APPLICATIONS

Extraction and Analysis of Vitamin B1 (Thiamine) from Guar Gum by HPLC-UV

Scott Krepich and Morgan Kramer
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Scott Krepich
Senior Field Application Scientist
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

Guar Gum is a natural substance made from guar beans, and is commonly used in the food industry as an emulsifier, stabilizer, and thickener. Quantifying components formulated in such can be a challenge due to the matrix viscosity and potentially interfering components.

Here we demonstrate an effective extraction for HPLC-UV quantification of Vitamin B1 in matrix, that may be expanded to cover additional water soluble vitamins.

LC Method Parameters

Column: Luna® Omega 3 µm Polar C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4760-E0
Mobile Phase: A: 20 mM Sodium phosphate, pH 3.5 with phosphoric acid
B: Methanol

Gradient:

Time (Min)	%B
0	0
15	60
15.1	0

Flow Rate: 0.5 mL/min
Injection Vol.: 1 µL
Temperature: 22˚C
Detector: UV @ 254 nm

Sample Preparation

1. Weigh out ~2 g of guar gum into a 125 mL Erlenmeyer flask
2. Add between 30-50 mL of the extraction solvent (see **Table 1** for trial matrix) MeOH-2 (Methanol : 1 % HCL in water 80:20)
3. Shake vigorously until all guar gum appears to have absorbed/ dissolved into the solvent
4. Sonicate for 10 minutes
5. Pass an aliquot (~2 mL) of the mixture through a PTFE syringe filter
6. Inject 1 µL of the filtrate using the HPLC method shown above

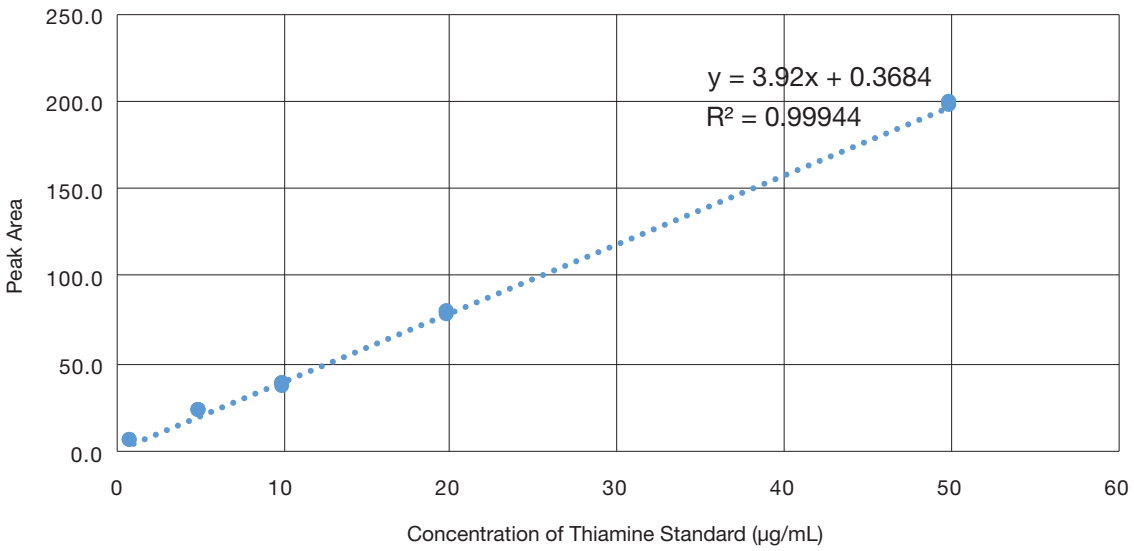
Table 1.
Extraction Solvent Trials

Extraction Labels	Solvents	Solvent Ratio	Volume added (mL)
MeOH	Methanol:1 % HCl in water	80:20	50
EtOH – Recrystallization*	1 % HCl in water	100	35
ACN H+	Acetonitrile + 0.1 % Acetic Acid	100	45
ACN -2	Acetonitrile: 1 % HCl in water	80:20	50
IPA	Isopropanol: 1 % HCl in water	80:20	50
EtOH -2	Ethanol: 1 % HCl in water	80:20	50
NaCl	5 % NaCl dissolved in 1 % HCl	100	150
MeOH -2*	Methanol: 1 % HCl in water	80:20	50
Acetone	Acetone: 1 % HCl in water	80:20	50
Water	Water	100	50
H+, H_2O	1 % HCl in water	100	50

* The EtOH and MeOH-2 –Recrystallization had an additional step where 5 mL of the dissolved guar gum sample was aliquoted into a test tube and cooled in an ice bath. Followed by the addition of 5 mL cooled ethanol to induce crystalization of undesired material.

APPLICATIONS

Figure 1.
Calibration Curve for Thiamine @ 254 nm



Note: Peak areas obtained by employing the above "Running Conditions" and relating peak areas obtained to concentration of known standards

Table 2.
Quantitation of Thiamine Recovered from Extraction

Extraction from A1973-VS5	Guar Powder (g)	Peak Area (B1)	Mass Thiamine (mg)	Projected Conc. (mg/lb)**	Ideal conc. Range (mg/lb)
Extractions Techniques					
MeOH	2.36	61.4	0.78	149.62	100-200
EtOH – Recrystallization	2.62	17.0	0.30	51.42	100-200
ACN H+	2.29	10.1	0.11	22.13	100-200
ACN -2	2.14	11.8	0.01	3.09	100-200
IPA	2.30	46.9	0.59	117.05	100-200
EtOH -2	1.94	14.3	0.18	41.55	100-200
NaCl	2.14	16.7	0.62	132.46	100-200
MeOH -2	3.11	87.6	1.11	162.49	100-200
Acetone	2.42	19.3	0.24	45.33	100-200
Water	2.42	59.8	0.76	141.85	100-200
H+, H_2O	2.43	80.5	1.02	190.66	100-200

Note: Quantitation was performed by relating peak area to concentration from the developed HPLC method and calibration curve **(Figure 1)**.
**Projected Conc. was calculated assuming each 100% recovery from each extraction. Values are reported as mg of thiamine per pound of guar powder.

Figure 2.
Separation Profile for MeOH-2 Extraction

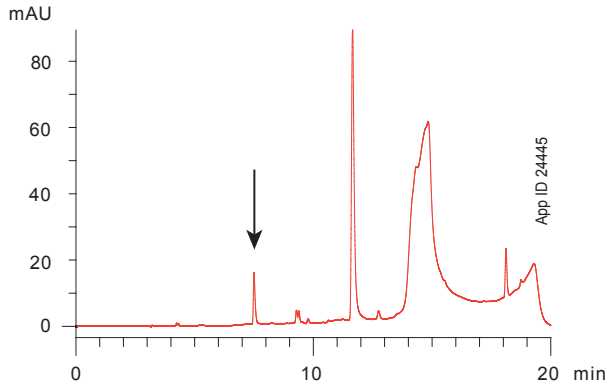


Figure 3.
Separation Profile for H+, H_2O Extraction

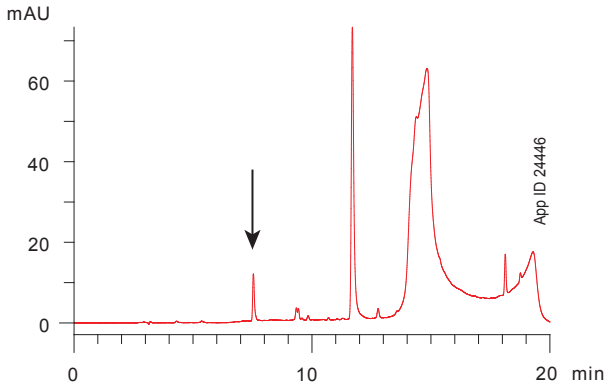


Figure 4.
Separation Profile for Isopropanol Extraction

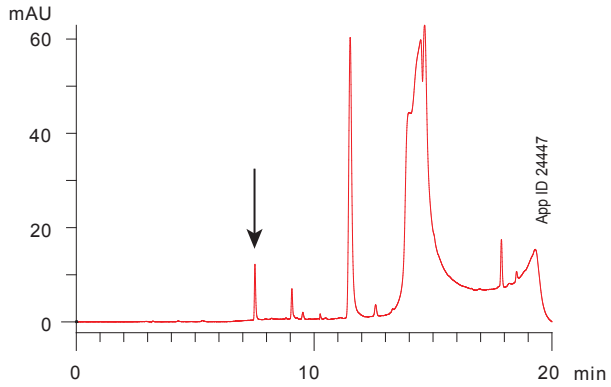
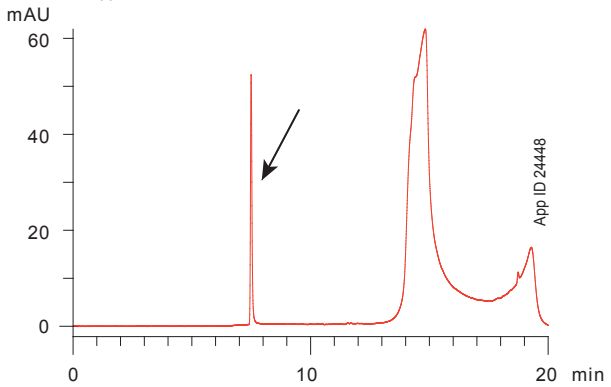


Figure 5.
Separation of Thiamine Standard 50 µg/mL



* The arrow identifies the Thiamine peak

Results and Discussion

Developed is a rapid, simple, and robust HPLC method for the separation and retention of Thiamine from a guar gum sample. The novel polar functionality within the Luna Omega Polar C18 stationary phase help improve retention and peak shape without the need for ion-pairing agents in the mobile phase.

The extraction procedures developed shone light onto which methodologies were most efficient in the extraction of thiamine from the matrix. In order to maintain a direct comparison among extraction techniques, only the guar gum sample labeled A 1973-VS5 was used for this analysis. The provided method, using and 80:20 mixture of Methanol to 1 % HCl was used as the comparison point for subsequent extractions. In **Figure 2**, the MeOH and MeOH-2 extractions are representative of the method being used by the client. These extractions gave results that led to a calculation of 149.62 and 162.49 µg/lb respectively for the guar gum sample. These results are consistent with the predicted range of 100-200 µg/lb.

Most of the extractions netted a lower yield than the MeOH and MeOH-2 extractions, though the H+, H_2O extraction managed to have a higher recovery than the MeOH extractions. The H+, H_2O extraction yielded results that allowed for the calculation of thiamine to be 190.66 µg/lb. This value is consistent with the predicted range, though, the higher thiamine concentration being calculated through this extraction indicates a greater recovery from this extraction technique. The calculated projected concentration of thiamine in the guar gum sample was done in order to compare the extraction techniques. A higher projected thiamine concentration as a result of the extraction, in turn, means a higher percentage of thiamine was recovered from the guar gum. The projected concentration was in order to compare the results gathered from the assay to the expected values provided by the client and as a way to monitor successful extractions.

Each of the extractions were done in duplicate, in order to ensure consistency in the results. Further work must be done in order to fully represent percent recovery, account for matrix effects, develop RSDs for the extractions, and to determine differences in recovery for the various samples. In conclusion, we provide a fast, robust, and ion-pairing agent free HPLC separation and quantitative assay for Thiamine. Additionally, we have laid the groundwork for further optimization of Thiamine extraction from the guar gum matrix.

APPLICATIONS

Luna Omega™ Ordering Information

1.6 µm Microbore and Minibore Columns (mm)								SecurityGuard ULTRA Cartridges (mm)
Phases	**50 x 1.0**	**100 x 1.0**	**150 x 1.0**	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Polar C18	00B-4748-A0	00D-4748-A0	00F-4748-A0	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
								for 2.1 mm ID

3 µm Minibore and MidBore™ Columns (mm)							SecurityGuard Cartridges (mm)
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0***
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
							for ID:	2.0 - 3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0***
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
				for ID:	3.2-8.0 mm

5 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**4 x 2.0***
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	AJ0-7600
				for ID:	2.0 - 3.0 mm

5 µm MidBore™ Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0***
Polar C18	00B-4754-Y0	00D-4754-Y0	00F-4754-Y0	AJ0-7600
			for ID:	2.0 - 3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0***
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
				for ID:	3.2-8.0 mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* 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.

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN53180617_W