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percentages of an organic modi er, which are suitable conditions for LC-MS/MS analysis [2-6].

Citation:

Remove matrix e ect

When urinesamples were analyzed under the selected conditions, the analyte peak shape deteriorated. It was thought that the peak deterioration might be caused by endogenous substances in the urine. Samples prepared with varying urine percentages from 0 to 5 %, keeping constant the concentration of the analyte, were analyzed, and the analyte peak shape deteriorated more with increasing urine percentages [Figure 7]. In addition, peak shape deterioration was also observed in standard samples at high analyte concentrations applied to the column [Figure 8]. Based on these observations, we reasoned tha the peak shape deterioration was caused by the total amount of ionic substances applied to the column, which max out the possible sites for ionic interaction. erefore, a process to remove highly ionized substances coexisting with the analyte was considered to be necessa before the sample was subjected to the PFPP column. When acidi ed aqueous bu er solution was used with a reversed-phase column for the process, the analyte peak shape was improved. However, the pea area was lower than those of the standard solution, suggesting that ion suppression occurred due to the co-elution of endogenous components probably participating in weakly hydrophobic interactions (data not shown).

e ion suppression could be avoided by adding acetonitrile to the bu er. A good peak shape without ion suppression was achieved with 5 mmol/L ammonium formate bu er (pH 2.9) with 10% acetonitrile content, suggesting that the components retained by the ionic and weakly hydrophobic interactions were removed.

Application of the method

e optimized method was validated for assaying the analyte in human urine in accordance with the FDA guidelines for bioanalytical method validation [7]. e LC-MS/MS system used in the validation study is depicted in Figure 3.

e b e l optimi (.575mu)-96(10%)-96(acetonitrile)]TJ T* [(content

in a mobile phase containing 5 mmol/L ammonium formate bu er (pH 2.9), the retention ability of the analyte on the PFPP column was compared with that on an ODS column (Luna C18 (2), 50 mm length x 2 mm, 5 µm, Phenomenex, CA, USA). e analyte was retained on the PFPP column at acetonitrile percentages of 85% or below but was not retained on the ODS column under any conditions studied [Figure 6]. At 80% acetonitrile or below, a good peak shape of the analyte was obtained. erefore, 80% acetonitrile containing 5 mmol/L ammonium formate bu er (pH 2.9) was selected as the mobile phase for the LC-MS/MS analysis. us, conditions enabling a high ionization e ciency and an adequate retention of the analyte were achieved with an acidic bu er solution and with high percentages of an organic component, which is highly advantageous for the use of a PFPP column with LC-ESI-MS analysis.

uorinated phenylalanyl derivative compound, GW823093, in human urine was developed using a PFPP analytical column connected in series with an ODS column employed for sample clean-up. e analyte was strongly retained on the PFPP column, and a good peak shape was obtained without ion suppression. e method was validated and successfully applied to a clinical study.

&RQÀLFW RI ,QWHUHVW

7KH DXWKRUV GHFODUHG QR FRQÀLFW RI LQWHUHVW

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1. Reta M, Carr PW, Sadek PC, Rutan SC (1999) Comparative Study of Hydrocarbon, Fluorocarbon, and Aromatic Bonded RP-HPLC Stationary Phases by Linear Solvation Energy Relationships. Anal Chem 71: 3484-3496.
2.