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Analysis and Quantitation of Pre-Workout Powder Components in Matrix by LC/UV using Luna® 3 µm HILIC HPLC Column

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Scott enjoys surfing and eating. He is crazy about chromatography, because his mom is really into CSI and thinks that is what he does.

Introduction

With the implementation of the food safety modernization act (FSMA) and new label claim verification requirements, attention to pre-workout supplements have increased in recent years. This newfound popularity has led to an increase in interest for the identification and quantitation of the key ingredients promoted on the labels for these pre-workout drinks. The understanding of what is contained within a consumable supplement is important to ensure that the product being consumed is accurately represented for both product safety and quality. In this work, we present a fast and highly efficient method for the identification and quantitation of four key ingredients of pre-workout powders using HILIC mode HPLC-UV/Vis analytical techniques.

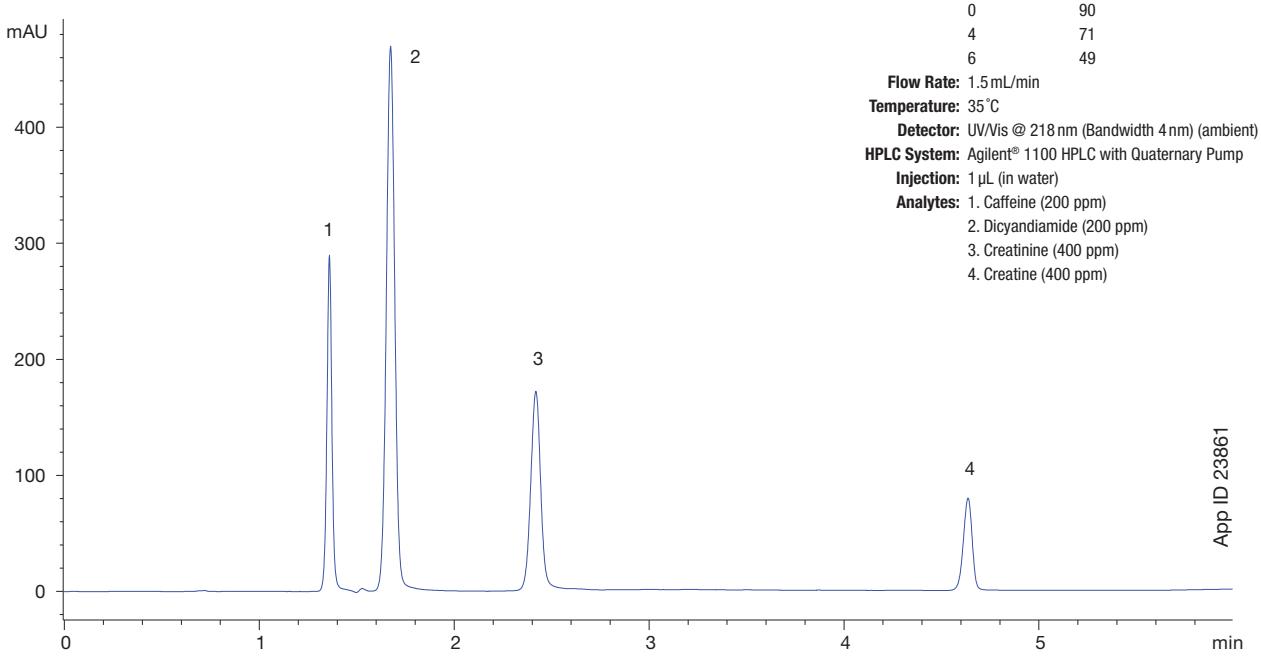
Materials and Methods

Analytical standards of Creatine, Creatinine, Caffeine and Dicyandiamide were purchased from Sigma-Aldrich, and were used for method development purposes. Each of these components were then made into stock solutions and injected at various concentrations to develop a standard calibration curve (**Figures 3-6**).

Sample Preparation

10 mg of Pre-Workout powder was dissolved into 1 mL of DI water and vortexed for 1 minute at 3000 rpm to ensure the sample was fully dissolved. The samples were then diluted to a concentration of 2.5 mg/mL with DI water for HPLC injection.

Figure 1.
Optimized Separation of 4 Key Components



HPLC Conditions

Column: Luna® 3 µm HILIC 200 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4449-E0
Mobile Phase: A: 1 mM Sodium Acetate pH 5.5 (pH Adjusted with acetic acid)
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	90
	4	71
	6	49

Flow Rate: 1.5 mL/min
Temperature: 35 ˚C
Detector: UV/Vis @ 218 nm (Bandwidth 4 nm) (ambient)
HPLC System: Agilent® 1100 HPLC with Quaternary Pump
Injection: 1 µL (in water)
Analytes: 1. Caffeine (200 ppm)
2. Dicyandiamide (200 ppm)
3. Creatinine (400 ppm)
4. Creatine (400 ppm)

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Figure 2.
Optimized method using variable standard concentrations in water

HPLC Conditions

Column: Luna® 3 µm HILIC 200 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4449-E0
Mobile Phase: A: 1 mM Sodium Acetate pH 5.5 (pH Adjusted with acetic acid)
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
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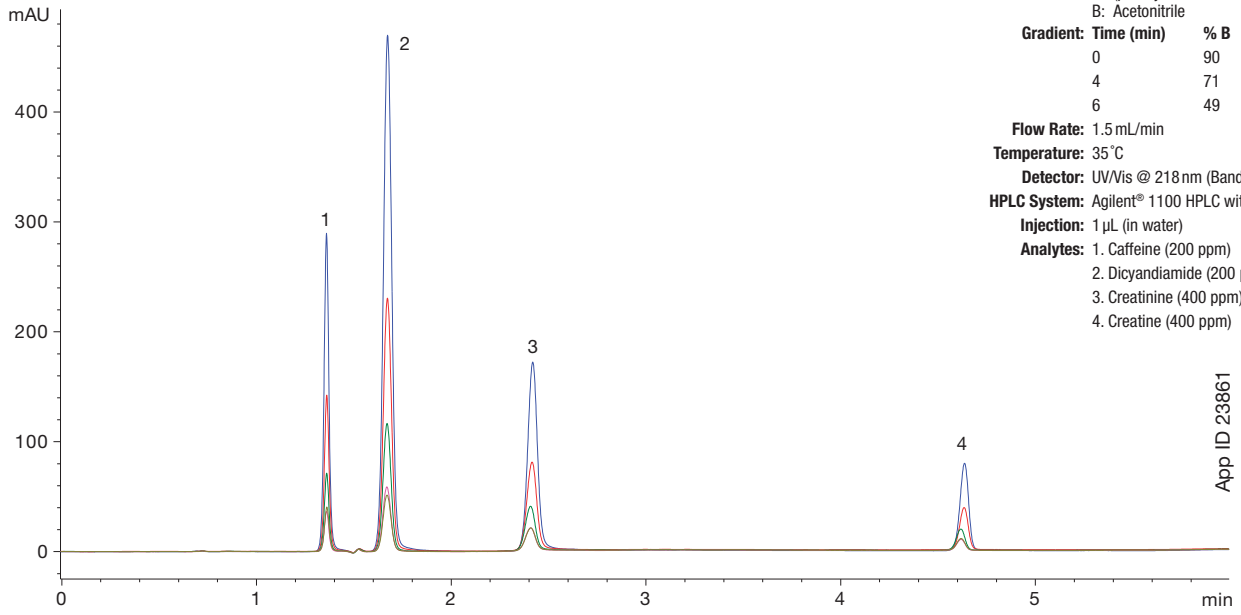
Injection: 1 µL (in water)
Analytes: 1. Caffeine (200 ppm)
2. Dicyandiamide (200 ppm)
3. Creatinine (400 ppm)
4. Creatine (400 ppm)



Blue: 400/200 ppm Red:200/100 ppm Green: 100/50 ppm Pink: 50/25 ppm Brown: 25/12.5 ppm
*X/Y Refers to the concentration of X (Creatine and Creatinine) and the concentration of Y (Caffeine and Dicyandiamide) in the mixture

Figure 3.
Calibration Curve for Caffeine 12.5 to 200 ppm

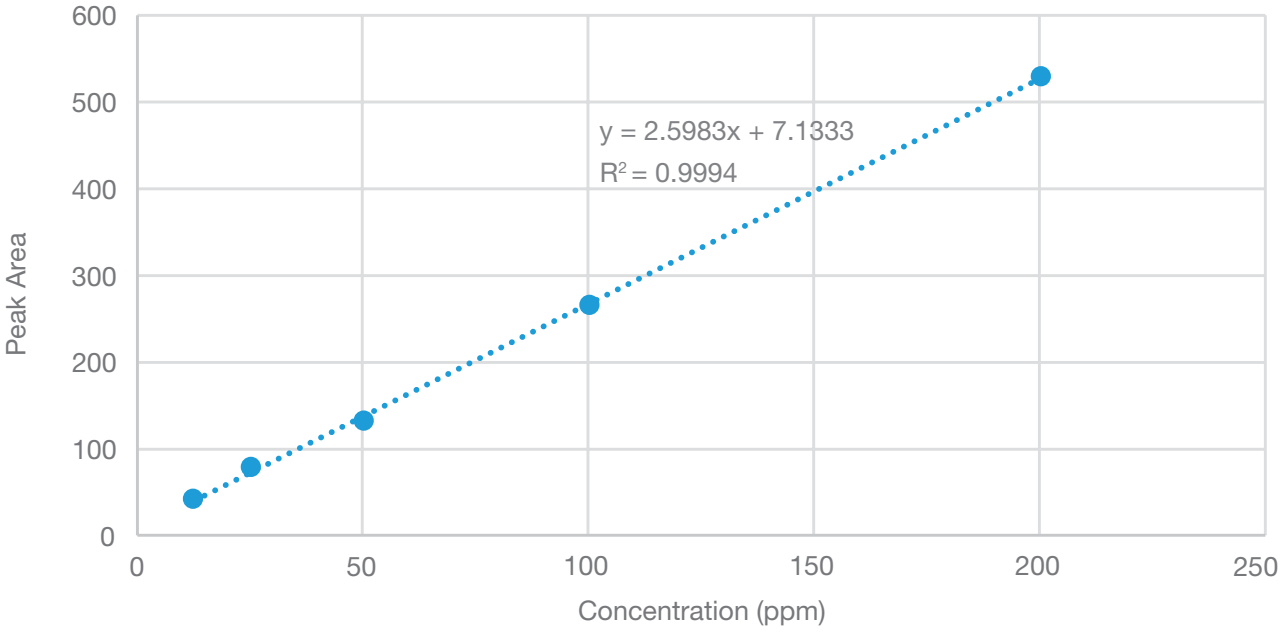


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Figure 4.
Calibration Curve for Dicyandiamide 12.5 to 200 ppm

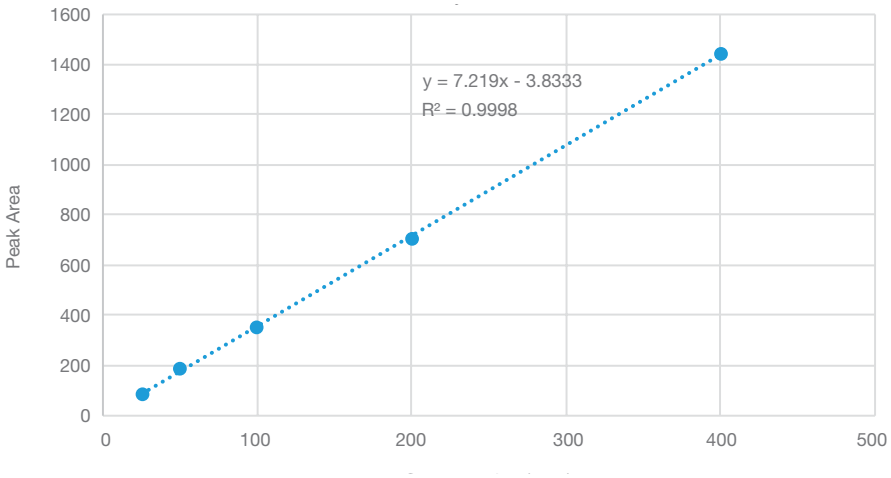


Figure 5.
Calibration Curve For Creatinine 25 to 400 ppm

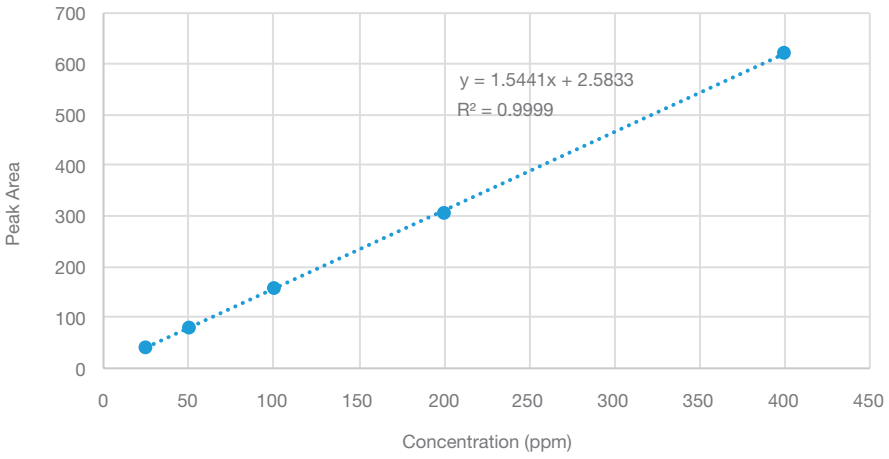


Figure 6.
Calibration Curve For Creatine 25 to 400 ppm

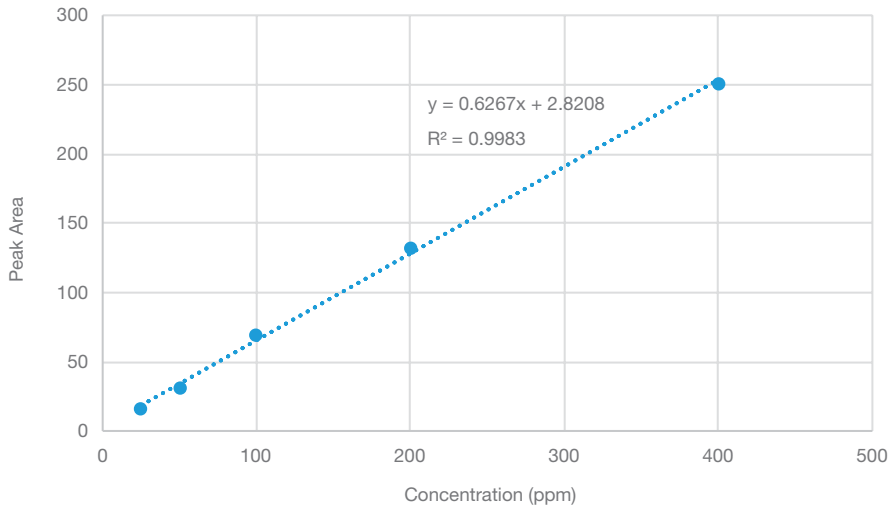
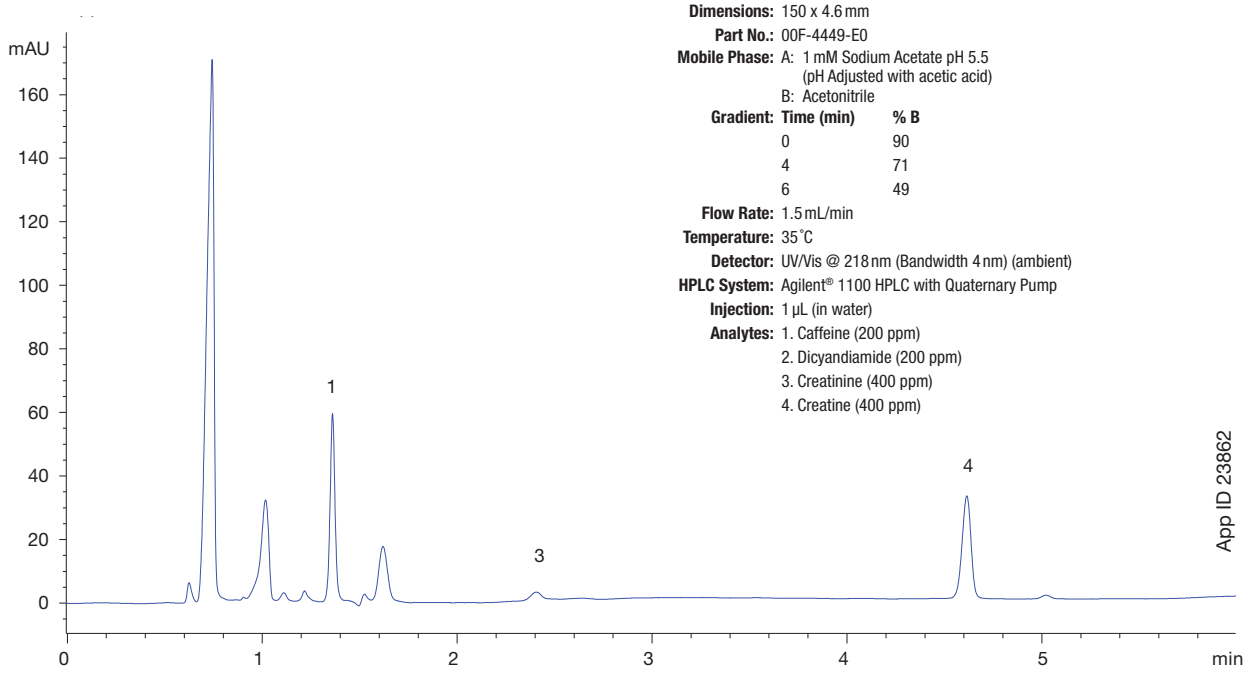


Figure 7.
C4 Pre-workout 1µL injection of 2.5 mg/mL



HPLC Conditions

Conditions for both separations:

Column: Luna® 3 µm HILIC 200 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4449-E0
Mobile Phase: A: 1 mM Sodium Acetate pH 5.5 (pH Adjusted with acetic acid)
B: Acetonitrile

Gradient:

Time (min)	% B
0	90
4	71
6	49

Flow Rate: 1.5 mL/min
Temperature: 35˚C
Detector: UV/Vis @ 218 nm (Bandwidth 4 nm) (ambient)
HPLC System: Agilent® 1100 HPLC with Quaternary Pump
Injection: 1 µL (in water)
Analytes: 1. Caffeine (200 ppm)
2. Dicyandiamide (200 ppm)
3. Creatinine (400 ppm)
4. Creatine (400 ppm)

Figure 8.
N.O. Xplode Pre-workout 1µL injection of 2.5 mg/mL

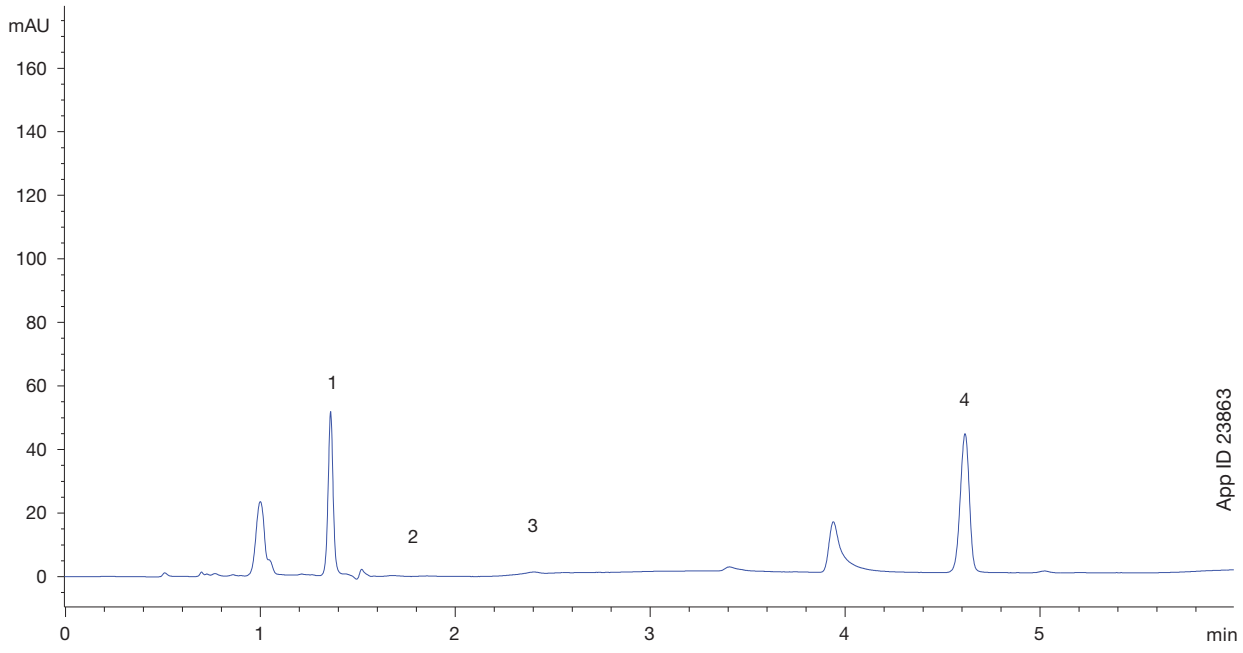
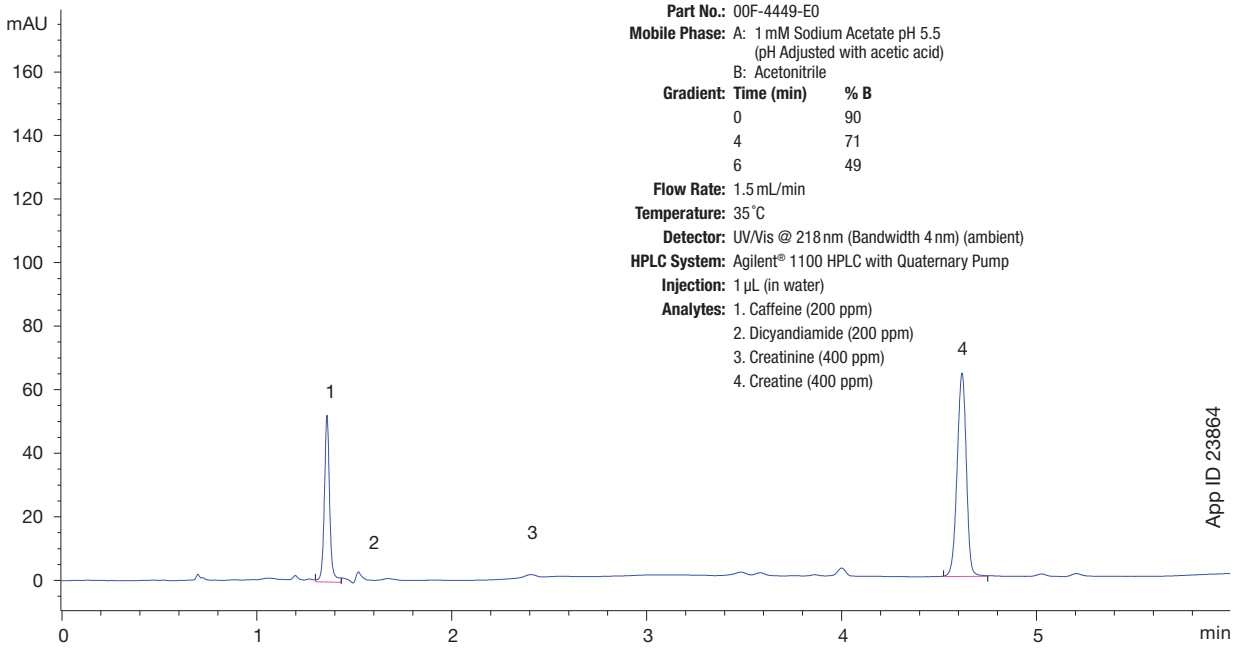


Figure 9.
Bang Pre-workout 1µL injection of 2.5 mg/mL



HPLC Conditions

Conditions for both separations:

Column: Luna® 3 µm HILIC 200 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4449-E0
Mobile Phase: A: 1 mM Sodium Acetate pH 5.5 (pH Adjusted with acetic acid)
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	90
	4	71
	6	49

Flow Rate: 1.5 mL/min
Temperature: 35˚C
Detector: UV/Vis @ 218 nm (Bandwidth 4 nm) (ambient)
HPLC System: Agilent® 1100 HPLC with Quaternary Pump
Injection: 1 µL (in water)
Analytes: 1. Caffeine (200 ppm)
2. Dicyandiamide (200 ppm)
3. Creatinine (400 ppm)
4. Creatine (400 ppm)

Figure 10.
Outlift Pre-workout 1µL injection of 2.5 mg/mL

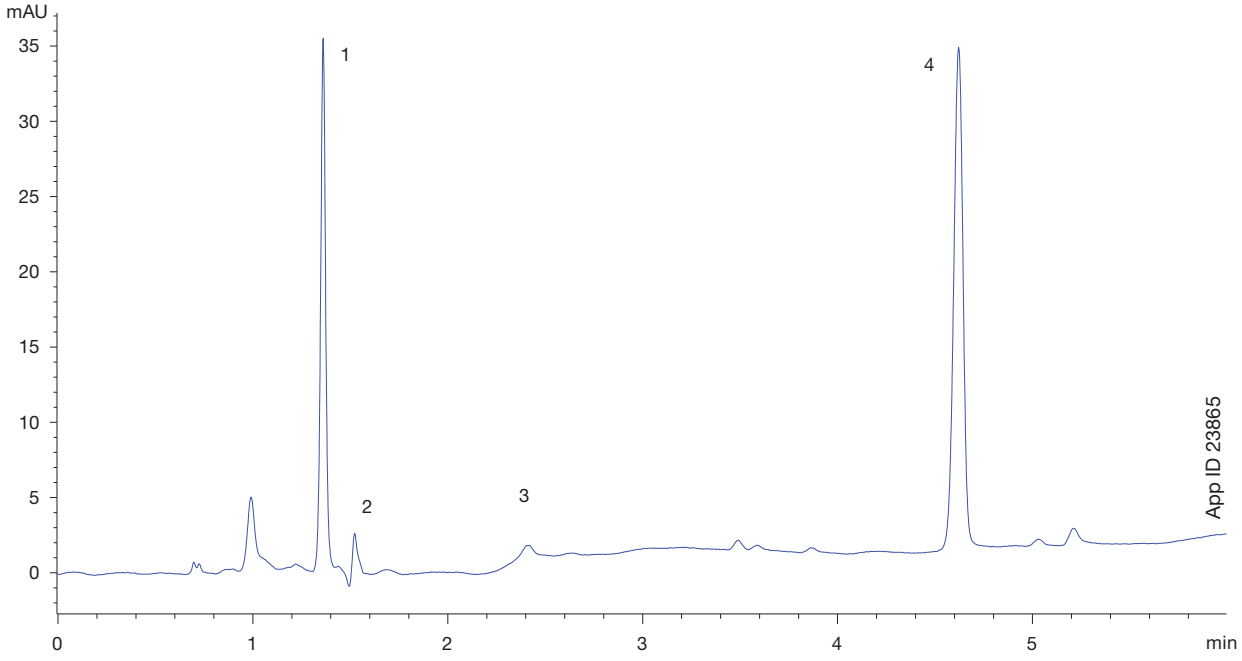


Table 1. Concentrations of matrix components present in a 1 µL injection of a 2.5 mg/mL matrix in water (0.0025 mg injection)

	Concentrations of Components (ppm)			
Sample	Caffeine	Dicyandiamide	Creatinine	Creatine
C4	40.2	0.0	8.1	162.1
N.O. Xplode™	33.1	0.8	0.1	223.8
Bang®	36.9	0.8	0.9	331.4
Outlift™	21.7	0.8	3.4	175.3

Table 2. Scaled up concentrations of matrix components accounting for serving size

	Mass concentration of matrix components (mg)				
Sample	Caffeine	Dicyandiamide	Creatinine	Creatine	Serving Size (mg)
C4	104.54	0.00	21.08	421.42	6500
N.O. Xplode	244.83	5.73	0.56	1656.40	18500
Bang	389.62	8.97	9.69	3499.43	26400
Outlift	222.54	7.71	34.60	1795.38	25600

Results and Discussion

With the recent increased interest in the analysis of Pre-Workout powders, a rapid and robust analytical technique was needed for the fast and efficient analysis of their key components. Due to the high polarity of the compounds present in these matrices, traditional reversed phase HPLC techniques can become rather difficult and time consuming, and in many cases, could even result in inaccurate quantitation and poor peak shape. Here we presented a highly selective, HILIC HPLC method that demonstrates a high degree of efficiency by exploiting these polar interactions between the stationary phase and the target analytes. The chromatography shown in **Figures 7-10** highlight the excellent speed and the high efficiency of this separation while maintaining good retention, peak resolution and peak symmetry.

Peak quantitation was completed by comparison of the peak areas demonstrated in **Figure 1**, to the peak areas of the identified peaks in each of the matrices (**Figures 7-10**). A calibration curve was then made for each analyte to determine the concentration for creatine, creatinine, caffeine, and dicyandiamide, present in each matrix. The amount of each analyte was initially quantitated for a 1 µL injection of a 2.5 mg/mL solution (**Table 1**). It was then scaled up to be proportional to the amount of one serving size of each of the Pre-Workout powders (**Table 2**).

By employing this HILIC approach, we were able to generate a method that quickly and efficiently separates the key components of the mixture. Using the Luna 3 µm HILIC 150 x 4.6 mm column we were able to extend a successful separation of chemical standards to analyzing popular market products.

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Luna® Ordering Information

3 µm Minibore Columns (mm)				SecurityGuard™ Cartridges (mm)
Phases	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0*
				/10pk
HILIC	00B-4449-B0	00D-4449-B0	00F-4449-B0	AJ0-8328
				for ID: 2.0-3.0 mm

3 µm MidBore™ and Analytical Columns (mm)					SecurityGuard Cartridges (mm)	
Phases	50 x 3.0	150 x 3.0	100 x 4.6	150 x 4.6	4 x 2.0*	4 x 3.0*
					/10pk	/10pk
HILIC	00B-4449-Y0	00F-4449-Y0	00D-4449-E0	00F-4449-E0	AJ0-8328	AJ0-8329
					for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm MidBore Columns (mm)		SecurityGuard Cartridges (mm)
Phases	150 x 3.0	4 x 2.0*
		/10pk
HILIC	00F-4450-Y0	AJ0-8328
		for ID: 2.0-3.0 mm

5 µm Analytical Columns (mm)				SecurityGuard Cartridges (mm)
Phases	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
				/10pk
HILIC	00D-4450-E0	00F-4450-E0	00G-4450-E0	AJ0-8329
				for ID: 3.2-8.0 mm

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

guarantee

If Luna analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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Disclaimer

Dimensions and chromatographic conditions are the same for all columns unless otherwise noted. Comparative separations may not be representative of all applications.

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6, 162, 362
CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

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