Talanta 171 (2017) 190–196

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

On-line Identification of chiral ofloxacin in milk with an extraction/ionization device coupled to Electrospray Mass Spectrometry

Jiang Wang, Xiao-Xiao Jiang, Wei Zhao*, Jun Hu, Qi-Yuan Guan, Jing-Juan Xu*, Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

ARTICLE INFO

Keywords:
Chiral analysis
Extraction/ionization device
Ofloxacin
Electrospray Mass Spectrometry

ABSTRACT

The direct separation and analysis of chiral drugs in the complex matrix systems are meaningful and challenging. As the most common broad-spectrum antibiotic, levofloxacin has a strong antibacterial ability, but its enantiomer, dextrofloxacin can cause serious harm to human health. In this work, we reported a rapid on-line extraction/ionization device coupled with Electrospray Mass Spectrometry (ESI-MS) for chiral analysis of ofloxacin enantiomers in complex matrix of milk. Since ofloxacin is difficult to dissolve in water and most organic solvents, the procedure of separating ofloxacin in complex system is often complicated. Using the homemade apparatus, the sample pretreatment process was greatly simplified. Milk sample was directly injected and chiral ofloxacin in the sample was extracted at PTFE membrane for further ionization. It took less than 10 s to finish all the procedures including sampling, extraction, reagents mixing, ionization and mass analysis. Utilizing reaction thermodynamics method, trimeric cluster ion $[Ni^{II}(ref)_2Ofloxacin\text{-}H]^+$ was formed and collisionally dissociated to get chiral resolution of levofloxacin and dextrofloxacin due to the different relative stabilities of the two diastereomeric clusters produced through the dissociation of Ni^{II} bound trimeric clusters. With the proposed method, qualitative and quantitative chiral analysis of ofloxacin in milk was successfully achieved in a simple and fast way.

1. Introduction

Chiral enantiomers, as one kind of the isomers, widely exist in nature and involve in biological activities. Since tartaric acid enantiomers was discovered by Louis Pasteur in 1848 [1], researches of chiral chemistry, such as reaction mechanism in chiral substances synthesis [2–4], functions of enantiomers in life activities [5,6], as well as the development of chiral drugs [7], have become hot topics and attracted considerable attentions. In pharmaceutical industries, more than 50% of the drugs currently in use are chiral products [8]. Although enantiomers have the same chemical structure, most chiral drugs exhibit significant differences in biological activities such as pharmacology, toxicology, pharmacokinetics, metabolism etc. [7,9,10]. For instance, as an artificially synthesized antibiotic, levofloxacin has strong antibacterial ability, but its enantiomer, dextrofloxacin not only has no antibacterial ability, but also exhibits central nervous system toxicity, cardiovascular toxicity, tendon/articular toxicity as well as hepatic toxicity. Therefore, it is important to promote the chiral separation and analysis in pharmaceutical industry to limit unwanted isomers [11,12].

Antibiotic abuse contributes to the development of antibiotic resistance, and may create multidrug-resistant bacteria, which cause great danger to human health and environment [13]. For example, as one of the most common broad-spectrum antibiotics, ofloxacin has been widely adopted in the process of cattle feeding, which limited the risk of cow disease but caused the antibiotic contamination in milk source. For our living environment, many countries have put forward more stringent requirements on the content of antibiotics, especially toxic enantiomers in food supply [14]. Common analytical methods of enantiomers include polarimetry, chiral column for MNR [15], circular dichroism [16,17], HPLC [6,18–21], CE [12,22] and etc. Compared to these techniques, mass spectrometry offers superior advantages of sensitivity, specificity and versatility to chemical analysis. In early 2000s, Cooks' group reported a breakthrough to test chiral chemicals via kinetics reaction based on tandem mass spectrometry [1,23,24]. In the novel method, the metal ions (M^{II}), the reference amino acid (ref) and the analyte molecules (A) formed the gas phase ion $[M^{II}(ref)2A_{L/D}\text{-}H]^+$. Due to the different steric effect of chiral structure (ref) between A_L and A_R, the proportions of collision-induced dissociation (CID)

* Corresponding authors.
E-mail addresses: weizhao@nju.edu.cn (W. Zhao), xujj@nju.edu.cn (J.-J. Xu).

http://dx.doi.org/10.1016/j.talanta.2017.04.071
Received 17 January 2017; Received in revised form 26 April 2017; Accepted 30 April 2017
Available online 01 May 2017

fragments of $[M^{II}(ref)2\text{-}H]^+$ and $[M^{II}(ref)A_{L/D}\text{-}H]^+$ were different, which made the separation and analysis of chiral compounds come true. Based on Cooks's strategy, Pohl developed a variant method for the identification and differentiation of enantiomeric pentose isomers [25]. The kinetic reaction strategy coupled mass spectrometry is also suitable for the identification of chiral drugs [26]. However, as well-known, direct mass spectrometric analysis of complex samples is very difficult. Lemr's group reported a nano-desorption electrospray for the chiral analysis of drugs in human blood samples without pre-treatment. It was quite innovative, but there were some drawbacks which limit its further application. On one hand, the set-up was laborious. On the other hand, continuous sampling couldn't be achieved using this method [27]. Therefore, there is a big challenge meanwhile good opportunity for the researchers to develop suitable method for fast separation and accurate detection of chiral antibiotics in complicated matrix.

In this work, we built a simple and universal sample extraction/ionization device for on-line analysis of chiral ofloxacin in milk via Electrospray Mass Spectrometry (ESI-MS). Since ofloxacin is hard to dissolve in water and common organic solvents, the sample pretreatment procedures including separation and purification could be very tedious. Our aim is to greatly simplify the procedure and make continuous sampling and on-line analysis possible. As shown in Fig. 1, two metal three-way valves are connected with a homemade two-way connector. A PTFE Microporous Membrane was installed in the device for the on-line sample pretreatment (membrane extraction). Milk and the extraction agent were flowing at different sides of the PTFE extraction membrane. Most proteins were blocked by the film, but small molecules as ofloxacin were extracted at the film and successfully passed through. Using electrospray ionization MS^2 with kinetic method, quick qualification and quantification of ofloxacin enantiomers was realized by adding central metal ions and reference ligands in the extraction solution to form mass-selected trimetric cluster ions by electrospray ionization. This strategy greatly simplified the detection procedures of extraction, reaction agent mixing and ionization with total detection time less than 10 s. The device could easily couple with commercial instruments such as ESI-MS and HPLE as an accessory. For the first time, milk was directly "injected" into the ion source for the mass spectrometric analysis of chiral drugs. For chiral analysis, the proposed method showed comparable reproducibility compared with traditionally high performance liquid chromatography (HPLC), but the sensitivity was much higher, which had good prospect for application.

2. Experimental section

2.1. Reagents and chemicals

Levofloxacin and ofloxacin bought from J & K Chemical Ltd. (≥98% purity), dextrofloxacin (≥98%) bought from Daicel Chiral Technologies (China) Co., Ltd. Methanol and acetic acid were HLPC grade and bought from ROE Scientific Inc. (Newark, U.S.A). Ethanol absolute

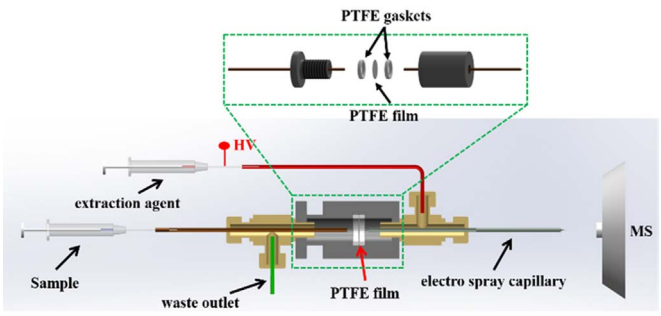

Fig. 1. Structure of the on-line extraction/ionization device coupled with ESI-MS.

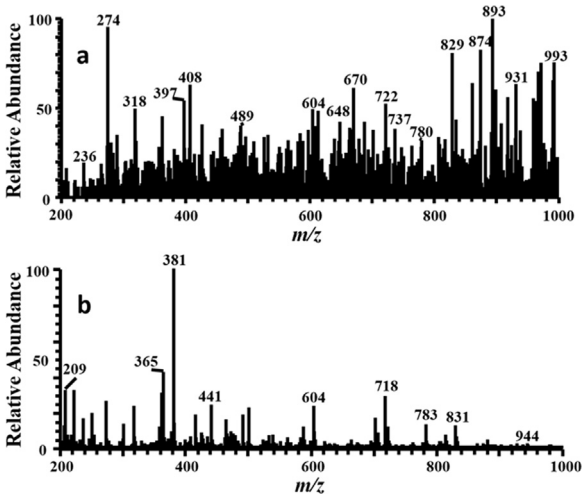

Fig. 2. MS spectra of (a) milk diluted 5 times with water, (b) milk extracted by methanol/water (1:1, V/V) using the device.

bought from Aladdin(≥99.7%, AR). L-phenylalanine and L- aspartic acid was bought from J & K Chemical Ltd. (≥98% purity). L-arginine, L-isoleucine, L-serine, Ninhydrin, $NiCl_2$ and $CuSO_4$ were purchased from Sigma-Aldrich (≥98% purity). Ultrapure water is home-made by a Simpak0r1 Millipore membrane filter system (Millipore, USA). Fresh MengNiu® milk (Neimenggu, China) was purchased in a local shop. Ofloxacin eye drops and levofloxacin hydrochloride eye drops were purchased from Bausch & Lomb Inc. and Yabang Co. Ltd., respectively. The PTFE extraction membranes (0.45 μm, aperture) were purchased from Taoyuan Medical Chemical Instrument Co. Ltd. (Haining, China).

The Cence H1650R high speed refrigerated centrifuge was from Hunan Xiang Yi Laboratory Instrument Development Co., Ltd. (Changsha, Hunan, China), working on 1500 r/min at 20 °C. Syringe pumps (Pump 11 Elite) were from Harvard Apparatus. MS experiments were conducted on an LTQ linear ion trap mass spectrometer (Thermo Fischer Scientific Co. Ltd, San Jose, CA, USA). Mass spectra were collected in *m/z* range of 100–1000. The high performance liquid chromatography (HPLC) experiments were conducted on the Agilent 1200 HPLC (Agilent technologies Co. Ltd, California, USA) with a 20 μL sample loop and reversed phase C18 chromatographic column (4.6×250 mm, 5 μm) from Waters Corp.

2.2. On-line analysis of ofloxacin via extraction/ionization device coupled ESI-MS

As shown in Fig. 2, the apparatus is consisted of two three-way valves connected with a homemade two-way connector. PTFE Microporous Membrane with pore size of 0.45 μm is compressed tightly in the middle of connector. To limit the dead-volume, both sampling and ionization spray capillaries are installed close to the membrane with distance less than 1 mm. For on-line measurement, sample is pumped through the three-way valve to the connector at 3 μL/min, meanwhile mobile phase containing extraction agents and reaction agents is flowing into the connector from the other valve at 4 μL/min. After membrane extraction, sample waste flows out through the outlet, and mobile phase containing the analyte passes through the capillary and is ionized at the spray tip under a high voltage of 3500 V.

2.3. Chiral resolution analysis by ESI-MS

For chiral analysis, 50 μM metal cation and 200 μM reference ligand are added in the mobile phase, and mixed with ofloxacin in the capillary. At high voltage of 3500 V, $[M^{II}(ref)_2Ofloxacin\text{-}H]^+$ is formed and entries into the MS analyzer. Through collision-induced