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A novel two-dimensional liquid chromatographic system for the online toxicity prediction of pharmaceuticals and related substances

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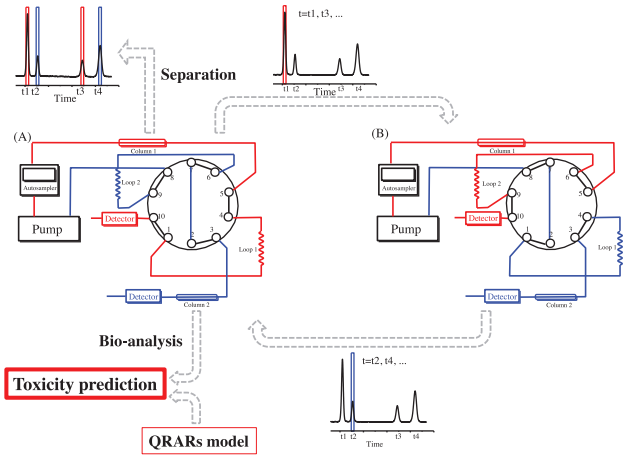
HIGHLIGHTS

- A novel two-dimensional liquid chromatographic system was developed.
- The 1st dimension was ODS to separate components in the sample.
- The 2nd dimension was biopartitioning micellar chromatography to predict toxicity.
- The system was used to screen toxicity of pharmaceuticals and related substances.
- It was promising for fast online toxicity screening of complex sample in one step.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a novel two-dimensional liquid chromatographic (2D-LC) system was developed for simultaneous separation and toxicity prediction of pharmaceutical and its related substances. A conventional ODS column was used on the 1st-D to separate the sample; while, bio-partitioning micellar chromatography served as the 2nd-D to predict toxicity of the components. The established system was tested for the toxicity of ibuprofen and its impurities with known toxicity. With only one injection, ibuprofen and its impurities were separated on the 1st-D; and LC50 values of individual impurity were obtained based on the quantitative retention–activity relationships, which agreed well with the reported data. Furthermore, LC50 values of photolysis transformation products (TPs) of carprofen, ketoprofen and diclofenac acid (as unknown compounds) were screened in this 2D-LC system, which could be an indicator of the toxicity of these TPs and was meaningful for the environmental monitoring and drinking water treatment. The established 2D-LC system was cost-effective, time-saving and reliable, and was promising for fast online screening of toxicity of known and unknown analytes in the complex sample in a single step. It may find applications in environment, pharmaceutical and food, etc.

Abbreviations: 2D-LC, two dimensional liquid chromatography; LC50, lethal concentration 50; TPs, transformation products; BMC, biopartitioning micellar chromatography; NSAIDs, non-steroidal anti-inflammatory drugs; IBU, ibuprofen; CAR, carprofen; KET, ketoprofen; DIC, diclofenac acid; QRARs, quantitative retention–activity relationships.

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1. Introduction

The quality of pharmaceuticals and the pollution of residual pharmaceuticals in the environment attracted heightened attention with the enhancing living standard of people. The related substances, e.g., impurity, were highly connected to the quality of pharmaceuticals. In addition, when pharmaceuticals were released into the environment as industrial wastewater, medicine waste, or as a result of improper handling of expired drugs and excretion by living creatures, they may exist as their original status or be converted into transformation products (TPs) under photolysis, oxidization and so on. Both impurities and TPs may be bioactive, and cause negative effects on humans and creatures. Hence, much attention was paid on the toxicity study of pharmaceutical impurities and TPs in the environment [1–3]. However, traditional methods for toxicity or bioactivity studies, such as animal and cell experiments [4–7], are always expensive, poorly reproducible, time-consuming and tedious. It is a great challenge to the toxicity research, as hundreds of thousands of various pharmaceuticals are currently and regularly used.

In the present study, a novel two-dimensional liquid chromatographic (2D-LC) approach was put forward for online prediction of toxicity of known and unknown analytes in the complex sample. A traditional reversed-phase system with an ODS column was used as the first dimension (1st-D) for separation of components in the complex sample, while a biopartitioning micellar chromatography (BMC) based on perfusive silica matrix as the second dimension (2nd-D) was modeled for toxicity prediction. In this manner, biochromatographic model (in vitro) instead of in vivo model accomplished fast toxicity screening of multiple components in complex samples in one single step. Biochromatography uses biomolecules or analogies as the stationary phase and simulated body fluid as the mobile phase [8,9]. Owing to the similarity of stationary phase and mobile phase to the real living conditions, biochromatography is able to simulate and predict the bioactivity or toxicity of the related analytes based on the retention time. BMC was an environmental-friendly biochromatographic system using micellar solution, especially including the non-ionic surfactant, polyoxyethylene (23) lauryl ether (brij-35), as mobile phase [10]. Because of its simple operation, good reproducibility and wonderful simulation ability, BMC was widely applied to screen active components of Traditional Chinese Medicines [11], and predict blood–brain barrier and skin permeability [12,13], oral absorption ability [13] and toxicity of chemicals [14–16].

Herein, non-steroidal anti-inflammatory drugs (NSAIDs), which are widely used for clinical purpose and reported to be present in the environment [17,18], were chosen as the target analytes. The toxicity of ibuprofen (IBU) and its six impurities (as known compounds), and photolysis TPs of carprofen (CAR), ketoprofen (KET) and diclofenac acid (DIC) (as unknown compounds) were screened in the novel established 2D-LC system. Impurities J, N and V of IBU were more toxic than IBU. All the studied TPs of KET were more toxic than the parent compound, but all the TPs of CAR and DIC were less toxic.

2. Experimental

2.1. Reagents and materials

HPLC grade methanol (MeOH), NaH_2PO_4, Na_2HPO_4, NaCl and ammonium acetate (NH_4Ac) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). DIC, IBU, fenbufen, sulindac acid, naproxen and probenecid were purchased from Hubei Jusheng Technology Co., Ltd. (Wuhan, China). KET and loxoprofen were purchased from Wuhan Fuxin Chemical Co., Ltd. (Wuhan, China). CAR was purchased from Sun Chemical Technology Co., Ltd. (Shanghai, China). Fluprofen and flurbiprofen were purchased from Hubei Xin Yuanshun Pharmaceutical Co., Ltd. (Wuhan, China). Zaltoprofen was purchased from Hubei Xin Tuokangchuyuan Biotechnology Co., Ltd. (Wuhan, China). Mefenamic acid, clofenamic acid, flufenamic acid and tolfenamic acid were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Indometin was purchased from Aladdin Industrial Corporation (Shanghai, China). Indoprofen was purchased from Sigma–Aldrich (St. Louis, MO, USA). Salicylic acid, aspirin and 4-aminosalicylic acid were purchased from Alfa aesar (Tianjin, China). All the compounds for modeling are listed in Table 1. Polyoxyethylene (23) lauryl ether (Brij-35) was purchased from J & K Technology Co., Ltd. (Beijing, China). IBU impurities, impurity B, E, N, V, I and J, whose properties are listed in Table 2, were obtained from the Hubei Instrument for Food and Drug Control (Wuhan, China).

Table 1
Compounds for the QRARs modeling.

Compounds	CAS NO	LC_{50} ($mg\,L^{-1}$)			
		Fish 96 h	Fish (SW) 96 h	Daphnid 48 h	Green aglae 96 h
Salicylic acid	69-72-7	156.34	67.31	52.49	235.76
Aspirin	50-78-2	777.32	1247.32	1773.85	886.69
Indoprofen	31842-01-0	493.19	437.06	467.78	16.63
4-Aminosalicylic acid	65-49-6	154.69	867.28	304.43	1546.58
KET	22071-15-4	264.08	334.45	164.45	179.46
Fenbufen	36330-85-5	180.91	229.34	114.58	134.08
Fluprofen	73231-34-2	145.62	290.63	212.48	2.69
Loxoprofen	68767-14-6	569.33	719.38	342.10	322.05
Sulindac	38194-50-2	26.36	33.64	18.47	32.83
Probenecid	57-66-9	195.87	174.58	142.90	7.47
Naproxen	22204-53-1	193.34	245.01	121.55	137.94
IBU	15687-27-1	41.56	52.89	27.85	41.13
Zaltoprofen	89482-00-8	63.33	80.57	42.33	61.93
Fubiprofen	51543-40-9	47.48	60.43	31.87	47.38
Phenol	108-95-2	38.35	18.90	9.30	44.82
DIC	15307-86-5	37.66	47.98	25.75	41.41
Indometacin	53-86-1	0.88	–	33.93	3.28
Clofenamic acid	13278-36-9	5.87	7.51	4.33	9.49
Mefenamic acid	61-68-7	2.25	2.89	1.73	4.51
Tolfenamic acid	13710-19-5	2.00	2.57	1.55	4.19
Flufenamic acid	530-78-9	3.45	4.42	2.62	6.48
CAR	53716-49-7	55.64	70.80	37.27	54.95