PBI Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO
Discovery Starts With Sample Preparation™

Volume 8, Issue 3 March 2014

PBI Reports Strong Fourth Quarter and Fiscal Year 2013 Financial Results

Dr. Vera Gross Presented Optimization of an HFIP/MTBE Fractionation Method for Sequential Extraction of Proteins, Lipids and Nucleic Acids for Systems Biology Applications at the 2014 MSACL Meeting

Pressure BioSciences, Inc. Reports Strong Fourth Quarter and Fiscal Year 2013 Financial Results

Total Revenue Up 64% (Q/Q) and 21% (Y/Y)
Products & Services Revenue Up 146% (Q/Q) and 29% (Y/Y)
Operating Loss Decreases in Both Q4 and FY 2013

South Easton, MA, April 1, 2014 -- Pressure BioSciences, Inc. (OTCQB: PBIO) ("PBI" or the "Company") today announced financial results for the three-month period and fiscal year ended December 31, 2013 and provided a business update.

Total revenue for the fourth quarter of 2013 was $354,052 compared to $216,032 for the same period in 2012, a 64% increase. Revenue from the sale of products and services was $300,628 for Q4 2013 compared to $122,285 for the same period in 2012, a 146% increase. Grant revenue in Q4 2013 was $53,424 compared to $93,747 for the same period in the prior year. Sales of consumables were $52,968 for the three months ended December 31, 2013 compared to $18,624 for the same period in 2012, an increase of 184%.

Operating loss for the fourth quarter of 2013 decreased to $900,110 from $1,129,750 for the same period in 2012, a reduction of 20%. Contributing to this decrease in operating loss was a quarter over quarter decrease in general and administrative expenses of $268,105, or 30%.

Loss per common share (basic and diluted) was $0.16 for the 2013 fourth quarter compared to $0.10 for the same period in 2012. The additional loss per common share was due primarily to the change in the fair value of the derivative instruments in Q4 2013.

Total revenue for the fiscal year 2013 was $1,503,288 compared to $1,238,217 for the fiscal year 2012, a 21% increase. Revenue from the sale of products and services was $1,046,678 for FY 2013 compared to $809,308 for FY 2012, a 29% increase. Grant revenue in FY 2013 was $456,610 compared to $428,909 for FY 2012. Sales of consumables were approximately $157,676 for FY 2013 compared to approximately $85,493 for FY 2012, an increase of 84%.

Operating loss for the fiscal year 2013 decreased to $3,327,192 from $3,463,642 for the fiscal year 2012, a reduction of 4%. Contributing to this decrease in operating loss were decreases in total operating expenses of $22,792 and increased gross margin of $113,658.

Loss per common share (basic and diluted) was $0.44 for 2013 compared to $0.43 for the same period in 2012.

Richard P. Thomley, Chief Financial Officer of PBI, said: "In addition to strong quarter over quarter (Q4 2013 vs. Q4 2012) revenue increases, both total revenue and products and services revenue showed substantive sequential increases for the year (FY 2013 vs. FY 2012), posting gains of 21% and 29%, respectively.

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Presented at the 2014 MSACL Meeting

Optimization of an HFIP/MTBE Fractionation Method for Sequential Extraction of Proteins, Lipids and Nucleic Acids for Systems Biology Applications

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Introduction

We have developed a new sample preparation technique which allows sequential isolation of proteins, nucleic acids and lipids from solid and liquid samples. The new method is based on one described in 2008 [1] that yielded good separation of proteins and lipids, but involved a lipid-partitioning solvent that was incompatible with many lipid analysis methods. Therefore, the new method described here takes advantage of the excellent protein and lipid solubilizing ability of hexafluoroisopropanol (HFIP), followed by lipid partitioning into methyl-tert-butyl ether (MTBE), a volatile solvent that is compatible with most downstream lipid analysis methods [2,3]. Initial sample homogenization is carried out in HFIP, a volatile organic solvent that has been previously shown to effectively solubilize proteins and lipids without the need for any added salts or detergents [1]. Nucleic acids, which are not soluble in HFIP, are separated by centrifugation. DNA and/or RNA can then be further purified from the nucleic acid pellet. MTBE is added to the lysate to precipitate the proteins. Water is used to induce phase separation of the lipid-containing MTBE phase from the residual HFIP/water phase. Due to its low density, the MTBE forms the upper layer after centrifugation, making lipid recovery more convenient than chloroform-based methods. This HFIP/MTBE method was previously used to extract lipids from neonatal fecal material for lipidomic profiling [4].

Click on Title for Printable Poster Format

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UPCOMING EVENTS

Scientific Meeting: PBI to showcase the Company's PCT-based systems and new products for sample preparation as well as Constant Systems' cell disruption equipment at the 10th annual **Protein and Antibody Engineering Summit – PEGS 2014** meeting, May 5-9, 2014 at the Seaport World Trade Center, Boston, MA.

Scientific Meeting: PBI to showcase the Company's PCT-based systems and new products for sample preparation as well as Constant Systems' cell disruption equipment at **62nd ASMS Conference on Mass Spectrometry and Allied Topics,** June 15 - 19, 2014 at the Baltimore Convention Center, Baltimore, MD.

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We achieved these results while also decreasing operating expenses. We believe that our financial performance for the fourth quarter and fiscal year 2013 reflect PBI's significant progress in our stated goal of developing a sound financial roadmap moving forward."

Mr. Richard T. Schumacher, President and CEO of PBI, commented: "During 2013, and the first three months of 2014, we achieved a number of notable financial and operational successes, including:

- We closed the first three tranches ($2.8M) of our Series K PIPE (initial goal $1.5M)
- We released our ultra-high pressure HUB880 instrument system to the market on time (Q1 2014)
- We closed our $2.0M Series J PIPE, slightly over-subscribed at $2.035M
- We reported increases in total revenue for Q1, Q2, Q3, and Q4 2013 vs. same quarters in 2012
- We announced a Core Technology Breakthrough enabling our PCT Platform to develop integration with the dominant laboratory high-throughput robotic pipetting and automation footprint used worldwide
- We reported on independent studies that showed our novel instrument systems could potentially (i) generate new insights into protein structure and function (for biomarker discovery and drug design); (ii) greatly accelerate rape kit testing; (iii) generate a new understanding of diseases and disorders related to the gastrointestinal tract through non-invasive means; and (iv) offer advantages in cancer, stem cell, and heart disease studies."

Mr. Schumacher continued: "We believe that our product and services revenue will continue to increase in 2014 and beyond; that the distinct advantages of our pressure cycling-based products will continue to be reported by scientists worldwide; that the number of applications and thus fields of use for our products will continue to increase; and that we will be successful in continuing to raise the funds as required, to help us achieve our goal of financial self-sufficiency and profitability, with steadily increasing shareholder value as we move forward."

About Pressure BioSciences, Inc.
Pressure BioSciences, Inc. ("PBI") (OTCQB: PBIO) develops, markets, and sells proprietary laboratory instrumentation and associated consumables to the estimated $6 billion life sciences sample preparation market. Our products are based on the unique properties of both constant (i.e., static) and alternating (i.e., pressure cycling technology, or PCT) hydrostatic pressure. PCT is a patented enabling technology platform that uses alternating cycles of hydrostatic pressure between ambient and ultra-high levels to safely and reproducibly control bio-molecular interactions. To date, we have installed over 250 PCT systems in approximately 160 sites worldwide. There are over 100 publications citing the advantages of the PCT platform over competitive methods, many from key opinion leaders. Our primary application development and sales efforts are in the biomarker discovery, drug discovery & design, and forensics areas. Customers also use our products in other areas, such as bio-therapeutics characterization, soil & plant biology, vaccine development, and counter-bioterror applications.

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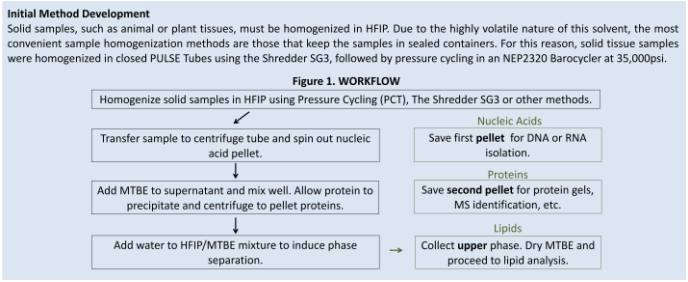
Optimization of an HFIP/MTBE Fractionation Method for Sequential Extraction of Proteins, Lipids and Nucleic Acids for Systems Biology Applications: Continued from Page 1, Column 2

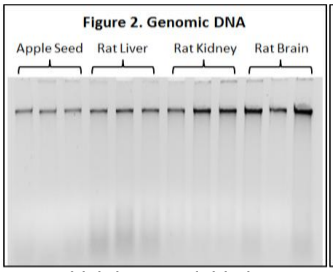


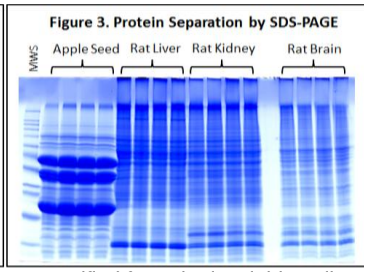
Reagent Ratios: A 4x volume of MTBE relative to HFIP resulted in the best lipid partitioning into the MTBE phase, as well as the best protein yield (Table 1). Subsequent phase separation induced by the addition of water was shown to be optimal with a 3x volume of water relative to the initial HFIP volume (not shown). Lower volumes of water led to poor phase separation, while larger volumes appeared to have no added benefit. The final optimized ratio was determined to be 1:4:3 HFIP:MTBE:Water.

Table 1. Optimization of HFIP:MTBE Ratio

HFIP:MTBE Ratio	Final Protein Extract (mg/ml in ProteoSolve-IEF)	Observations
1:1	0.9	Much of the protein pellet remains undissolved
1:2	1.3	
1:3	2.4	Much of the protein pellet is dissolved
1:4	2.9	
1:5	2.9	

Protein and DNA: Various animal and plant tissues were used to validate protein and DNA extraction using the HFIP/MTBE method





DNA, which is not soluble in HFIP, was purified from the insoluble pellets after the first centrifugation, and visualized on agarose gels (Figure 2). The protein pellets were solubilized directly in laemmli buffer and visualized by SDS-PAGE (Figure 3).

Lipids: Fluorescently labeled phosphatidylcholine was spiked into tissue lysates in HFIP. As expected, the fluorescent signal was found to partition almost entirely into the MTBE phase, with negligible signal detected in the HFIP phase after partitioning (Figure 4). Other fluorescently labeled lipids were also tested (Figure 5) and confirm that lipids of different classes are correctly partitioned into the MTBE. Further testing confirmed that the fluorescent lipids are recovered almost exclusively from the MTBE phase (Figure 6, n=3 per sample).

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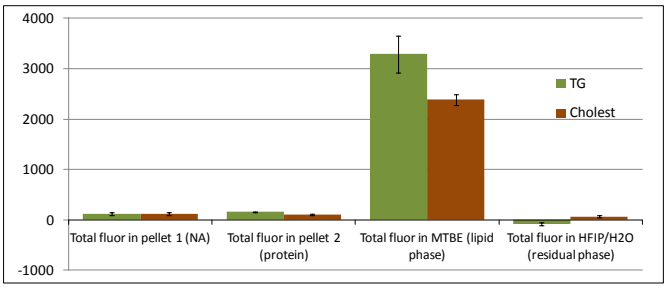
Figure 4. Labeled Phosphatidylcholine Partitioning in Tissue Lysates

	Sample:	Rat Spleen	Mouse Brain	Rat Adipose	Neonatal Human Stool	Rat Sk. Muscle	Rat Liver	Apple seed	Control
HFIP/MTBE Method	MTBE (upper) Phase	1.3×10^4	1.3×10^4	1.8×10^4	1.7×10^4	1.4×10^4	1.3×10^4	1.7×10^4	1.2×10^4
	HFIP/Water (lower) phase	0	0	$<0.07 \times 10^4$	$<0.07 \times 10^4$	0	$<0.07 \times 10^4$	$<0.07 \times 10^4$	$<0.01 \times 10^4$
Bligh-Dyer Method Control	Chloroform (lower) Phase	1.5×10^4							
	Methanol/Water (upper) Phase	0							

Figure 5. Labeled Lipids Partitioning in Blood lysate

Fluorescent Lipid:	Phosphocholine	Triglyceride (TG)	Cholesterol	Free Fatty Acids
MTBE (upper) Phase	1.4×10^4	4.1×10^4	4.5×10^4	4.1×10^4
HFIP/Water (lower) phase	0	0.3×10^4	0.2×10^4	0.4×10^4

Figure 6. Tracking Labeled Lipids Throughout the Protocol



Further lipid extraction validation was performed to demonstrate that the pool of lipids obtained by the HFIP/MTBE method is comparable to that obtained using a standard methanol/DCM extraction. Rat serum samples extracted using the two methods were compared by LC-MS (Figure 7). The results demonstrate that the two methods are essentially equivalent in the amount and type of lipids recovered. In addition, the effect of pressure cycling (PCT) was examined (Figure 8), and the results confirm that pressure cycling appears to have no deleterious effect on lipid extraction and detection, confirming that this method of sample disruption can be used to homogenize solid samples without leading to any artifactual results. SDS-PAGE of proteins extracted from the same serum samples is shown in Figure 9.

Figure 7. Serum Lipid Analysis in Positive (7A) and Negative (7B) Ionization Mode. The HFIP/MTBE method (no PCT) is compared to a standard methanol/DCM method.

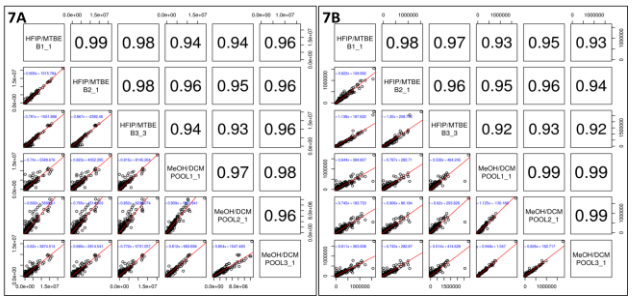


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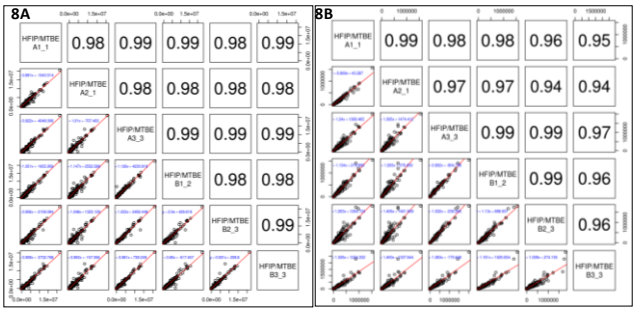
Figure 8. Serum Lipid Analysis in Positive (8A) and Negative (8B) Ionization Mode. HFIP/MTBE method performed with (A1, A2, A3) and without (B1, B2, B3) pressure cycling

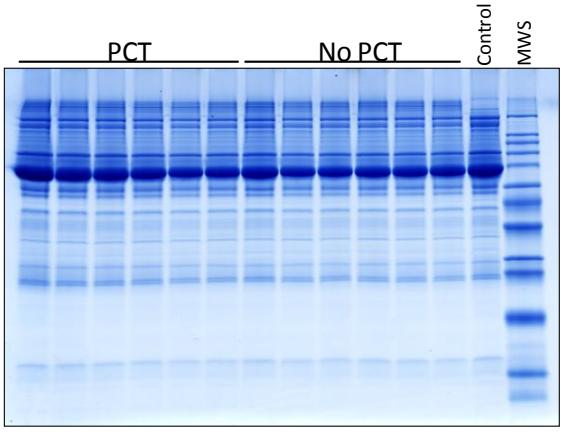


Figures 7 and 8 show scatter plots along with regression lines and coefficients of correlation. The samples (n=3 of each) were quantified using Thermo Sieve software and the correlation analysis was done in R.

Serum lipid extraction method

Aliquots of pooled rat serum were spiked with an internal standard mix and processed using the HFIP/MTBE method with or without pressure cycling in PCT MicroTubes as follows: To each 30µl serum aliquot, 110µl of HFIP was added and the samples were either subjected to pressure cycling (20 cycles at 35,000psi at ambient temp), or vortexed vigorously (n=6 each). The samples were transferred to centrifuge tubes and the PCT MicroTubes were rinsed with 90ul additional HFIP to ensure complete sample recovery. Proteins were precipitated by addition of 800µl MTBE (the amount of nucleic acids in this serum was too low for recovery by this method). The proteins were pelleted by centrifugation and the resulting pellets were dissolved directly in Laemmli buffer and separated by SDS-PAGE (Figure 9). After centrifugation, the supernatant was transferred to a second tube and phase separation was induced by addition of 600µl dH_2O. Phases were separated by centrifugation and the upper MTBE phase was collected and dried prior to LC-MS. Control samples extracted using methanol/DCM were from the same pool of rat serum and were extracted by conventional methods without pressure cycling.

Figure 9. SDS-PAGE Separation of Rat Serum Proteins.



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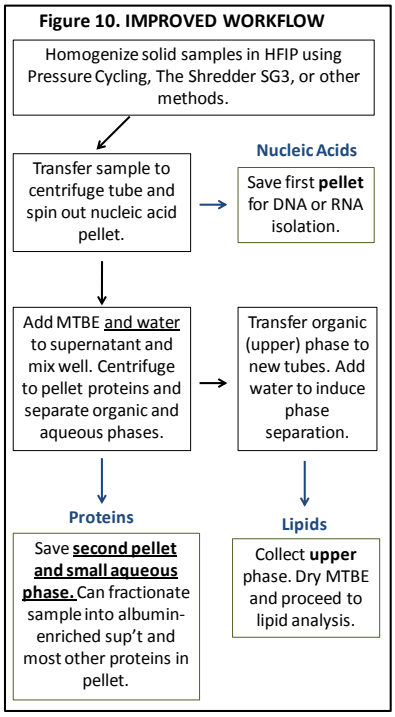
Optimization for Improved Protein Recovery. During method development, we observed that the protein pellet was often difficult to dissolve, even in reagent containing urea and CHAPS detergent (ProteoSolve-IEF). Addition of a variety of detergents to the protocol did not significantly improve protein pellet solubility (data not shown). A slight method modification that involves the addition of a small amount of water to the precipitation reaction (Figure 10) was found to significantly improve protein yield by improving the solubility of the protein pellet.

Figure 10. IMPROVED WORKFLOW



The addition of water to the precipitation step at the same time as the MTBE forms a small aqueous phase below the HFIP/MTBE organic phase and contains some of the most soluble sample proteins (e.g., albumin). This has the added benefit of fractionating the sample proteins into a serum albumin-enriched supernatant fraction and a serum albumin-depleted pellet fraction (Figure 11). The total protein yield is greater than obtained using the original protocol (Figure 12), due in large part to the greater solubility of the recovered protein pellet.

Figure 11. SDS-PAGE Separation of Rat Spleen Proteins

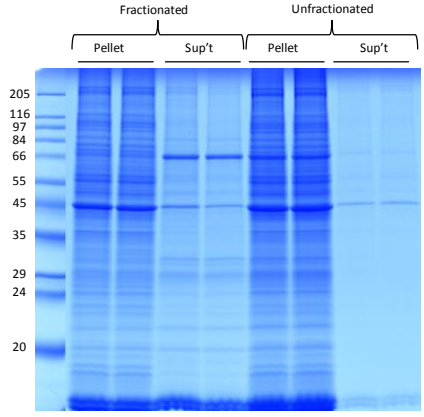


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Figure 12. Protein Yield Quantification

	Soluble protein recovered in pellet (µg)	Soluble protein recovered from total supernatant after drying (µg)
Original Method (no water)	654	0
Water added (Fractionated)	607	197
Water added (Unfractionated)	855	6

Conclusions

The HFIP/MTBE method described here can be used to sequentially extract nucleic acids, proteins and lipids from solid or liquid samples for systems biology studies. The use of the volatile HFIP and MTBE solvents allows for efficient mass spectrometry-compatible protein and lipid extraction, without the use of detergents, salts or other non-volatile additives. The lipids extracted by this method are comparable to those extracted by a standard method that utilizes methanol and DCM

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Forward Looking Statements

This press release contains forward-looking statements. These statements relate to future events or our future financial performance and involve known and unknown risks, uncertainties and other factors that may cause our or our industry's actual results, levels of activity, performance or achievements to be materially different from any future results, levels of activity, performance or achievements expressed, implied or inferred by these forward looking-statements. In some cases, you can identify forward-looking statements by terminology such as "may," "will," "should," "could," "would," "expects," "plans," "intends," "anticipates," "believes," estimates," "predicts," "projects," "potential" or "continue" or the negative of such terms and other comparable terminology. These statements are only predictions based on our current expectations and projections about future events. You should not place undue reliance on these statements. In evaluating these statements, you should specifically consider various factors. Actual events or results may differ materially. The Company's financial results for the quarter and fiscal year ended December 31, 2013 may not necessarily be indicative of future results. These and other factors may cause our actual results to differ materially from any forward-looking statement. These risks, uncertainties, and other factors include, but are not limited to, the risks and uncertainties discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2013, and other reports filed by the Company from time to time with the SEC. The Company undertakes no obligation to update any of the information included in this release, except as otherwise required by law.

To view the Consolidated Statement of Operations, please click on this link:

PRESSURE BIOSCIENCES, INC. AND SUBSIDIARY CONSOLIDATED STATEMENT OF OPERATIONS (UNAUDITED)

For more information about PBI and this press release, please click on the following link:

http://www.pressurebiosciences.com