1.0 Purpose

The purpose of this procedure is to define (HP)TLC methods and conditions for the qualitative analysis of botanicals and their extracts in raw materials.

2.0 Scope

This procedure applies to the identification of all botanicals and complex materials. This SOP only applies to identification of raw materials using certified standards.

3.0 Responsibility

3.1 It is the responsibility of QC and analytical chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of Analytical Development to ensure that (HP)TLC is appropriate for ID.

3.4 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **TLC** – Thin Layer Chromatography

4.2 **HPTLC** – High Performance Thin Layer Chromatography

4.3 **QC** – Quality Control

4.4 **ID** – Identification

5.0 References

5.1 D-742-F1, Form, TLC Botanical Identification Test Methods

5.2 D-742-F2, Form, TLC Developing Solution Recipes

6.0 Equipment and Materials

6.1 Supplies

6.1.1 Beaker – 500mL, 250mL, and 100mL

6.1.2 Whatman Filter Paper (4in diameter)

6.1.3 500mL Bottle with Cap

6.1.4 Pipettes – 10mL, 1mL, and 10µL

6.1.5 Pipette Tips – 10mL, 1mL, and 20µL

6.1.6 Petri Dish

6.1.7 Silica Gel 60 F254, 2.5cm x 7.5cm and 5cm x 10cm (HP)TLC Plates

6.1.8 Scintillation Vials

6.1.9 2.0mL Eppendorf Tubes

6.1.10 Syringe – 3mL

6.1.11 Syringe Filters – Nylon and Polypropylene

6.1.12 Graphite Pencil

6.1.13 Capillary – 4" TLC Spotting Tubes

6.1.14 Aluminum Foil

6.2 Equipment

6.2.1 Vacuum Oven

6.2.2 Cannon Rebel3 EOS Digital Camera

6.2.3 ChromaDoc-IT 125 Imaging System

6.2.4 Chromato-Vue C-75 UV Viewing Cabinet

6.2.5 Sonication Bath

6.2.6 Hot Plate

6.2.7 Fume Hood

6.2.8 Eppendorf Tube Rack

7.0 General Method

Note: Reference Form D-742-F1 Botanical Identification Test Methods for Test Conditions

7.1 Chamber Preparation

7.1.1 Prepare a development chamber using a 250mL or 500mL beaker sealed with aluminum foil.

7.1.2 Fold a 4in diameter piece of Whatman filter paper or equivalent into a rack to support the plate and place into the covered beaker. Trim the paper as necessary to fit into the beaker.

7.1.3 Add 10mL or 15mL of mobile phase per D-742-F1 to the covered beaker. Equilibrate to room temperature.

7.2 TLC Plate Preparation

7.2.1 Draw a line ~1cm from the bottom of the plate with pencil, then place three hash marks, each ~8mm apart, to identify the lanes for a 2.5" x 7.5" plate. A line should be placed ~1cm from the top of the plate to mark the end point of the solvent front.

7.2.2 Each hash mark should be numbered from 1 to 3 beginning on the left.

7.2.3 In the 1cm space at the top of the plate, the label information can be added. This includes the name of the botanical, the R number tested, and the date.

7.2.4 Place the labeled (HP)TLC plate without sample into the chamber with mobile phase and allow the solvent front to migrate to the top of the plate.

7.2.5 Dry the plate ~10 minutes on the hot plate in the fume hood.

7.2.6 Allow the dried plate to cool.

7.3 Choosing a Mobile Phase

7.3.1 Choosing a solvent system as a mobile phase requires some knowledge of TLC. If all spots have an RF of 1, the mobile phase is too polar and its polarity needs to be reduced. If all spots have an RF of 0, then the mobile phase is too non-polar and the polarity needs to be increased.

7.3.2 Form D-742-F1 TLC Botanical Identification Test Methods has some commonly used botanicals along with their mobile phases, dissolution media and developing solutions.

7.3.2.1 This should be used as a guide. Deviations from this are acceptable and should be noted on the write-up.

7.3.3 As needed, new materials will be compiled and added to Form F1 in groups.

7.4 Sample Preparation

7.4.1 Pre-label the scintillation vials and Eppendorf tubes to identify the samples.

7.4.2 Weigh 250mg of sample and transfer to the scintillation vial.

7.4.3 Add 2.5mL of the appropriate dissolution solution per Form D-742-F1 to the vial and cap.

7.4.4 Sonicate both samples and standards for ~15 minutes in a sonication bath.

7.4.5 Remove the samples from the bath and allow the samples to cool for ~10 minutes.

7.4.6 Transfer supernatant into a pre-labeled Eppendorf tube.

7.4.7 Centrifuge for ~6 minutes at 500RPM in an Eppendorf microfuge.

7.4.8 Transfer supernatant #2 to a fresh pre-labeled Eppendorf tube.

7.4.9 If necessary, the sample can be dried or concentrated in the vacuum oven (botanical test specific) or other method (like evaporation with nitrogen).

7.5 Standard Preparation

7.5.1 If the standard in its whole form is suitable for the identification then use section 7.3 for standard preparation.

7.5.2 If the standard in its whole form is not a suitable reference then refer to the manufacturer extract conditions per form D-742-F1 TLC Botanical Identification Test Methods of the raw material. Extract the standard under the same conditions to create a reference material.

7.5.3 As needed, general manufacturer extraction conditions for the raw material will be added to Form D-742-F1 TLC Botanical Identification Test Methods listed under the appropriate botanical.

7.6 Plate Spotting

7.6.1 Place the stenciled plate onto a hot plate pre-equilibrated to ~105°C.

7.6.2 Some volatile components of a botanical sample or standard may not do well under heated conditions and may need to be air dried.

7.6.3 Using a capillary tube, spot the sample onto the plate slowly with small incremental volumes. Be sure to add spots directly on top of old spots. Offset spots will not give good results. Allow each addition to dry before adding the next increment. Keep spots ≤ 2mm in diameter. Add appropriate volume of sample/standard to each lane as follows:

7.6.3.1 Lane 1: xµL Standard

7.6.3.2 Lane 2: xµL Sample A

7.6.3.3 Lane 3: xµL Standard or xµL Sample B

7.7 Plate Developing

7.7.1 Place cooled TLC plate into the chamber and cover.

7.7.2 Allow the solvent front to migrate to the top line then remove the plate from the chamber.

7.7.3 Heat dry the plate for approximately ten minutes before reading. This may be accomplished by placing onto a hot plate. Some volatile components of a botanical sample or standard may not do well under heated conditions and may need to be air dried.

7.8 Plate Reading

7.8.1 Plates should be evaluated visually for similarity between standard and test material during each interval of the plate reading process.

7.8.1.1 Visual assessment of the slide during each step in the development process is the primary means for determining the similarity between standard and test material.

7.8.1.2 Photographs and printouts of the plate are used for tracking purposes.

7.8.2 Plates can be evaluated from the back (glass slide) of the plate if the band patterns are faint.

7.9 Photo Documentation

7.9.1 Take a digital picture in JPEG form of the TLC plate in ambient light using the <close> setting in the dial.

7.9.2 Place TLC into the viewing cabinet with the visible and UV lights on.

7.9.3 Put the camera in <night> picture mode or <manual> shutter mode (for control of exposure time) using the dial.

7.9.4 Put the camera into manual focus mode by setting the switch to <MF>.

7.9.5 Zoom the camera to maximize picture size by turning the zoom adjuster.

7.9.6 Focus the camera manually by hand turning the focus adjuster until the image is clear and readable.

7.9.7 Set the UV to 365nm.

7.9.8 Turn off the visible light.

7.9.9 Take picture.

7.9.10 Switch the UV to 254nm.

7.9.11 Take picture.

7.9.12 If test conditions require adding a developing solution per form D-742-F2 Developing Solution Recipes to enhance the picture, follow the instructions on forms D-742-F1 TLC Botanical Identification Test Methods and D-742-F2 Developing Solution Recipes.

7.10 RF Value documentation

7.10.1 If required, RF Values can be documented for tracking purposes and easier comparison.

7.10.2 The **Rf value** is defined as the ratio of the distance moved by the solute and the distance moved by the solvent along the plate and is derived by the formula:

7.10.3 RF values should be above 0.05 and below 0.95. Values of 0 or 1 should not be considered in determining matches between standards and samples.

$$\textbf{RF Value} = \frac{\textbf{Distance component travelled}}{\textbf{Distance solvent traveled (from spot location)}}$$

7.11 Picture Handling and Labeling

7.11.1 Pictures should be transferred from the SD card to a computer and archived in a dedicated folder.

7.11.2 Adjust contrast, rotate and crop the pictures as needed to maximize quality and readability.

7.11.3 Label the files as appropriate, indicating sample information and reading conditions.

7.12 Report Generation

7.12.1 An electronic report will be generated containing the following information:

7.12.1.1 Name of Botanical (latin name) and part of plant

7.12.1.2 R# of Raw Material

7.12.1.3 Identification or Raw Material with extract ratio

7.12.1.4 Identification of each lane

7.12.1.5 Identification of each plate (exposure + developing conditions)

7.12.1.6 A composite photograph (containing up to four different types of exposure + developing conditions)

7.12.1.7 Extraction conditions of raw material (if available)

7.12.1.8 Standard used (vendor, part#, name, extract ratio, lot#) as necessary

7.12.1.9 If required, record RF Values of major spots

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	02/04/14	New	14-0109	B. Johns
1	10/24/14	Reduced lanes from 5 to 3 for 2.5"x7.5" plate. Updated format. Added 250mL beaker option.	14-0840	B. Johns
2	04/08/19	Scheduled review: Added RF value requirement. Added volatile botanical component warnings. Added more stains to F2	19-0241	J. Maignan
3	02/28/23	Scheduled review: updated logo and format. Revised responsibility section. Aligned with current practices.	CC-23-0098	K. Burris

Botanical Name	Scientific Name	Mobile Phase	Stationary Phase	Dissolution Media	SOP Deviation	Standard	Developing Solution	Mfr Extract Conditions
Acai Palm Fruit	*Euterpe oleracea*	Ethyl acetate/Acetic acid/formic acid/H2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	15uL sample, 12 uL Standard	Chromadex Acai Palm Fruit, Part #00031071	*p*-anisaldehyde/ H_2SO_4	N/A
African Mango	*Irvingia Gabonesis Fruit*	Chloroform/Ethyl acetate/Formic acid [3:4:2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	[10X] std and sample	Nutracargo, Irvingia Gaonensis Powder, Part# 26309	10% H_2SO_4 in ETOH	N/A
Alfafa Leaf	*Medicago Sativa*	Chloroform/AcOH/MeOH/H_2O [6.4/3.2/1.2/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Alfalfa Leaf, Part# 00031108	*p*-anisaldehyde/ H_2SO_4	N/A
Aloe Vera Leaf Resin	*Aloe barbadensis*	Ethyl acetate/MeOH/H_2O [10.0/1.4/1.0]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Aloe Vera Leaf Resin, Part# 00031140	KOH Spray Reagent *(Visible Light)*	N/A
American Ginseng Root	*Panax quinquefolius*	Chloroform/MeOH/H_2O [7.3/3.0/0.4]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	70% ETOH	N/A	Chromadex Ginseng American, Part# 00030676	Vanillin/ H_2SO_4	N/A
Amla Fruit (Indian Gooseberry)	*Phyllanthus emblica*	Toluene/Ethyl Acetate/Formic Acid/MeOH [6/6/1.6/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Amla Fruit, Part#31310	Vanillin/ H_2SO_4	N/A
Ashwagandha Root	*Withania somnifera*	Toluene/Ethyl Acetate/Formic Acid [10/3/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Ahwagandha Root Lot#00030048-119	10% H_2SO_4 in ETOH	N/A
Asian Ginseng Root	*Panax ginseng*	Butanol/Ethyl Acetate/H_2O [20/5/10]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	70% ETOH	NA	Chromadex Asian Ginseng Root, Part# 00030303	*p*-anisaldehyde/ H_2SO_4	N/A
Astaxanthin	*Haematococcus pluvialis*	25% Acetone / 75% Hexane	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% Acetone	Dry at 70°C and resuspend in 100uL Ethyl Ether	Changsha Huir Biological-Tech, Haematococcus pluvians, 1.5% astaxanthan	N/A	N/A
Astragalus Root	*Astragalus membranaceus*	Chloroform/MeOH/H_2O [9/4/0.5]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	Develop in 250mL beaker, 7.5mL mobile phase, covered in foil, Concentrate standard 5X as necessary	Chromadex Astragalus Root, Part# 00030073	5% H_2SO_4 in MeOH + Visible light	H_2O. Heat at 70°C for 45 minutes. Maltodextrin- 10:1 10%, 5:1 15%
Bacopa Monnieri	*Bacopa Monnieri*	Ethyl Acetate/MeOH/H2O [7/2/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	2-5uL Sample 10uL Standard	Chromadex Bacopa Whole Plant, Part# ASB-00030842-005	*p*-anisaldehyde/ H_2SO_4	N/A
Beet Root / Beet Juice	*Beta vulgaris*	Ethyl acetate/Acetic acid/formic acid/H2O [10/1.1/1.1/2.6]	Silica Gel50, F254, 5cm X 10cm HP-TLC plate	100% MeOH	[5X] std and sample	Spectrum Beet Root Powder, Part# B1724	Vanillin/ H_2SO_4	N/A
Bioperine (Black Pepper)	*Piper nigrum*	Toluene/Ethyl Formate/Formic Acid [5/4/1]	Silica Gel50, F254, 5cm X 10cm HP-TLC plate	100% MeOH	2uL Standard, 1uL Sample	Chromadex Pepper Fruit BRM Lot#00031130-361	Vanillin/ H_2SO_4	N/A
Bitter Orange Fruit	*Citrus x aurantium*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Bitter Orange Fruit, Part# 00030102	Natural Products Spray Reagent + 365nm or 5% H2SO4 in MeOH	N/A
Blue Vervain Aerial	*Verbena hastata*	Ethyl acetate/AcOH/formic acid/H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Blue Vervain, Part#AS-00030157-005	5% H_2SO_4 in MeOH	N/A
Buckthorn Bark	*Rhamnus frangula*	Ethyl acetate/MeOH/H_2O [10/1.35/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Buckthorn Bark, Part#ASB-00031106-005	KOH Spray Reagent *(Visible Light)*	N/A
Cascara Sagrada	*Frangula purshiana*	Ethyl acetate/MeOH/H_2O [10/1.35/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Alkemist, Frangula purshiana, Lot# C15209CRB	Natural Products Spray Reagent + 365nm or 5% H2SO4 in MeOH	N/A
Cayenne Pepper (Paprika)	*Capsicum annuum*	Isopropyl Ether 100%	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	Dichloromethane	N/A	Chromadex Paprika Fruit BRM, Part#ASB-00031164-005	5% H_2SO_4 in MeOH	N/A

Chamomile Flower	*Matricaria recutita*	Ethyl acetate/AcOH/formic acid/H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Chamomile Flower BI, Part#ASB-00030848-005	5% H_2SO_4 in MeOH	N/A
Chaste Tree Fruit	*Vitex agnus-castus*	Ethyl acetate/MeOH/H_2O [7.7/1.5/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Chaste Tree Fruit, Part# 00030780	*p* -anisaldehyde/ H_2SO_4	N/A
Cinnamon Bark	*Cinnamomum burmannii*	Toluene/Ethyl Acetate [8/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	Hexanes	15uL Sample	Chromadex Cinnamon Bark, Part#31013	KOH Spray Reagent *(Visible Light)*	N/A
Coleus Forskohli	*Plectranthus Barbatus Root*	Toluene/ Ethyl Formate/ Formic Acid [5/5/1.5]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	5uL Standard, 2uL Sample	Chromadex Coleus Forskohlii, Part# ASB-00031089-005	*p* -anisaldehyde/ H_2SO_4	N/A
Cranberry Fruit	*Vaccinium Oxycoccus*	Dichloromethane/Ethyl Acetate/Formic Acid [6:10:1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	15uL Sample	Nutracargo, Cranberry 4-1 Pwdr Ext, Part#FRX523	10% H_2SO_4 in ETOH	
Damiana Leaf	*Turnera diffusa*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Damiana Leaf, Part#36102	10% H_2SO_4 in ETOH	N/A
Dandelion Root	*Taraxacum officinale*	Chloroform/EtOH [9.5/0.5]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Dandelion Root, Part# 00030180	Vanillin/ H_2SO_4	N/A
Echinacea purpurea Root	*Echinacea purpurea*	Ethyl Acetate/Methylethyl ketone/Formic acid/H_2O [7.5/4.5/1.5/1.5]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Echinaea purpurea, Part# 00030216	Natural Products Spray Reagent + 365nm or 5% H2SO4 in MeOH	N/A
Eleuthro/Siberian Ginseng Root	*Eleutherococcus senticosus*	Chloroform/AcOH/MeOH/H_2O [6.4/3.2/1.2/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Eleuthro Root, Part# 00030301	5% H_2SO_4 in MeOH *(Visible Light)*	ETOH/H_2O [40/60]. Heat at 70°C for 30 minutes. Maltodextrin- 5:1 10%
Epimedium Leaf / Horny Goat Weed	*Epimedium spp.*	Ethyl acetate/AcOH/Formic acid/ H_2O [7.7/1.5/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Epimedium Leaf Part# 00030230	Natural Products Spray Reagent + 365nm or 5% H2SO4 in MeOH	N/A
Fennel Seed	*Foeniculum vulgare*	Toluene/Ethyl Formate/Formic Acid [5/4/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Spectrum Fennel Seed Lot#QR0019	Vanillin/ H_2SO_4	N/A
Flax Seed	*Linum Usitatissimum Seed*	Toluene/ Ethyl Acetate [9.5/0.5]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	Dichloromethane	5uL Standard and Sample	Chromadex Flax Seed Part# 00030250	Vanillin/ H_2SO_4	N/A
Gentian Root	*Gentiana lutea*	Ethyl acetate/MeOH/H_2O [7.7:1.5/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	[20X] Samples	Chromadex Gentian Root Part# 00031099	Vanillin/ H_2SO_4	N/A
Ginger Root	*Zingiber officinale*	Toluene/Ethyl Acetate/AcOH [17.5/8.25/0.75]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	Dichloromethane	N/A	AHP Zingiber officinale Root Part# 3096	Vanillin/ H_2SO_4	N/A
Gingko Biloba Leaf	*Gingko biloba*	Chloroform/AcOH/MeOH/H_2O [6.4/3.2/1.2/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	5uL Standard, 10uL [3X] Sample	Chromadex Ginkgo Biloba, Part# 00030831	Natural Products Spray Reagent + 365nm or 5% H_2SO_4 in MeOH	ETOH/H_2O [75/25]. Heat at 75°C for 30 minutes. Maltodextrin- 15:1 15%, 50:1 5%
Goji Berry	*Lycium barbarum*	Ethyl acetate/Chloroform/Formic acid [3/2/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	80% MeOH	[10X] std and sample	AHP Lycium barbarum Fruit, Part# 3425	5% H_2SO_4 in MeOH	N/A
Goldenseal Root and Rhizome	*Hydrastis canadensis*	Ethyl Acetate/MeOH/Formic Acid/H_2O [10/2/1/0.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	Start with samples at 10µL, 5µL, 3µL	Goldenseal Root and Rhizome, Part# 00030829	Ninhydrin *(Visible Light)*	N/A
Gotu Kola Herb	*Centella asiatica*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	90%MeOH	N/A	Spectrum Gotu Kola Herb, Part#G1309	*p* -anisaldehyde/H_2SO_4	N/A
Green Tea Leaf	*Camellia sinensis*	Chloroform/ethyl formate/formic acid/methanol [5/4/2/2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	80% MeOH	N/A	Green Tea Leaf, Part# 00030330	Vanillin/ H_2SO_4	N/A

Guarana Seed	*Paullinia cupana Kunth [Sapiindaceae]*	Chloroform/Ethyl Acetate/Formic acid/MeOH [5/4/2/2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	1%NH_4OH in MeOH	15uL Sample	Chromadex Guarana Seed, Part# 30335-064	*p*-anisaldehyde/H_2SO_4	N/A
Horsetail Aerial Parts	*Equisetum arvense*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	Start with samples at 10µL, 5µL, 3µL	Chromadex Horsetail Aerial Parts, Part# 00030347	Natural Products Spray Reagent +365nm or 5% H_2SO_4 in MeOH	H_2O. Heat at 70°C for 45 minutes. Maltodextrin- 7:1 10%
Kiwi Fruit	*Actinidia deliciosa*	Hexane/Ethyl Acetate [4/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	Ethyl Acetate	[20X]+20uL Sample	AHP Kiwi Fruit, Product Code BRM-ActDel, Lot#3674	5% H_2SO_4 in MeOH	N/A
Leuzea root	*Rhaponticum Carthamoides*	Chloroform/MeOH [8:2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	Develop in 250mL beaker, 7.5mL mobile phase, covered in foil, Concentrate extracts 10X	Vendor Supplied	Vanillin/ H_2SO_4	H_2O. Heat at 70°C for 45 minutes. Maltodextrin- 2:1 20%, 10:1 6%, 11:1 5%, 4:1 15%
Licorice Root	*Glycyrrhiza glabra*	Ethyl Acetate/AcOH/Formic Acid/H2O [15/1/1/2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Licorice Root VBRM Lot#00030774-005	*p*-anisaldehyde/H_2SO_4	N/A
Lovage Root	*Levisticum officinale*	Toluene/Ether/AcOH [5/5/0.5]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Lovage Root, Part# 00031186	KOH Spray Reagent *(Visible Light)*	N/A
Maca Root	*Lepidium meyenii Walp.*	Chloroform/AcOH/MeOH/H_2O [6.4/3.2/1.2/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Alkemist Labs Lepidium meyenii Walp. Root Lot#AP23809-002CRB	Vanillin/ H_2SO_4	N/A
Mangosteen Fruit Rind	*Garcinia mangostana*	Chloroform/ethyl formate/formic acid/methanol [5/4/2/2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Mangosteen Fruit Rind, Part#00030992	*p*-anisaldehyde/H_2SO_4	N/A
Milk Thistle Seed	*Silybum marianum*	Chloroform/Acetone/Formic acid [7.5/1.65/0.85]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Milk Thistle Seed, Part# 0003041	Vanillin/ H_2SO_4	ETOH/H_2O [70/30]. Heat at 65°C for 45 minutes. Maltodextrin- 12:1 10%
Psyllium Husk	*Plantago psyllium*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	10uL Standard, 20uL sample	Chromadex Psyllium Husk RGBRM, Part#ASB-00030580-005	*p*-anisaldehyde/H_2SO_4	N/A
Pumpkin Seed	*Cucerbita pepo*	Toluene/Ethyl Acetate [9/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	AHP Cucerbita pepo Lot#3388	*p*-anisaldehyde/H_2SO_4	N/A
Red Clover Flower	*Trifolium pratense*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Red Clover, Part#00030620	Natural Products Spray Reagent, 365nm	N/A
Red Yeast Rice	*Monascus purpureus*	Chloroform/Ethyl formate/Formic acid/Methanol [5/4/2/2]	Silica Gel60, F254, 5.0cm X 10cm HP-TLC plate	100% MeOH	[5X] std and sample	Chromadex Red Yeast Rice, Part# 00030823	Vanillin/ H_2SO_4	N/A
Reishi Mushroom	*Ganoderma lucidum*	Dichloromethane/methanol [9/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	Develop in 250mL beaker, 7.5mL mobile phase, covered in foil, Concentrate extracts 10X	AHP, Reishi Mushroom, Part#947	5% H_2SO_4 in MeOH + Visible light	H_2O. Heat at 70°C for 30 minutes. Maltodextrin- 10:1 10%
Rhodiola Root	*Rhodiola rosea*	Ethyl Acetate/MeOH/H_2O/Formic Acid [77:13:10:2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	Develop in 250mL beaker, 7.5mL mobile phase, covered in foil, Concentrate extracts 10X	Chromadex Rhodiola Root, Part# 00030699	Vanillin/ H_2SO_4	ETOH/H_2O [30/70]. Heat at 65°C for 45 minutes. Maltodextrin- 10:1 5%, 5:1 10%
Sage Leaf	*salvia officinales*	Dichloromethane	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A		*p*-anisaldehyde/H_2SO_4	N/A
Salvia Miltiorrhiza Root	*Salvia Miltiorrhiza Root*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Chinese Salvia Root Part# ASB-00031077-005	10% H_2SO_4 in ETOH	N/A
Saw Palmetto	*Serenoa repens*	Chloroform/ETOH/AcOH [9.5/0.5/0.1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Saw Palmetto Part#00030725	*p*-anisaldehyde/H_2SO_4	N/A
Senna Leaf	*Senna Alexandrina*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Senna Leaf Part# 00031117	*p*-anisaldehyde/H_2SO_4	N/A
Schisandra Fruit	*Schisandra chinensis*	Toluene/Ethyl Acetate/AcOH [17.5/8.25/0.75]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	AHP, Schisandra Berry, Part# 2851	*p*-anisaldehyde/H_2SO_4	ETOH/H_2O [70/30]. Heat at 70°C for 45 minutes. Maltodextrin- 5:1 10%.
Skullcap Root	*Scutellaria lateriflora*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	5uL Sample	AHP Ulmus rubra Inner Bark, Part#1270	Natural Products Spray Reagent, 365nm	N/A
Slippery Elm Bark	*Ulmus rubra*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	3uLStandard 2X Sample	AHP Ulmus rubra Inner Bark, Part#1270	*p*-anisaldehyde/H_2SO_4	N/A

Sweet Wormwood	*Artemisia Annua Herb*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Sweet Wormwood Herb Part# ASB-00030633-005	*p*-anisaldehyde/H_2SO_4	N/A
Tribulus Terrestris Fruit	*Tribulus terrestris*	Chloroform/MeOH/H_2O [13/7/2]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	70% EtOH	N/A	Chromadex Tribulus Terrestris, Part# 00030850	*Vanillin* /H_2SO_4	H_2O. Heat at 70°C for 45 minutes. Maltodextrin- 5:1 10%
Turmeric Root	*Curcuma longa*	Chloroform/ETOH/AcOH [9.5/0.5/0.1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex Tumeric Root, Part# 00030813	*Vanillin* /H_2SO_4	N/A
Valerian Root	*Valeriana officinales*	Hexane/Ethyl Acetate/AcOH [13/7/0.1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	Dichloromethane	5uL Standard, 10uL Sample	Chromadex Valerian Root BRM, Part#ASB-00035900-005	*p*-anisaldehyde/H_2SO_4	N/A
White Kidney Bean	*Phaseolus Vulgaris L.*	Chloroform/MeOH/H2O [8/2/0.25]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	[10X] Std [20X] Sample	Alkemist Labs Phaseolus Vulgaris L. [Fabaceae] Bean, Part# KHY27612CRB	10% H_2SO_4 in ETOH	N/A
White Oak Bark	*Quercus alba*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	[20X] Samples	Chromadex White Oak Bark Part# 00030779	*p*-anisaldehyde/H_2SO_4	N/A
White Willow Bark	*Salix Alba Bark*	Ethyl acetate/MeOH/H_2O [7.7:1.5/0.8]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% MeOH	N/A	Chromadex White Willow Bark Part# 00035950	*p*-anisaldehyde/H_2SO_4	N/A
Wild Yam Root	*Dioscorea villosa*	Chloroform/MeOH/H2O [6.4/5/1]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	70% EtOH	N/A	Chromadex Wild Yam Root BRM, Part#ASB-00031070-296	*Vanillin* /H_2SO_4	N/A
Yerba Mate	*Ilex paraguariensis*	Ethyl acetate/AcOH/Formic acid/ H_2O [10/1.1/1.1/2.6]	Silica Gel60, F254, 2.5cm x 7.5cm TLC plate	100% Water	N/A	Chromadex Mate Leaf Part#00030830	*p*-anisaldehyde/H_2SO_4	N/A

Acronyms Used

IPA - Isopropyl Alcohol
MeOH - Methanol
ETOH - Ethanol
H_2SO_4 - Sulfuric Acid
AcOH - Acetic Acid
AHP - American Herbal Pharmacopoeia

Chemicals	Use	Recipe
p-Anisaldehyde/H_2SO_4	*General Purpose Stain*	Add 0.5mL *p*- Anisaldehyde in 50mL Glacial AcOH and 1mL H_2SO_4. Plates are developed by incubating at 105°C until bands appear maximally. Background can be reducing with humidity.
KOH Spray Reagent	*Anthraquinones-red, Anthrones-yellow/365, Coumarins-blue/365*	5% or 10% Ethanolic potassium hydroxide (KOH). Saturate plate and allow developing at room temperature or 105°C. Evaluate using visible light and 365nm.
Ninhydrin Reagent	*Good for amino acids*	Dissolve 750mg of ninhydrin in 50mL *n*- butanol and add 3mL AcOH. Allow plate to incubate at 105°C for up to 10 minutes until maximum color appears.
Vanillin/ H_2SO_4	*Steroids / General reagent*	Add 0.5g vanillin to 80mL H_2SO_4 and 20mL EtOH. Allow plate to incubate at 105°C for up to 10 minutes.
Natural Products Reagent	*Flavonoids, aloin*	1% diphenylboric acid-β-ethylamino ester (Z-aminoethyldiphenylborinate) in MeOH. 5% Polyethylene Glycol-4000 in ETOH. Spray with 1st reagent followed by 2nd reagent. Dry and read at 365nm.
5% H_2SO_4 in MeOH	*General purpose stain*	Add 5mL H_2SO_4 to 95mL MeOH. Allow plate to incubate at 105°C for up to 10 minutes. Read under visible light.
$KMnO_4$	*Olefins and other readily oxidized groups*	Dissolve 1.5g $KMnO_4$, 10g K_2CO_3, and 1.25mL 10% NaOH in 200mL water
Cerium Sulfate	*General stain, particularly useful for alkaloids.*	Make an aqueous solution of 10% Cerium (IV) sulfate and 15% H_2SO_4.
Morin Hydrate	*General reagent. Fluorescently active.*	Make up a 0.1 wt% solution in methanol.
Cerium Molybdate	*General purpose stain. Requires heating to visualize. aka Hanessian's stain*	Dissolve 0.5g $Ce(NH_4)_2(NO_3)_6$ and 24.0 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. Carefully add 28 mL H_2SO_4, stir for 1 hour and filter if necessary.
2,4-DNP	*aldehydes and ketones*	Dissolve 12 g of 2,4- dinitrophenylhydrazine, 60 mL of H_2SO_4, and 80 mL of H_2O in 200 mL 95% EtOH.
Bromocresol Green	*Acidic (pKA < 5) groups*	Add 0.04g bromocresol green to 100 mL absolute EtOH. Slowly drip in a 0.1M solution of NaOH until the solution just turns pale blue.
Phosphomolybdic Acid	*General purpose*	Dissolve 10 g PMA in 100 mL absolute ethanol

Acronyms Used

MeOH - Methanol

EtOH - Ethanol

H_2SO_4 - Sulfuric Acid

AcOH - Acetic Acid

KOH - Potassium Hydroxide

$KMnO_4$ -Potassium Permanganate

K_2CO_3 - Potassium Carbonate

$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ - Ammonium Molybdate Tetrahydrate

NaOH - Sodium Hydroxide

$Ce(NH_4)_2(NO_3)_6$ Ammonium Cerium (IV) Nitrate