) and the potential for oral-cavity residue from the ODT formulation are the primary limitations of this feasibility study. The ODT formulation was selected to reflect its increasing use in Japanese clinical practice, particularly for patients with dysphagia. Although mouth rinsing was implemented to mitigate contamination, ODT residue remains a potential confounding factor. Future confirmatory studies should ideally use capsules to eliminate this risk or incorporate early post-dose rinse-fluid testing to verify the absence of residue.

These findings provide initial evidence that structured saliva collection can yield clinically interpretable pregabalin concentrations. Future studies should establish actionable saliva-to-plasma conversion thresholds using larger, multi–time-point cohorts— particularly in older adults and patients with renal impairment — to define decision-making targets and to assess clinical outcomes.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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