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Two-dimensional liquid chromatography/mass spectrometry/mass spectrometry separation of water-soluble metabolites

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ABSTRACT

Off-line two-dimensional liquid chromatography with tandem mass spectrometry detection (2D-LC/MS–MS) was used to separate a set of metabolomic species. Water-soluble metabolites were extracted from *Escherichia coli* and *Saccharomyces cerevisae* cultures and were immediately analyzed using strong cation exchange (SCX)–hydrophilic interaction chromatography (HILIC). Metabolite mixtures are well-suited for multidimensional chromatography as the range of components varies widely with respect to polarity and chemical makeup. Some currently used methods employ two different separations for the detection of positively and negatively ionized metabolites by mass spectrometry. Here we developed a single set of chromatographic conditions for both ionization modes and were able to detect a total of 141 extracted metabolite species, with an overall peak capacity of *ca.* 2500. We show that a single two-dimensional separation method is sufficient and practical when a pair or more of unidimensional separations are used in metabolomics.

1. Introduction

Typically, metabolite extracts have been analyzed with a single chromatographic dimension [1–8]. Chromatography paired with mass spectrometry (MS) has become a valuable technique for metabolomics due to the ability to isolate compounds via chromatography and to detect with high sensitivity over a large dynamic range. Recently, a variety of both gas chromatography (GC) and liquid chromatography (LC)–MS metabolomic assays have been devised. The GC–MS assays are ideally suited to quantifying a large number of non-polar and/or derivatized metabolites [9,10], but are less effective for measuring highly polar analytes, such as phosphorylated compounds. Techniques that utilize liquid chromatography have not shown the ability to measure as large a set of compounds, but are very well suited to measure compounds whose structures are more diverse, including highly polar analytes such as the nucleotide triphosphates [11–18]. LC–MS techniques designed to measure highly polar metabolite concentrations almost exclusively rely on electrospray ionization (ESI) to convert the metabolites into gas-phase ions [19]. With ESI, the possibility of competitive ionization from high abundance species can complicate quantitation [20]; however, the introduction of isotope-labeled internal standards can be used to validate endogenous metabolite concentrations and overcome artifacts introduced via competitive ionization [21]. Current LC–MS metabolomic techniques fall into two broad categories: (1) those that detect and measure a predetermined set of metabolites in a sample (targeted experiments) and (2) those that attempt to detect and measure every metabolite from a sample (untargeted experiments). For this work, we sought to develop a technique complimentary to the targeted techniques developed by Rabinowitz and co-workers at Princeton University. These LC–MS/MS methods utilize a triple quadrupole MS for metabolite quantitation, and they measure at least one metabolite from each core pathway. Much work has been done to ensure that the extracted metabolite pool accurately reflects biologically relevant concentrations and that compounds present in the sample solution do not result from degradation [22–28]. After screening ~20 separation conditions, their initial method used an aminopropyl (HILIC) column with identical separation conditions for both the positive and negative ion mode analyses [1]. However, their current chromatographic methods utilize an aminopropyl column, operated in HILIC mode, and a octadecyl (C18) reverse phase column, containing an embedded polar group, for the separation of metabolites to be detected in positive- and negative-ion mode, respectively [1,29]. The negative-ion mode separation relies on the use of tributylamine as an ion-pairing reagent, thus requiring that positive- and negative-ionization mode experiments be run on separate instruments or

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with separate tubing to avoid complications from incompatible ion-pairing reagents. This directly suggests an opportunity for improvement of these separations by developing a comprehensive method employing both ionization modes under a single set of chromatographic conditions. However, compromises must be made due to the complex nature of metabolites (e.g. acids, bases, neutrals, etc.) [30]. To this end, we used off-line two-dimensional liquid chromatography with tandem mass spectrometry detection (2D-LC–MS/MS) to separate a set of metabolomic species from *Escherichia coli* and *Saccharomyces cerevisae* extracts.

Multidimensional chromatography is well-suited for metabolite mixtures as the range of components varies widely with respect to polarity and chemical makeup [17,31–33]. In Tolstikov et al. [33], the authors were able to obtain complementary separations of metabolites using reversed-phase and HILIC capillary columns. The overwhelming obstacle for creating an off-line 2D-LC/MS–MS method in our approach is the consolidation of the resolving powers of both the liquid chromatography and mass spectrometry dimensions. Analysts can rely heavily on the resolving power of a mass spectrometer, but this would involve using chromatography to resolve easily ionized metabolites that otherwise might suppress one another's ionization. This is an acceptable usage of separation science, however the chromatograms produced are often far from desirable. Other approaches and considerations were made based on previous work in our group [34–37], and the decision to utilize off line 2D-LC was based on its relative simplicity.

In this work, we used strong cation exchange (SCX) liquid chromatography and hydrophilic interaction liquid chromatography (HILIC) to separate mostly polar, water-soluble metabolites extracted from *E. coli* and *S. cerevisae*. Selective reaction monitoring (SRM) detection was used based on fragmentation transitions from standards in Bajad et al. [1]. SRM detection is ideal for directed metabolite detection due to the specificity and lower limit of detection. Here, we show that a two-dimensional separation method is sufficient and practical to replace a pair or more of unidimensional separations used in metabolomics. The comprehensive 2D-LC method was designed as what can be described as a "work horse" approach. This is when a first dimension is short and few fractions were collected, relying on the second dimension and detector for much of the separation power. This conscious decision was made with respect to desiring a high-throughput method necessary for processing a large number of metobolomic samples and the relative instability of several metabolites. Furthermore, we have the luxury of relying on a detection method that provides additional dimensionality to the separations. This means that some chromatographic performance can be sacrificed to reduce the overall analysis time and still acquire a large amount of analytical data.

2. Experimental

2.1. Materials, reagents

HPLC grade water and acetonitrile as well as reagent grade ammonium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA, USA). Reagent grade ammonium formate and LC/MS grade formic acid were obtained through Sigma-Aldrich (St. Louis, MO, USA). The first dimension colum used in this work was a 150 mm × 4.6 mm Luna SCX (Phenomenex, Torrence, CA, USA). This column consists of fully porous, 5 μm particles with bonded aryl-SO_3^- groups. The second dimension column was a 150 mm × 3.0 mm Luna HILIC (Phenomenex). The packing material of the HILIC column consisted of 3 μm fully porous silica diol particles.

2.2. Microbial strains and growth conditions

Wild type *E. coli* strain BW25113 was purchased from the Coli Genetic Stock Center at Yale University. Cultures of *E. coli* were grown in minimal media at 37 °C with aeration until to mid to late exponential phase (Optical Density at 600 nm (OD_{600}) = 0.915 at the time of extraction). *S. cerevisae* was purchased from Carolina Biological (Burlington, NC, USA) and grown in Yeast Mold Broth at 30 °C with aeration to an OD_{600} of 0.373 before extraction. The metabolites were extracted as explained in the *metabolite extraction* section.

2.3. Metabolite extraction

Metabolites were extracted from *E. coli* and *S. cerevisae* cells similar to the method described in Bajad et al. [1] and Lu et al. [38]. For the *E. coli*, a 48 mL culture was centrifuged (6 min, 3×10^3 rcf, 4 °C) to concentrate the bacteria cells. The supernatant was discarded and the pellet was resuspended in extraction solvent (1300 μL, 2:2:1 ACN:MeOH:H_2O with 0.1 M formic acid). Due to added complications caused by the numerous metabolites in rich media, *S. cerevisae* cultures were vacuum filtered directly onto a nylon filter (Part #: R04SP04700, GE Osmonics, Minnetonka, MN) before extraction to remove excess media and to collect the cells. The filter was then immediately placed face down in a petri dish containing 1300 μL of extraction solvent to sample the metabolome. Both microorganisms were allowed to incubate in the extraction solvent for 15 min at 4 °C. For *S. cerevisae* samples, the filters were then rinsed with the previously added extraction solvent by pipetting a steady stream over the surface of the filter for about 2 min to resuspend the cells in the extraction solution. After incubation, the suspensions containing the metabolites were transferred to 1.5 mL centrifuge tubes, and any remaining cell debris was removed by centrifugation (5 min, 16.1 3×10^3 rcf, 4 °C). An 840 μL aliquot of the supernatant was mixed with 72 μL of 15% bicarbonate (to neutralize the solution), 5 μL of 4.25 mM Tris and 5 μL of 1.7 mM benzoic acid (here benzoic acid and Tris are added as internal standards). The extracts were stored at 80 °C until use.

2.4. Instrumentation and data handling

Experiments were performed on a Thermo Finnigan (San Jose, CA, USA) Surveyor liquid chromatograph equipped with a quaternary pump, autosampler, and convective column oven. The chromatograph was connected to a TSQ Quantum Discovery Max (Thermo Finnigan) triple quadrupole mass spectrometer for detection. Both the chromatograph and mass spectrometer were controlled simultaneously by Thermo's Xcalibur 2.0 software. Eluates were introduced into the electrospray ionization (ESI) chamber through a 0.1 mm i.d. fused silica capillary. The ESI source spray voltage was set to 4500 V for detection in positive mode and 3000 V in negative mode. A sheath gas of nitrogen (40 psi) was used to dry the excess solvent and the capillary inlet temperature was set to 290 °C. Argon was used as the collision gas, set at 1.5 mTorr. Data was collected in the selective reaction monitoring (SRM) mode where parent ions are selected by the first quadrupole, fragmented by collision induced dissociation (CID) in the second and the product ions are detected in the final quadrupole. Each SRM was scanned for 0.015 s over a range of 1 m/z unit. Parameters for the SRM transitions were used from Bajad et al. [1] and the supplemental information provided therewith.

2.5. Experimental setup

In the first dimension, a 100 μL volume of the sample mixture was injected onto the strong cation exchanger column. The mobile

phases for the cation exchange gradient separation were (mobile phase A) 75:25 H_2O:ACN and (mobile phase B) 75:25 400 mM NH_4HCO_2:ACN. The volatile buffer, 400 mM NH_4HCO_2 (ammonium hydroxide-formic acid buffer) was prepared by titrating 400 mM ammonium hydroxide to pH 3.25 with formic acid. Since the pKa of formic acid is 3.75, only 25% of the formic acid is deprotonated and combined with the ammonium cation, the total cationic strength of this buffer adds up to 725 mM. For the cation exchange gradient, mobile phase B was increased linearly from 2–35% in 5 min and held at 35% until all components were eluted with a constant flow rate of 0.4 mL/min. The fractions from the first dimension were collected using a Gilson F203 fraction collector (Gilson, Middleton, WI, USA), retrofitted with F203b internal hardware. Eighteen equal volume fractions were collected from 2.5 to 8.5 min across the first dimension. Fractions were collected in 300 μL vials and loaded into the autosampler quickly, where they were chilled to 4 °C until injection.

For the second dimension, 50 μL injections were made onto the silica diol column. The HILIC separation used a gradient of (mobile phase A) 95:5 ACN:100 mM ammmonium formate, pH 3.2, and (mobile phase B) 50:50 ACN:10 mM ammonium formate, pH 3.2. This mobile phase system was chosen to provide a constant 5 mM concentration of ammonium formate throughout the second dimension separation. The second dimension gradient consisted of two linear increases of mobile phase B operated at a constant flow rate of 0.25 mL/min. The gradient was initially increased from 15–35% mobile phase B in the first 2 min, followed by an increase from 35–100% mobile phase B from 2–9 min. Mobile phase B was held at 100% for 1 min then returned to the initial conditions for re-equilibration of the column.

3. Results and discussion

Initially, the experiment was designed as a HILIC separation followed by a reverse phase dimension. These dimensions are quite orthogonal, which is critical to a two-dimensional separation [39]. The importance of orthogonality is to utilize the entire separational space provided by the dimensions. In two-dimensional chromatography, the peak capacity (the number of peaks that can be uniformly resolved in a chromatographic space) of the dimensions is, in principle, multiplicative. This means, two paired dimensions quickly generate a high power of separation, assuming the entire separational space is used. Our choice for a HILIC-RP separation, however, proved to be an unsuccessful choice when the second dimension chromatograms were reviewed. Even though a reverse phase column with an embedded polar group was used, the polar nature of the compounds used here resulted in mostly unresolved peaks. The poor resolution could also be attributed to the transfer of fractions with a high organic solvent content from the first dimension. We decided to use an unconventional, yet necessary route to not only pair strong cation exchange and HILIC, but to use volatile buffers in both dimensions so that de-salting was not necessary before detection by mass spectrometry. Choosing to order the dimensions as strong cation exchange followed by HILIC was a pragmatic decision. The HILIC dimension used here is much more efficient and can be re-equilibrated quicker than the cation exchanger, both of which are important when considering a second dimension.

3.1. Design

To design the experiment, we used a sub-set of metabolite standards as a test solution to ensure the elution of all metabolites of interest in a reasonable time frame. We also constrained ourselves that the number of fractions to be taken from the first dimension must be low. This constraint reduces the length of time necessary to analyze all the fractions, but increases the dilution factor of each

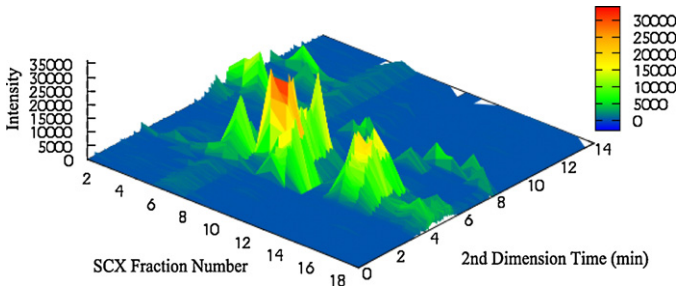

Fig. 1. Three-dimensional depiction of the two-dimensional chromatogram from metabolites detected in negative mode ionization.

compound [37]. Also, we chose not to use a flow splitter before the ion source, which means that the second dimension flow rate must be relatively low considering the columns used in this work. Therefore, we set out to make a second dimension that is the "work horse" for the overall separation. Requiring that the first dimension only separates components of the mixture into manageable groups to be analyzed in the second dimension. A "work horse" type multidimensional separation is ideal for simple and quick method development, especially in the case where a very sensitive, multiplex detector is used. Fig. 1 shows a three-dimensional chromatogram of metabolites detected in negative-ionization mode. The metabolites detected show a wide range of concentrations which make extracting much useful information from the plots about the metabolites difficult. Therefore, in Figs. 2 and 3 we have represented the approximate locations of metabolites in a

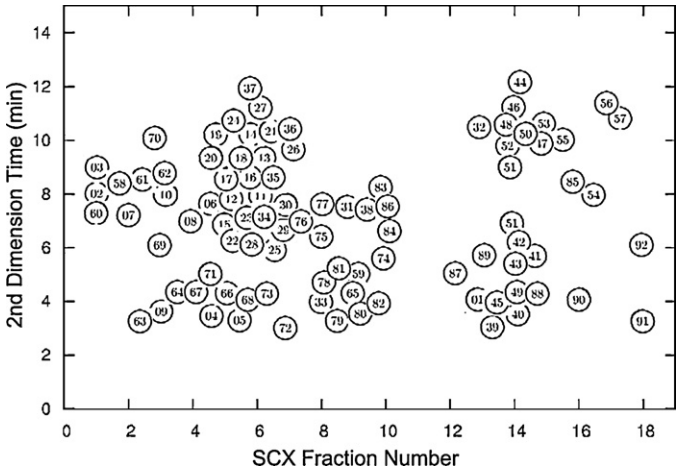

Fig. 2. Spotting arrangement of *E. coli* metabolites detected in both positive and negative ionization modes. 01-glutamine; 02-lysine; 03-ornithine; 04-aspartate; 05-betaine; 06-betaine aldehyde; 07-o-acetyl-L-serine; 08-urea; 09-proline; 10-thymine; 11-isoleucine; 12-octanyl-homoserine; 13-acetyl CoA; 14-CoA; 15-cyclic-di-GMP; 16-dephospho CoA; 17-FAD; 18-glutathione; 19-glycerophosphocholine; 20-proponyl CoA; 21-succinyl CoA; 22-TMP; 23-UMP; 24-1-methylhistidine; 25-acetylcarnitine; 26-acetyllysine; 27-arginine; 28-N-carbamyl-aspartate; 29-carnitine; 30-choline; 31-dimethylglycine; 32-histidine; 33-imidazole; 34-imidazole acetic acid; 35-1-methyladenosine; 36-GMP; 37-NADP; 38-creatine; 39-indole; 40-epinephrine; 41-N-acetyl-L-lysine; 42-N-acetyl-glutamine; 43-N-acetyl-glutamate; 44-nicotinamide; 45-pyridoxamine; 46-ADP; 47-AMP; 48-ATP-gamma-S; 49-Biotin; 50-CMP; 51-dAMP; 52-IMP; 53-NAD; 54-serine; 55-dCMP; 56-oxidized glutathione; 57-glucosamine-6-phosphate; 58-2,3-dihydroxybenzoic acid; 59-myo-inositol; 60-orotic acid; 61-UDPAG; 62-UDPG; 63-aconitate; 64-isoketovalerate; 65-maleic acid; 66-fumarate; 67-malate; 68-phenylpyruvate; 69-phosphoenolpyruvate; 70-dephospho CoAII; 71-kynurenic acid; 72-4-hydroxybenzoic acid; 73-lactate; 74-citrate; 75-D-glyceraldehyde-3-phosphate; 76-DHAP; 77-glycerol-phosphate; 78-glucono-lactone; 79-isocitrate; 80-pantothenic acid; 81-glyceric acid; 82-oxaloacetate; 83-cAMP; 84-pyridine-dicarboxylate; 85-glucose-1-phosphate; 86-succinyl/methylmalonyl CoA; 87-xanthine; 88-nicotinate; 89-inosine; 90-hypoxanthine; 91-fructose-bis-phosphate; 92-gluconate.

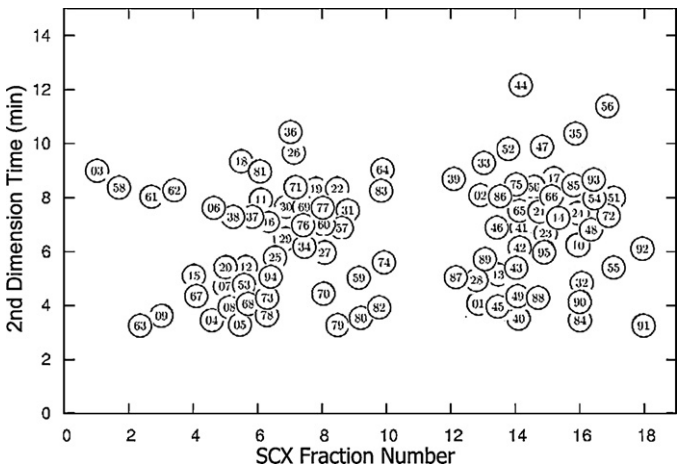

Fig. 3. Spotting arrangement of *yeast* metabolites detected in both positive and negative ionization modes. 01-glutamine; 02-4,5-dihydroxy-2,3-pentanedione; 03-ornithine; 04-aspartate; 05-betaine; 06-betaine aldehyde; 07-2-oxoglutatrate; 08-4-hydroxybenzoate; 09-proline; 10-adenosine; 11-iso-leucine; 12-allantoate; 13-5′-methylthioadenosine; 14-asparagine; 15-acetyl-CoA; 16-adenine; 17-alanine; 18-glutathione; 19-cytidine; 20-carbamoyl aspartate; 21-cytosine; 22-D-hexose-phosphate; 23-dihexose; 24-citrulline; 25-acetylcarnitine; 26-acetyllysine; 27-D-glyceraldehyde-3-phosphate; 28-folate; 29-carnitine; 30-choline; 31-dimethylglycine; 32-DL-pipecoline; 33-glucosamine; 34-erythrose-4-phosphate 35-flavone; 36-GMP; 37-glucose-6-phosphate; 38-fructose-6-phosphate 39-GABA; 40-epinephrine; 41-N-acetyl-L-lysine; 42-N-acetyl-glutamine; 43-N-acetyl-glutamate; 44-nicotinamide 45-pyridoxine; 46-glutamate; 47-AMP; 48-guanosine; 49-biotin; 50-methionine; 51-phenylalanine; 52-IMP; 53-phenylpropiolate; 54-serine; 55-riboflavin; 56-oxidized glutathione; 57-methylcysteine; 58-methylmalonate; 59-myo-inositol; 60-N-acetylglucosamine-1-phosphate; 61-UDPAG; 62-UDPG; 63-aconitate; 64-NAD; 65-N-carbamoyl-L-aspartate; 66-nicotinamide ribotide; 67-malate; 68-phenylpyruvate; 69-sedoheptulose-7-phosphate; 70-succinate; 71-trehalose-6-phosphate; 72-typtophan; 73-lactate; 74-citrate; 75-tyrosine; 76-DHAP; 77-glycerol-3-phosphate; 78-pyruvate; 79-isocitrate; 80-pantothenate; 81-S-adenosyl-L-homocystein; 82-oxaloacetate; 83-cAMP; 84-S-ribosyl-homocystein; 85-glucose-1-phosphate; 86-threonine; 87-xanthine; 88-nicotinate; 89-inosine; 90-hypoxanthine; 91-fructose-1,6-bis phosphate; 92-gluconate; 93-valine; 94-UTP; 95-xanthosine.

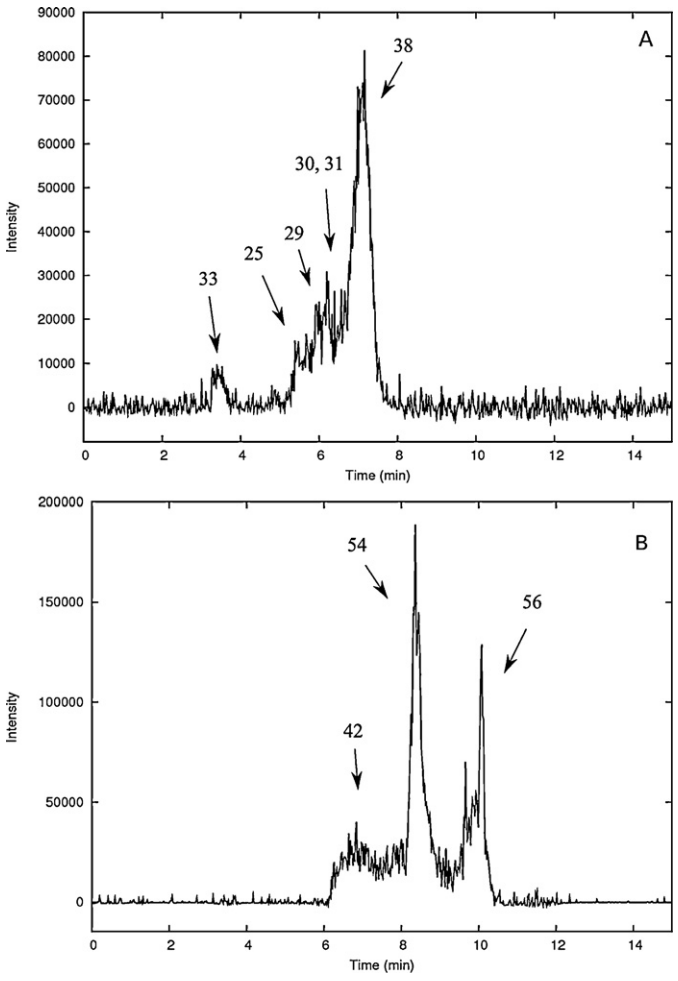

Fig. 4. Second dimension chromatogram of (A) fraction 9 and (B) 17, recorded in positive-ionization mode ((A) 25-acetylcarnitine; 29-carnitine; 30-choline; 31dimethylglycine; 33-imidazole; 38-creatine; (B) 42-N-acetyl-glutamate; 54-serine; 56-oxidized glutathione).

two-dimensional space, based on their relative maximum concentrations. As expected, many of the metabolites detected are found in a few contiguous fractions, but our plot demonstrates the high orthogonality between the two separation dimensions and shows grouping of structurally similar metabolites.

3.2. Experimental results

In total, 141 metabolite species were detected (92 and 95 for *E. coli* and *S. cerevisae* respectively with 46 metabolites in common), the list of which are in the captions of Figs. 2 and 3. These metabolites represent a wide range of metabolite classes from amino acids to sugars and vitamins. From Figs. 2 and 3, the orthogonality between the two dimensions is apparent. This is near the best case scenario for a two-dimensional separation, where the analytes are spread over nearly the entire separational space. The quantity of metabolites detected is considerable based on the extraction method, which preferentially extracts highly polar metabolites. Further considering that *E. coli* in this study was grown on minimal media, this result is promising. In general, microorganisms grown in a well-fed environment should be able to allocate their energy differently, resulting a higher quantity of metabolites. The methods used here were also designed such that positive and negative mode detection would be possible utilizing a single set of chromatographic conditions. Typical analysis of metabolites from a bacteria extract requires *ca.* 2 h for positive and negative mode detection using different columns and conditions for both cases. In this work, the second-dimension separation takes about 15 min per fraction for a total analysis time of 4.5 h. The 4.5 h cycle is performed for one ionization mode, then repeated from another. Although our approach still requires two separate columns and conditions, detection is only required in a single dimension. In the case where an analyst is interested in a subset or class of metabolites, our method could be ideal. The first dimension here could be used to fractionate particular metabolites into groups and only targeted groups would require collection and analysis using the second dimension. If a heart-cutting method was used, the total analysis time could be significantly reduced. Such a procedure could be highly productive for metabolic flux studies [40,41].

Methods used from others have produced acceptable results, but at some consequences. Two methods in particular were used, one utilized an aminopropyl column at a high pH and another used a reverse phase column and an ion-pairing reagent, tributylamine. The aminopropyl columns were degraded quickly and tributylamine proved extremely difficult to remove from the HPLC system. The two-dimensional method described here could result in a higher throughput of samples with a dedicated LC–MS system (for the second dimension) because the first dimension could be operated off-line with only a gradient pump, an injector, and a fraction collector. Figs. 4 and 5 show detection of positive and negative mode metabolites collected under the same separation conditions. These are typical chromatograms of fractions where compounds

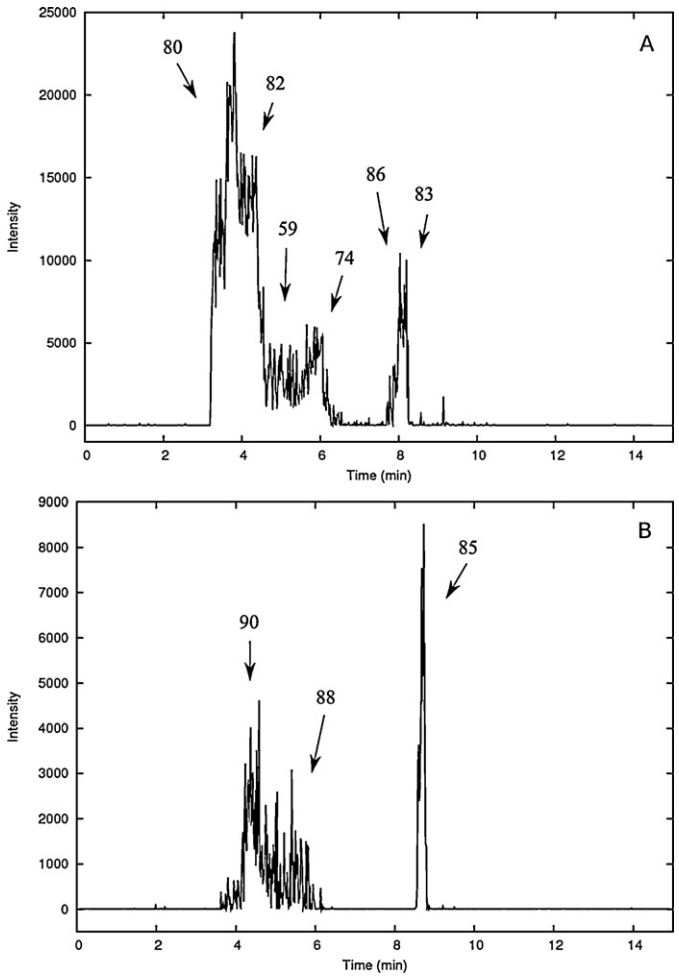

Fig. 5. Second dimension chromatograms of (A) fraction 10 and (B) fraction 16, recorded in negative-ionization mode ((A) 59-myo-inositol; 74-citrate; 80-pantothenic acid; 82-oxaloacetate; 83-cAMP; 86-succinyl/methylmalonyl CoA; (B) 85-glucose-1-phosphate; 88-nicotinate; 90-hypoxanthine).

may still co-elute, but the number of co-eluting compounds is significantly reduced such that mass spectrometry can easily deconvolute the spectra. The peaks shown illustrate a wide range of absolute and local metabolite concentrations. Assessing the chromatographic separation, we have calculated the two-dimensional peak capacity to be *ca.* 2500 (neglecting the undersampling and dimension orthogonality) if we conservatively assume the peak capacity of the mass spectrometer to be five (based on observed co-eluting metabolites detected), although the mass spectrometer can easily handle many more compounds.

3.3. Improving results

The most significant drawback in using multidimensional approaches to detect metabolites of relatively low concentrations in the sample is dilution. This issue is not without some remedies that can be implemented. First, larger sample sizes can be injected into the first dimension column. Injection of a larger initial sample into the first dimension should be allowable in most cases without significant problems, as long as this column is not highly overloaded. However, injecting a higher volume into the second dimension when using a 3.0 mm i.d. column could be detrimental to the second separation. Care must be taken to ensure the effect of sample diluent-induced peak profile distortion via injecting one mobile phase, which behaves as a strong eluent additive in the subsequent mobile phase, is negligible [37,42].

Second, the number of fractions taken can be increased. More fractions could be collected from the first dimension column. Although the absolute signal for peaks in the second dimension would be lower, the dilution factor for each peak would also be lowered. If the number of fractions taken were to be reduced, the aggregate analysis time can be significantly reduced at the cost of a decreased signal.

Thirdly, the diameter of the second dimension column could be reduced to lower the elution volume. This is an excellent option, considering that mass spectrometry is used for detection. Decreasing the column diameter will increase the ionization efficiency, thereby increasing the detector signal [43,44]. A favorable consequence of reducing the second dimension column diameter is that the second dimension column can be eluted at a faster flow velocity, insofar as the corresponding inlet pressure remains acceptable. Since we are working with an acetonitrile-rich second dimension mobile phase, the second dimension fractional survey time (the time required to analyze a fraction and re-equilibrate the second dimension) could then be cut roughly in half. Another consideration is that both the first and second-dimension gradients could be optimized for particular metabolites of interest or to significantly reduce the overall analysis time.

Although much work has been done in the metabolomics field using unidimensional chromatography, the resolution of components of such complex mixtures would be improbable, at best. If a unidimensional approach was undertaken, the resolving power will be significantly lower than that of a two-dimensional approach; however, this result can be acceptable if, in compensation, the degree of dilution of some critical analytes is reduced. Unfortunately, this is rarely the case in metabolomics because we desire to identify and study many parts of a metabolome concurrently. In this work we have chosen a two-dimensional approach because of the complexity of the sample and greatly value the resolution of individual metabolites. If a unidimensional approach is preferred, we recommend modifying the HILIC second dimension used here or the approach of Bajad et al. [1]. Using the conditions provided here, the gradient length of the separation should be increased to 40 min or more (until an acceptable resolution is achieved), which would maintain the advantage of using only one phase system for both positive and negative ion detection.

4. Conclusions

A successful method of analysis of *E. coli* and *S. cerevisae* metabolites by comprehensive, two-dimensional liquid chromatography with MS/MS detection was developed. This is a step forward in consolidating detection of many water-soluble compounds from microorganisms. The method developed permits MS detection in both positive and negative modes of ionization and works well under a single set of chromatographic conditions for the polar metabolites of interest. In future work, similar conditions will be used to detect (semi-quantitatively) other metabolites of interest to monitor metabolite production. Although, the mixtures of metabolites are quite complex, two-dimensional liquid chromatography will be the best choice to separate the mixture components quickly.

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