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Enantioselective determination of ibuprofen in saliva by liquid chromatography/tandem mass spectrometry with chiral electrospray ionization-enhancing and stable isotope-coded derivatization

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ABSTRACT

A method was developed and validated for the enantioselective determination of trace ibuprofen (IBU) in saliva using liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) combined with the derivatization using a chiral ESI-enhancing reagent, (*S*)-1-(4-dimethylaminophenylcarbonyl)-3-aminopyrrolidine (DAPAP), and its isotope-coded analog, $^{2}H_{4}$-DAPAP (*d*-DAPAP). The DAPAP-derivatization enabled the highly sensitive detection [detection limit, 0.15 fmol (equivalent to 30 fg of intact IBU) on the column] and complete separation (resolution 3.1) of the IBU enantiomers. The use of *d*-DAPAP significantly improved the assay precision and accuracy; the intra- ($n = 5$) and inter-assay ($n = 5$) relative standard deviations did not exceed 6.2%, and good accuracy (101.3–106.1%) was obtained. The developed method was successfully applied to the quantitative analysis of IBU in saliva. Using this method, salivary concentration–time profiles of each enantiomer after a single oral administration of the racemic IBU to healthy subjects were obtained. The area under the salivary concentration-time curve of the (*S*)-enantiomer was ca. twice that of the (*R*)-enantiomer due to the unidirectional chiral inversion of the (*R*)- to (*S*)-enantiomer in vivo. Thus, saliva-based noninvasive pharmacokinetic analyses of IBU enantiomers were achieved by this method.

1. Introduction

Although ibuprofen [(*R*,*S*)-2-(4-isobutylphenyl)propionic acid, IBU], a non-steroidal anti-inflammatory drug (NSAID), is usually used as a racemic mixture, the pharmacodynamics, pharmacokinetic and toxicological properties of the two enantiomers are different in vivo [1]. (*S*)-IBU exerts its anti-inflammatory effect by inhibiting cyclooxygenase-1 and -2, while (*R*)-IBU is much less potent and effectively undergoes a unidirectional chiral inversion by α-methylacyl-CoA racemase in vivo [1–3]. Therefore, it is useful to monitor the enantiomers in biological fluids not only to keep the drug therapy safe and effective, but also to study the metabolic inversion of IBU in vivo.

Saliva has recently been attracting attention as a new tool in clinical examinations and therapeutic drug monitoring [4,5], because saliva offers an easy, noninvasive, stress-free and real-time repeated sampling whereas blood collection is either undesirable or difficult. Saliva analysis has a great potential to facilitate clinical research. Although a method using high-performance liquid chromatography with chemiluminescence detection for the analysis of IBU in saliva is described in the literature [6], to the best of our knowledge, there is no report of the enantioselective determination of IBU in saliva.

Liquid chromatography/electrospray-tandem mass spectrometry (LC/ESI-MS/MS) has been used for the analysis of IBU in biological fluids [2,7–9]. The enantioselective analyses of IBU using LC/ESI-MS/MS are based on the use of a chiral stationary phase (CSP) column [2,8] or on the diastereomeric derivatization using a chiral reagent (chiral derivatization) [9]. The CSP column is comparatively expensive and sometimes applicable only in the normal-phase mode, which is not always suitable for biological sample analyses. On the contrary, the method based on chiral derivatization is more practical for the LC/ESI-MS/MS of IBU in biological samples, because this method uses a conventional reversed-phase column, such as an octadecylsilyl (ODS)-silica column. Furthermore, the ESI-detection responses of carboxy group-containing compounds, including IBU, can be enhanced by the derivatization [10–16]. We recently developed a novel chiral derivatization reagent, (*S*)-1-(4-dimethylaminophenylcarbonyl)-3-aminopyrrolidine (DAPAP), which was very effective for the

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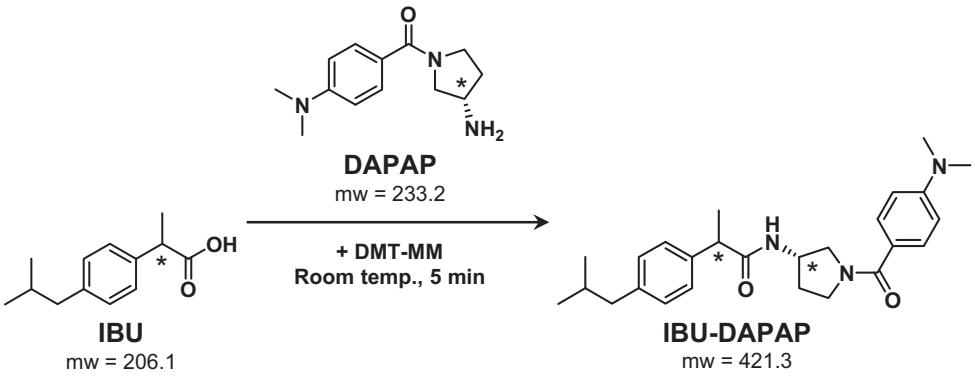

Fig. 1. Derivatization reaction scheme of IBU with DAPAP. * indicates an asymmetric carbon.

enantiomeric separation and sensitive ESI-MS/MS detection of IBU (Fig. 1) [16].

The matrix effects caused by endogenous components often occur during the determination of a low abundant analyte using LC/ESI-MS/MS. The ionization efficiency is also sometimes different between two single runs even for the same analyte. A stable isotope-labeled analog of the analyte is widely used as an internal standard (IS) to overcome the matrix effects and run-to-run ionization differences for an accurate quantification. However, the stable isotope-labeled IBU is very expensive and its synthesis is not always easy. The stable isotope-coded derivatization is an alternative way to introduce a stable isotope-coded moiety to the analyte, and the resulting derivative can be used as a substitute for a stable isotope-labeled IS [17,18]. The general outline for the enantioselective determination of salivary IBU is as follows. IBU in the saliva sample is derivatized with DAPAP, while a standard IBU is separately derivatized with ^{2}H-coded DAPAP and spiked into the sample solution prior to the analysis. Since the isotopic pairs of the derivatives elute at the almost same time in a single run, the matrix effects and ionization process for the DAPAP-derivatized IBU are expected to be identical with the ^{2}H-coded DAPAP-derivatized IBU. Thus, the stable isotope-coded derivatization can improve the analytical performance and precision without a stable isotope-labeled IS. DAPAP contains the dimethyamino-group, and therefore, its ^{2}H-coded analog can be easily prepared.

Based on this background information, in this study, we developed a method for the enantioselective determination of IBU in saliva by LC/ESI-MS/MS combined with the chiral, ESI-enhancing and stable isotope-coded derivatization. The matrix effects and run-to-run ionization differences were reduced by the use of the derivative with $^2H_4$-DAPAP (*d*-DAPAP). The application of the method to the analysis of saliva samples obtained from healthy subjects who had orally taken racemic IBU was also described.

2. Experimental

2.1. Chemicals and reagents

Racemic IBU and (*S*)-IBU were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma–Aldrich Japan (Tokyo, Japan), respectively. A stock solution of racemic IBU was prepared as a 100 μg/mL solution in acetonitrile. Subsequent dilutions were carried out with acetonitrile to prepare 0.40, 1.0, 2.0, 4.0, 10, 20 and 40 ng/mL solutions. DAPAP was synthesized in our laboratories as previously reported [16]. $^2H_4$-DAPAP (*d*-DAPAP) was synthesized from $^2H_4$-4-dimethylaminobenzoic acid [19]; this starting material was condensed with (*S*)-3-(*tert*-butoxycarbonylamino)pyrrolidine and then converted to *d*-DAPAP as previously reported [16]. The isotopic purity of *d*-DAPAP was greater than 99.9%; the $^2H_3$-, $^2H_2$-, $^2H_1$- and $^2H_0$-forms were not detected at all by ESI-MS. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) was from Kanto Chemicals (Tokyo). All other reagents and solvents were of LC/MS or analytical grade.

2.2. LC/ESI-MS/MS

LC/ESI-MS/MS was performed using a Shimadzu LCMS-8030 triple quadrupole mass spectrometer connected to a Shimadzu LC-20AD chromatograph (Kyoto, Japan). A YMC-Pack Pro C18 RS column (5 μm, 150 × 2.0 mm i.d.) was used at the flow rate of 0.2 mL/min and 40 °C. The mobile phase was acetonitrile–10 mM ammonium formate (11:9, v/v) (isocratic elution). The derivatized IBU was analyzed in the positive-ion mode. The MS conditions were as follows: interface voltage, 4.5 kV; Q1 pre-rod bias voltage, −16 V; Q3 pre-rod bias voltage, −30 V; collision energy, 18 eV; nebulizer gas flow rate 3 L/min; drying gas flow rate 15 L/min; desolvation line temperature, 250 °C; heat block temperature, 400 °C and collision gas, 230 kPa. The SRM transitions (precursor and product ions) were *m*/*z* 422.3 → 148.1 and *m*/*z* 426.3 → 152.2 for IBU-DAPAP and IBU-*d*-DAPAP, respectively. LabSolutions software (version 5.53 SP3, Shimadzu) was used for the system control and data processing.

2.3. Collection and pretreatment of saliva

Healthy male volunteers (22–35 years old) took a non-prescription drug (EVE® sugar-coated tablet, SSP Co., Ltd., Tokyo) which contains racemic IBU (150 mg), then 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 h later their saliva (ca. 1 mL) was directly collected into a collecting tube (without a collection device). The saliva samples were stored at −18 °C until used. The volunteers ingested no food and beverage within 1 h prior to the saliva collection. The volunteers also did not brush their teeth within 1 h prior to the sample collection to avoid contamination of the saliva with blood. The volunteers understood the purpose and significance of this experiment and donated their saliva after signing an agreement.

After thawing, the saliva samples were centrifuged at 1000 × *g* for 10 min to precipitate the denatured mucins and solids. The supernatant (20 μL) was diluted with 0.02 M acetic acid (100 μL), and IBU was extracted with ethyl acetate (100 μL). Fifty microliters of the ethyl acetate solution were transferred to another test tube and the solvent was then evaporated under an N_2 gas stream. The residue was subjected to the derivatization with DAPAP [16]; solutions of DMT-MM in methanol (10 mM, 10 μL) and DAPAP (10 μg) in methanol (10 μL) were successively added to the residue, and then the mixture was stored at room temperature for 5 min. The racemic IBU (100 pg) was derivatized with *d*-DAPAP and 1/20th of