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Development and Validation of a Liquid Chromatography-Mass Spectrometry Assay for the Quantitation of IPTG in *E. Coli* Fed-Batch Cultures

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IPTG (Isopropyl-β-D-1-thiolgalactopyranoside) is a gratuitous inducer commonly used for the overexpression of heterologous recombinant proteins in *Escherichia coli*. A reliable method has been developed for the determination of IPTG in *E.coli* fed-batch fermentation samples with a minimal sample treatment. Analysis was performed in single ion monitoring positive mode using ESI source. The extracted ion was 261 *m/z* and the retention time of IPTG was 12.4 min with a total run time in samples of 30 min. The flow was directed to mass spectrometer 11 min after the start of the run and diverted from mass spectrometer after 14.5 min in order to avoid interference of salts and other metabolites. The assay was validated for medium and intracellular matrices and linear calibration curves of 3 orders of magnitude were obtained ($R^2 \geq 0.99$). Quality control samples were analyzed and showed precision and accuracy within the limits according to FDA Guidelines for analytical method validation. Recovery for both matrices was between 95.8 and 113.5%. The limit of detection (LOD) was set at 0.02 μM being the 0.1% of the lowest IPTG concentration used for induction of recombinant protein overexpression. The developed procedure has been applied to determine the IPTG distribution profiles in medium and intracellular samples in high cell density induced cultures for the production of the recombinant protein rhamnulose-1-phosphate aldolase (RhuA).

Among many systems available for heterologous protein production, the Gram-negative bacterium *Escherichia coli* remains one of the most attractive because of its ability to grow rapidly and at high cell density cultures on inexpensive substrates, its well characterized genetics and the availability of increasing number of cloning vectors and mutant host strains.[1]

Expression systems derived from the *lac* operon are the most employed ones. In that case, Isopropyl-β-D-1-thiolgalactopyranoside (IPTG) is the commonly used gratuitous inducer for intracellular protein expression because it is a synthetic analog of lactose which binds and inactivates the *lac* repressor.[2]

IPTG is a nine carbon sugar with a high polarity, soluble in water and it has a molecular weight of 238.3 $g \cdot mol^{-1}$. It has seven rotable bonds, five H^+ acceptors and four H^- donors are predicted.[3]

Several strategies for cell growing are commonly used such as batch cultivations in which IPTG inducible promoters are pulse induced reaching high IPTG concentrations inside the reactor,[4,5] while fed-batch strategies can be induced by pulses[6−8] or adding IPTG continuously[9,10] in order to induce recombinant protein production.

Some publications report that the probability for the inducer to bind the repressor depends on the inducer concentration inside the cell.[11] Other authors mention that there are "stochastic" events involved to explain why, at some points, just by chance, the cell becomes induced.[12] Hence, optimization of IPTG concentration for recombinant protein expression is an important factor to be considered.[2,13] It is common to set an in excess concentration of IPTG to ensure the total induction of the system in small scale experiments. A wide range of IPTG concentrations, ranging from 0.005 to 5 mM have been reported, but overexpression is commonly induced at IPTG concentrations of 500−1000 μM.[2] Nevertheless under these conditions, growth inhibition occurs as a result of the metabolic burden. When working with strong promoters, much lower concentrations (in the range between 50 and 100 μM) have been shown to be enough in order to obtain the maximum specific activity and higher concentrations did not

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lead to an increase of specific activity for the strain studied.[8,14] In process scale bioreactors, with high cell density cultures, an excess of IPTG implies an increase in both production and downstream costs. In most cases, where target proteins are of pharmaceutical use, they need to be produced free of potential impurities.

On the other hand, optimization of the expression processes need an in-deep knowledge of the system by elucidating the relative influence of the IPTG transport mechanisms (active transport and diffusion) from the medium to the cell. This is a requirement for determining the conditions for fully induction and, conversely, maximum productivity.

All the above points emphasize the importance of an accurate analysis of both extra and intra cellular IPTG concentration.

Several high-performance liquid chromatography (HPLC) methods are available for sugars and sugar-derived compounds determination. As many of them have poor chromophores, the detection methods include either the formation of fluorescent derivatives,[15−17] electrochemical detection[18−20] or mass spectrometry (MS) detection.[21−23] An HPLC method for IPTG and monothioglycerol (MTG) detection and measurement in biopharmaceuticals using pulsed electrochemical detection (PED) has been reported.[24] Nevertheless, the authors applied the methodology only to MTG-containing real samples. Despite the significant importance of IPTG dosage and high cost of the inducer, it was not found in the literature any experimental data related to detection or quantification of IPTG in fermentation broths whether in medium or inside the cells in order to provide an understanding of the IPTG transport and intracellular concentrations needed for optimal induction of recombinant proteins.

This study describes a specific and reliable method for the determination of IPTG in fermentation samples—medium and intracellular—by HPLC coupled to mass spectrometry (MS), using validation procedures and with minimal sample treatment. Culture broths and cellular lysates are complex matrices. In order to avoid interferences in the probe mainly due to high salts concentration and other metabolites, the flow was directed to the MS after the column just before elution of IPTG and diverted from the MS after the analyte of interest is detected.

The proposed methodology will be applied to the determination of IPTG distribution in two high-cell density fed-batch fermentations of *Escherichia coli* carried out at different inducer concentrations for the production of the recombinant protein rhamnulose-1-phosphate aldolase (RhuA).

MATERIALS AND METHODS

Chemicals and Reagents. IPTG (Isopropyl β-D-1-thiogalactoryranoside) was purchased from Sigma-Aldrich and the stock solution was prepared by dissolving 2.38 g into 100 mL miliQ water and filter-sterilized through a MillexGS 0.22 μm filter from Millipore in order to obtain a stock solution of 100 mM. Medium composition was described elsewhere.[14] Formic acid (98% purity) was supplied from Panreac, and miliQ water was employed for HPLC analysis.

Bacterial Strain and Growth Conditions. *E.coli* M15ΔGlyA-[pREP4] derived from K-12 harboring the plasmid pQαβrham was used for the production of RhuA under the control of the strong promoter T5. Construction of the vector is described elsewhere.[25] This expression system is based on glycine auxotrophy to ensure plasmid stability in order to avoid antibiotic supplementation.[26]

Substrate limiting fed-batch cultivations have been previously described.[14] The specific growth rate (μ) was kept constant by setting an exponential feeding profile.[27]

Samples from different fermentations were used for IPTG analytical method development and validation.

Two fed-batch fermentations were employed for medium and intracellular IPTG monitoring during induction of recombinant protein production at $\mu = 0.22\ h^{-1}$ but varying the induction conditions. Induction of RhuA was carried at 20 (culture 1) and 45 (culture 2) $g \cdot L^{-1}$ DCW (biomass dry cell weight) by a pulse of 100 mM IPTG stock solution in order to obtain a final concentration of IPTG inside the reactor of 20 and 70 μM respectively. IPTG distribution in both medium and inside the biomass as well as RhuA production were assessed through the induction period.

Analysis of Bacterial Cultures. Growth of *E.coli* was monitored by optical density measurements at a wavelength of 600 nm using a spectrophotometer (Uvicon 941 Plus, Kontrol). Samples were diluted with distilled water until the final $OD_{600\ nm}$ value was within the range of 0.3−0.9. Biomass was expressed as dry cell weight (DCW), 1 $OD_{600\ nm}$ is equivalent to 0.3 $g \cdot L^{-1}$ DCW.[8]

For determination of glucose concentration in medium, 1 mL of culture was centrifuged. The supernatant was filtered using a 0.45 μm filter and it was determined enzimaticaly by means of YSI 2070.

Cell disruption and quantitation of RhuA concentration are described elsewhere.[14] Total protein content was determined by means of Bradford Method using Coomassie Protein Assay Reagent Kit (Thermo Scientific, U.S.).

To determine the percentage of RhuA among the rest of intracellular soluble proteins, NuPAGE 12% Bis-Tris gels were performed according to manufacturer's instructions (Invitrogen, U.S.) and quantified by Kodak Digital Science 1D 3.0.2 densitometry software.

IPTG Analysis. *Instrumentation.* IPTG analysis was performed on a Shimadzu Prominence (Columbia, MD) liquid chromatograph with an UV/vis detector operating at a wavelength of 210 nm coupled to a mass spectrometer Shimadzu 2010A equipped with an ESI (electro spray ionization) interface and single quadrupole and using a LC-10AD solvent delivery system (pump A and B). A Shimadzu FCV-20H2 valve unit was used in order to

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divert the flux. The injection was made with a Shimadzu SIL-10AD automatic injector and data analysis were processed with Lab Solutions 3.04 software. Samples were kept in the autoinjector at room temperature.

Sample Preparation. Samples were withdrawn from the reactor and the biomass was separated at 14.000 rpm for 4 min. Supernatant was filtered using a 0.45 μm filter, diluted until the measurement of IPTG concentration in the medium was within the calibration curve range and latterly stored at −20 °C until analysis.

For determination of intracellular IPTG concentration, biomass was washed and adjusted to an OD_{600} 3−4 with distilled water. Cell suspensions were placed in ice and sonicated with four 15 s pulses with 2 min intervals in ice between each pulse using a Vibracell model VC50 (Sonics & Materials). Disrupted samples were centrifuged at 14.000 rpm for 10 min in order to remove the cell debris. Supernatant was filtered using a 0.45 μm filter and stored at −20 °C until IPTG analysis.

Chromatographic and Mass Spectrometer Conditions. The mobile phase was prepared from formic acid in miliQ water at pH 3 isocratic mode; 1 h of equilibration was required before the first injection. The flow rate was 0.6 mL·min^{-1}. Ten μL of samples/standards were directly injected on a 300 mm ×7.8 mm ICSep ICE-Coregel 87H3 column purchased from Transgenomic.

ESI source and positive ionization mode were used. Nitrogen was used as the nebulizing and drying gas. The MS data acquisition with the ESI mode was carried out under the following conditions: probe high voltage, 1.6 kV; nebulizing gas (N_2) flow rate, 1.5 L·min^{-1}; curved desolvation line (CDL) temperature, 250 °C; heat block temperature, 200 °C; acquisition mode, SCAN between 100−400 m/z. Column and autoinjector were kept at room temperature. As retention time of IPTG was 12.4 min, the flow was directed to mass spectrometer 11 min after the start of the run and diverted from the mass spectrometer after 14.5 min. The total run time was 30 min.

During method development all temperatures, ionization mode, injection volume, and probe high voltage were varied but the above values resulted the optimal conditions for largest peak areas.

Method Validation. A full validation according to FDA guidelines[28] was carried out for the assay of IPTG in medium and intracellular samples from *E. coli* fed-batch cultivations by means of determining selectivity, accuracy and precision, linearity, recovery, and stability.

Selectivity. Samples from 10 different fermentations before induction were extracted and analyzed for the assessment of potential interferences with endogenous substances. The apparent response at the retention time of IPTG was compared to lower limit of quantitation (LLOQ) and to standard samples. LLOQ was defined according to the FDA Guide for Bioanalytical Method Validation.[28] The limit of detection (LOD) was calculated using quality control samples (QCs) of 0.02 μM ($n = 5$) which is the lowest concentration of analyte which can be detected. In chromatography this limit is 3 times the signal-to-noise ratio. Experiments were carried out to investigate matrix effects to ensure that precision, selectivity and sensitivity were not compromised.

Accuracy and Precision. In order to validate the method for IPTG, intraday accuracy and precision were evaluated in medium and intracellular samples by analysis at several concentration levels on the same day of QCs prepared by spiking samples with known concentration of IPTG. Three different concentration (0.1, 1, 10 μM for medium and 0.1, 1, 5 μM for intracellular) levels were selected in order to cover the entire range of the calibration curve. Analysis was performed by preparing five samples at each concentration level and concentrations were back-calculated from the calibration curve. To calculate the interday accuracy and precision, the intraday assay was repeated on three consecutive days. Accuracy was determined as the ratio between the back-calculated concentration and the theoretical value expressed in terms of percentage. The relative standard deviation (RSD) was used as a measure of precision.

Recovery. In order to assess any possible matrix effects (ion suppression or ion enhancement), recovery experiments were tested by spiking samples at three different concentration levels, 0.1, 5, and 10 μM for medium samples; 0.15, 0.6, and 5 μM for intracellular samples. The analysis was done per duplicate and recovery was calculated comparing the area of QCs and standards.

Calibration Curve. The calibration curve was prepared for the concentrations of 0.05, 0.1, 0.5, 1, 5, and 10 μM for medium QCs and 0.05, 0.15, 0.31, 0.62, 1.25, 2.5, and 5 μM for intracellular QCs ($n = 2$, at each level). The analysis was done using the peak area of IPTG plotted against concentration. Calibration curve parameters, lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were defined according to the FDA Guide for bioanalytical method validation[28] as the lowest and highest points of the calibration curve, respectively at which a compound can be measured with accuracy between 80 and 120%.

Stability. Freeze and Thaw Stability. Stability of the analyte was studied by analyzing low (0.1 μM) and high (5 μM) concentration QCs per triplicate for both matrices, that were frozen overnight at normal storage temperature (−20 °C) and then thawed unassisted at room temperature. When completely thawed, the samples were refrozen at the same temperature for 24 h and thawed. The freeze−thaw cycle was repeated two more times and after the third cycle, the samples were analyzed. The concentration of analyte in the samples was calculated employing the daily calibration curve. This data was used to support need for repeat analysis.

Stock Solution Stability. The stability of the stock solution was evaluated at room temperature for 8 h using concentrations of 0.1, 5, and 10 μM ($n = 5$, at each concentration level). Coefficient of variance was used as a validation parameter according to FDA Guidelines.[28]

Autoinjector Stability. Stability of medium and intracellular QCs in autoinjector was done for over 24 h at two concentration levels (0.1 and 5 μM) by injecting the same vial at intervals of 2 h. This analysis provides useful data in situations likely to be encountered during actual sample handling and analysis run time.

RESULTS AND DISCUSSION

Chromatography and Detection. Injection of standards of IPTG in the mass spectrometer along with the mobile phase with a positive ion interface was done and the adduct $[M\text{-}Na]^+$ was observed being m/z 261.1. Analysis temperature, nebulizer gas,

(28) Guidance for Industry: bioanalytical method validation, FDA, US Department of Health and Human Services, 2001, http://www.fda.gov/cder/guidance/4252fnl.pdf (accessed September 30, 2009).

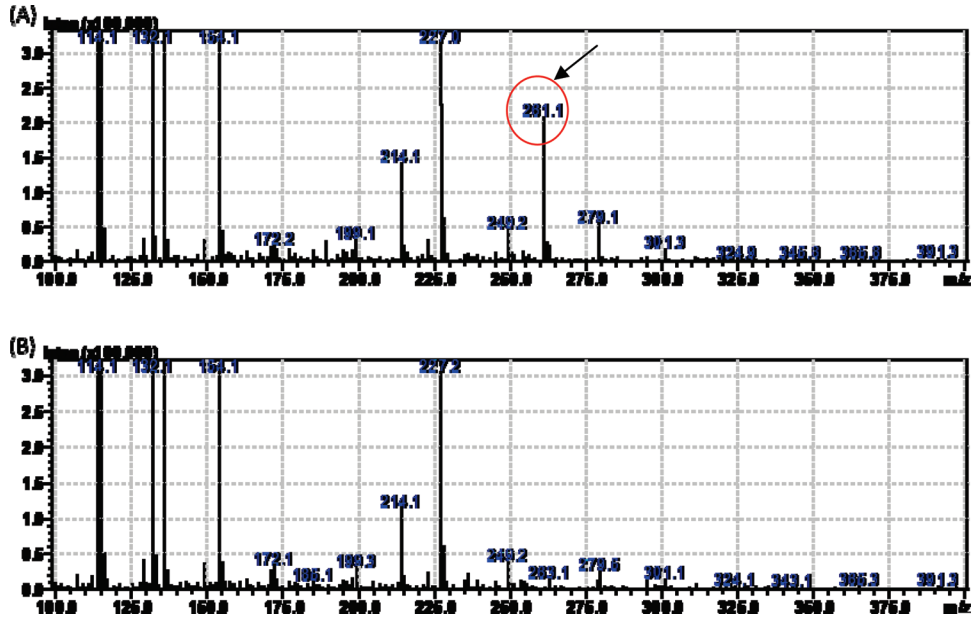

Figure 1. Full mass spectra of aqueous standard with (A) and without IPTG (B).

and ESI temperature were tuned to optimize specificity and selectivity of m/z 261.1. The full scan mass spectra of aqueous solution with and without IPTG are shown in Figure 1 (A) and (B) respectively.

Observed retention time was 12.4 min with a total run time of 30 min in medium and intracellular samples. As shown in Figure 2, medium (A) and intracellular (B) blanks were tested for endogenous interference and no additional peaks due to endogenous substances were observed that would interfere the detection of IPTG. Figure 2(C) shows a chromatogram of a standard sample containing 10 μM of IPTG.

Validation Study. *Specificity.* The apparent response at the retention time of IPTG was compared to the lower limit of quantification (LLOQ). None of the 10 blanks from fermentation samples had any interference at the retention time of IPTG. Signal to noise ratio was 5:1 at the lower limit of quantification in QCs which was 0.05 μM while lower limit of detection (LLOD) was 0.02 μM for medium and intracellular.

Accuracy and Precision. Intraday precision was determined as the standard deviation of an analytical run divided by the mean of the run at each level of the QCs. Interday precision was obtained as the ratio between the standard deviation of the three analytical runs and the overall mean. The data obtained for IPTG was within the acceptable limits to meet guidelines for bioanalytical validation.[28] Data for accuracy and precision are shown in Table 1.

Recovery Data. Percentage of IPTG recovery was measured as the ratio between the peak area of QCs and the peak area of standard controls at each concentration level ($n = 2$). The mean recoveries and coefficients of variance (CV %) in medium and intracellular matrices are shown in Table 2.

Calibration Curve. Assays in medium and intracellular QCs were linear over the validated concentration ranges of 0.05−10 and 0.05−5 μM, respectively. The best fit for both calibration curves was obtained by using a linear regression. Equation of calibration curves were $y = 2 \times 10^{-6} \cdot x - 0.065$ ($R^2 = 0.998$) for medium and $y = 7 \times 10^{-6} \cdot x - 0.11$ ($R^2 = 0.996$) for intracellular matrices, were $y =$ IPTG concentration and $x =$ peak area. The calibration curves accuracy is presented in Table 3. Results were calculated by plotting the peak area vs. the nominal concentration. Results at lowest concentration (0.05 μM) met the criteria for LLOQ.[28]

Freeze/Thaw Stability. The freeze−thaw stability of IPTG in medium and intracellular was determined by measuring the accuracy and precision of QCs that underwent three freeze−thaw cycles. Data for freeze/thaw analysis are shown in Table 4. The results showed that IPTG was stable in medium and intracellular according to FDA Guidelines criteria.[28]

Stock Solution Stability. Standard vials at three concentration levels; 0.1, 1, and 10 μM ($n = 5$ at each concentration level) were injected for stock solution stability for 8 h. Accuracy (%) and coefficient of variation (%) were 102 ± 4, 102 ± 3, and 104 ± 2 respectively.

Autoinjector Stability. Stability of samples stored in the autoinjector was determined over a period of 24 h by injecting the same sample at 2 h intervals. Data for the percentage of reduction in levels of IPTG from baseline (0 h) are shown in Table 5. The results show a minimal reduction in levels of IPTG when compared with samples analyzed at time zero in medium and intracellular matrices.

Application to Fermentation Samples. The method was applied to the study of IPTG distribution in two *E.coli* fed-batch fermentations under substrate limiting conditions (see Materials and Methods). Properly diluted medium and intracellular samples were analyzed separately thus, four different runs were carried out. Each run involved six calibration standards of different concentrations injected before and after samples, two QCs among samples to ensure reliable and accurate quantitation of the metabolite of interest and the corresponding samples analyzed per duplicate. Non induced samples were analyzed as negative control. Accuracy of QCs was within the range of 85−115%. Accuracy and precision of calibration curve were also in agreement with the international guidelines.[28]

Figure 3 presents the results of IPTG concentration profile in the medium and inside the microbial cell. Observing the distribu-

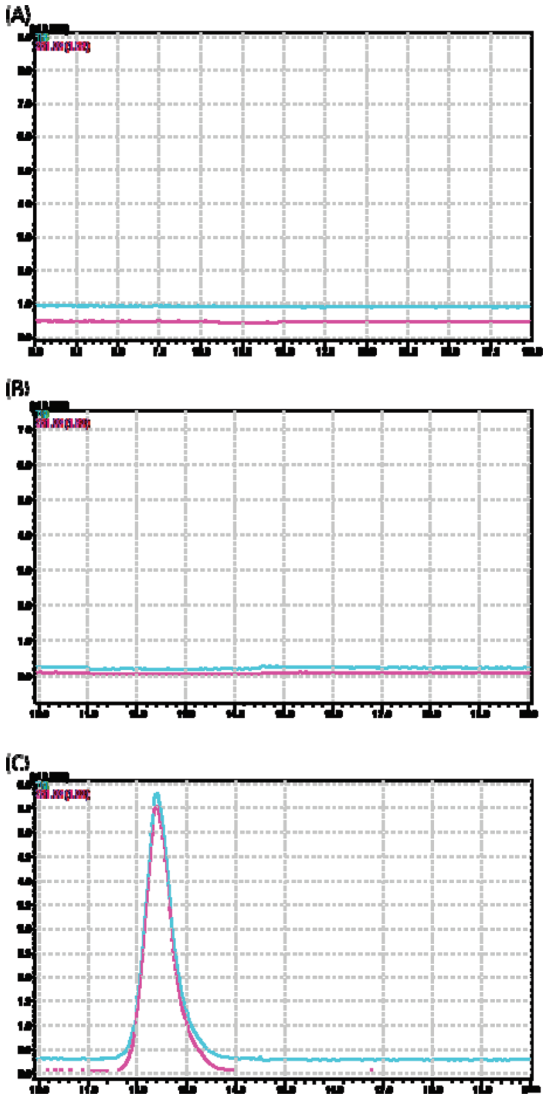

Figure 2. SIM (single ion monitoring) chromatograms at 261 *m/z*. Ten μL injection medium blank (A), 10 μL injection intracellular blank (B), calibration standard IPTG 10 μM (C).

Table 1. Intra-Day and Inter-Day Precision and Accuracy for Medium and Intracellular Matrices

	nominal concentration (μM)					
	medium QCs			intracellular QCs		
	0.1	1	10	0.1	1	5
intraday run						
mean ($n = 5$)[a]	0.107	1.03	10.5	0.086	0.97	4.7
SD	0.001	0.03	0.1	0.006	0.04	0.3
CV (%)	2.0	3.0	1.0	7.0	5.0	7.0
accuracy (%)	108.0	103.0	105.0	86.0	98.0	94.0
interday run						
mean ($n = 15$)[a]	0.107	1.03	10.5	0.086	1.04	4.9
SD	0.002	0.06	0.6	0.007	0.08	0.3
CV (%)	2.0	6.0	6.0	8.0	8.0	7.0
accuracy (%)	107.0	103.0	105.0	86.0	105.0	99.0

[a] Measured IPTG concentration (μM).

Table 2. Recovery Data for IPTG

	nominal concentration (μM)	recovery (%)	CV (%)
medium matrix $n = 2$	0.1	99.0	5,0
	5	96.0	1.0
	10	97.0	10.0
intracellular matrix $n = 2$	0.15	100.0	4.0
	0.6	114.0	11.0
	5	97.0	9.0

Table 3. Back-Calculated Concentrations from Calibration Curves ($n = 2$)

medium QCs						
nominal concentration (μM)	0.05	0.1	0.5	1	5	10
average	0.057	0.11	0.57	1.30	6.0	11.3
SD	0.01	0.02	0.05	0.03	0.3	0.6
C.V. (%)	18.4	15.0	8.02	2.60	5.4	5.2

intracellular QCs							
nominal concentration (μM)	0.05	0.15	0.31	0.62	1.25	2.5	5
average	0.09	0.22	0.38	0.74	1.45	2.90	5.1
SD	0.01	0.005	0.03	0.01	0.02	0.06	0.2
C.V. (%)	11.8	2.480	6.80	1.37	1.14	1.90	4.0

Table 4. Freeze-Thaw Precision and Accuracy for IPTG in Medium and Intracellular Matrices

	nominal concentration (μM)			
	medium QC		intracellular QC	
	0.1	5	0.1	5
IPTG (cycle 1)				
mean ($n = 3$)[a]	0.084	4.57	0.09	4.6
SD	0.008	0.07	0.01	0.1
CV (%)	9.0	2.0	12.0	3.0
accuracy (%)	88.0	109.0	111.0	108.0
IPTG (cycle 3)				
mean ($n = 3$)[a]	0.104	5.65	0.086	5.60
SD	0.008	0.37	0.006	0.39
CV (%)	8.0	7.0	8.0	6.0
accuracy (%)	104.0	113.0	86.0	112.0

[a] Measured IPTG concentration (μM).

Table 5. Autoinjector Stability of Medium and Intracellular Samples[a]

	nominal concentration (μM)			
	medium QC		intracellular QC	
time (hours)	0.1 (PDB)	5 (PDB)	0.1 (PDB)	5 (PDB)
0 ($n = 1$)	0	0	0	0
0–8 ($n = 5$)	−7.40	−2.81	−1.40	+2.41
8–16 ($n = 4$)	−11.96	−8.70	−6.10	+4.87
16–24 ($n = 3$)	−9.51	−3.26	−4.72	−1.83

[a] PDB: percentage deviation from baseline (0 h).

tion of IPTG in the medium, it is noticeable that the depletion rate of inducer in *culture 1* (A) induced at 20 μM is much lower and smooth than in *culture 2* (B) induced at 70 μM. These concentrations correspond to a ratio between inducer/biomass of 1 and 1.5 μmol IPTG·g^{-1} DCW respectively. In the second case, IPTG rapidly decreases in medium. Regarding intracellular concentrations, it can be observed that IPTG can be considered to be almost constant in terms of μM. It is well-known that active transport occurs and that IPTG induces its

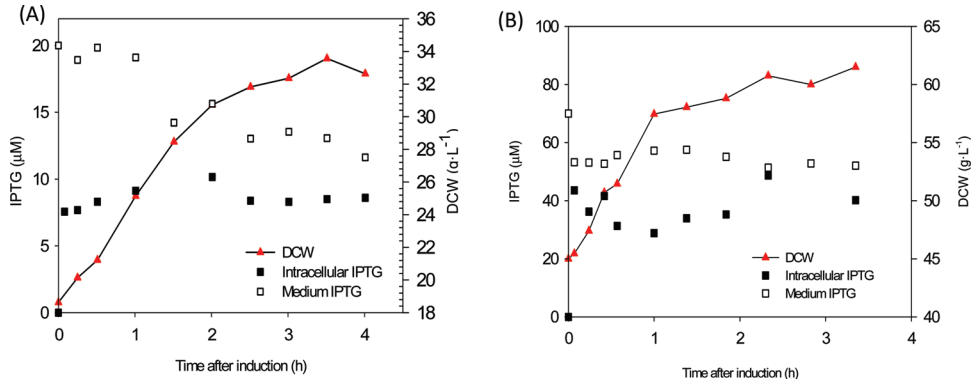

Figure 3. IPTG concentration distribution in medium and intracellular after induction of RhuA overexpression along with the growing profile in *culture 1* (A) and *culture 2* (B). Note: IPTG concentration at time of induction has been estimated from the added IPTG amount.

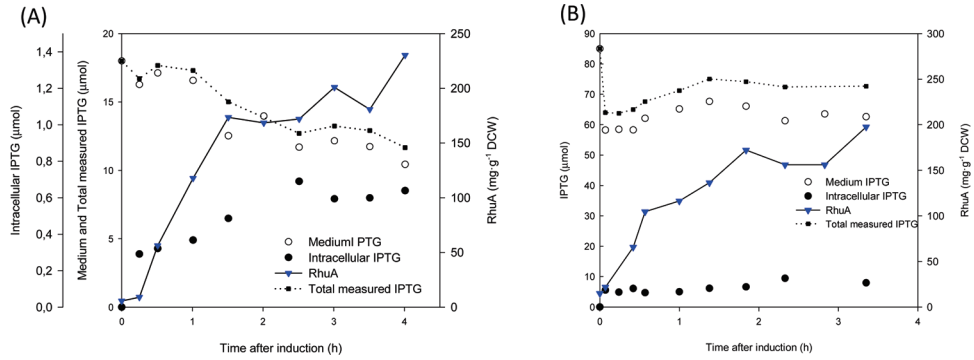

Figure 4. Distribution of IPTG in terms of micromoles in medium and intracellular together with the production of RhuA and mass balances in *culture 1* (A) and *culture 2* (B). Note: IPTG amount at induction time was calculated according to the added one.

own active transport[29] as well as IPTG diffuses across the cell membrane depending on the medium concentration.[30] These may explain the fact that IPTG in medium drops differently in the studied cultures. Besides, at the end of the cultures the approximate concentration of IPTG in medium and inside the biomass is 11 and 8 μM respectively for *culture 1* and 52 and 40 μM respectively for *culture 2*.

Since the volume of cultures is not constant in fed-batch operation, the measured IPTG concentrations were converted to micromoles of IPTG in order to determine the fraction of IPTG present in extra and intracellular environments. The estimation of the fraction of the total volume occupied by cells and medium was calculated by assuming a cellular volume of 0.0023 $L \cdot g^{-1}$ DCW.[31]

In Figure 4, total IPTG in medium and intracellular are presented for *culture 1* (A) and *culture 2* (B) together with specific aldolase amount. It is to be noticed that the intracellular IPTG increases in *culture 1*. By the contrary, it remains almost constant for *culture 2* which was induced at higher concentration. Although the measured intracellular inducer concentration differs in 1 order of magnitude between *culture 1* and *culture 2*, similar specific RhuA amount were obtained in accordance with the dependence of the protein levels on the ratio between IPTG and the biomass concentration at induction time,[10] which are relatively close (1 and 1.5 respectively). Figure 4 shows the calculation of mass balances of total measured IPTG (μmol) by adding the measured extra and intracellular amounts, as well as the removed IPTG when sampling. As can be seen, there is a lack of IPTG, in both cultures. As an average, it accounts approximately for 0.15−0.20 μmol of IPTG per each gram of DCW. It is known that IPTG binds the transmembrane protein *lac*-permease which is involved in the active transport of lactose and IPTG.[30] It also binds irreversibly the cytoplasmatic protein *lacI*[32] which is the lac operon repressor[33] and there are reported other unspecific bounds.[34,35] The lack of IPTG could be due to an efficiency of cell disruption lower than 100% or more likely, to the non measured bound inducer.

CONCLUSIONS

A reliable and fast method for IPTG quantitation in *E.coli* fed-batch fermentation samples by HPLC-MS with minimal sample treatment has been developed and validated. This assay is suitable for medium and intracellular samples without significant interferences and it has been demonstrated to be reproducible and

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accurate. Calibration curves cover 3 orders of magnitude showing an excellent linearity and having a detection limit of 0.02 μM in both matrices for the analyte of interest.

The method has been demonstrated to be able for IPTG determination in real fermentation samples at biomass concentration levels employed in industrial production of proteins.

Further data provided from the analysis of IPTG will lead to uncover the lack of knowledge on IPTG distribution in *E.coli* cultures and how induction of recombinant proteins with IPTG can be optimized. It is expected to be a helpful tool for modeling induced growth behavior from experimental data and moreover, elucidation of IPTG transport mechanisms across the cell will be studied.

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