Application of an LC-MS/MS Method for the Simultaneous Quantification of Human Intestinal Transporter Proteins Absolute Abundance using a QconCAT Technique

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Application of an LC–MS/MS method for the simultaneous quantification of human intestinal transporter proteins absolute abundance using a QconCAT technique


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Abstract

Transporter proteins expressed in the gastrointestinal tract play a major role in the oral absorption of some drugs, and their involvement may lead to drug–drug interaction (DDI) susceptibility when given in combination with drugs known to inhibit gut wall transporters. Anticipating such liabilities and predicting the magnitude of the impact of transporter proteins on oral drug absorption and DDIs requires quantification of their expression in human intestine, and linking these to data obtained through in vitro experiments. A quantitative targeted absolute proteomic method employing liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) together with a quantitative concatenation (QconCAT) strategy to provide proteotypic peptide standards has been applied to quantify ATP1A1 (sodium/potassium-ATPase; Na+/K-ATPase), CDH17 (human peptide transporter 1; HPT1), ABCB1 (P-glycoprotein; P-gp), ABCG2 (breast cancer resistance protein; BCRP), ABCG2 (multidrug resistance-associated protein 2; MRP2) and SLC51A (Organic Solute Transporter subunit alpha; OST-α), in human distal jejunum (n = 3) and distal ileum (n = 1) enterocyte membranes. Previously developed selected reaction monitoring (SRM) schedules were optimised to enable quantification of the proteotypic peptides for each transporter. After harvesting enterocytes by calcium chelation elution and generating a total membrane fraction, the proteins were subjected to proteolytic digestion. To account for losses of peptides during the digestion procedure, a gravimetric method is also presented. The linearity of quantifying the QconCAT from an internal standard (correlation coefficient, R² > 0.998) and quantification of all target peptides in a pooled intestinal quality control sample (R² > 0.980) was established. The assay was also assessed for within and between-day precision, demonstrating a <15% coefficient of variation for all peptides across 3 separate analytical runs, over 2 methods. The methods were applied to obtain the absolute abundances for all targeted proteins. In all samples, Na+/K-ATPase, HPT1, P-gp and BCRP were detected above the lower limit of quantitation (i.e., >0.2 fmol/µg membrane protein). MRP2 abundance could be quantified in distal jejunum but not in the distal ileum sample. OST-α was not detected in 2 out of 3 jejunum samples. This study highlights the utility of a QconCAT strategy to quantify absolute transporter abundances in human intestinal tissues.

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1. Introduction

Successful prediction of drug absorption, disposition and drug–drug interactions (DDIs) is a critical component of drug development. Drug transporter proteins functionally expressed in the plasma membrane of enterocytes can facilitate or hinder drug absorption and influence drug disposition in polypharmacy [1]. To estimate the influence of transporter protein function on drug absorption, disposition and DDIs, strategies incorporating physiologically based pharmacokinetic (PBPK) modelling approaches are
used [2]. Considerable challenges have been encountered in achieving good predictions when including transporter functionality from \textit{in vitro} data within IVIVE-PBPK frameworks [3]. This is, in part, based on a lack of high quality data accurately representing transporter abundance levels in a variety of organs. This is pertinent given that whole body PBPK models require the incorporation of organ-specific transporter expression including the intestine, that are typically based on gene expression (i.e., mRNA) or immunoblotting analyses [4,5].

Recently, studies have employed LC–MS/MS-based quantitative targeted absolute proteomic strategies, to quantify the absolute abundances of transporter proteins in human intestines by an absolute quantification (AQUA) strategy for generating synthetic isotope labelled peptides [6–8]. The QconCAT technique is an alternative method for generating proteotypic isotope labelled peptide standards [9]. An artificial protein is constructed within an \textit{Escherichia coli} host vector and expressed, with stable isotope enrichment of growth media. The extracted artificial protein is subjected to proteolytic digestion strategies to yield equimolar concentrations of standard peptides for quantification of target proteins [10]. This approach has recently been applied to simultaneously quantify several human hepatic cytochrome P450 (CYP450) and uridine 5′-diphospho glucuronosyltransferase (UGT) drug metabolising enzymes and corresponding correlation profiles have been highlighted [10]. In addition, the construction of a transporter protein-specific QconCAT (‘TransCAT’) has recently been described [11]. However, the validation of these methods, in terms of quality control procedures, and their application for quantification of several transporter proteins by LC–MS/MS has yet to be reported.

The aim of this study was to validate an LC–MS/MS method to quantify the absolute abundances of ATP1A1 (sodium/potassium-ATPase; Na/K-ATPase), CDH17 (human peptide transporter 1; HPT1), ABCB1 (P-glycoprotein; P-gp), ABCG2 (breast cancer resistance protein; BCRP), ABCC2 (multidrug resistance-associated protein 2; MRP2) and SLC31A (organic solute transporter subunit alpha; OST-a) in human enterocyte membrane preparations using the TransCAT construct.

2. Materials and methods

2.1. Materials/reagents

All chemicals and reagents unless specified were either from Sigma–Aldrich (Poole, Dorset, UK), or the supply source can be obtained from previous studies [10,11]. The details of the design, expression in \textit{E. coli} and purification of the TransCAT synthesis have been described previously, including that of the non-naturally occurring peptide (NNOP) peptide calibrator Glu-Fib (EGVNDEEGFSAR), incorporated into the construct to enable quantification of the TransCAT [11].

2.2. Intestinal Tissue

Human intestinal tissue was obtained after informed consent from patients undergoing elective intestinal surgery at Salford Royal NHS Foundation Trust. Prior ethics committee approval had been granted by the North West Research Ethics Committee (12/NW/0306). Patients suffering from inflammatory bowel disease (i.e., Crohn’s disease or ulcerative colitis) and/or known to be affected by hepatitis B were excluded from participation. Donor demographics and recent drug history are provided in Table S1, Supporting Information.

2.3. Tissue preparation, marker enzyme activity enrichment

Immediately after resection the macroscopically healthy margins of resected intestinal tissues were opened to expose the mucosa and were then washed in ice-cold 0.9% NaCl. The tissue was then transferred in ice-cold oxygenated small bowel ringer (NaCl (121 mM), NaHCO3 (25.1 mM), KHCO3 (1.6 mM), KH2PO4 (0.2 mM), K3HPO4 (1.2 mM), CaCl2 (1.2 mM), MgCl2 (1.2 mM) and D-glucose (10 mM)), which had been equilibrated to pH 7.4 by bubbling with 5% CO2/95% O2. Enterocytes were harvested by elution from the mucosa by calcium chelation. Briefly, the mucosa was separated from the underlying bowel wall by blunt dissection and then placed onto perspex tissue adaptors (constructed by the Medical Physics Dept, Salford Royal Hospital Trust, Salford, UK), with 8 or 16 cm² apertures (typically 16–24 cm² mucosal surface area was used, see Table S1, Supporting Information) to expose the mucosa to chelating solutions. The time from bowel resection to securing the tissue in adaptors was always less than 1 h. All solutions used for chelation were made in a base buffer consisting of NaCl (112 mM), KCl (5 mM), HEPES (20 mM), pH 7.1 (Tris). The adaptor-secured mucosa was washed twice in base buffer (4°C) and immersed in a sodium citrate solution (27 mM), pH 7.1 with a protease inhibitor cocktail at 4°C for 30 min, followed by two washes in base buffer (4°C). Chelation commenced when the adaptor-secured mucosa was incubated in an EDTA-buffer (EDTA (30 mM), heparin (10 U/mL), dithiothreitol (DTT, 1 mM) and protease inhibitors), pH 7.1 at 4°C and stirred at 250 rpm for 40 min at 4°C. The tissue was repeatedly flushed with a 10 mL syringe in EDTA buffer to harvest chelated enterocytes. The chelated material was washed by centrifugation twice at 2000 x g (10 min, 4°C). The enterocytes were stored at ~80°C.

The enterocyte pellet was re-suspended in TSEM buffer (Tris–HCl (10 mM), sucrose (250 mM), EGTA (0.1 mM), MgCl2 (0.5 mM), pH 7.4) at 4°C and homogenised on ice with a Dounce hand-held homogeniser for a minimum of 75-strokes. The absence of intact enterocytes was confirmed by microscopy. A previously described differential centrifugation procedure to obtain a total membrane (TM) fraction for Caco-2 cells was employed for the enterocytes in this study [11]. Protein content was determined using a BCA assay.

The activity of alkaline phosphatase (AP, EC 3.1.3.1), a plasma membrane marker, was utilised to assess enrichment of the TM fraction compared with the homogenised enterocytes in 7 samples (donor information supplied in Table S2, Supporting Information). The liberation of p-nitrophenyl + inorganic phosphate (p-NP + Pi) by alkaline phosphatase from p-nitrophophylphosphate (p-NPP) is measured in fresh assay buffer (Glycine (100 mM), 1 mM MgCl2, pH 8.8) in samples containing 10 μg protein against a p-NP standard curve for 60 min at 37°C. The reaction was stopped by NaOH (1 M) and was read on a plate reader at 405 nm for 0.1 s per sample.

2.4. Proteolytic digestion

An adapted in-solution digest was developed, based on established methods [12]. Protein samples (typically 50 μg) and the TransCAT (5 μL, 1/10 diluted stock) were suspended in ([NH4]HCO3 (25 mM), pH 8), denatured with 10% (w/v, final volume) sodium deoxycholate (DOC) and incubated at room temperature for 10 min. Samples were reduced by DTT (60 mM) at 56°C for 20 min and subsequently alkylated by iodoacetamide (15 mM) in the dark at room temperature for 30 min. The DOC level was reduced to 1% (w/v) with (NH4)HCO3 (25 mM) and 1 μL of Lys-C (1 μg/μL) was added. The mixture was incubated at 30°C for 4 hours. Trypsin (2.5 μL at 1 μg/μL; Roche Applied Sciences, Mannheim, Germany) was then added, and the mixture incubated for 18 h at 37°C. To precipitate the DOC and acidify the digest, trifluoroacetic acid was added.
(0.1–0.5%, v/v) to achieve the optimal pH of 3, and was chilled at 4 °C for 30 min. After two 14,000 × g spins, the supernatant containing the peptides was removed and evaporated by vacuum centrifugation with adjustment to 3% acetonitrile and stored at −20 °C.

2.5. Gravimetric determination of peptide content

To account for losses of peptides through the digestion procedure, i.e., the DOC precipitation stage and any additional losses resulting from non-specific binding to preparatory/LC–MS/MS tubes and during pipetting, a gravimetric method using an analytical balance was developed to determine the protein content entering the LC system. The protein content relates the abundance in fmol of the target peptide in the digest to abundances per μg in the ‘Protein’ matrix under study (fmol/μg). In order to derive peptide mass by gravimetric means, there are a series of instances in which sample tubes and their contents mass (μg) and volumes are measured (see Supporting Information).

2.6. LC–MS/MS analysis

A typical mixture of: digested sample, standard (QconCAT) and NNOP (calibrator peptide) (‘the sample’) was prepared as follows: 18 μL of digested membrane protein and standard with 2 μL of stock NNOP/Glu-Fib (25 pmol/μL) which was diluted 1/100,000 in 3% acetonitrile to provide a final concentration of 0.238 fmol/μL after correction for Glu-Fib stock purity (95%).

Samples (8 μL injection) were analysed by LC–MS/MS as previously described [10,11]. Peptides were eluted with a gradient of 3–60% acetonitrile over 40 min, followed by a ramp to 95% acetonitrile for 5 min, then a return to starting conditions for 10 min to re-equilibrate the system. In a departure from the previous studies [10,11], Skyline version 2.5.0.6079 was used (MacCross Lab Software, Seattle, USA). The selected transitions are given in Table S3 in the Supporting Information. For each transition, the dwell time was set manually at 0.15 s.

2.7. Method validation

To validate the methods for quality control, linear regression analysis was employed to evaluate; (1) the linearity of the calibrator NNOP, and (2) the sample peptides in relation to the NNOP dilution factor [10]. To assess NNOP linearity, a calibration curve was prepared using the synthetic ‘light’ Glu-Fib and a pre-digested TransCAT construct to calculate the light to heavy peak area under the curve ratio over a 100-fold range. A pool of 3 digested samples was generated to evaluate sample peptide to QconCAT dilution factor linearity in a similar manner to that previously described for CYP3A4 and CYP3A5 [10]. Assay precision was assessed in 5 samples with 3 separate injections per sample, spanning 2 days for each peptide. The within-day (intra-day) and between-day (inter-day) precision was assessed in two separate runs for 5 samples. The impact of inter-operator differences when manually evaluating the co-elution profile boundaries that enable Skyline to calculate the light-to-heavy peak area of selected peptides was also assessed. Two independent operators performed this analysis.

Transporter abundances were determined from TransCAT-derived proteotypic standards as previously described for CYP...
and UGT enzymes [10], and the equation is given in Fig. 1. The ratios of native (light) and standard (heavy) selected transitions for each peptide were calculated in Skyline. The ‘Protein’ term in Equation S14 (Supporting Information) reflects the gravimetrically determined protein content (Supporting Information) and not the nominal protein content entering the digestion procedure. All results are given as mean abundance (fmol/μg total membrane protein) ± standard deviation.

3. Results and discussion

The aim of this study was to optimise and validate an LC–MS/MS-based method to quantify the absolute abundance of 6 key membrane transporter proteins in human intestinal samples with isotope labelled proteotypic peptides generated using a QconCAT strategy [11]. A schematic to describe the workflow of this study is provided in Fig. 1.

3.1. Harvesting intestinal enterocytes and marker enzyme enrichment

A calcium chelating agent, EDTA, was used to liberate the enterocytes from the basal lamina propria by securing the stripped intestinal mucosa in specialised adaptors to maximise EDTA exposure of the luminal mucosal surface. A histological inspection of distal ileal mucosa (Fig. 2) showed that enterocytes were removed from the underlying lamina propria layer after elution. The TM protein yield for the distal jejunum (n = 3) was 22.37 ± 4.57 μg/cm² and for the single distal ileum, 16.61 μg/cm², considerably lower values than those obtained after mucosal scraping [13]. This is likely to result from the scraping technique sampling the underlying interstitium.

Enrichment in the activity of a plasma membrane marker, alkaline phosphatase, was assessed in the starting total protein and TM fraction. A 3.64 ± 1.74-fold increase in the activity of alkaline phosphatase in the TM fraction relative to the total protein homogenate (n = 7) confirmed an increase in the purity of the TM with plasma membrane components (Fig. 3).

3.2. LC–MS/MS analysis

Upon LC–MS/MS analysis, only one peptide for each transporter protein was suitable for quantification and can be used, to varying extents, for quantification in other mammalian species used as preclinical models in drug development (Table 1). Peptide selection was further based on a combination of in silico selection criteria; the identification of the 3 transitions that gave the highest signal intensity using synthesised sequence-equivalent unlabelled peptides (Maxi SpikeTides; JPT Peptide Technologies GmbH, Berlin, Germany), and identifying previously optimised product ion transitions in intestinal TM digests. A SRM method was developed to simultaneously detect three singly charged product ions of each of the selected peptides in a single analytical run (Table S3, Supporting Information). For the NNOP (Glu-Fib) and HPT1, two product ion transitions were of sufficient intensity to develop the finalised SRM method.

3.3. Method validation

The linearity of measurement of the QconCAT concentration from the co-elution of the internal QconCAT calibrator peptide NNOP (EGVNDNEEGFFSAR) was established over a greater than 100-fold range of QconCAT to NNOP ratio in the assay mix, with R² close to 1 (Figure S1a, Supporting Information). The linearity of peptide abundances in a pooled sample of 3 intestinal TM digests was established over a 14-fold range (R² ≥ 0.980) (Figure S1b, Supporting Information). The lower limit of quantitation (LLOQ) was determined in the biological matrix at the lowest protein
Fig. 3. Alkaline phosphatase activity enrichment in the TM fraction compared to the original starting homogenate total protein fraction. The assay was performed with proteins from jejunum (n = 3), ileum (n = 2) and distal colon (n = 2) during enterocyte chelation optimisation experiments (see Table S2 in Supporting Information).

Table 1
Species specificity of the selected transporter protein proteotypic peptides. A 100% agreement in sequence identity between the native protein and the proteotypic peptide is denoted by a check/tick mark (✓).

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Peptide sequence</th>
<th>Homo sapiens</th>
<th>Macaca fascicularis</th>
<th>Canis familiaris</th>
<th>Mus musculus</th>
<th>Rattus norvegicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na/K-ATPase</td>
<td>IVEIFFNSTK</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>HPT1</td>
<td>AENPEPEFGV</td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>P-gp</td>
<td>AGAVAEVLAAR</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MRP2</td>
<td>LVNDIFTFSQQLK</td>
<td>✓</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>BCRP</td>
<td>VIQELGLDK</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>OST-α</td>
<td>YTADILLEVELK</td>
<td>✓</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Contents injected (0.23 μg) as 0.2 fmol/μg. Within-day (intra-day) and between-day (inter-day) precision was assessed in 5 intestinal TM digests. The precision (coefficient of variation of the mean values) over 3 analytical runs between 2 separate days was <15%, within current FDA bio-analytical guidelines [14]. The relative errors (RE) within-day and between-day were <±15%, with the exception of MRP2 and OST-α that were below the LLOQ in some samples and displayed RE <±20% (Table 2).

Previous characterisation of the TransCAT [11] using the reported dual enzyme digestion strategy confirmed proteolysis reached steady state. Another study using the same protocol reported the same result [10]. Assessment of the stability and recovery of peptides is also required by FDA guidelines on bioanalytical method validation. Although these were not covered, steps were taken to limit known causes of degradation of peptides by analysing within a short time of preparation and limiting the number of freeze–thaw cycles to less than 3. Also, peptide loss in the assay was estimated using the developed gravimetric method. For transporter proteins, establishing accuracy in an AQUA or QconCAT assay is difficult to implement due to absence of standard reference materials comprising protein standards of known concentration in suitable biological matrices and has only been demonstrated in a single study for proteo–liposomes containing P-gp [15], but is not routinely performed. However, the optimal approach to determine accuracy is to run biological matrices under study against samples containing a known concentration of the purified target protein. Collectively, it is the lack of available standards of known concentrations, and the requirement for an internal standard to quantify the QconCAT concentration, that creates a significant challenge to implement the accuracy strategies employed in the literature, especially for drug transporter proteins. Recently, accuracy measurements for a QconCAT construct have been performed for CYP3A4 and CYP3A5 in commercially available liver microsomes, in which the enzyme abundances were measured by immunoblotting and ELISA [10]. Thus, given the assumption that there is equimolar release of standard peptides during overnight digestion, accurate stoichiometric determination of light and heavy peak signal intensity should permit peptide quantification with sufficient accuracy.

The potential loss of peptides during the DOC precipitation step, or due to non-specific binding to liquid handling equipment, was accounted for by a gravimetric procedure based on the masses of sample tubes, peptide solutions and precipitates. The total TM

Table 2
Precision (coefficients of variation, CV, %) analysis is provided for transporter proteins in human intestinal total membrane (TM) digests (n = 5) over 3 sample runs, on 2 separate days. Within and between-day differences are provided as relative errors (RE, %) in two separate sample runs.

<table>
<thead>
<tr>
<th>Protein</th>
<th>All runs</th>
<th>Within-day</th>
<th>Between-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision (%)</td>
<td>RE (%)</td>
<td>RE (%)</td>
</tr>
<tr>
<td>TransCAT</td>
<td>5.2–11.3</td>
<td>5.3 to 10.8</td>
<td>–8 to 12.5</td>
</tr>
<tr>
<td>Na/K-ATPase</td>
<td>5.4–12.7</td>
<td>6.7 to 13.1</td>
<td>–3.5 to 14.5</td>
</tr>
<tr>
<td>HPT1</td>
<td>1.4–11.4</td>
<td>–2.7 to 7.3</td>
<td>–10.4 to 10.2</td>
</tr>
<tr>
<td>P-gp</td>
<td>2.5–14.7</td>
<td>0.9 to 12.8</td>
<td>–2 to 13.9</td>
</tr>
<tr>
<td>MRP2</td>
<td>4.3–10.3</td>
<td>4.8 to 18.6</td>
<td>–7.5 to 3.8</td>
</tr>
<tr>
<td>BCRP</td>
<td>2.3–8.0</td>
<td>–0.2 to 9.9</td>
<td>–11 to 10.3</td>
</tr>
<tr>
<td>OST-α</td>
<td>4.7–11.5</td>
<td>–8.7 to 15.4</td>
<td>–10.3 to 6.1</td>
</tr>
</tbody>
</table>

* Precision for one of the samples was based on two runs, as run 3 represented an outlier for MRP2.
protein entering the digest was 50 μg for all samples of which 32 μg (32.01 ± 3.37 μg peptide, n = 4) was recovered giving an estimated recovery of 64%, which was applied as a correction factor to all peptide abundances.

Inter-operator differences in abundance quantification were assessed between 2 independent analysts for 104 co-elution profiles (7 peptides in 5 samples). In >99% of cases, the abundance values determined by each operator were within a pre-defined threshold of 1.25-fold difference between operators. These quality control steps are rarely performed within the drug metabolising enzymes and transporters proteomics field and are essential for comparability of work over a longer period of time and between different laboratories employing similar techniques.

3.4. Application of methods

This study provides a validated LC–MS/MS method using QconCAT derived proteotypic standard peptides to measure the abundances of 6 key drug transporter proteins in human distal jejunum (n = 3) and distal ileum (n = 1), adding to the limited data currently available that quantifies the absolute abundance of transporter proteins in the human intestine using targeted proteomic techniques [6–8]. The advantage that the QconCAT technology provides over conventional QTAG techniques is the ability to use one standard for a host of target proteins, making it more amenable to simultaneous multiplexed quantitative analysis, in addition to the sustainability of the expressed standard and transferability of the QconCAT vector providing an unlimited source of standard peptides in different laboratories, for lower cost when analysing ≥ 10 proteins [16].

The abundances of the 6 quantified transporter proteins are shown in Fig. 4. The basal membrane marker protein Na/K-ATPase showed a considerably higher membrane expression than the other proteins under study. The cadherin-like peptide transporter that also possesses pharmacological relevance [17], HPT1, was selected for analysis as it was anticipated to be a highly abundant apical/lateral membrane marker protein in the intestine, which was confirmed. Of the apical efflux transporter proteins studied in the distal jejunum, the rank order of mean expression is BCRP > P-gp > MRP2, similar to the rank observed in duodenal samples in which the absolute abundance was determined with an S-tagging quantitative immunoblot approach [13]. Yet, in a targeted proteomic strategy, MRP2 was shown to possess the highest abundance compared to P-gp and BCRP [6–8]. In our single distal ileum sample, MRP2 abundance was below the limit of quantification. The mean absolute levels of P-gp and BCRP in jejunum differ by approximately 3 and 7-fold respectively, as does the rank order of expression for P-gp, BCRP and MRP2 between this study and studies centred at The University of Greifswald [6–8]. These differences may arise from inherently different biological expression between the tissues, or from differences in the techniques applied to obtain abundances.

![Fig. 4. Absolute abundances of transporter proteins in human distal jejunum and ileum TM fractions. (A) The transporter protein abundances in distal jejunum from 3 donors, measured in 3 separate analytical runs. (B) The transporter abundances in an ileum sample from a single donor, measured in 3 separate analytical runs. Data are expressed as mean ± standard deviation of biological samples. The text above the bars is the mean abundance of the transporter protein. BLQ denotes that the protein abundance was below the limit of quantification (<0.2 fmol/μg membrane protein). For OST-α, data for the jejunum is based on a single donor as its abundance was below the limit of quantification for the other two biological samples.](image-url)
The OST-α subunit forms a dimeric molecule with the OST-β subunit, which is functionally expressed in the basal membrane of enterocytes and has been shown to participate in endogenous compound and digoxin transport [18,19]. The OST-α subunit was expressed at relatively low levels, and could not be detected in 2 of the 3 jejunum samples. The assay may find greater utility when applied to terminal ileum where OST-α/β is likely to be expressed at higher levels due to its involvement in bile acid reabsorption.

The influence on recent drug administration should also be considered when determining transporter abundances. The 4 donors had all been administered a variety of drugs in the recent past which may have influenced transporter expression (Table S1, Supporting Information). Characterising protein abundances in tissues originating from a diseased population is critical to generating PBPK models in clinical sub-populations for which the drugs are primarily indicated.

4. Conclusion

This study has optimised LC–MS/MS targeted proteomic methods for quantifying drug transporter protein absolute abundance using a QconCAT strategy. The methods were applied to measure Na/K-ATPase, HPT1, P-gp, BCRP, MRP2 and OST-α in eluted human enterocyte total membrane fractions. This study provides the basis to develop further SRM methods for quantification of numerous other proteins of pharmacological relevance in many different organs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2015.02.043.

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