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(54) **Title**: MASS SPECTROMETRY QUANTITATION OF P450 PROTEIN ISOFORMS IN HEPATOCYTES

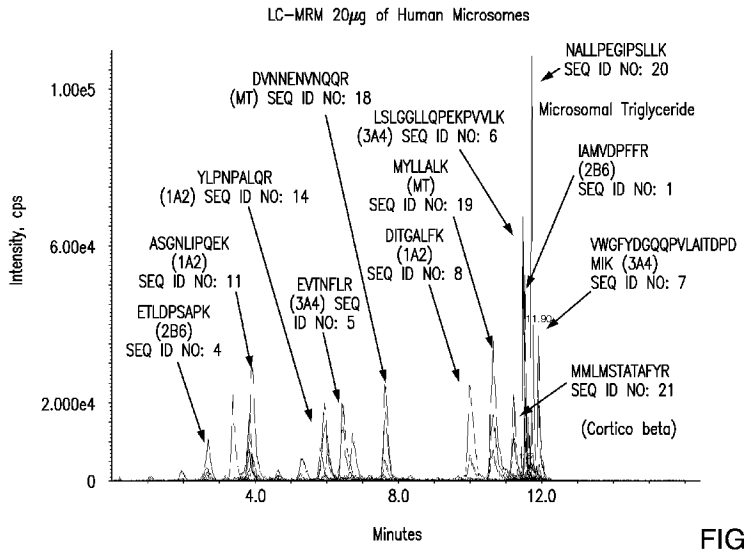

FIG. 1

(57) **Abstract**: A method for screening a drug for cytochrome P45O (CYP) induction is provided and can include incubating the drug with a microsome-containing biological sample and then quantitating at least one cytochrome P45G isoform. The isofoms can be selected from 2B6, 3A4,] A2, and 3A5 isoforms. In some embodiments, the method uses liquid chromatography tandem mass spectrometry (LC-MSMS). A quantitated value can be compared to a threshold value and the drug can be determined to exhibit an acceptable CYP induction potential when the quantitated value does not exceed the threshold value. Isolated peptides are also provided.

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MASS SPECTROMETRY QUANTITATION OF P450 PROTEIN ISOFORMS IN HEPATOCYTES

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims the benefit of the earlier filing dates of U.S. Provisional Patent Applications Nos. 61/252,648, filed October 17, 2009, and 61/252,430, filed October 16, 2009, both of which are incorporated herein in their entireties by reference.

FIELD

[002] The present teachings relate to cytochrome P450 enzymes (CYPs) and detection of enzymes using mass spectrometry.

BACKGROUND

[003] Cytochrome P450 enzymes (CYPs) are major drug metabolizing enzymes and experimental pharmaceutical compounds are generally evaluated for their CYP induction potential early in the development process. Measurement of a CYP induction profile in response to a chemical can be used as a fundamental aspect of drug safety evaluation, but expression of these proteins is regulated by transcriptional, post transcriptional and translational mechanisms. As a result, mRNA-based assays are not reliable predictors of CYP induction. Further, CYPs exhibit extensive amino acid sequence homology, particularly within subfamilies, so that P450 protein expression methods are also poorly discriminatory.

SUMMARY

[004] According to various embodiments of the present teachings, a method of quantitating the 1A2, 2B6, 3A4, and 3A5 isoforms of cytochrome P450 enzymes (CYPs) is provided without the need for any chemical labeling. Isoform-specific tryptic peptides can be observed in liquid chromatography-Tandem Mass Spectrometry (LC-MSMS) analysis of

samples derived from hepatocytes, for example, in microsomes, along with their optimal Q1 and Q3 transitions. Those observed peptides and transitions, can be used to enable a reliable CYP quantitation of the isoforms 1A2, 2B6, 3A4, and 3A5.

[005] According to various embodiments, a set of peptides and optimal MRM transitions are provided as "house keeping" microsomol proteins whose concentrations are unaffected by drug incubation. The set can be used as normalization proteins for quantitative anlysis. In some embodiments, quantitation can be performed by spiking into the sample heavy forms of the isoform-specific peptides, for example, forms that have been enriched with C13 and/or N15. FIG. 1 shows a typical MRM analysis for CYP 1A2, CYP2B6, and CYP3A3/3A4 from human microsomes, wherein some of the isoform-specific peptides have been labeled in FIG. 1. The optimized transitions for each of a set of observed, most sensitive isoform-specific peptides, according to various embodiments of the present teachings, is shown in Tables 1-4 below.

BRIEF DESCRIPTION OF THE DRAWINGS

[006] The present teachings will be described with reference to the accompanying drawings. The drawings are intended to illustrate, not limit, the present teachings.

[007] FIG. 1 shows an MRM analysis of 20µg of a microsomal preparation for CYP 1A2, CYP 2B6, and CYP 3A4 (extracted ion chromatogram (XIC) of + MRM (412 pairs)).

[008] FIG. 2 shows an MRM analysis of 1 µl of a microsomal preparation for CYP 2B6 (extracted ion chromatogram of + MRM (15 pairs)).

[009] FIG. 3 shows an MRM analysis of 2 µg of a microsomal preparation for CYP 1A2 (extracted ion chromatogram of + MRM (57 pairs)).

[0010] FIG. 4 shows an MRM analysis of 1 µl of a microsomal preparation for CYP 3A4 (extracted ion chromatogram of + MRM (34 pairs)).

[0011] FIG. 5A shows an MRM analysis for 30 µl of a microsomal preparation for 3A5 (extracted ion chromatogram of + MRM (48 pairs)).

[0012] FIG. 5B shows an MRM analysis for 30 µl of a microsomal preparation for 3A5 (extracted ion chromatogram of + MRM (48 pairs)).

[0013] FIG. 5C shows an MRM analysis for 30 µl of a microsomal preparation for 3A5 (extracted ion chromatogram of + MRM (48 pairs)).

[0014] FIG. 6 shows a graph showing protein expression changes observed in a hepatocyte sample preparation treated with inducers for CYP 2B6, using RNA assays, enzyme activity assays and the protein quantitation method of the present teachings.

[0015] FIG. 7 shows a graph showing protein expression changes observed in a hepatocyte sample preparation treated with inducers for CYP 1A2, using RNA assays, enzyme activity assays and the protein quantitation method of the present teachings.

[0016] FIG. 8 shows a graph showing protein expression changes observed in a hepatocyte sample preparation treated with inducers for CYP 3A4, using RNA assays, enzyme activity assays and the protein quantitation method of the present teachings.

DETAILED DESCRIPTION

[0017] According to various embodiments, a method for screening a drug for cytochrome P450 (CYP) induction is provided that comprises incubating the drug with a microsome-containing biological sample and quantitating at least one CYP isoform. In some embodiments, the isoforms can comprise one or more isoform selected from 2B6, 3A4, 1A2, and 3A5 isoforms. The method can comprise using a liquid chromatography tandem mass spectrometry (LC-MSMS) technique to quantitate the amount of each isoform. The quantitated value of each can be compared to a threshold value, and the drug can be identified as having an acceptable CYP induction potential when the quantitated value does not exceed

the threshold value. The threshold value can be selected or predetermined based on a desired CYP induction potential or based on the CYP induction potential of one or more different drugs, similar or non-similar to the drug being screened. In some embodiments, the microsome-containing biological sample can be derived from a mammal, from a primate, or from a human.

[0018] According to various embodiments, the drug can be incubated with a sample containing human hepatocytes. In some embodiments, after incubation with the drug, the sample containing human hepatocytes can be used to obtain at least one microsome fraction by, for example, 16 G centrifugation. The microsome fraction can be analyzed for CYP induction by the drug, by detecting isolated peptides specific to CYP (isoform-specific peptides). According to some embodiments, after incubation with the drug, the sample containing human hepatocytes can be used to obtain at least one S9 fraction by, for example, 9 G centrifugation. The S9 fraction can be analyzed to detect CYP induction by the drug, for example, by detecting isolated peptides specific to CYP (isoform-specific peptides). According to some embodiments, the microsome fraction or the S9 fraction can be analyzed using a liquid chromatography tandem mass spectrometry (LC-MSMS) technique in order to quantitate at least one CYP isoform. The quantitated value of each can be compared to a threshold value, and the drug can be identified as having an acceptable CYP induction potential when the quantitated value does not exceed the threshold value.

[0019] According to various embodiments, a method is provided for directly analyzing CYP from hepatocytes. In some embodiments, antibody peptides can be used to pull the isoform-specific peptides directly out of hepatocytes. According to some embodiments, using antibody peptides to pull the isoform-specific peptides directly out of hepatocytes would have the advantage of not needing to prepare S9 or microsome fractions, and would require less hepatocyte cells for drug incubation.

[0020] In some embodiments, the method comprises comparing detected induction to a control. For example, because little or no drug induction of CYPs is desirable, a threshold can be set such that the drug must show less than (<) 40% induction compared to the positive control, to be considered acceptable. In some embodiments, the drug must show less than (<) 30% induction compared to the positive control, to be considered acceptable. In other embodiments, the drug must show less than (<) 20% induction compared to the positive control, to be considered acceptable.

[0021] According to various embodiments, a method for determining an amount of at least one isoform of cytochrome P450 (CYP) in a sample, is provided. The method can comprise the use of a mass spectrometry technique, wherein the at least one isoform of cytochrome P450 comprises at least one of CYP 2B6, CYP 3A4, CYP 1A2, and CYP 3A5. The mass spectrometry technique can comprise a tandem mass spectrometry (MS/MS) technique and/or a liquid chromatography tandem mass spectrometry (LC-MS/MS) technique. In some embodiments, the technique comprises an LC-MS/MS technique and the use of a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

[0022] FIG. 1 shows a typical MRM analysis for CYP 1A2, CYP 2B6, and CYP 3A4, as well as the "housekeeping" microsomal proteins (Microsomal GST, Corticosteroid 11 beta, and Microsomal Tryglyceride), from a microsomal sample preparation prepared as described below in the Examples. The quantity of CYP 1A2, CYP 2B6, and CYP 3A4 can be determined by, for example, Isotope Dilution Mass Spectrometry, wherein the sample preparation is spiked with heavy forms of the isoform-specific peptides. The quantity of CYP 1A2, CYP 2B6, and CYP 3A4 can also be determined using other conventional methods known in the art. In some embodiments, the method uses LC-MSMS with multiple reaction monitoring (MRM) quantitation of the isoform-specific peptides and isotope-coded affinity tags (ICAT) to generate a CYP induction profile. The method can use, for example,

approaches similar to the approaches presented by Pennington et al. to quantitate isoform-specific cysteine-containing peptides labeled with ICAT as described in Proteomics, 6(6), pages 1934-1947 (March 2006), which is incorporated herein in its entirety by reference.

[0023] FIG. 2 shows a typical MRM analysis for CYP 2B6 from the microsomal preparation prepared as described herein. Isolated peptides comprising the amino acid sequence of SEQ ID NOS: 1, 2, 3, or 4 identified in Table 1 below are specific to CYP 2B6.

[0024] FIG. 3 shows a typical MRM analysis for CYP 1A2 from the microsomal preparation prepared as described herein. The isolated peptides comprising the amino acid sequence of SEQ ID NOS: 8, 9, 10, 11, 12, 13, or 14 identified in Table 3 below are specific to CYP 1A2.

[0025] FIG. 4 shows a typical MRM analysis for CYP 3A4 from the microsomal preparation prepared as described herein. The isolated peptides comprising the amino acid sequence of SEQ ID NOs: 5, 6, or 7 identified in Table 2 below are specific to CYP 3A4. It should be understood that peptides comprising the amino acid sequence of SEQ ID NOS: 5, 6, or 7 can also be used to identify and/or quantify CYP 3A3.

[0026] The isolated peptides comprising the amino acid sequence of SEQ ID NOs: 15, 16, or 17 identified in Table 4 below are specific to CYP 3A5.

[0027] FIGs. 5A-5C show three different panes of a typical MRM analysis for CYP 3A5 from the microsomal preparation prepared as described herein. Each pane relates to specific transitions used for the particular peptide. The isolated peptides comprising the amino acid sequence of SEQ ID NOS: 15, 16, or 17 are specific to CYP 3A5.

[0028] According to various embodiments, the method can comprise determining an amount of CYP 2B6 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 2B6, for example, one or more of the isoforms comprising the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 identified herein.

The amount can be determined using a triple quadrupole instrument and Multiple Reaction Monitoring (MRM). In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 1 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 548/911, 548/681, or 548/566, wherein the term "about" as used herein means within a range of +/- one (1) atomic mass unit. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 2 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 494/777, 494/437, or 494/874. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 3 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 421/508, 421/607, or 421/694. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 4, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 479/499, 479/614, or 479/727.

[0029] According to various embodiments, the method can comprise determining an amount of CYP 3A4 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 3A4, comprising the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7 identified herein. The method can use, for example, a triple quadrupole instrument and Multiple Reaction Monitoring (MRM). In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 5 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 440/549, 440/650, or 440/532. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 6 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 704/794, 704/929, 564/689, 564/745, or 564/790. In some embodiments, the isolated peptide

can comprise the amino acid sequence of SEQ ID NO: 7 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 798/819, 798/932, or 798/1004.

[0030] According to various embodiments, the method can comprise determining an amount of CYP 1A2 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 1A2, comprising the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14, identified herein. The method can use, for example, a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

[0031] In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 8 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 432/636, 432/535, or 432/478. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 9 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 482/800, 482/628, or 482/743. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 10 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 491/721, 491/834, or 491/535. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 11 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 528/501, 528/614, or 528/727. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 12 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 571/783, 571/971, 571/1028, 381/587, 381/474, or 381/375. In some embodiments, the isolated peptide can comprise the amino acid sequence of SEQ ID NO: 13 identified herein, and the method

can comprise monitoring precursor-product ion pair transitions having an m/z value of about 695/695, 695/837, or 695/950. In some embodiments, the isolated peptide can comprise the isolated peptide comprises the amino acid sequence of SEQ ID NO: 14 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 536/795, 536/584, or 536/698.

[0032] According to various embodiments, the method can comprise determining an amount of CYP 3A5 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 3A5, comprising the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17 identified herein. The method can use, for example, a triple quadrupole instrument and Multiple Reaction Monitoring (MRM). In some embodiments, the isolated peptide can comprise an amino acid sequence of SEQ ID NO: 15 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 468/581, 468/679, or 468/736. In some embodiments, the isolated peptide can comprise an amino acid sequence of SEQ ID NO: 16 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 470/494, 470/608, or 470/722. In some embodiments, the isolated peptide can comprise an amino acid sequence of SEQ ID NO: 17 identified herein, and the method can comprise monitoring precursor-product ion pair transitions having an m/z value of about 589/747, 589/696, or 589/647.

[0033] According to various embodiments of the present teachings, a kit is provided that can comprise one or more of the isolated peptides specific to one or more of cytochrome P450 (CYP) isoform CYP 2B6, CYP 3A4, CYP 1A2, and CYP 3A5. For example, the kit can comprise one or more isolated proteins specific to CYP 2B6, comprising the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4. The kit can comprise one or more isolated proteins specific to CYP isoform CYP 3A4, comprising the

amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7. The kit can comprise one or more isolated proteins specific to CYP isoform CYP 1A2, comprising the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14. The kit can comprise one or more isolated proteins specific to CYP isoform CYP 3A5, comprising the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17.

[0034] In some embodiments, the kit can comprise at least one isolated peptide specific to each of CYP isoforms CYP 2B6, CYP 3A4, CYP 1A2, and CYP 3A5. For example, the kit can comprise each of the isolated peptides of SEQ ID NOS: 1-17 identified herein, and further can comprise instructions for measuring Q1 and Q3 transition values for each of the isoform-specific peptides. The kit can comprise enzyme digestion components including buffers and enzymes, other buffers, and optionally other reagents and/or components. In some embodiments, the kit can comprise, for example, a homogeneous assay such that the user need only add a sample. In some embodiments, the kit can comprise calibration or normalization reagents or standards. Information pertaining to instrument settings that can or should be used to perform an assay can also be included in the kit. Information pertaining to sample preparation, operating conditions, volumetric amounts, temperature settings, and the like, can be included with the kit.

[0035] According to various embodiments, different transitions can be used to measure and benchmark assay results, depending on various factors. Accordingly, the kit can comprise different transition values and/or suggested settings, useful to make comparative measurements between a sample and one or more control reagents. The kit can include instructions to measure specific pairs of transition values, for example, the Q1/Q3 transition pair, or the values of one or more different transition pairs.

[0036] The kit can be packaged in a hermetically sealed container containing one or more

regent vessels and appropriate instructions. An electronic medium can be included in the kit, having stored thereon electronic information pertaining to one or more assays, measurement values, transition pairs, operating instructions, software for carrying out operations, a combination thereof, or the like.

EXAMPLES

[0037] The present teachings can be even more fully understood with reference to the examples and resulting data that follow. In the examples below and the results shown in the attached drawing figures, a CYP induction study was undertaken as follows.

Hepatocyte Treatment

[0038] Primary cultures of human hepatocytes were treated for 72 hours with the prototypical liver enzyme inducers 3-methylcholanthrene (3-MC, 2 μM), phenobarbital (PB, 1 mM), or rifampicin (RIF, 10 μM) to enhance the expression and activity of CYP1A2, CYP2B6, and CYP3A4, respectively. The CYP quantitation method of the present teachings was used to determine the amount of CYP in the sample after treatment with the liver enzyme inducers. In addition, CYP activity was measured by metabolite formation from selective substrates (phenacetin, bupropion and testosterone, respectively) and mRNA was measured by qRT-PCR (Taqman®, Applied Biosystems).

Microsome Preparation

[0039] Microsomal subcellular fractions were prepared by lysing treated hepatocytes in homogenization buffer (50 mM TRIS-HCl, pH 7.0, 150 mM KCl, 2 mM EDTA) followed by centrifugation at 9,000 x g for 20 minutes at 4°C. The supernatant (S9 fraction) was then spun at 100,000 x g for 60 minutes at 4°C. The resulting microsomal pellet was resuspended in 0.25 M sucrose and stored at -80°C until analysis.

Tryptic Digestion

[0040] To 100ul of each microsomal preparation, 5ul of 2% SDS was added, followed by 10ul of 50mM TCEP and incubated at 60°C for 1 hr. 5ul of 0.1M MMTS was added and incubated at room temperature for 10 minutes. 100 ul of 100mM TRIS (pH = 8.50) was then added followed by 50ug of trypsin and the resultant solution was digested overnight at 37°C. One (1) ng of each isotopically enriched synthetic peptide was added to the digest and analyzed by LC-MS.

Chromatography

[0041] Chromatography was performed using an Agilent 1100 system (Agilent of Santa Clara, California) coupled to a C18 Jupiter Proteo 50 x 2.0 mm column (Phenomenex of Torrance, California). The gradient was 5-40% B over 15 minutes with A consisting of 2% ACN, 0.1% formic and B consisting of 90% ACN, 10% H2O, 0.1% formic acid. Flow rate was 700µL/min.

Mass Spectrometry

[0042] Samples were analyzed on a Applied Biosystems MDS SCIEX 4000 QTRAPTM LC/MS/MS system, using a Turbo VTM source and Analyst 1.5. For quantitation, scheduled MRM (sMRM) was used to maximize dwell time on each transition.

Data Processing

[0043] Quantitative data was processed using MultiQuantTM 1.2 software available from Applied Biosystems, LLC of Foster City, California..

Results

[0044] FIG. 6 is a graph comparing the changes in expression of CYP 2B6 observed using the RNA assay, the CYP activity assay (designated "enzyme activity assay" in the figures), and a CYP quantitation method of the present teachings (designated "protein assay" in the figures). The 3-MC is a vehicle control and induces basal levels of CYP 2B6. The PB is a prototypical

inducer for CYP 2B6, by CAR nuclear receptor activation. The RIF is also a known inducer of CYP 2B6. As is shown, protein expression changes observed in the CYP activity assay and RNA assay generally mirror the expression changes observed using a CYP quantitation method of the present teachings.

[0045] FIG. 7 is a graph comparing the changes in expression of CYP 1A2 observed using the RNA assay, the CYP activity assay (designated "enzyme activity assay" in the figures), and the CYP quantitation method of the present teachings (designated "protein assay" in the figures). The 3-MC is a prototypical inducer of CYP 1A2 by AhR nuclear receptor activation. The PB and RIF are vehicle controls inducing basal levels of 1A2, if any. As is shown, protein expression changes observed in the CYP activity assay and RNA assay generally mirror the expression changes observed using a CYP quantitation method of the present teachings, except that the RNA assay for the sample treated with 3MC exhibits very high levels of RNA. The RNA assay cannot accurately quantify proteins because not all mRNA is converted to protein.

[0046] FIG. 8 is a graph comparing the changes in expression of CYP 3A4 observed using the RNA assay, the CYP activity assay (designated "enzyme activity assay" in the figures), and a CYP quantitation method of the present teachings (designated "protein assay" in the figures). The 3-MC minimally induces CYP 3A4. The PB significantly induces 3A4. The RIF is a prototypical inducer of CYP 3A4 by PXR nuclear receptor activation. As is shown, protein expression changes observed in the CYP activity assay and RNA assay generally mirror the expression changes observed using the CYP quantitation method of the present teachings.

[0047] Table 1 below shows sequences of the peptides determined, according to the present teachings, to be specific to cytochrome P450 (CYP) isoform CYP 2B6, along with their optimal MRM Q1, Q3 transitions. According to various embodiments, these observed peptides and transitions can be used to enable a reliable CYP quantitation of the isoform CYP

2B6.

TABLE 1

2B6 Human	Q1	Q3
IAMVDPFFR	548.3	911.4
(SEQ ID NO: 1)	548.3	681.3
	548.3	566.3
IPPTYQIR	494.3	777.4
(SEQ ID NO: 2)	494.3	437.7
	494.3	874.5
FSVTTMR	421.2	508.4
(SEQ ID NO: 3)	421.2	607.3
	421.2	694.4
ETLDPSAPK	479.2	499.3
(SEQ ID NO: 4)	479.2	614.3
	479.2	727.4

[0048] Table 2 below shows sequences of the peptides determined, according to the present teachings, to be specific to cytochrome P450 (CYP) isoform CYP 3A4, along with their optimal MRM Q1, Q3 transitions. According to various embodiments, these observed peptides and transitions can be used to enable a reliable CYP quantitation of the isoform CYP 3A4.

TABLE 2

3A4 Human	Q1	Q3
EVTNFLR	439.7	549.3
(SEQ ID NO: 5)	439.7	650.5
	439.7	532.3
LSLGGLLQPEKPVVLK	704.4	794.5
(SEQ ID NO: 6)	704.4	929.8
LSLGGLLQPEKPVVLK +3	564.3	789.5
(SEQ ID NO: 6)	564.3	745.9
	564.3	689.4
VWGFYDGQQPVLAITDPDMIK	798.4	819.4
(SEQ ID NO: 7)	798.4	932.5
	798.4	1003.5

[0049] Table 3 below shows sequences of the peptides determined, according to the present teachings, to be specific to cytochrome P450 (CYP) isoform CYP 1A2, along with their optimal MRM Q1, Q3 transitions. According to various embodiments, these observed peptides and transitions can be used to enable a reliable CYP quantitation of the isoform CYP 1A2.

TABLE 3

1A2 Human	Q1	Q3
DITGALFK	432.7	636.4
(SEQ ID NO: 8)	432.7	535.3
	432.7	478.3
YGDVLQIR	482.3	800.5
(SEQ ID NO: 9)	482.3	628.4
	482.3	743.4
FLWFLQK	491.3	721.4
(SEQ ID NO: 10)	491.3	834.4
	491.3	535.3
ASGNLIPQEK	528.7	501.2
(SEQ ID NO: 11)	528.7	614.4
	528.7	727.4
IGSTPVLVLSR +2	571.4	783.5
(SEQ ID NO: 12)	571.4	971.6
	571.4	1028.6
IGSTPVLVLSR +3	381.1	587.4
(SEQ ID NO: 12)	381.1	474.3
	381.1	375.2
SPPEPWGWPLLGHVLYGK	695.4	695.9
(SEQ ID NO: 13)	695.4	837.5
	695.4	950.5
YLPNPALQR	536.3	795.4
(SEQ ID NO: 14)	536.3	584.3
	536.3	698.4

[0050] Table 4 below shows sequences of the peptides determined, according to the present teachings, to be specific to cytochrome P450 (CYP) isoform CYP 3A5, along with their optimal MRM Q1, Q3 transitions. According to various embodiments, these observed peptides and transitions can be used to enable a reliable CYP quantitation of the isoform CYP 3A5.

TABLE 4

3A5	Q1	Q3
SLGPVGFMK	**468.5**	**581.5**
(SEQ ID NO: 15)	**468.5**	**678.1**
	468.5	**735.5**
DTINFLSK	**469.5**	**494.3**
(SEQ ID NO: 16)	**469.5**	**608.4**
	469.5	**721.5**
GSMVVIPTYALHHDPK	**589.2**	**746.5**
(SEQ ID NO: 17)	**589.2**	**696**
	589.2	**646.5**

[0051] Table 5 below shows sequences of the peptides along with their optimal MRM Q1, Q3 transitions for the house-keeping microsomal protein Microsomal GST. According to various embodiments, the concentration of this observed peptide is unaffected by drug incubation and thus the peptide can be useful as a normalization protein to enable reliable CYP quantitation.

TABLE 5

Microsomal GST	Q1	Q3
DVNVENVNQQR	657.8	758.3
(SEQ ID NO: 18)	657.8	887.5
	657.8	545.3
MYLLALK	426.3	720.5
(SEQ ID NO: 19)	426.3	557.4
	426.3	444.3
NALLPEGIPSLLK	682.9	1066.7
(SEQ ID NO: 20)	682.9	727.5
	682.9	953.6

[0052] Table 6 below shows sequences of the peptides along with their optimal MRM Q1, Q3 transitions for the house-keeping microsomal protein Microsomal Triglyceride. According to various embodiments, the concentration of this observed peptide is unaffected by drug incubation and thus the peptide can be useful as a normalization protein to enable reliable CYP quantitation.

TABLE 6

Microsomal Tryglyceride	Q1	Q3
MMLMSTATAFYR	711.8	829.4
(SEQ ID NO: 21)	711.8	916.2
	711.8	1047.5

[0053] Table 7 below shows sequences of the peptides along with their optimal MRM Q1, Q3 transitions for the house-keeping microsomal protein Corticosteroid 11 beta. It should be understood that the Q1 and Q3 masses for the peptide of SEQ ID NO: 22 in Table 7 refers to the MMTS alkylated peptide and that changing to a different alkylating reagent will change the Q1, Q3 masses. In addition, alkylating reagents other than MMTS can be used. According to various embodiments, the concentration of this observed peptide is unaffected by drug incubation and thus the peptide can be useful as a normalization protein to enable reliable CYP quantitation.

TABLE 7

Corticosteroid 11 beta	Q1	Q3
EECALEHK	**547.3**	**615.4**
(SEQ ID NO: 22)	**547.3**	**686.4**
	547.3	**835.4**
FALDGFFSSIR	**630.3**	**928.4**
(SEQ ID NO: 23)	**630.3**	**1041.5**
	630.3	**813.4**

[0054] The observed isoform-specific tryptic peptides that are detected using LC-MSMS analysis of microsomes, along with their optimal Q1, Q3 transitions, enable a method for CYP quantitation of the isoforms 1A2, 2B6, 3A4, and 3A5 without the need for any chemical labeling approaches.

[0055] Other embodiments of the present teachings will be apparent to those skilled in the art from consideration of the present specification and practice of the present teachings disclosed herein. It is intended that the present specification and examples be considered as exemplary only.

WHAT IS CLAIMED IS:

1. An isolated peptide specific to cytochrome P450 (CYP) isoform CYP 2B6, comprising the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

2. An isolated peptide specific to cytochrome P450 (CYP) isoform CYP 3A4, comprising the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.

3. An isolated peptide specific to cytochrome P450 (CYP) isoform CYP 1A2, comprising the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

4. An isolated peptide specific to cytochrome P450 (CYP) isoform CYP 3A5, comprising the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17.

5. A method for determining an amount of at least one isoform of cytochrome P450 (CYP) in a sample, comprising the use of a mass spectrometry technique, wherein the at least one isoform of cytochrome P450 comprises at least one of CYP 2B6, CYP 3A4, CYP 1A2, and CYP 3A5.

6. The method of claim 5, wherein the mass spectrometry technique comprises a tandem mass spectrometry (MS/MS) technique.

7. The method of claim 5, wherein the mass spectrometry technique comprises a liquid chromatography tandem mass spectrometry (LC-MS/MS) technique.

8. The method of claim 7, wherein said LC-MS/MS technique comprises the use of a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

9. The method of claim 5, wherein the determining comprises determining an amount of CYP 2B6 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 2B6, comprising the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, using a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

10. The method of claim 9, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 1, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 548/911, 548/681, or 548/566.

11. The method of claim 9, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 2, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 494/777, 494/437, or 494/874.

12. The method of claim 9, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 3, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 421/508, 421/607, or 421/694.

13. The method of claim 9, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 4, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 479/499, 479/614, or 479/727.

14. The method of claim 5, wherein the determining comprises determining an amount of CYP 3A4 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 3A4, comprising the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7, using a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

15. The method of claim 14, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 5, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 440/549, 440/650, or 440/532.

16. The method of claim 14, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 6, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 704/794, 704/929, 564/689, 564/745, or 564/790.

17. The method of claim 14, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 7, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 798/819, 798/932, or 798/1004.

18. The method of claim 5, wherein the determining comprises determining an amount of CYP 1A2 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 1A2, comprising the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14, using a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

19. The method of claim 18, wherein the isolated peptide comprises the amino acid

sequence of SEQ ID NO: 8, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 432/636, 432/535, or 432/478.

20. The method of claim 18, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 9, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 482/800, 482/628, or 482/743.

21. The method of claim 18, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 10, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 491/721, 491/834, or 491/535.

22. The method of claim 18, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 11, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 528/501, 528/614, or 528/727.

23. The method of claim 18, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 12, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 571/783, 571/971, 571/1028, 381/587, 381/474, or 381/375.

24. The method of claim 18, wherein the isolated peptide comprises the amino acid sequence of SEQ ID NO: 13, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 695/695, 695/837, or 695/950.

25. The method of claim 18, wherein the isolated peptide comprises the amino acid

sequence of SEQ ID NO: 14, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 536/795, 536/584, or 536/698.

26. The method of claim 5, wherein the determining comprises determining an amount of CYP 3A5 in the sample by detecting an isolated peptide specific to cytochrome P450 (CYP) isoform CYP 3A5, comprising the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, using a triple quadrupole instrument and Multiple Reaction Monitoring (MRM).

27. The method of claim 26, wherein the isolated peptide comprises an amino acid sequence of SEQ ID NO: 15, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 468/581, 468/679, or 468/736.

28. The method of claim 26, wherein the isolated peptide comprises an amino acid sequence of SEQ ID NO: 16, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 470/494, 470/608, or 470/722.

29. The method of claim 26, wherein the isolated peptide comprises an amino acid sequence of SEQ ID NO: 17, and the method comprises monitoring precursor-product ion pair transitions having an m/z value of about 589/747, 589/696, or 589/647.

30. A method for screening a drug for cytochrome P450 (CYP) induction, comprising:

incubating the drug with a microsome-containing biological sample;

quantitating at least one cytochrome P450 isoform selected from the group of 2B6, 3A4, 1A2, and 3A5 isoforms, using liquid chromatography tandem mass spectrometry (LC-

MSMS);

comparing the quantitated value to a threshold value;

determining that the drug has an acceptable CYP induction potential when the quantitated value does not exceed the threshold value.

31. The method of claim 28, wherein the microsome-containing biological sample is derived from a human.

32. A kit comprising one or more isolated peptides specific to CYP isoform CYP 2B6, CYP 3A4, CYP 1A2, or CYP 3A5, comprising one or more of the amino acid sequences of SEQ ID NOS: 1-17.

33. The kit of claim 32, comprising one or more amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

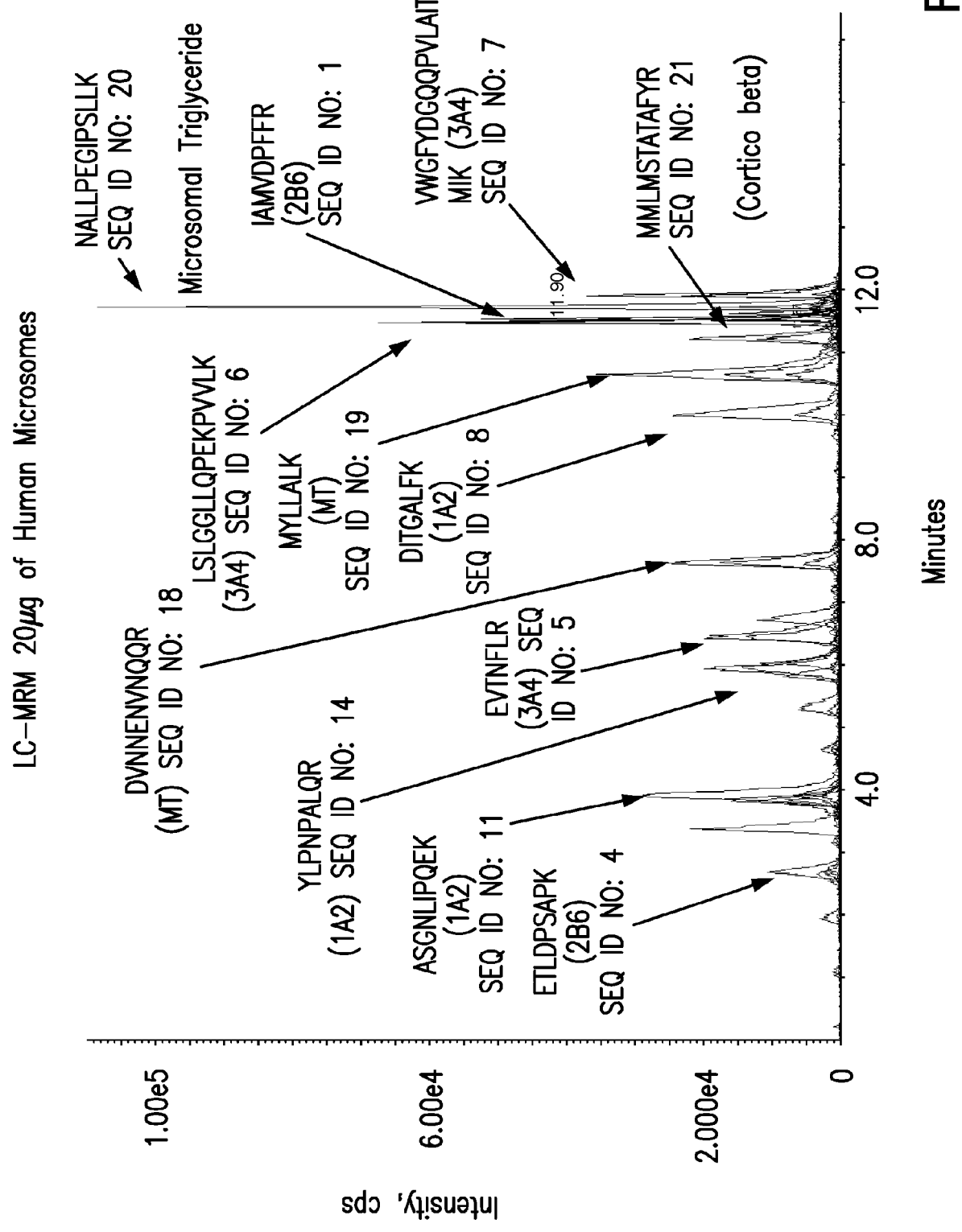
34. The kit of claim 32, comprising one or more amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.

35. The kit of claim 32, comprising one or more amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

36. The kit of claim 32, comprising one or more amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17.

37. The kit of claim 32, comprising a homogeneous assay.

38. The kit of claim 32, comprising information pertaining to transition pairs to be used as settings in a mass spectrometry instrument, for carrying out an assay using the kit.


LC–MRM 20μg of Human Microsomes
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Microsomal Triglyceride
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(2B6)
SEQ ID NO: 1
VWGFYDGQQPVLAITDPD
MIK (3A4)
SEQ ID NO: 7
11.90
MMLMSTATAFYR
SEQ ID NO: 21
(Cortico beta)
DVNNENVNQQR
(MT) SEQ ID NO: 18
LSLGGLLQPEKPVVLK
(3A4) SEQ ID NO: 6
MYLLALK
(MT)
SEQ ID NO: 19
DITGALFK
(1A2)
SEQ ID NO: 8
YLPNPALQR
(1A2) SEQ ID NO: 14
EVTNFLR
(3A4) SEQ
ID NO: 5
ASGNLIPQEK
(1A2)
SEQ ID NO: 11
ETLDPSAPK
(2B6)
SEQ ID NO: 4
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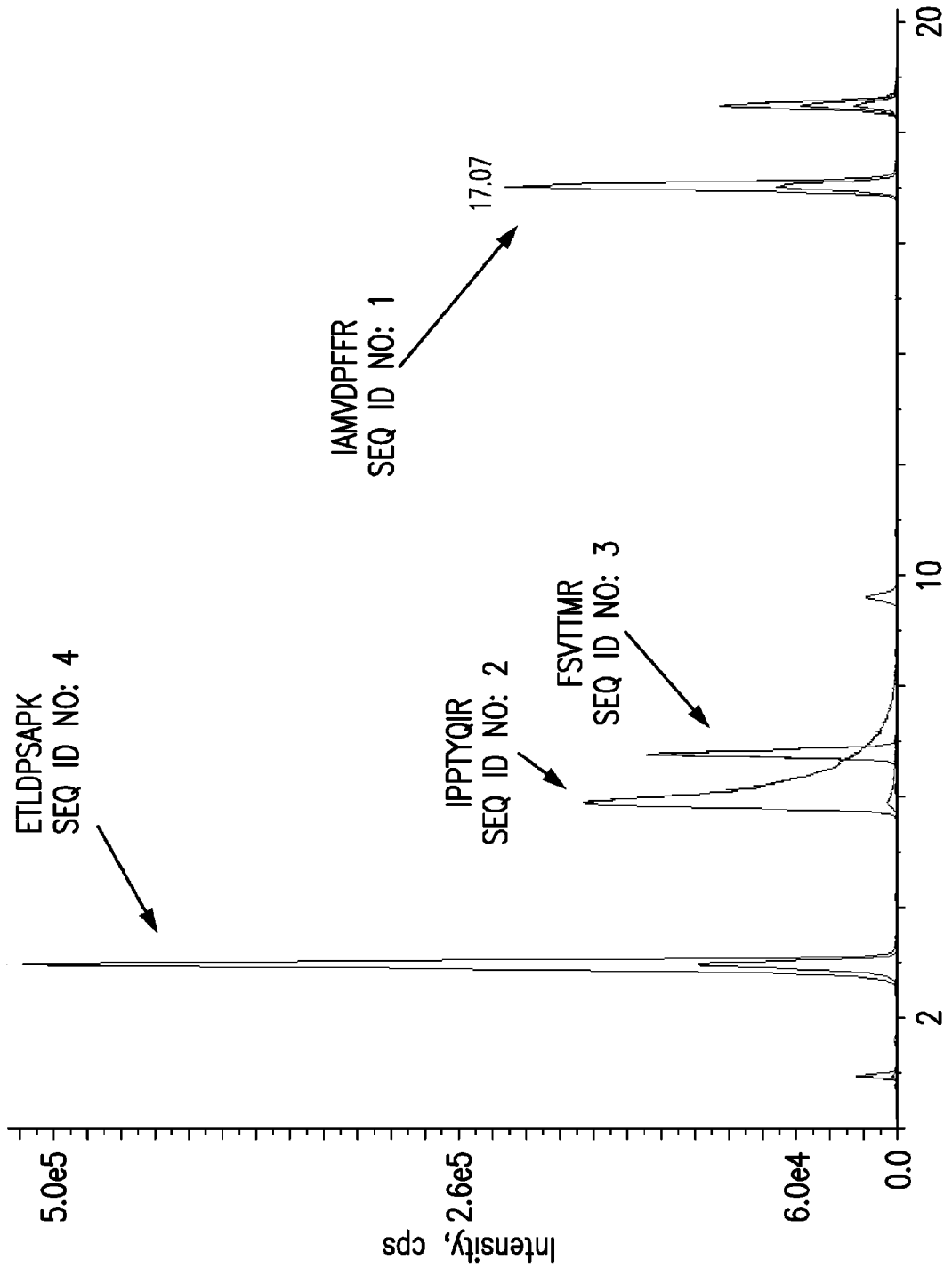
12.0
Minutes

FIG. 1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SEQ ID NO: 2
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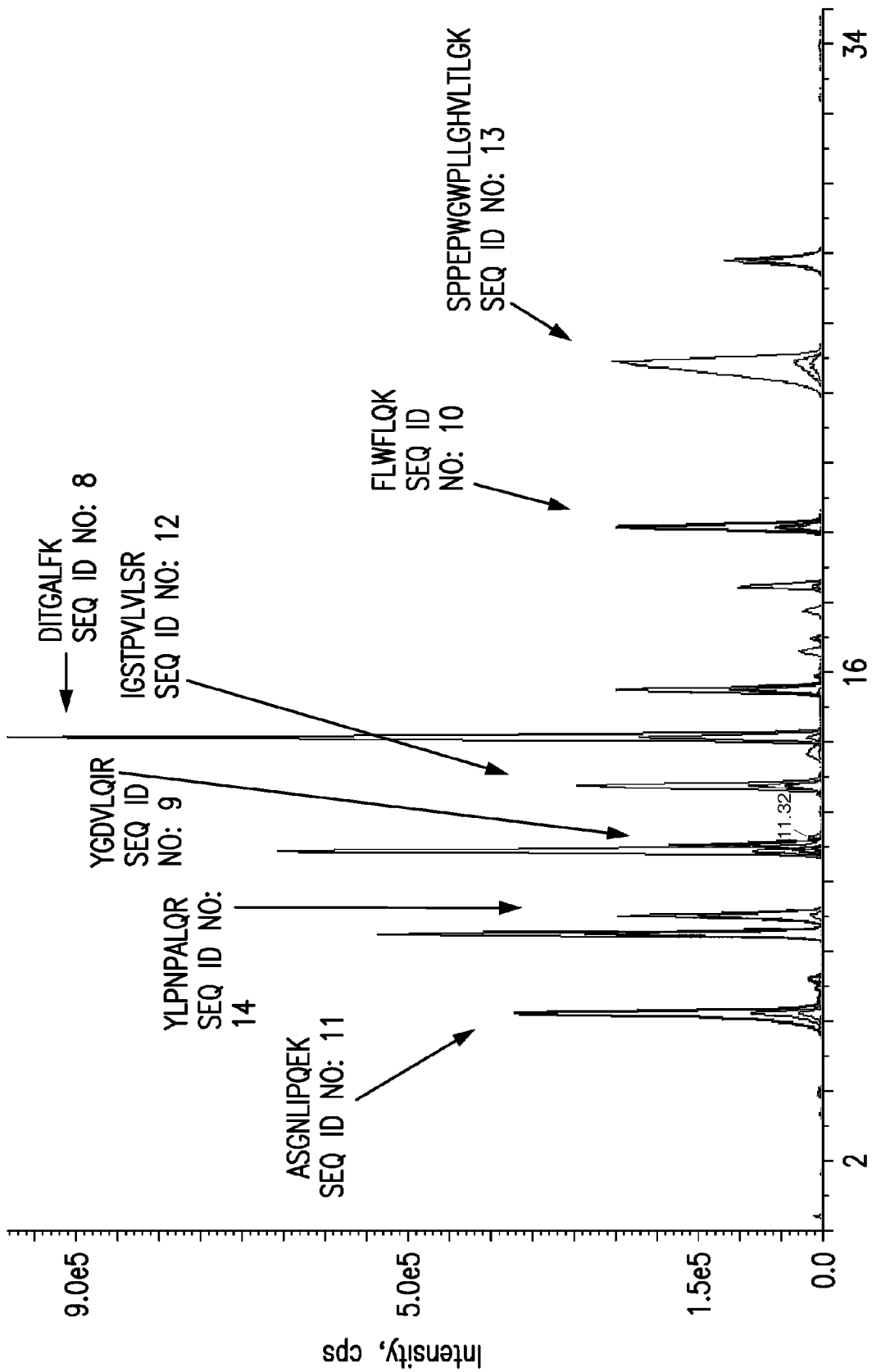
2
10
20
Minutes

FIG. 2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SEQ ID NO: 12
YGDVLQIR
SEQ ID
NO: 9
YLPNPALQR
SEQ ID NO:
14
ASGNLIPQEK
SEQ ID NO: 11
FLWFLQK
SEQ ID
NO: 10
SPPEPWGWPLLGHVLTLGK
SEQ ID NO: 13
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Intensity, cps
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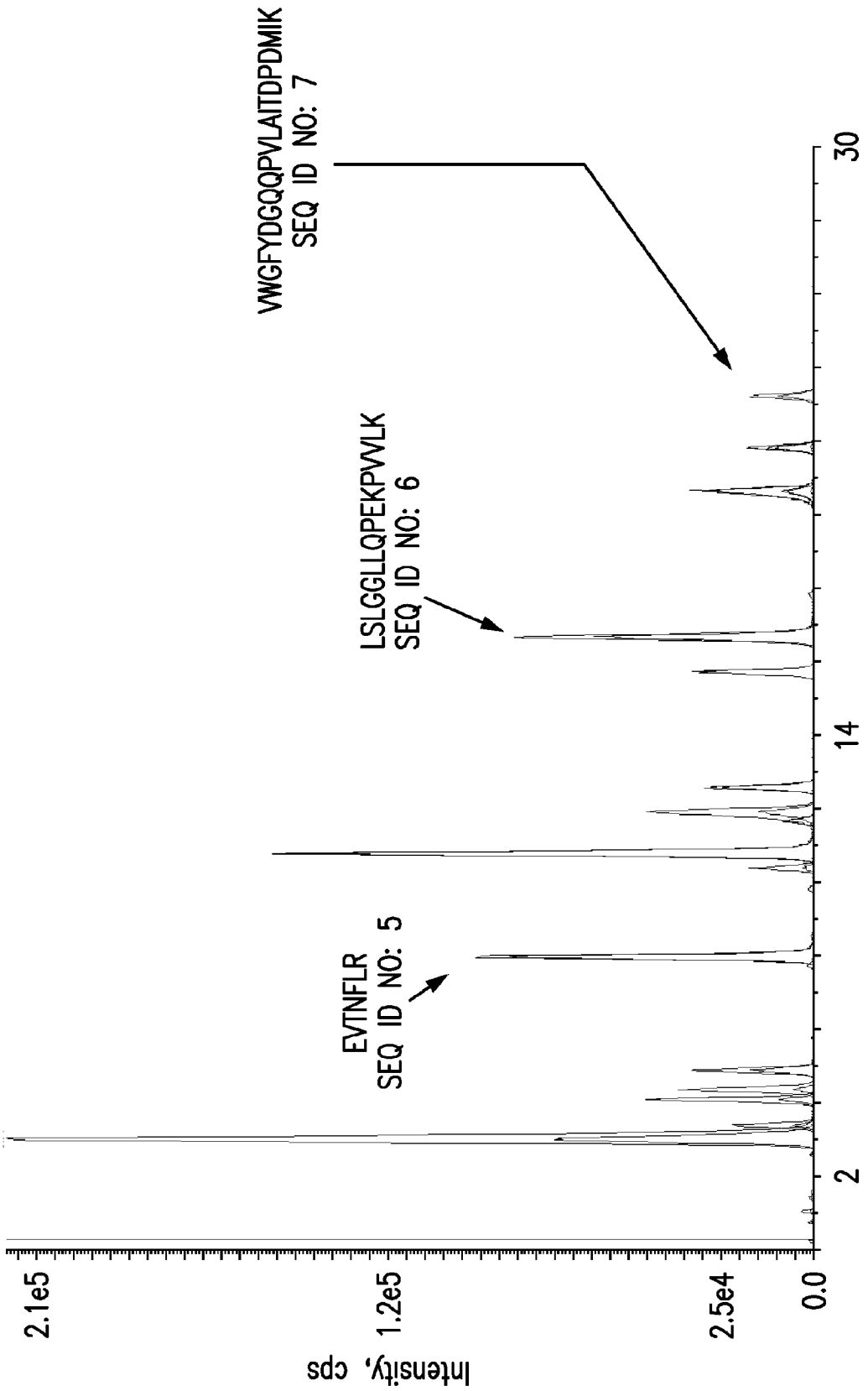
16
34
Minutes

FIG. 3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SEQ ID NO: 6
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14
30
Minutes

FIG. 4

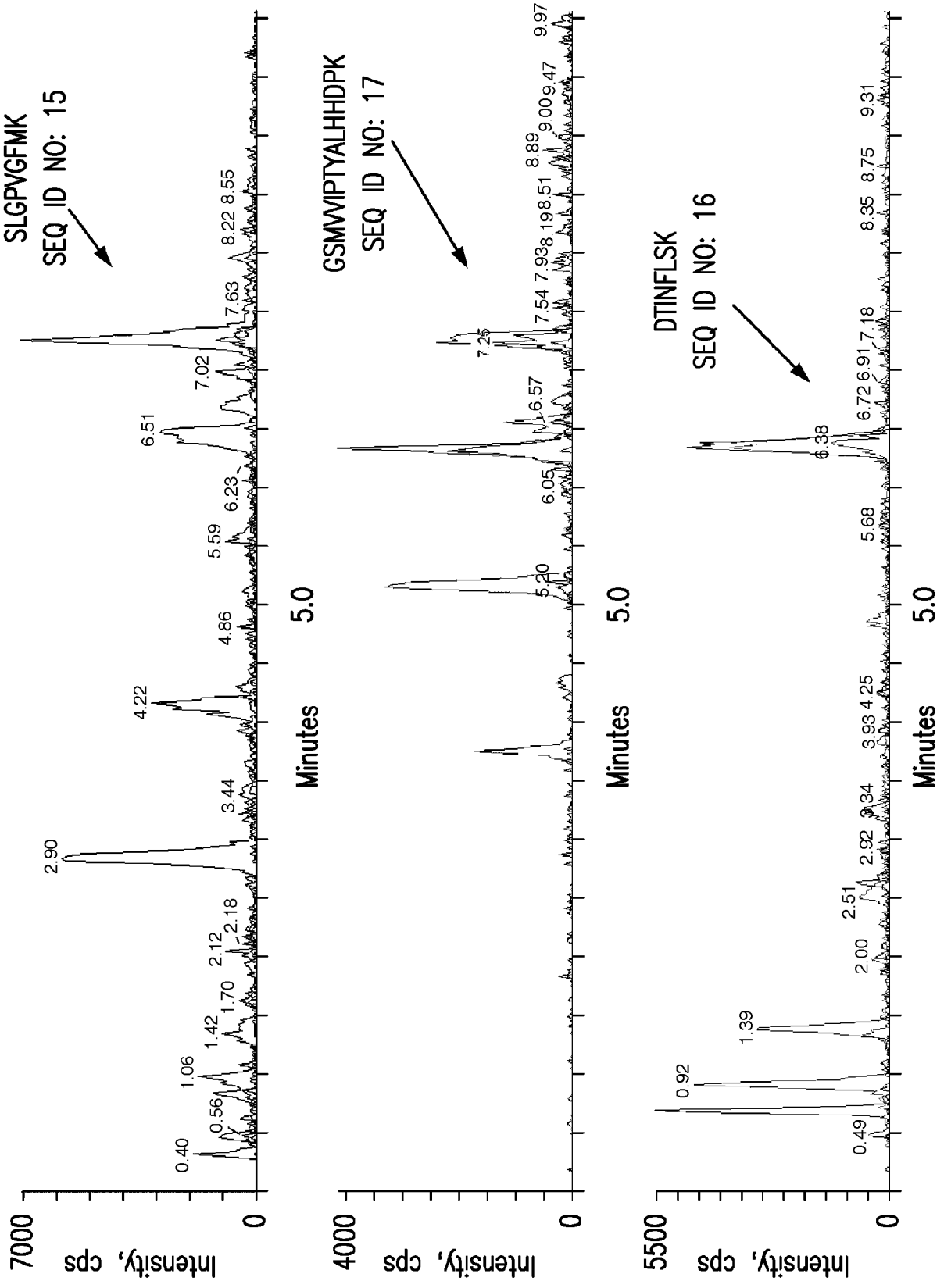
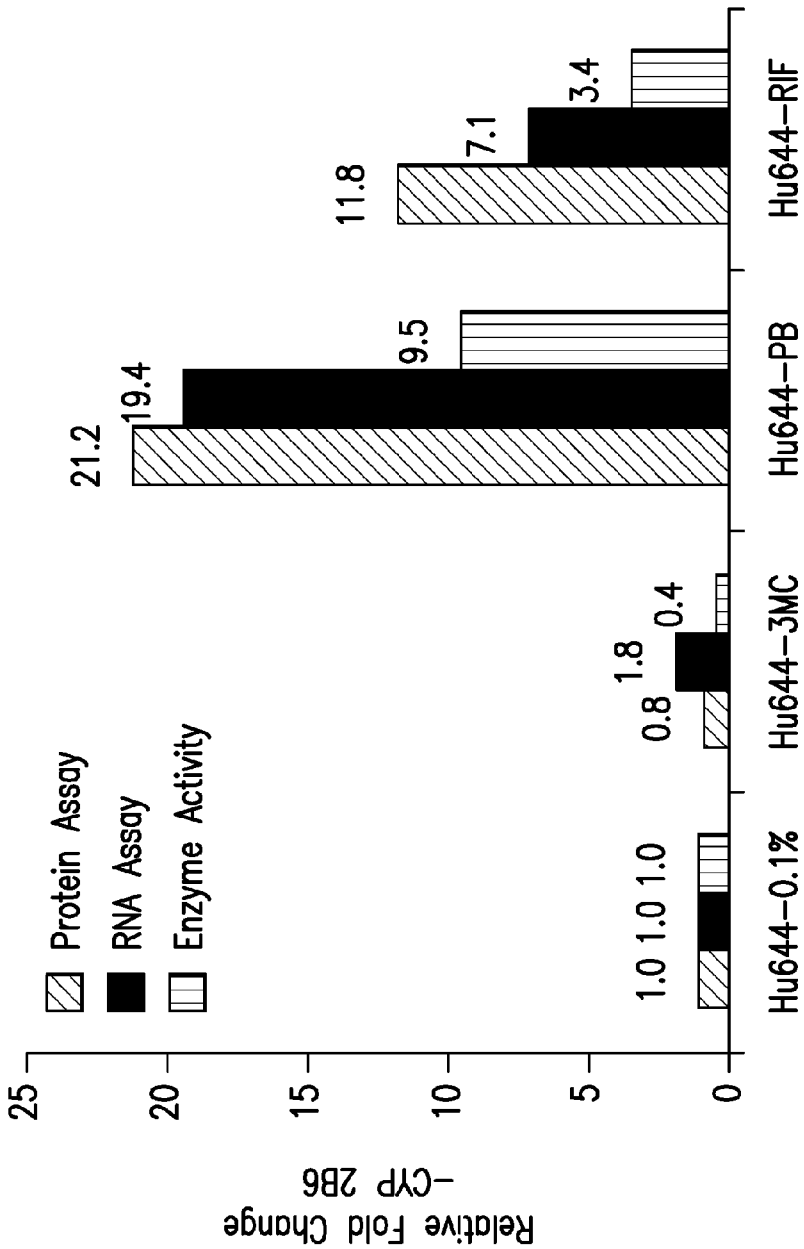


FIG. 5A

FIG. 5B

FIG. 5C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-CYP 2B6
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20
15
10
5
0
Protein Assay
RNA Assay
Enzyme Activity
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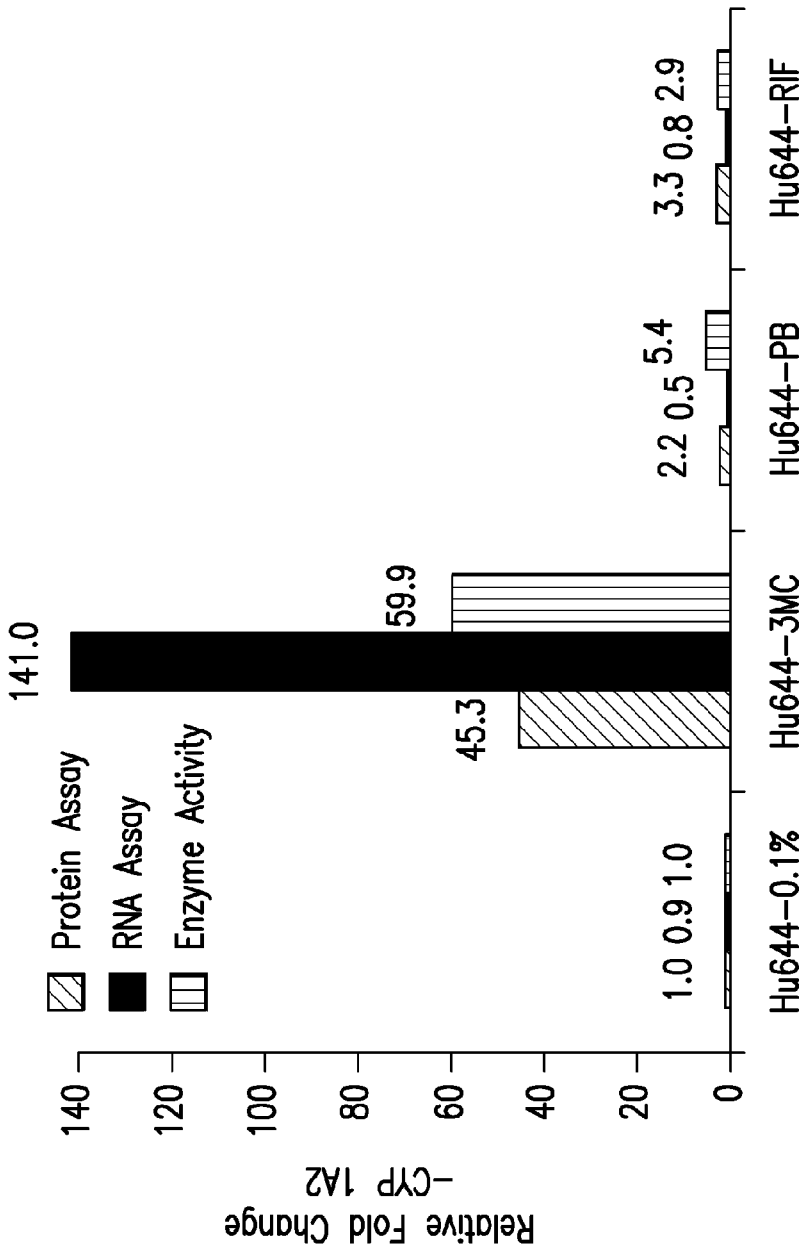
0.8
1.8
0.4
21.2
19.4
9.5
11.8
7.1
3.4
Hu644-0.1%
DMSO
Hu644-3MC
Hu644-PB
Hu644-RIF

FIG. 6


Relative Fold Change
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120
100
80
60
40
20
0
Protein Assay
RNA Assay
Enzyme Activity
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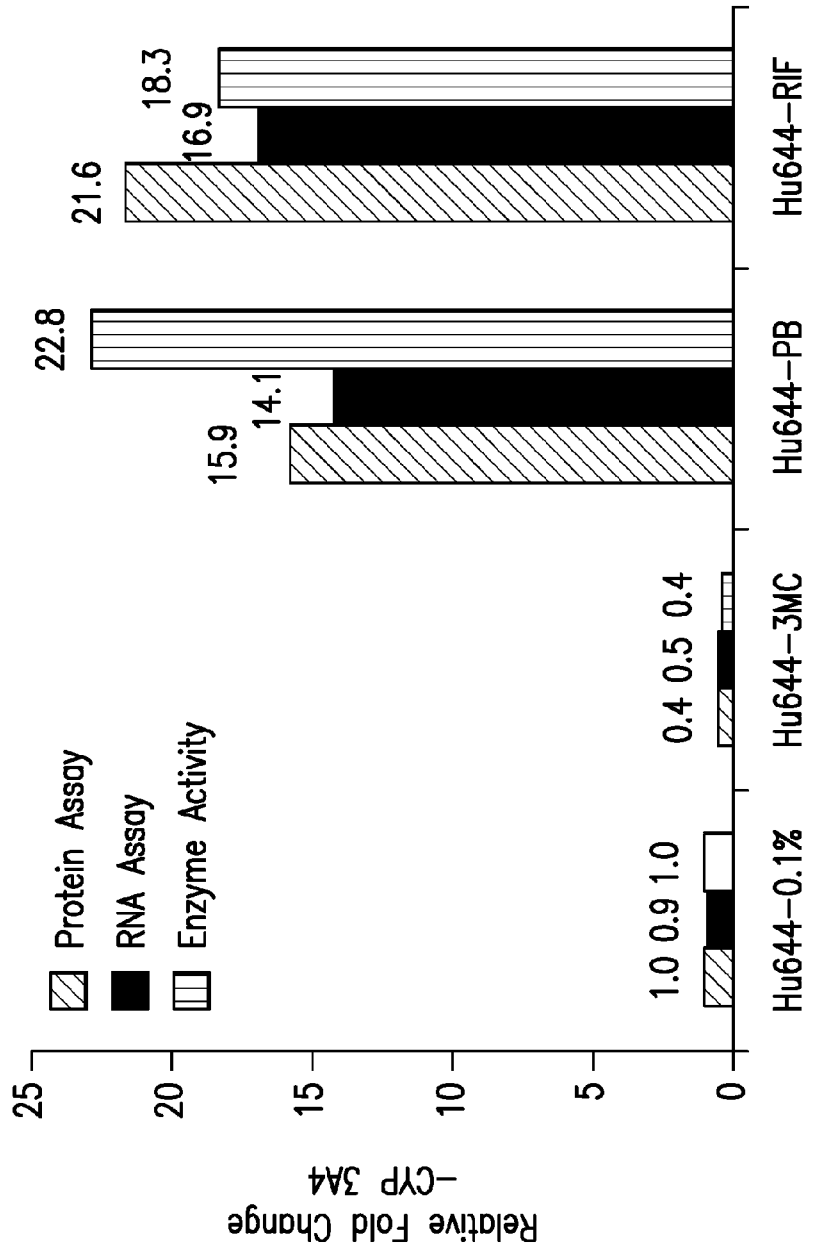
59.9
45.3
1.0 0.9 1.0
2.2 0.5 5.4
3.3 0.8 2.9
Hu644-0.1%
DMSO
Hu644-3MC
Hu644-PB
Hu644-RIF

FIG. 7


Relative Fold Change
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20
15
10
5
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Protein Assay
RNA Assay
Enzyme Activity
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0.4 0.5 0.4
15.9
14.1
22.8
21.6
16.9
18.3
Hu644-0.1%
DMSO
Hu644-3MC
Hu644-PB
Hu644-RIF

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/52665

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 7/00; G01N 30/72, 33/00 (2011.01)
USPC - 530/300; 436/86, 173
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 530/300; 436/86, 173

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 530/300; 436/86, 173 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PubWEST (PGPB, EPAB, JPAB, UPST); Google Scholar; GenCore Sequence Search (AA)
Search terms: mass spectrometry (MS); triple quadrapole MS, multiple reaction monitoring (MRM); absolute quantitation, quantitative proteomics; cytochrome P450, CYP B26; SEQ ID NO: 1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 2007/0092926 A1 (ALTERMAN et al.) 26 April 2007 (26.04.2007). Especially para [0010-0013], pg 9 table, SEQ ID NO: 43	1,5-7 ---------- 8-10, 32, 33, 37, 38
Y	US 2008/0206737 A1 (HUNTER) 28 August 2008 (28.08.2008). Especially para [0019], [0029], [0041], [0056], [0143], [0152], [0153], sheet 6 fig 6.	8-10, 32, 33, 37, 38
A	LANGENFIELD et al. Quantitative analysis of highly homologous proteins: the challenge of assaying the "CYP-ome" by mass spectrometry. Anal Bioanal Chem November 2008 Vol 392 No 6 Pages 1123-1134. Especially pg 1128 right col, pg 1130 fig 4.	1, 5-10, 32, 33, 37, 38

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2011 (22.02.2011)	14 MAR 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/52665

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☒ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☒ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore ver 6.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/52665

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1, 5-13, 32-33 and 37-38, directed to an isolated peptide specific to cytochrome P450 isoform CYP 2B6, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3 and 4, and a method for determining an amount of a P450 isoform by detecting said peptide via mass spectrometry; limited to SEQ ID NO: 1. (Note: each of claims 11, 12, and 13 will only be searched in event that the applicant elects to pay for additional searches of each of SEQ ID NOs: 2, 3, and 4, respectively).

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 5-10, 32-33 and 37-38; limited to SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box III: Lack of Unity of Invention

Group II: claims 2, 5, 14-17, 32 and 34, directed to an isolated peptide specific to cytochrome P450 isoform CYP 3A4, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, and 7 and a method for determining an amount of a P450 isoform by detecting said peptide via mass spectrometry; limited to SEQ ID NO: 5. (Note: each of claims 16 and 17 will only be searched in event that the applicant elects to pay for additional searches of each of SEQ ID NOs: 6 and 7, respectively).

Group III: claims 3, 5, 18-25, 32 and 35, directed to an isolated peptide specific to cytochrome P450 isoform CYP 1A2, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13 and 14 and a method for determining an amount of a P450 isoform by detecting said peptide via mass spectrometry; limited to SEQ ID NO: 8 (Note: each of claims 20, 21, 22, 23, 24 and 25 will only be searched in event that the applicant elects to pay for additional searches of each of SEQ ID NOs: 9, 10, 11, 12, 13 and 14, respectively).

Group IV: claims 4, 5, 26-29, 32 and 36, directed to an isolated peptide specific to cytochrome P450 isoform CYP 3A5, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 16, and 17 and a method for determining an amount of a P450 isoform by detecting said peptide via mass spectrometry; limited to SEQ ID NO: 15 (Note: each of claims 28 and 29 will only be searched in event that the applicant elects to pay for additional searches of each of SEQ ID NOs: 16 and 17, respectively).

Group V: claims 30 and 31, directed to a method for screening a drug for cytochrome P450 (CYP) induction, comprising: incubating the drug with a microsome-containing biological sample; quantitating at least one cytochrome P450 isoform selected from the group of 2B6, 3A4, 1A2, and 3A5 isoforms, using liquid chromatography tandem mass spectrometry (LC-MS) and determining that the drug has an acceptable CYP induction potential when the quantitated value does not exceed the threshold value.

The inventions listed as Groups I - V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the claims of Groups I-IV is an isolated peptide and method of quantitating the peptide in a sample, wherein each Group is directed to a different specific peptide from a cytochrome P450 isoform - not required by the claims of Group V. The special technical feature of the Group V claims is a method for screening a drug for cytochrome P450 (CYP) induction - not required by the claims of Groups I-IV.

The only common technical element shared by the above groups is that they are related to quantitation of cytochrome P450 peptides using mass spectrometry. Each of Groups I, II, III and IV further comprises the common technical element wherein the peptides are related to CP450 isoforms 2B6, 3A4, 1A2 and 3A5, respectively. These common technical elements do not represent an improvement over the prior art of US 2007/0092926 A1 to Alterman et al. (see abstract, SEQ ID NO: 43 in comparison to Applicants' SEQ ID NO: 1, as well as SEQ ID NO: 172 in comparison to Applicants' SEQ ID NO: 5, SEQ ID NO: 1 in comparison to Applicants' SEQ ID NO: 8 and SEQ ID NO: 180 in comparison to Applicants' SEQ ID NO: 17). Therefore, the inventions of Groups I-V lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.