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Screening and Quantitation of Amino Acids and Other Nutrients In Spent Media

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Abstract

This poster demonstrates the usage of Agilent 1290 Infinity II LC system coupled with Ultivo LC/TQ Mass Spectrometry system to screen major nutrients as present in Spent Media. The method provide fast separation with low ppb level quantitation solution for researchers from fermentation industry.

Introduction

The spent medium is useful for some industries as a nutrient and for others it is a discarded liquor. Recent years has seen interest in knowing the components of such viscous liquids to understand nutritional uptakes from cultures at various stages of growth including amino acids, vitamins, sugars etc.

This poster describes a solution to the challenging task of screening constituents of spent medium by making usage of Agilent AdvanceBio MS Spent Media columns for normal phase separation of amino acids and small, polar metabolites in media samples [1]. The zwitterionic phase bonded onto superficially porous silica particles supported efficient and reproducible separations of small, charged molecules[2]. The Ultivo LC/TQ system supported quantitation of 24 analytes of interest focus in MRM scan mode.

Figure 1: Ultivo LC/TQ and Advance Bio columns

Sample Preparation

Amino Acid Supplement Kit (Agilent P No 5062-2478), Amino Acids Standard (Agilent P No 5061-3330) and Vitamin B compounds were diluted with 1% FA in 50/50 ACN/H2O for stock and working concentration. The spent media samples were diluted upto 100X.

Reagents and Chemicals

All LCMS grade chemical were purchased from Honeywell.

Ultivo LC/TQ Conditions

Ionization Source = Agilent Jet Stream
Nebulizer Gas = 20psi
Drying Gas = 12L/min at 150° C
Sheath Gas = 12L/min at 390° C
Capillary Voltage = +/- 2000 V
Nozzle Voltage = +/- 0 V

UHPLC Conditions

Mobile Phase A = 20mM Amm Acetate with 0.1%FA
Mobile Phase B = 20mM Amm Acetate in 90% ACN

Parameter	Value
Column	Agilent AdvanceBio MS Spent Media, 2.1x100 mm (Agilent P No - 675775-901)
Flow Rate	300 µl/min
Injection Vol	10 µL
Column Temp.	25^0 C

Time (min)	% B
0.0	100
11.5	70
12.0	40
13.0	40
13.5	100
20.0	100

Table 1: HPLC parameters and gradient program

In this study 21 amino acids and 3 compounds from Vit B (table 2) showed good chromatographic separation in a total runtime of 20 min, as seen in fig 2. A calibration plot with minimum 5 level was generated from 1ppb to 1ppm with variable LOQs of 1ppb to 20ppb and R2 values between 0.993 to 0.999 with representative plots shown in Fig 3.

#	Analyte	#	Analyte	#	Analyte
1	Alanine	9	Glutamine	17	Phenylalanine
2	Arginine	10	Histidine	18	Proline
3	Asparagine	11	Hydroxyproline	19	Sarcosine
4	Aspartic Acid	12	Leucine	20	Serine
5	Cyanocobalamin	13	Lysine	21	Threonine
6	Cystine	14	Methionine	22	Tryptophan
7	Folic Acid	15	Nicotinic Acid	23	Tyrosine
8	Glutamic Acid	16	Norvaline	24	Valine

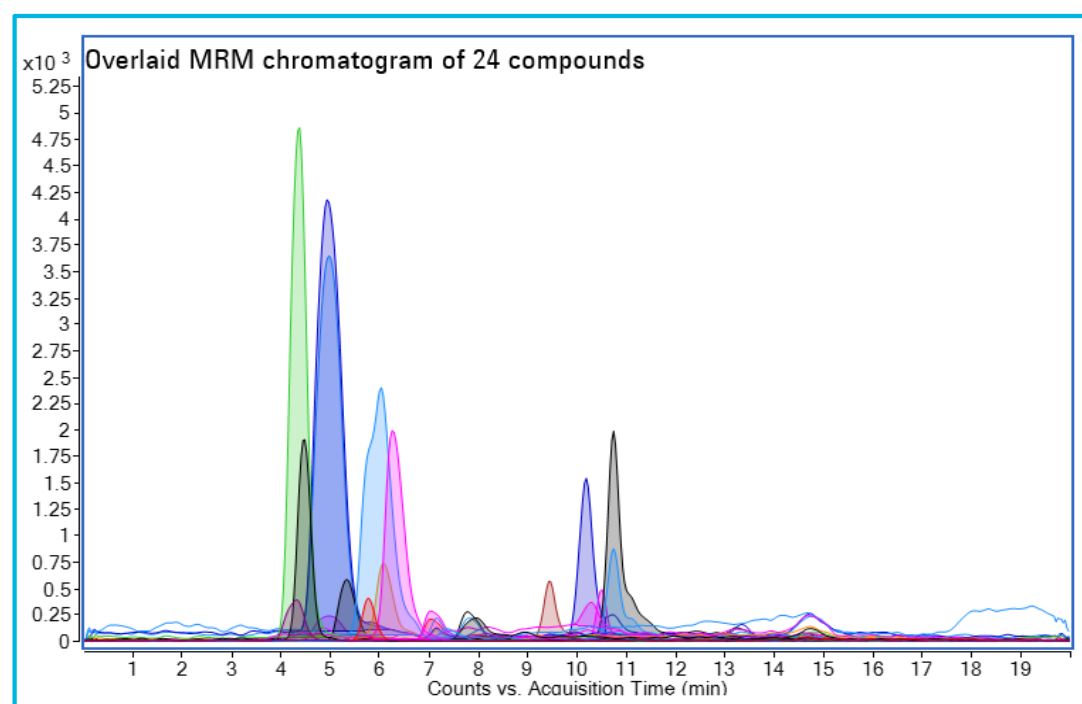Table 2: 24 Compounds as quantified in methodology

Figure 2: Chromatographic separation of 24 analytes

Spent media samples were taken at every 24 hour across 10 days, labelled as S1 to S10. The 24 nutrients were quantified in 10x, 100x diluted spent media samples. TIC profiles as seen in fig 4 confirm that there are differences in abundance of nutrients on Zero time (100x_Med) vs 8 day (S8_100).
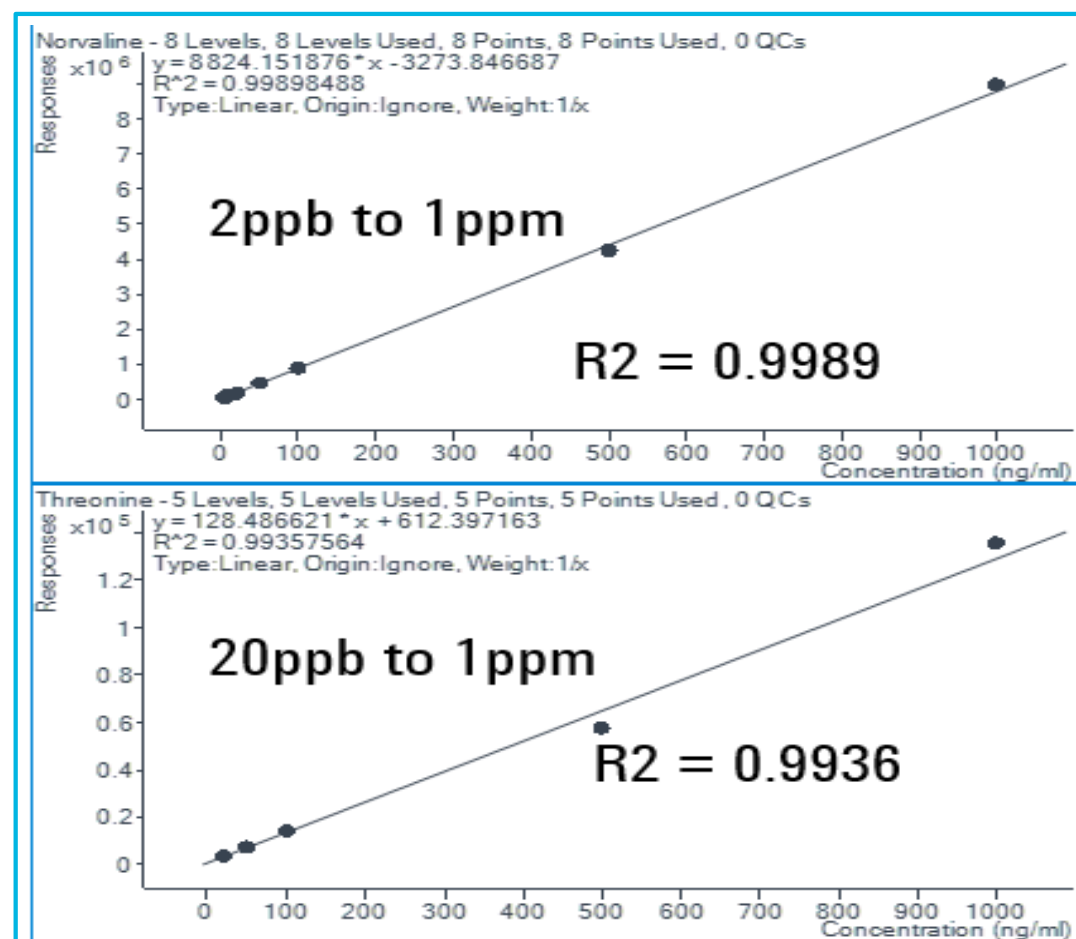

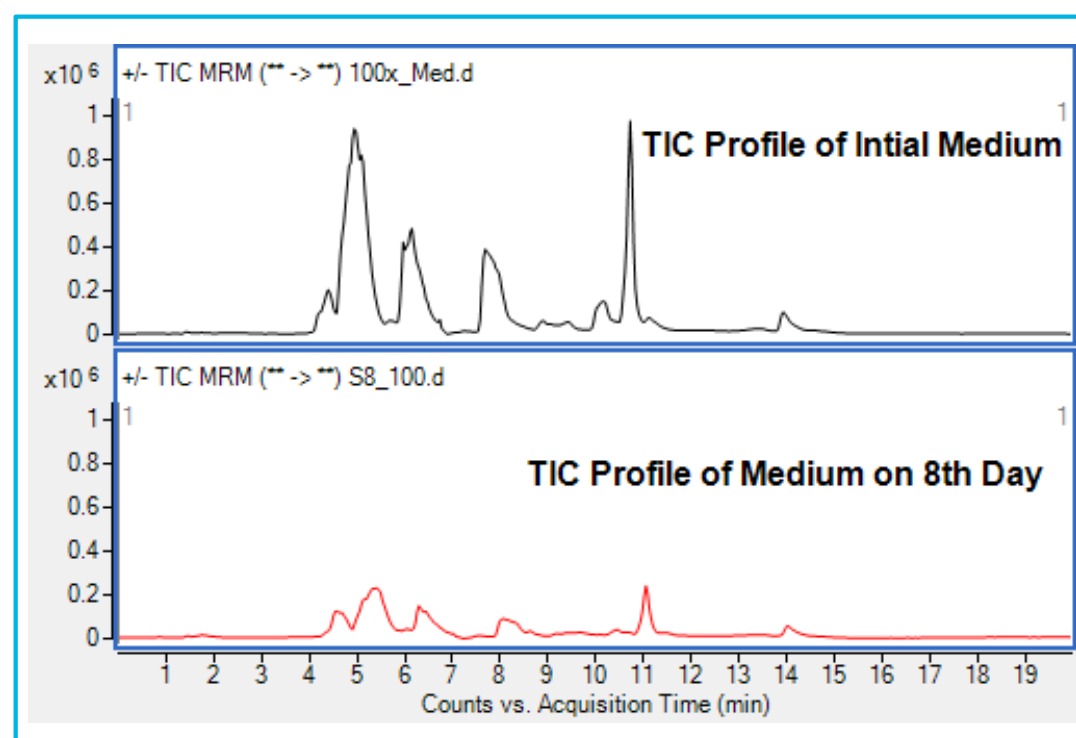Figure 3: Calibration plots with R2 from 0.993 to 0.999

Figure 4: TIC profile comparison of Spent Media sample at Initial Medium *s 8th day.

The 24 analytes had variable responses for 100X dilution as seen for 8th day sample in Fig 5. All analytes, present in Initial sample to 10 days samples, were quantified using Mass Hunter Quant-My-Way s/w with ± 20% accuracy and Qualifier/Quantifier Ion response ratio. Bar chart of analyte vs media sample, as plotted for Aspartic Acid, Methionine, Proline and Sarcosine as representative plots confirms the behavior, as seen in fig 6.
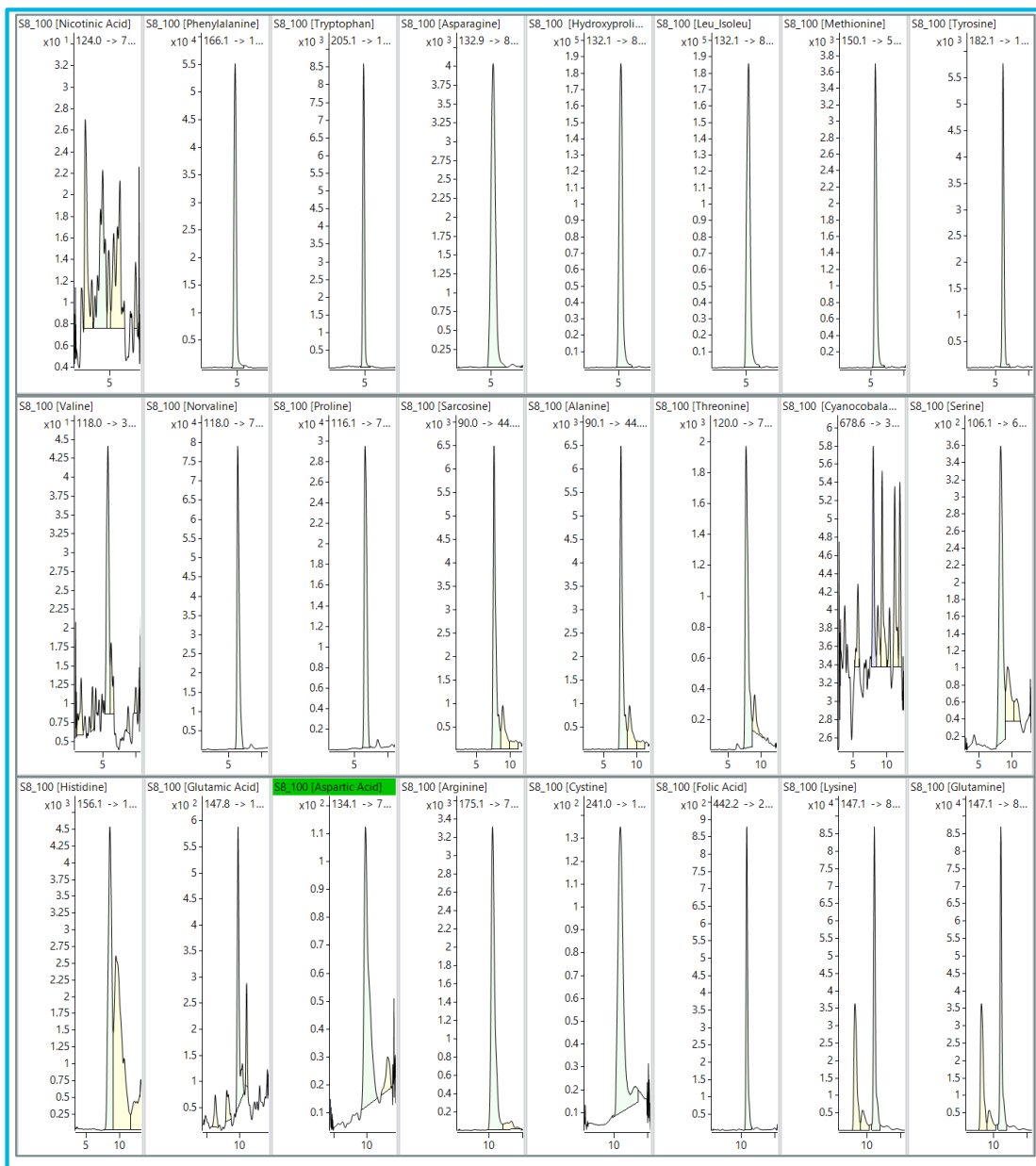
Figure 5: MRM Chromatogram from 100x diluted Spent Media sample on day 8, having good response of 22 metabolites.

Conclusions

- Low ppb (picogram quantity) level analytical sensitivity of nutrients attained using Triple Quadrupole LC/TQ.

- Fast chromatographic separation is achieved for amino acids and vitamin B compounds.

- A cost effective and quick method requiring minimal sample preparation is proposed, since derivatization steps are not used.

- Expected variations in concentration level from ppb to ppm are well estimated.

- Spent Media samples must be 100 times diluted.

- Single dual polarity LC/TQ method for analytes.

- Method can be utilized by academia, research and other fermentation-based laboratories.

References

1. Agilent AdvancedBio workflows for spent media analysis; Agilent Publication No 5991-8817EN

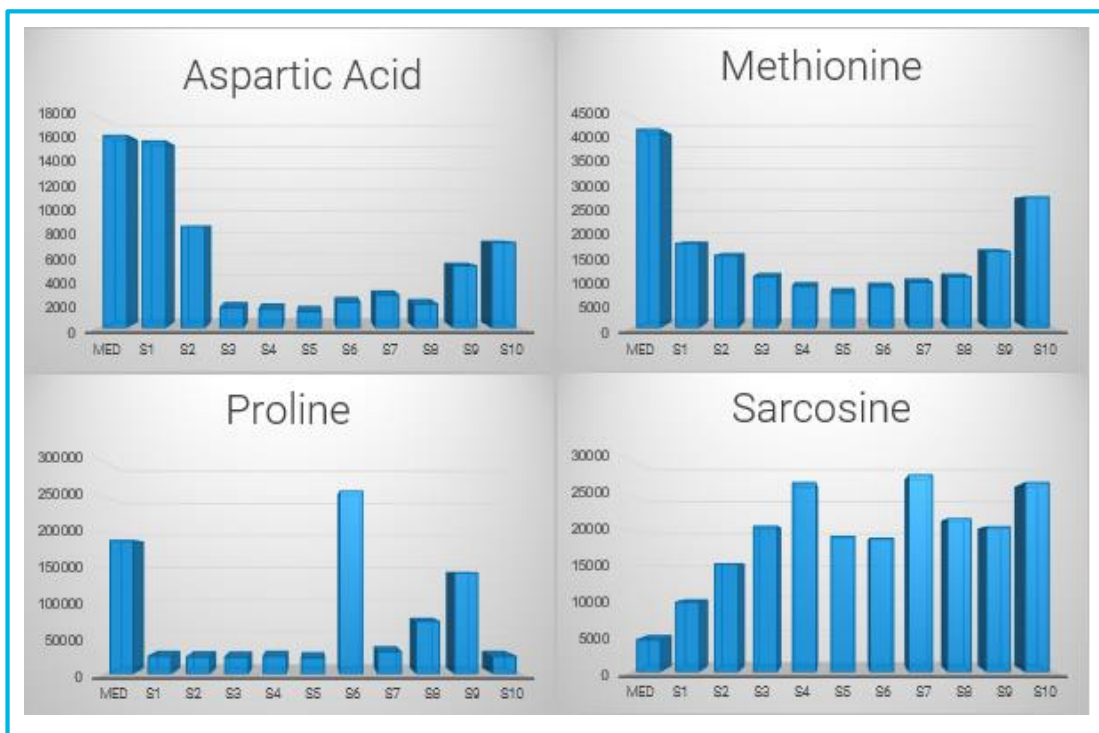2. Agilent AdvanceBio MS Spent Media Column, User Guide; Agilent Publication No 820120-015

Figure 6: Varibale amount of 4 representative nutrients in ng/ml (ppb) from initial stage (Med) to day 10 (S1, S2…S10) of spent media.

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