Journal of Chromatography A, 1478 (2016) 10–18

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Full length article

Establishment of quantitative retention-activity model by optimized microemulsion liquid chromatography

Liyuan Xu[a,d], Haoshi Gao[b], Liangxing Li[a], Yinnong Li[a], Liuyun Wang[a], Chongkai Gao[c], Ning Li[a,*]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, 510006, China
[b] Department of Analytical Chemistry, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, 110016, China
[c] Department of Pharmaceutics, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, 510006, China
[d] School of Management Engineering, Hua Xia Technical College, Guangzhou, 510935, China

ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form 1 November 2016
Accepted 3 November 2016
Available online 4 November 2016

Keywords:
Chromatography
Microemulsion
Permeability
Artificial membrane, QRAR model

ABSTRACT

The effective permeability coefficient is of theoretical and practical importance in evaluation of the bioavailability of drug candidates. However, most methods currently used to measure this coefficient are expensive and time-consuming. In this paper, we addressed these problems by proposing a new measurement method which is based on the microemulsion liquid chromatography. First, the parallel artificial membrane permeability assays model was used to determine the effective permeability of drug so that quantitative retention-activity relationships could be established, which were used to optimize the microemulsion liquid chromatography. The most effective microemulsion system used a mobile phase of 6.0% (w/w) Brij35, 6.6% (w/w) butanol, 0.8% (w/w) octanol, and 86.6% (w/w) phosphate buffer (pH 7.4). Next, support vector machine and back-propagation neural networks are employed to develop a quantitative retention-activity relationships model associated with the optimal microemulsion system, and used to improve the prediction ability. Finally, an adequate correlation between experimental value and predicted value is computed to verify the performance of the optimal model. The results indicate that the microemulsion liquid chromatography can serve as a possible alternative to the PAMPA method for determination of high-throughput permeability and simulation of biological processes.

1. Introduction

Although the number of new drugs discovered has been high since the 1990s, many drugs eventually fail in later stages of development, which can result in losses of millions of dollars for pharmaceutical companies. Development of a cost effective, high throughput, and highly predictive model for drug absorption is required to guide drug design in the early stages of drug discovery [1–3]. To address this challenge, high throughput tools for the determination of absorption potential have been considered in drug development [4,5]. Among the routes for absorption of drugs through membranes and tissues, passive diffusion is the most common. In this sense, intestinal permeability is a key parameter for the selection of compounds for drug discovery [6].

Two popular tools used assessing in vitro absorption/permeability are Caco-2 cells, which is a continuous cell of heterogeneous human epithelial colorectal adenocarcinoma cells, developed by the Sloan-Kettering Institute for Cancer Research through research conducted by Dr. Jorgen Fogh, and the parallel artificial membrane permeability assay (PAMPA). Among the in vitro systems used to predict drug absorption in humans, Caco-2 monolayers are reliable. Even though this method expresses some important drug transporters, its use as a high throughput tool is limited by a long cell-growth cycle and high implementation costs [7–10]. For this reason, artificial membranes have been investigated as an alternative model for GI membrane simulation. The PAMPA has been used to measure the permeability and evaluate the absorption of drugs with diverse structures across a membrane via the transcellular route. The PAMPA was proposed by Kansy et al. in 1998, and uses a hydrophobic filter coated with lecithin in an organic solvent [11–14]. The technique is rapid, simple, and has higher precision beween laboratories than cell culture studies. However, its default is that it only allows observation of the passive permeability component [15–18].

In recent years, biopartitioning chromatography methods have been used as in vitro methods to mimic the interactions between

* Corresponding author.
E-mail address: 13724117338@163.com (N. Li).

http://dx.doi.org/10.1016/j.chroma.2016.11.005
0021-9673/

Table 1
The compositions of the mobile phases used in this study.

Mobile phase	Surfactant (w/w)	Oil phase (w/w)	Butanol (w/w)	Buffera (w/w)
MP1	6.00%Brij35	0.80% *n*-Octanol	6.60%	86.60%(pH 7.4)
MP2	4.84%Brij35-1.16 SDS	0.80% *n*-Octanol	6.60%	86.60%(pH 7.4)
MP3	3.30%SDS	0.80% *n*-Octanol	6.60%	89.30%(pH 7.4)
MP4	6.00%Brij35	0.80% cyclohexane	6.60%	86.60%(pH 7.4)
MP5	6.00% Brij35	0.80% *n*-heptane	6.60%	86.60%(pH 7.4)
MP6	6.00%Brij35	0.80% *n*-octane	6.60%	86.60%(pH 7.4)
MP7	6.00%Brij35	0.80% ethyl acetate	6.60%	86.60%(pH 7.4)
MP8	6.00% Brij35	0.80% *n*-Octanol	6.60%	86.60%(pH 6.8)
MP9	6.00%Brij35	0.80% *n*- Octanol	6.60%	86.60%(pH 5.0)
MP10	6.00%Brij35	0.80% *n*-Octanol	6.60%	86.60%(pH 4.2)
MP11	6.00% Brij35	0.80% *n*-Octanol	6.60%	86.60%(pH 3.0)

[a] Buffer is 0.05 mol/L potassium dihydrogen phosphate solution adjusted to pH 7.4, 6.8, 5.0, 4.2, 3.0 with 1 mol/L sodium hydroxide solution or phosphate.

Table 2
Well-to-Well Reproducibility.

$logP_e$	1	2	3	Average $logP_e$	RSD (%)
Propranolol	−5.02	−5.05	−5.04	−5.04	0.3
Verapamil	−5.08	−5.22	−5.12	−5.14	1.4
Metoprolol	−5.95	−6.06	−5.95	−5.99	1.0
Theophylline	−6.93	−6.87	−6.96	−6.85	1.2

Table 3
$logP_e$ values for four reference compounds from experiment and reference.

Drugs name	$logP_{e(exp)}$	$logP_{e(ref)}$	difference
Propranolol	−5.04	−5.00 [26]	0.04
Verapamil	−5.22	−5.30 [26]	0.08
Metoprolol	−5.99	−6.25 [5]	0.26
Theophylline	−6.89	−6.92 [27]	0.03

[a] From ref [26].
[b] From ref [5].
[c] From ref [27].

drug molecules and biological membranes. They are useful for high-throughput screening and evaluation of new compounds during the drug discovery process [19]. Biopartitioning micellar chromatography is the most popular among these methods, and has been used to model many biopartitioning processes, including protein-drug binding [20], skin permeability of drugs [21], drug absorption in humans [22], pharmacological activity [23], and pharmacokinetics and pharmacodynamics [24]. Among the biopartitioning micellar chromatography systems, the use of microemulsion mobile phases in HPLC provides several advantages in terms of predicting the drug membrane permeability properties. These include the ability to control experimental conditions, good stability, unique separation selectivity, and enhanced detection sensitivity. Furthermore, the use of a microemulsion mobile phase consisting of a surfactant, oil phase and cosurfactant leads to the formation of a membrane structure. Microemulsion liquid chromatography (MELC) has been successfully applied as a model system to predict many physicochemical and ADME parameters [25]. Despite its successful application in several biologically important processes, there have been very few reports on the use of MELC to predict the permeability of drugs.

In this paper, we proposed and developed a MELC system to predict the permeability of components from Commercially available drugs. The microemulsion systems were optimized in terms of the correlation between the logarithm of the chromatographic retention factor (log*k*) and effective permeability coefficient, including the types of surfactant, oil and the pH. Quantitative retention-activity relationship (QRAR) models were established and evaluated to screen the MELC system. Finally, the optimal MELC system was used to establish a more accurate prediction model via regression of back propagation neural networks (BPNNs) and support vector machine (SVM). The aim of this work was to develop and validate an efficient QRAR model for absorption/permeability evaluation of drugs using MELC.

2. Experimental

2.1. Chemicals and reagents

The water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). High purity grade polyoxyethylene lauryl ether (Brij35) and sodium dodecyl sulfate (SDS) were obtained from AMRESCO (Solon, OH, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. The high purity grade phosphatidylcholine was purchased from the Shenyang Tianfeng Bio-pharmaceuticals Co. Ltd (Shenyang, China). Dimethyl sulfoxide (DMSO) and Dodecane were from Aladdin Industrial Inc. (Shanghai, China). All other reagents used were analytical grade. Potassium dihydrogen phosphate, sodium hydroxide solution, phosphate were obtained from Fuchen Chemical Reagents Factory (Tianjin, China). Cyclohexane, *n*-octane, *n*-heptane, *n*-octanol, *n*-butanol, and ethanol ethyl acetate were purchased as the analytical grade from Baishi Chemical Co. Ltd (Tianjin, China). Hydrocortisone(1), hydrocortisone acetate(2), antipyrine(3), ibuprofen(4), ketotifen(5), indomethacin(6), aspirin(7), naproxen(8), piroxicam(9), diclofenac(10), caffeine(11), fluconazole(12), valsartan(13), verapamil(14), theophylline(15), barbiturate(16), phenobarbital(17), phenytoin(18), amobarbital(19), diazepam(20), nitrazepam(21), clonazepam(22), propranolol(23), metoprolol(24), trimethoprim(25), ranitidine(26), gliclazide(27), glipizide(28), promethazine(29), hydrochlorothiazide(30), indapamide(31), lidocaine(32), secobarbital(33), prazosin(34), amidopyrine(35), diphenhydramine(36), phenylbutazone(37), naloxone(38), chlorphenamine(39), salbutamol(40), sulfamethoxazole(41), metronidazole(42), atenolol(43), testosterone(44), metformin(45), acyclovir(46), chloramphenicol(47), captopril(48) were crude materials and provided by the Pharmaceutical Laboratory of Guangdong Pharmaceutical University (HPLC, PC > 95.0%, Guangzhou, China). The number in parenthesis corresponds to the analyte identification numbers in tables of this article.

2.2. Instruments and conditions

A Shimadzu (Kyoto, Japan) HPLC system equipped with a LC-15C pump, a SPD-M20A PDA detector, a SIL-10AF automatic sampler and a CTO-10As column thermostat was applied to the chromatographic measurements. A Odyssil C18 columns (150mm × 4.6 mm, i.d. 5 μm Agela, Tianjin, China) was utilized in this study. The compositions of microemulsion mobile phases used for chromatographic analysis are summarized in Table 1.The flow rate was