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# Determination of ibuprofen enantiomers in breast milk using vortex-assisted matrix solid-phase dispersion and direct chiral liquid chromatography

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ABSTRACT

A mixture of β-cyclodextrin (β-CD) and primary and secondary amine (PSA) sorbents was employed for the extraction and quantification of ibuprofen enantiomers from human breast milk, combining a vortex-assisted matrix solid-phase dispersion method (MSPD) and direct chiral liquid chromatography (CLC) with ultraviolet detection (UV). The MSPD sample preparation procedure was optimized focusing on both the type and amount of dispersion/sorption sorbents and the nature of the elution solvent, in order to obtain acceptable recoveries and avoiding enantiomer conversion. These MSPD parameters were optimized with the aid of an experimental design approach. Hence, a factorial design was used for identification of the main variables affecting the extraction process of ibuprofen enantiomers. Under optimum selected conditions, MSPD combined with direct CLC-UV was successfully applied for ibuprofen enantiomeric determination in breast milk at enantiomer levels between 0.15 and 6.0 $\mu g\,g^{-1}$. The proposed analytical method also provided good repeatability, with relative standard deviations of 6.4% and 8.3% for the intra-day and inter-day precision, respectively.



## 1. Introduction

Ibuprofen (IB), (*R,S*)-2-(4-isobutylphenyl)propionic acid, is a non-steroidal anti-inflammatory drug (NSAID) commercialized as racemic mixture with an annual global production reported in gigagrams [1]. However, its anti-inflammatory action is mainly associated with the (+)-(*S*)-enantiomer. Ibuprofen undergoes stereoselective metabolism, resulting in stereoselective pharmacokinetics parameters, with higher plasma and urinary concentrations for the (+)-(*S*)-isomer [2].

The main difficulties in analyzing non-steroidal anti-inflammatory drugs, such as ibuprofen, in food and biological samples are due to the tight non-covalent interactions established with matrix proteins and the amount of occurring fatty material. Several strategies have been used to extract NSAIDs depending on determination technique; for liquid chromatography separation of NSAIDs in bovine milk and muscle tissue the extraction procedure included deproteinization/extraction with organic solvent and solid phase extraction (SPE) clean-up on OASIS® [poly(*N*-vinylpyrrolidone-divinylbenzene)] cartridges [3]. By gas chromatography (GC) separation technique, solid phase microextraction (SPME) and derivatization to ethyl esters was used to determine NSAIDs in bovine milk [4]. Azzouz et al. have also determined pharmacologically active substances, including IB, in cow, goat and human breast milk by GC with mass spectrometry (MS) detection. After milk deproteinization, sample enrichment and clean-up was done by continuous SPE using the Oasis® sorbent [5].

Like other analytical methods, traditional SPME and SPE have some drawbacks. SPME includes high time consumption, fiber breakage, stripping of coatings, low operating temperature and instability. On the other hand, SPE is one of the most commonly used clean-up techniques, but it involves several steps and requires longer time and higher organic solvent volumes than other modern techniques recently reported. To overcome these drawbacks, methods such as dispersive solid-phase extraction (DSPE) have been developed. This rapid and simple technique was proposed by Anastassiades et al. for food and environmental samples, which was included as a novel clean-up procedure for the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique [6]. DSPE is based on the addition of the sorbent material into an extract aliquot to remove the matrix interferences, which is then separated from the extract bulk by centrifugation. Thus, DSPE avoids passing the

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extract through SPE columns or cartridges, using smaller quantities of both sorbent and solvents and saving time and labor.

Other extraction methods such as magnetic solid phase extraction (MSPE) and matrix solid phase dispersion (MSPD) have also been described to avoid mentioned drawbacks. MSPE has received considerable attention because of its rapid, effective and unique mechanism of solid-liquid separation under a magnetic field. This means that the magnetic supports tagged with the analytes can be isolated from large volumes of sample solutions by a magnet, avoiding other steps such as centrifugation or filtration [7,8]. Thus, Ghorbani et al. have employed ultrasound-assisted MSPE with LC for microextraction of ibuprofen and naproxen from cow milk, human urine, river water and well water [9]. Four NSAIDs, included ibuprofen, have been determined too by MSPE followed by LC with UV detection in wastewater effluents [8]. Other authors have reported the excellent extraction performance of a β-cyclodextrin polymer for MSPE, due to its ability to bind selectively organic molecules into its hydrophobic cavity and to form stable host-guest inclusion complexes [7].

On the other hand, MSPD is an extraction process based on the dispersion of solid adsorbent in the sample. It was developed by Barker et al. for the isolation of drugs from bovine muscle [10], and resulted in a useful tool for the residue extraction from solid, semi-solid, viscous, and mainly proteinaceous and fatty biological matrices [11–16]. Although MSPD was initially developed for extracting solid samples, now it is applied to viscous and complex samples such as milk [16]. MSPD is simple, faster than SPE and it does not require specific equipment and extraction conditions (room temperature and atmospheric pressure) to preserve analytes from degradation and denaturation [2,15,16].

Despite the cited advantages, MSPE and; specially, MSPD sample pretreatment are not commonly used for determination of isomers in general or enantiomers in particular. Characterization and determination of isomers from different honeysuckle samples was carried out using trace MSPD with a β-cyclodextrin [17], and Zhan et al. determined enantiomers of organochlorine pollutants in oil seeds using *n*-hexane/dichloromethane as the eluting solvent (the eluent was evaporated with nitrogen at 30 °C and the residue was re-dissolved in isooctane before CG determination with electron capture detection using a chiral column) [11]. In general, special attention was paid to compatibility between sample preparation and chiral separation in order to get appropriate enantioresolution [18–20].

Several chiral stationary phases (CSPs) are available for ibuprofen enantioseparation by liquid chromatography (LC). Stationary phases of phenylcarbamates of amylose have been employed in normal phase mode using hexane-isopropanol-trifluoroacetic acid mixtures as the mobile phase, modified with a solution of ammonium acetate in methanol before entering into the electrospray interface of the mass spectrometry system [21]. Vancomycin antibiotic stationary phases have also been used for the separation of ibuprofen enantiomers using methanol modified with 4 mM ammonium acetate ($NH_4Ac$) as the mobile phase [22]. A stationary phase based on *(R)*-1-naphthyl-glycine 3,5-dinitrobenzoic acid was used for separation of ibuprofen, ketoprofen and naproxen enantiomers in wastewater using a mobile phase based on 90% (v) tetrahydrofuran and 10% (v) ammonium acetate in methanol [23]. In addition, the enantiomeric separation of ibuprofen has been achieved using a protein-based α-acid glycoprotein (AGP) stationary phase [24,25], which offers some advantages such as the use of aqueous mobile phases.

In this work, we report the development of a vortex assisted MSPD method for the extraction of ibuprofen enantiomers from breast milk, and its determination by direct chiral LC using an α-acid glycoprotein based CSP. The effect of several experimental parameters, such as sorbent type, dispersant amount and nature of extraction solvent, were optimized to obtain the maximum recovery for each enantiomer while maintaining the enantioresolution and the enantiomeric fraction (EF) value at 0.5, typical for racemic mixtures. Different sorbents were selected from preliminary experiments, and the adequate sorbent amounts for the MSPD extraction of ibuprofen enantiomers were optimized by experimental design.

To the best of our knowledge, the combination of vortex enhanced MSPD and chiral LC is introduced for the first time in this work as an efficient method for extraction of ibuprofen enantiomers from breast milk samples. Only two studies reported elsewhere have determined IB in human milk from lactating women, but (*R*)- and (*S*)-IB are not distinguished. The first one is a pharmacokinetic study [26] and it has been taken as a reference for establishing sample spiked levels. The other one has determined IB by continuous SPE followed by GC–MS (5).

## 2. Material and methods

### 2.1. Reagents and materials

All reagents and solvents were of analytical grade, and purified water from a Milli-Q system was used (Millipore, Bedford, MA, USA). Methanol (MeOH) and 2-propanol of gradient-HPLC grade were supplied by Scharlab (Barcelona, Spain). Sodium sulphate was supplied by Probus (Badalona, Spain), Florisil® (magnesium silicate, 60–100 mesh) was purchased from Carlo Erba Reagents (Sabadell, Spain), Discovery® DSC-18 polymerically bonded octadecyl endcapped (70 Å pore size), Discovery® DSC-SAX polymerically bonded quaternary amine, Discovery® DSC-WCX polymerically bonded ethylenediamine triacetic acid, diatomaceous earth, primary and secondary amine (PSA)-bonded silica were provided by Supelco (Bellfonte, PA, USA). Chirobiotic™ T (teicoplanin) (5 μm) was purchased from Advanced Separation Technologies Inc. (Whippany, NJ, USA); α- and β- cyclodextrin were obtained from Serva Feinbiochemica (Heidelberg, Germany).

The target analyte (*R,S*)-2-(4-(2-methylpropyl)phenyl)propanoic acid (*rac*-IB, 98% pure) and (*S*)-2-(4-(2-methylpropyl)phenyl)propanoic acid (*S*-IB, 99% pure) were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Analyte stock solutions (300 mg $L^{-1}$) were prepared in methanol and stored in the dark at 4 °C for three months maximum. Fresh working standard solutions were prepared by suitable dilution of stock solutions as required. In order to prevent the possible analyte degradation, working solutions were daily prepared.

### 2.2. Equipment

The chiral chromatographic separation was made using $\alpha_1$-acid glycoprotein Chiral-AGP™ column (100 × 3.0 mm, 5 μm) supplied by Chrom-Tech (Cheshire, U.K.) in a HPLC chromatograph consisting of an injection valve with a 20 μL sample loop (Rheodyne, Cotati, CA, USA), a gradient pump 125 S Solvent Module System Gold (Beckman, Fullerton, CA, USA) and a Beckman UV 166 Detector System Gold (11 μL, 1 cm pathlength), both interfaced to a computer equipped with a Gold Nouveau Chromatography Workstation Software (v 1.6) for chromatographic data processing.

MSPD of ibuprofen from milk samples was carried out by using an ultrasound bath provided by P-Selecta and a vortex mixer from VELP Scientifica (Usmate, Italy). An Unicen centrifuge model 21 supplied by Ortoalresa (Madrid, Spain) was used for centrifugation of milk extracts.

### 2.3. Assisted MSPD procedure

Half a gram of milk, 0.30 g diatomaceous earth, 0.30 g $Na_2SO_4$, 0.26 g (PSA)-bonded silica and 0.021 g β-cyclodextrin were thor-