Journal of Chromatography B 1061–1062 (2017) 356–363

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Development of a rapid and sensitive multiple reaction monitoring proteomic approach for quantification of transporters in human liver tissue

Li Wang[a,*], Kasi J. Rubadue[b], Jeffrey Alberts[a], David W. Bedwell[a], Kenneth J. Ruterbories[a]

[a] Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, United States
[b] Advanced Testing Laboratory, Cincinnati, OH, United States

ARTICLE INFO

Keywords:
Human hepatic transporters
Quantitative proteomics
Supercharging reagents
Fast LC
Protein solubilizers

ABSTRACT

With increasing knowledge on the role of hepatic transporters in drug disposition, numerous efforts have been described to quantify the expression of human hepatic transporters. However, reported quantitative proteomic approaches often require long analysis times. Additionally, greater assay sensitivity is still necessary for less abundant transporters or limited quantity of samples (e.g. hepatocytes and liver tissue). In the present study, an LC–MS/MS method for rapid and simultaneous quantification of 12 hepatic transporters (BCRP, BSEP, MATE1, MRP2, MRP3, MRP4, NTCP, OATP1B1, 1B3, 2B1, OCT1, and P-gp) was developed. Using a high LC flow rate (1.5 mL/min) and fast LC gradient (4 min total cycle time), the run time was markedly reduced to 4 min, which was much shorter than most previously published assays. Chromatographic separation was achieved using ACE UltraCore SuperC18 50 mm × 2.1 mm 5-μm HPLC column. In addition, greater analytical sensitivity was achieved with both high LC flow rate/fast LC gradient and post-column infusion of ethylene glycol. The on-column LLOQ for signature peptides in this method ranged from 0.194 to 0.846 femtomoles. The impact of five protein solubilizers, including extraction buffer II of ProteoExtract Native Membrane Protein Extraction Kit, 3% (w/v) sodium deoxycholate, 20% (v/v) Invitrosol, 0.2% (w/v) RapiGest SF, and 10% (w/v) formamide on total membrane protein extraction and trypsin digestion was investigated. Sodium deoxycholate was chosen because of good total membrane protein extraction and trypsin digestion efficiency, as well as no significant MS interference. Good precision (within 15% coefficient of variation) and accuracy (within ± 15% bias), and inter-day trypsin digestion efficiency (within 28% coefficient of variation) was observed for quality controls. This method can quantify human hepatic transporter expression in a high-throughput manner and due to the increased sensitivity can be used to investigate the down-regulation of hepatic transporter protein (e.g., different ethnic groups and liver disease patients).

1. Introduction

Increasing evidence from both in vitro and in vivo studies indicates the critical role of hepatic transporters in mediating the flux across the basolateral and apical membranes of hepatocytes for a broad array of drugs [1,2]. By affecting the hepatic concentration of a drug, transporters impact not only pharmacokinetics (e.g. distribution, metabolism, and biliary excretion), but also pharmacodynamics and toxicity. Therefore, quantitative assessment of the contribution of hepatic transporters in drug disposition will definitely improve safety and efficacy assessment. However, in contrast to drug metabolizing enzymes, such as cytochrome P450, in vivo specific probes are not available for most transporters [3]. To address this issue, a number of studies demonstrated that the protein expression of transporters could be used to quantitatively evaluate the role of transporters in drug disposition through physiologically based pharmacokinetic modelling [4–7].

Over the last decade, quantitative proteomics, using surrogate signature peptides and liquid chromatography-mass spectrometry (LC–MS/MS) have been increasingly applied for hepatic and intestinal transporter protein quantification [8–13]. While exhibiting improved selectivity, sensitivity, and reproducibility compared with conventional Western Blotting, there are some limitations with reported methods. One limitation is that a long run time is often required. According to recent publications on hepatic/intestinal transporter protein

Abbreviations: BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CE, collision energy; HSA, human serum albumin; LLOQ, lower limit of quantification; MRP, multidrug resistance-associated protein; NTCP, sodium-taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; OCT1, organic cation transporter 1; P-gp, P-glycoprotein; RT, retention time; QC, quality control; SIL, stable isotope-labeled

* Corresponding author at: Eli Lilly and Company, Corporate Center, Drop Code 0710, Indianapolis, IN 46285, United States.
E-mail address: lwang@lilly.com (L. Wang).

http://dx.doi.org/10.1016/j.jchromb.2017.07.051
Received 30 March 2017; Received in revised form 9 June 2017; Accepted 28 July 2017
Available online 01 August 2017
1570-0232/

Table 1
A comparison in assay characteristics between the current and previous LC–MS/MS-based analytical methods on quantification of the expression of hepatic/intestinal transporter.

Transporters	Calibrator preparation matrix	LC Flow rate (μL/min)	On-column LLOQ (femtomoles)	Run time (min)	Single or Multiple signature peptides used	References
BCRP, BSEP, MATE1, MRP2, MRP3, MRP4, NTCP, OATP1B1, 1B3, 2B1, OCT1, and P-gp	Trypsin digestion buffer	1500	0.19–0.84	4	Single	This study
BCRP, BSEP, MATE1, MRP2, MRP3, MRP4, NTCP, OATP1B1, 1B3, 2B1, OCT1, and P-gp	Trypsin digestion buffer	300	1.31–6.41	29	Single	[4]
MRP2	Digested HSA[a]	400	2.5	> 15.1	Single	[9]
ASBT, BCRP, MRP2, MRP3, OATP1A2, 2B1, OCT1, OCT3, PEPT1, and P-gp	Digested HSA[a]	500	2	18	Single	[10]
BCRP, MRP2, NTCP, OATP1B1, 1B3, 2B1, OCT1, and P-gp	Trypsin digestion buffer	0.2	1–2	44	Single	[11]
OATP1B1, 1B3, and 2B1	Digested membrane protein of HEK cells	350	0.05	15.5	Multiple	[12]
OATP1B1, 1B3, 2B1, and P-gp	Digested mouse plasma	400	1–33	13.5	Mixed	[14]
BCRP and BSEP	Trypsin digestion buffer	400	0.0006 and 2.5	20	Single	[23]
BCRP, MRP2, MRP3, and P-gp	NR[b]	15	0.2	35	Multiple	[24]
BCRP	Trypsin digestion buffer	500	0.12	15.8	Single	[21]
OATP1B1, 1B3, 2B1, and P-gp	Trypsin digestion buffer	400	0.2	25	Multiple	[7]
54 transporters	Digested membrane protein of *E. coli* cells	10	1–3	30	Single	[8]

[a] Human serum albumin.
[b] Not reported.

quantification (shown in Table 1), the shortest run time was 13.5 min (for four transporters), while some methods even required ~30 min run time. Another limitation is that greater assay sensitivity might still be needed to determine the expression of low abundance hepatic transporters (e.g. BCRP and P-gp), quantify biosamples (e.g. hepatocytes and liver tissue) with limited availability, or investigate the change in hepatic transporter expression in special populations (e.g., different ethnic groups and liver disease patients), in which down-regulation of hepatic transporter expression occurs. For example, reduced hepatic transporter expression was observed in liver tissue from subjects with liver cirrhosis, hepatitis, and non-alcoholic fatty liver disease [4,14]. In addition, different protocols/reagents have been used in the sample preparation and LC–MS/MS quantification, which could lead to marked between-laboratory variability in the expression data. For example, digested human serum albumin, mouse plasma, *E. coli* cell membrane proteins, and protein-free matrix were used to prepare calibrators [8,10,14]. Various protein solubilizers, including extraction buffer II of ProteoExtract Native Membrane Protein Extraction Kit, sodium deoxycholate, and RapiGest SF surfactant were used for total membrane protein extraction and trypsin digestion [4,12,14]. Unfortunately, the impact of using different protocols/reagents on transporter expression quantification results had not been well studied, making it difficult, if not impossible, to compare data between laboratories.

In the present study, an LC–MS/MS method for rapid and simultaneous quantification of 12 hepatic transporters (BCRP, BSEP, MATE1, MRP2, MRP3, MRP4, NTCP, OATP1B1, 1B3, 2B1, OCT1, and P-gp) was developed and validated. The run time was markedly reduced from published methods to 4 min and the assay sensitivity was improved by using high LC flow rate, fast LC gradient, and a supercharging reagent, ethylene glycol. Additionally, five protein solubilizers were tested for their influence on total membrane protein extraction and trypsin digestion efficiency, and sodium deoxycholate showed favorable effects on these two sample preparation steps. This validated assay was successfully applied to analyze the expression of transporter proteins in human liver tissue.

2. Materials and methods

2.1. Chemicals and reagents

Synthetic signature peptides and corresponding stable isotope-labeled (SIL) peptides (AQUA QuantPro, > 97% purity and ± 25% precision) as internal standards were obtained from New England Peptides (Boston, MA) and Thermo Fisher Scientific (Rockford, IL), respectively.

The transporter protein sequence was obtained from Uniprot (www.uniprot.org) and the uniqueness of selected signature peptides was verified through homology analysis in Protein Prospectus (http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=mshomology). Iodoacetamide, dithiothreitol, BCA protein assay kit, and in-solution trypsin digestion kit were purchased from Pierce Biotechnology (Rockford, IL). Ammonium bicarbonate (99.7% purity) was purchased from MP Biomedicals (Santa Ana, CA). The ProteoExtract Native Membrane Protein Extraction Kit including protease inhibitor cocktail was obtained from Calbiochem (Temecula, CA). Sodium deoxycholate (97% purity), formic acid (≥98% purity), 2-nitroanisole (≥99% purity), propylene carbonate (99.7% purity), and sulfolane (99% purity) were purchased from Sigma–Aldrich (St. Louis, MO). RapiGest SF surfactant was obtained from Waters (Milford, MA). Invitrosol LC/MS protein solubilizer was purchased from Invitrogen (Carlsbad, CA). Formamide (≥99.5% purity) and ethylene carbonate (≥99%) were obtained from ACROS Organics (Geel, Belgium). HPLC-grade acetonitrile (99.9% purity) and methanol (99.9% purity) were purchased from OmniSolv (Charlotte, NC). Ethylene glycol (≥99% purity) and dimethyl sulfoxide (≥99.9% purity) were purchased from Fischer Scientific (Fair Lawn, NJ). Deionized water was obtained from a Milli-Q Plus water purifying system Millipore (Bedford, MA).

2.2. Human liver tissue

Fifteen human liver tissues were obtained from various sources including Medical College of Wisconsin (Milwaukee, WI), Medical College of Virginia (Richmond, VA), Indiana University School of Medicine (Indianapolis, IN), University of Pittsburgh (Pittsburgh, PA), BD Gentest (Woburn, MA), and Xenotech (Lenexa, Kansas). The subjects were 13 Caucasian and 2 subjects for whom the ethnic background was not available. Subject age range was 1–75 years and comprised of 6 women, 7 men, and 2 subjects whose gender information was not reported. Detailed demographic characteristics of the donors are shown in Supplementary Table 1. These livers were obtained under protocols approved by the appropriate committees for the conduct of human research.