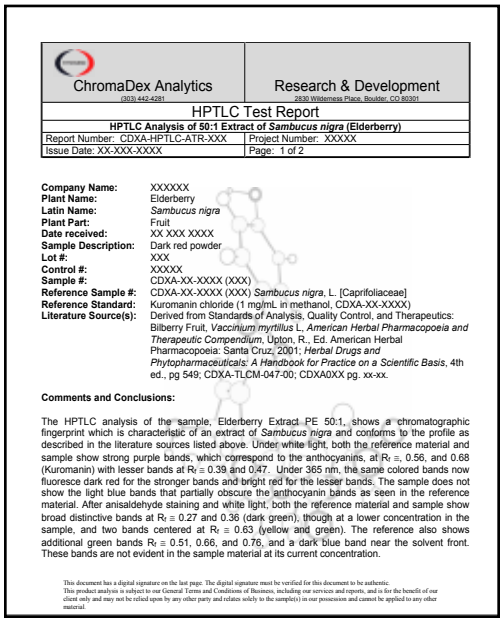
ChromaDex™ Report Types

HPTLC Analytical Test Report Example

- Designed for qualitative work.
- Provides a fingerprint for botanicals.



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HPTLC Test Report

HPTLC Analysis of 50:1 Extract of *Sambucus nigra* (Elderberry)

Report Number: CDXA-HPTLC-ATR-XXX	Project Number: XXXXX
Issue Date: XX-XXX-XXXX	Page: 1 of 2

Company Name: XXXXXX
Plant Name: Elderberry
Latin Name: *Sambucus nigra*
Plant Part: Fruit
Date received: XX XXX XXXX
Sample Description: Dark red powder
Lot #: XXX
Control #: XXXXX
Sample #: CDXA-XX-XXXX (XXX)
Reference Sample #: CDXA-XX-XXXX (XXX) *Sambucus nigra*, L. [Caprifoliaceae]
Reference Standard: Kuromanin chloride (1 mg/mL in methanol, CDXA-XX-XXXX)
Literature Source(s): Derived from Standards of Analysis, Quality Control, and Therapeutics: Bilberry Fruit, *Vaccinium myrtillus* L, *American Herbal Pharmacopoeia and Therapeutic Compendium*, Upton, R., Ed. American Herbal Pharmacopoeia: Santa Cruz, 2001; *Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis*, 4th ed., pg 549; CDXA-TLCM-047-00; CDXA0XX pg. xx-xx.

Comments and Conclusions:

The HPTLC analysis of the sample, Elderberry Extract PE 50:1, shows a chromatographic fingerprint which is characteristic of an extract of *Sambucus nigra* and conforms to the profile as described in the literature sources listed above. Under white light, both the reference material and sample show strong purple bands, which correspond to the anthocyanins, at R_f ≈, 0.56, and 0.68 (Kuromanin) with lesser bands at R_f ≈ 0.39 and 0.47. Under 365 nm, the same colored bands now fluoresce dark red for the stronger bands and bright red for the lesser bands. The sample does not show the light blue bands that partially obscure the anthocyanin bands as seen in the reference material. After anisaldehyde staining and white light, both the reference material and sample show broad distinctive bands at R_f ≈ 0.27 and 0.36 (dark green), though at a lower concentration in the sample, and two bands centered at R_f ≈ 0.63 (yellow and green). The reference also shows additional green bands R_f ≈ 0.51, 0.66, and 0.76, and a dark blue band near the solvent front. These bands are not evident in the sample material at its current concentration.

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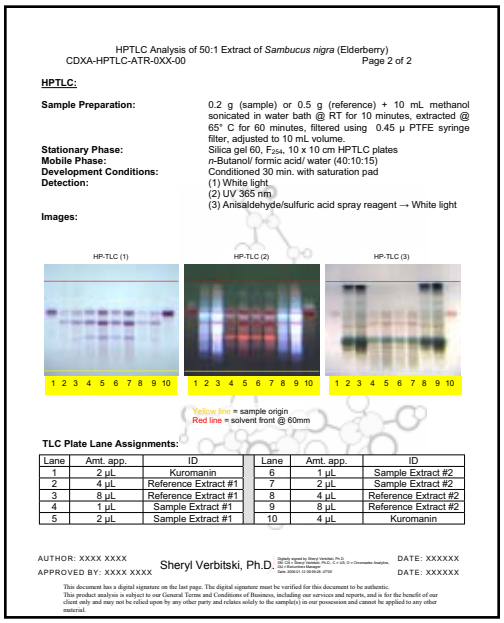

HPTLC Analysis of 50:1 Extract of *Sambucus nigra* (Elderberry)
CDXA-HPTLC-ATR-0XX-00 Page 2 of 2

HPTLC:

Sample Preparation: 0.2 g (sample) or 0.5 g (reference) + 10 mL methanol sonicated in water bath @ RT for 10 minutes, extracted @ 65° C for 60 minutes, filtered using 0.45 µ PTFE syringe filter, adjusted to 10 mL volume.
Stationary Phase: Silica gel 60, F_{254}, 10 x 10 cm HPTLC plates
Mobile Phase: *n*-Butanol/ formic acid/ water (40:10:15)
Development Conditions: Conditioned 30 min. with saturation pad
Detection: (1) White light
(2) UV 365 nm
(3) Anisaldehyde/sulfuric acid spray reagent → White light

Images:

HP-TLC (1) HP-TLC (2) HP-TLC (3)

1 2 3 4 5 6 7 8 9 10

Yellow line = sample origin
Red line = solvent front @ 60mm

TLC Plate Lane Assignments:

Lane	Amt. app.	ID	Lane	Amt. app.	ID
1	2 µL	Kuromanin	6	1 µL	Sample Extract #2
2	4 µL	Reference Extract #1	7	2 µL	Sample Extract #2
3	8 µL	Reference Extract #1	8	4 µL	Reference Extract #2
4	1 µL	Sample Extract #1	9	8 µL	Reference Extract #2
5	2 µL	Sample Extract #1	10	4 µL	Kuromanin

AUTHOR: XXXX XXXX Sheryl Verbitski, Ph.D. DATE: XXXXXX
APPROVED BY: XXXX XXXX DATE: XXXXXX

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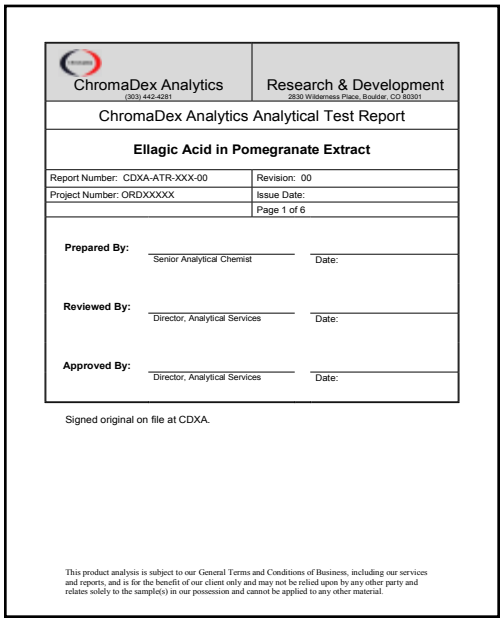
Analytical Test Report (ATR) - Page 1 & 2

- Highest level of detail provided on reports.
- Provides step-by-step instructions to perform the assay.
- Listing of lot numbers of standards, reagents, chromatograms of standards and samples.



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ChromaDex Analytics Analytical Test Report

Ellagic Acid in Pomegranate Extract

Report Number: CDXA-ATR-XXX-00	Revision: 00
Project Number: ORDXXXXX	Issue Date:
	Page 1 of 6

Prepared By: _____ Senior Analytical Chemist Date: _____
Reviewed By: _____ Director, Analytical Services Date: _____
Approved By: _____ Director, Analytical Services Date: _____

Signed original on file at CDXA.

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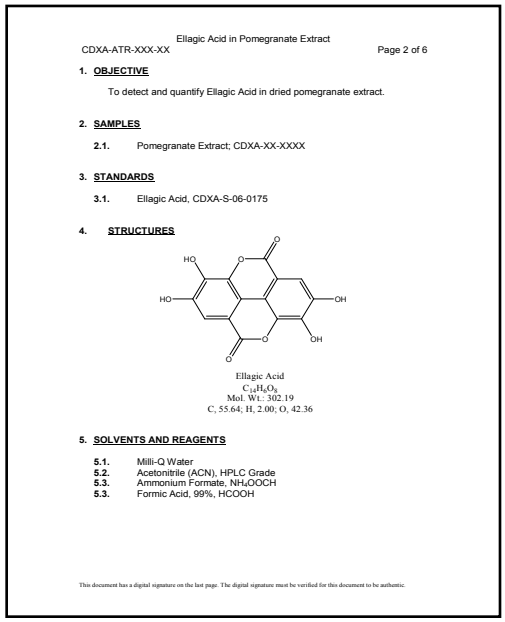

Ellagic Acid in Pomegranate Extract
CDXA-ATR-XXX-XX Page 2 of 6

1. OBJECTIVE

To detect and quantify Ellagic Acid in dried pomegranate extract.

2. SAMPLES

2.1. Pomegranate Extract; CDXA-XX-XXXX

3. STANDARDS

3.1. Ellagic Acid, CDXA-S-06-0175

4. STRUCTURES

Ellagic Acid
$C_{14}H_6O_8$
Mol. Wt.: 302.19
C, 55.64; H, 2.00; O, 42.36

5. SOLVENTS AND REAGENTS

5.1. Milli-Q Water
5.2. Acetonitrile (ACN), HPLC Grade
5.3. Ammonium Formate, NH_4OOCH
5.3. Formic Acid, 99%, HCOOH

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Analytical Test Report (ATR) - Page 3-6

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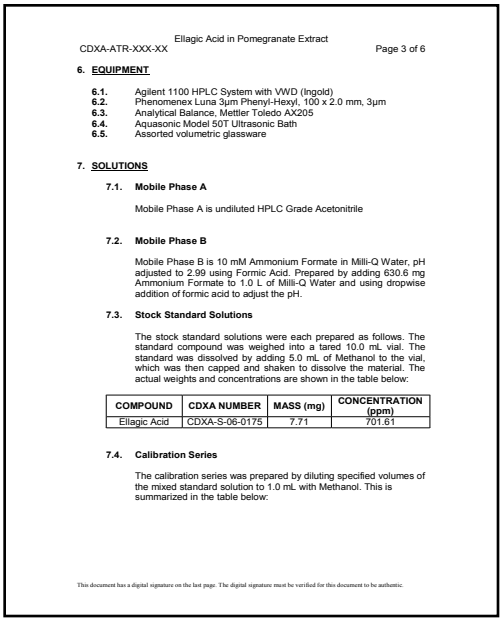

Ellagic Acid in Pomegranate Extract
CDXA-ATR-XXX-XX Page 3 of 6

6. EQUIPMENT

6.1. Agilent 1100 HPLC System with VWD (Ingold)
6.2. Phenomenex Luna 3µm Phenyl-Hexyl, 100 x 2.0 mm, 3µm
6.3. Analytical Balance, Mettler Toledo AX205
6.4. Aquasonic Model 50T Ultrasonic Bath
6.5. Assorted volumetric glassware

7. SOLUTIONS

7.1. Mobile Phase A

Mobile Phase A is undiluted HPLC Grade Acetonitrile

7.2. Mobile Phase B

Mobile Phase B is 10 mM Ammonium Formate in Milli-Q Water, pH adjusted to 2.99 using Formic Acid. Prepared by adding 630.6 mg Ammonium Formate to 1.0 L of Milli-Q Water and using dropwise addition of formic acid to adjust the pH.

7.3. Stock Standard Solutions

The stock standard solutions were each prepared as follows. The standard compound was weighed into a tared 10.0 mL vial. The standard was dissolved by adding 5.0 mL of Methanol to the vial, which was then capped and shaken to dissolve the material. The actual weights and concentrations are shown in the table below:

COMPOUND	CDXA NUMBER	MASS (mg)	CONCENTRATION (ppm)
Ellagic Acid	CDXA-S-06-0175	7.71	701.61

7.4. Calibration Series

The calibration series was prepared by diluting specified volumes of the mixed standard solution to 1.0 mL with Methanol. This is summarized in the table below:

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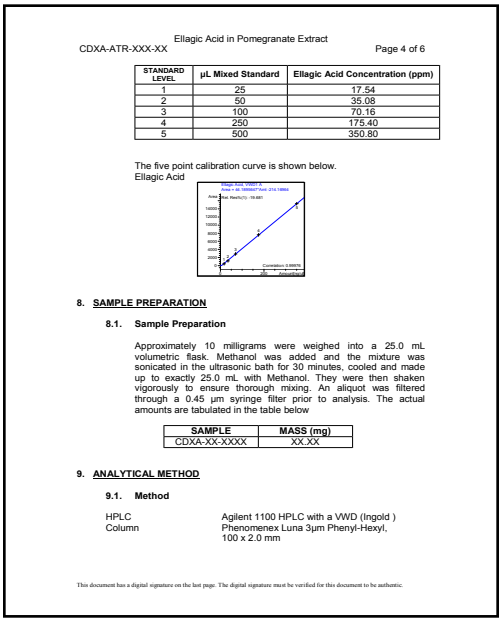

Ellagic Acid in Pomegranate Extract
CDXA-ATR-XXX-XX Page 4 of 6

STANDARD LEVEL	µL Mixed Standard	Ellagic Acid Concentration (ppm)
1	25	17.54
2	50	35.08
3	100	70.16
4	250	175.40
5	500	350.80

The five point calibration curve is shown below.
Ellagic Acid

8. SAMPLE PREPARATION

8.1. Sample Preparation

Approximately 10 milligrams were weighed into a 25.0 mL volumetric flask. Methanol was added and the mixture was sonicated in the ultrasonic bath for 30 minutes, cooled and made up to exactly 25.0 mL with Methanol. They were then shaken vigorously to ensure thorough mixing. An aliquot was filtered through a 0.45 µm syringe filter prior to analysis. The actual amounts are tabulated in the table below

SAMPLE	MASS (mg)
CDXA-XX-XXXX	XX.XX

9. ANALYTICAL METHOD

9.1. Method

HPLC: Agilent 1100 HPLC with a VWD (Ingold)
Column: Phenomenex Luna 3µm Phenyl-Hexyl, 100 x 2.0 mm

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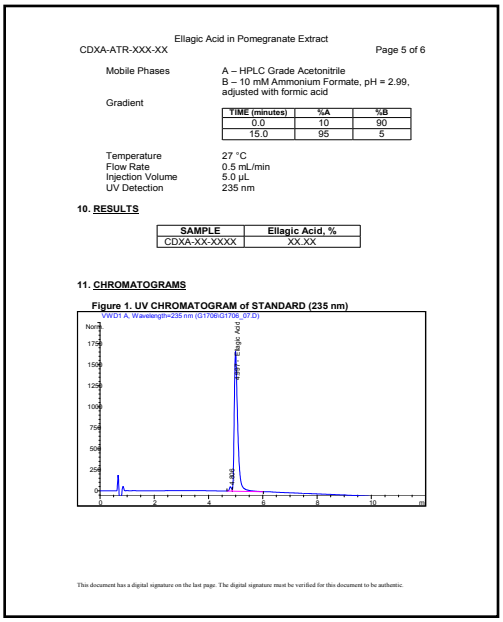
- Structure(s)
- Reagent List
- Equipment List
- Calibration Data
- Sample Preparation
- Method
- Results
- Data
- Reference Chromatogram
- Sample Chromatogram
- References
- History of Revisions



Ellagic Acid in Pomegranate Extract
CDXA-ATR-XXX-XX Page 5 of 6

Mobile Phases: A – HPLC Grade Acetonitrile
B – 10 mM Ammonium Formate, pH = 2.99, adjusted with formic acid

Gradient

TIME (minutes)	%A	%B
0.0	10	90
15.0	95	5

Temperature: 27 °C
Flow Rate: 0.5 mL/min
Injection Volume: 5.0 µL
UV Detection: 235 nm

10. RESULTS

SAMPLE	Ellagic Acid, %
CDXA-XX-XXXX	XX.XX

11. CHROMATOGRAMS

Figure 1. UV CHROMATOGRAM of STANDARD (235 nm)

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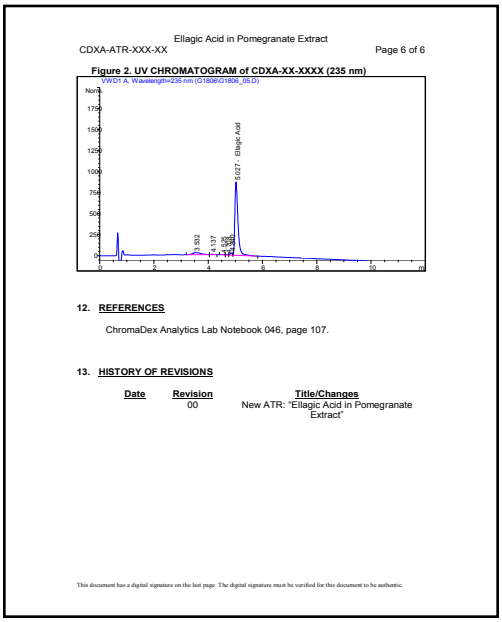

Ellagic Acid in Pomegranate Extract
CDXA-ATR-XXX-XX Page 6 of 6

Figure 2. UV CHROMATOGRAM of CDXA-XX-XXXX (235 nm)

12. REFERENCES

ChromaDex Analytics Lab Notebook 046, page 107.

13. HISTORY OF REVISIONS

Date	Revision	Title/Changes
	00	New ATR: "Ellagic Acid in Pomegranate Extract"

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