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TEST REPORT

Client: Hangzhou New Asia International Co., Ltd.

Address: 189 FengQi East Road, TSN Plaza 4-706, Hangzhou, 310020, China

1. SAMPLE DESCRIPTION

Product Name	Grape Seed Extract
Batch Number	GRS-160705
AIE Number	16070101
Sample Package	PE bag
Sample Appearance	Reddish brown powder
Sample Received	2016.07.01
Start of Analysis	2016.07.01
End of Analysis	2016.07.12

Report Authorization: Date：2016.07.12

Vincent Huang– Quality and Technical Director

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2. RESULTS

Test Items		Results	Units	Methods
Identification		Positive by HPTLC and HPLC	/	USP
Proanthocyanidins		99.3	%	UV (beta-Smith)
Oligomeric Proanthocyanidins(OPC)		77.5	%	USP
Heavy Metals	As	ND (<0.005)	ppm (mg/kg)	ICP-MS
	Pb	0.136	ppm (mg/kg)	
	Cd	ND (<0.005)	ppm (mg/kg)	
	Hg	ND (<0.005)	ppm (mg/kg)	
Microbiological Testing	Total Plate Count	<10	cfu/g	USP
	Mold	<10	cfu/g	
	Yeast	<10	cfu/g	

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3. METHOD OF HPTLC IDENTIFICATION

3.1 SAMPLE PREPARATION

Dissolve a quantity of sample in methanol, using sonication, to obtain a solution having a concentration of about 5 mg/mL. Centrifuge if necessary, and use the clear supernatant.

Note: Prepare fresh.

3.2 CHROMATOGRAPHIC CONDITIONS

Plate: Silica gel 60, Merck [Part #:1.05629.0001, Lot#:HX55154629]

Load: 15µl

Mobile Phase: acetone, toluene, and formic acid (15:15:5)

Note: Mobile phase must be made fresh prior to use. Mix well before use.

Development Chamber: Saturated Twin Trough (saturation time 20 minutes)

Spray Reagent: Dissolve about 100 mg of vanillin in 3 mL of methanol using sonication. Add about 3 mL of hydrochloric acid, dilute with methanol to 10 mL, and carefully mix under cold water.

Note: Prepare fresh.

Evaluation: Once development is complete, remove the plate and then allow the plate to dry. After spraying with the Spray reagent, place the plate in the oven at 105°C for 3-5 minutes. View the plate in visual light.

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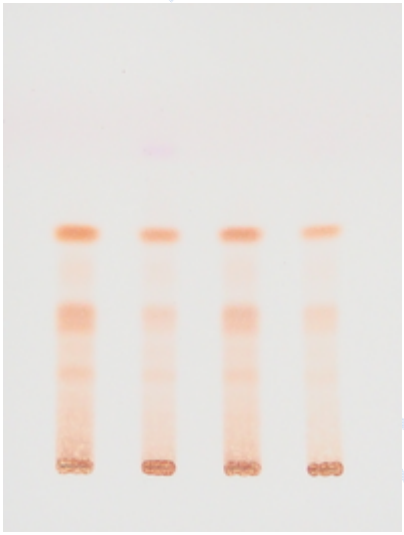
4. CHROMATOGRAM OF HPTLC CHROMATOGRAM



Trace 1, 3: Vitis vinifera Seed (Grape seed)

Trace 2, 4: GRS-160705

CONCLUSION: The test sample is characteristic of an extract derived from Vitis vinifera seed.

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5. METHOD OF PROANTHOCYANIDINS

5.1 REAGENTS

5.1.1 5%(0.6N) HCL/n-BuOH Solution: Put 70ml n-BuOH into 100ml brown volumetric flask, add 5.0ml hydrochloric acid (12N), cool to the room temperature, add n-BuOH to the volume, shake up.

5.1.2 2% Ammonium iron(III) sulfate Solution: Accurately weigh the Ammonium iron(III) sulfate 2.0g, put it into 100ml brown volumetric flask, add HCL 2ml, cool to the room temperature, add distilled water to the volume, shake up.

5.2 SAMPLE PREPARATION

5.2.1 Accurately weigh the sample 10mg, transfer to 100ml brown volumetric flask, add MeOH 80ml, ultrasonic solvent, cool to the room temperature, add MeOH to the volume, mix well.

5.2.2 Put the solution as follows into 10ml brown volumetric flask:

1.0ml Sample Solution

6.0ml 5%(0.6N) HCL/n-BuOH Solution

0.2ml 2% Ammonium iron(III) sulfate Solution

Make sure no reagent volatilize

5.2.3 Put the 10ml brown volumetric flask (B) in a water bath at 100±2℃ for 40min;

5.2.4 Cool it in tap water bath (room temperature) for 20min

5.3 EXPERIMENT

Measure the absorbance of the Sample Solution at 546nm using a blank solution.

5.4 CALCULATION

$$\text{Proanthocyanidins}(\%) = A \times D \times 1000/(W \times 275)$$

W=weight (mg) A= absorbance D= dilution (720)

275 is the test date of proanthocyanidins (standard)

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6. HPLC METHOD OF OLIGOMERIC PROANTHOCYANIDINS

6.1 REAGENT

Solution A: Use acetonitrile.

Solution B: Use a 0.3% aqueous solution of 85% phosphoric acid.

Solvent: Prepare a mixture of Solution A and Solution B (1:9).

6.2 SAMPLE PREPARATION

Dissolve, using sonication, a weighed quantity of sample in Solvent to obtain a solution having a known concentration of about 5 mg/mL. Centrifuge, and use the clear supernatant.

6.3 STANDARD PREPARATION

Dissolve, using sonication, a weighed quantity of USP Grape Seeds Oligomeric Proanthocyanidins RS in Solvent to obtain a solution having a known concentration of about 5 mg/mL. Centrifuge, and use the clear supernatant.

6.4 HPLC CONDITIONS

Column: Hypersil ODS C18 (4.6×250 mm) 5μm

Column temperature:25℃

Wavelength: 278nm

Flow rate: 1.0 ml/min

Injection volume: 10μl

Mobile phase: See the gradient table below

Time (min)	Solution A (%)	Solution B (%)
0	10	90
45	20	80
65	60	40
66	10	90
85	10	90

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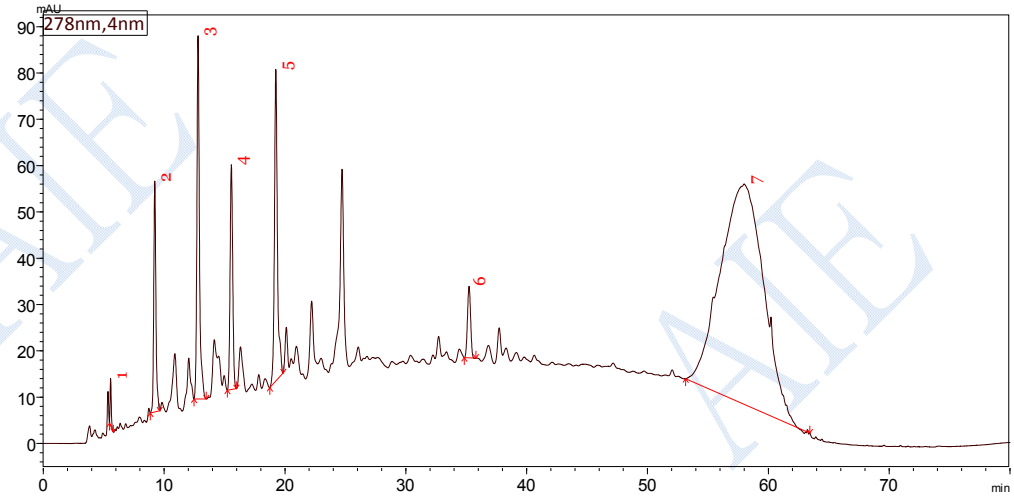
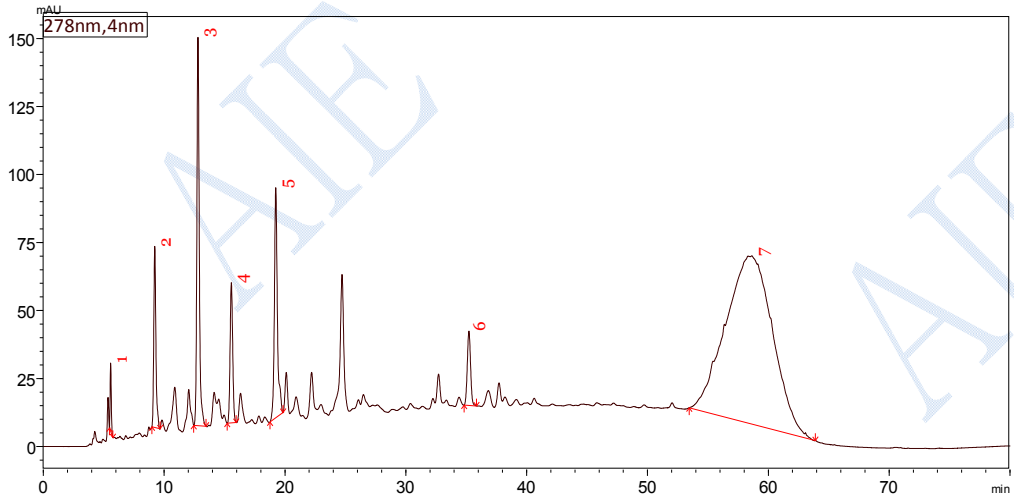
7. CHROMATOGRAMS OF OLIGOMERIC PROANTHOCYANIDINS BY HPLC

GRS-160705



Standard



1 = gallic acid
2 = procyanidin B1 (proanthocyanidin dimer B_1)
3 = (+)-catechin
4 = procyanidin B2 (proanthocyanidin dimer B_2)
5 = (-)-epicatechin
6 = (-)-epicatechin-3-O-gallate
7 = oligomeric proanthocyanidins

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8. SAMPLE PICTURE