

SEMI-QUANTITATIVE SEPARATION OF CANNABINOIDS USING RP-TLC PLATES

ABSTRACT

Described is a RP-TLC method that allows rapid (~ 10 min) separation, identification, and semi-quantitation of Cannabinoids from Hemp (*Cannabis sativa* var. *Indica*) and Marijuana (*Cannabis sativa* var. *Sativa*)

OBJECTIVE

To separate, identify, and semi-quantitate Cannabinoid Neutrals using RP-TLC plates

MATERIALS

Provided with the Cannabis TLC Kit

- 2733187 – C18-W Aluminum Backed TLC Plates
- 1 gram of Fast Blue BB (Store this in the freezer)
- 1 gram of Potassium Hydroxide
- TLC-Rocket
- 814022 - Glass Capillaries
- Four Vials for Mixing and Storage

Provided by the Customer

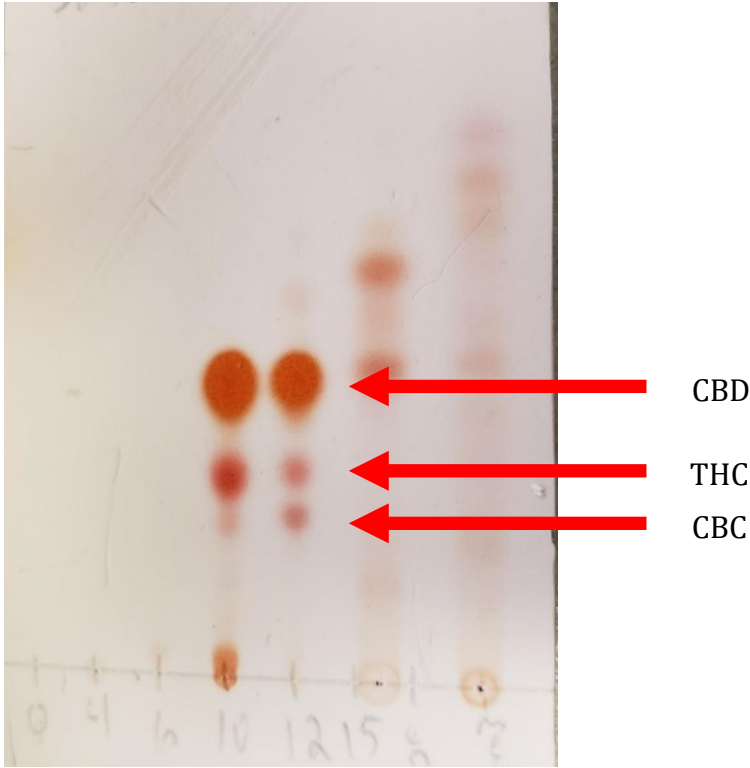
- Water (Chromatographic Quality)
- Methanol (Chromatographic Quality)
- Heat Gun
- 20mL Liquid Scintillation Vials
- Airbrush or Sprayer (Sorbtech)

PROCEDURE

1. Dissolve the sample in a solvent that will evaporate (Methanol, Ethanol, Chloroform, Hexane) at a concentration of about 1mg/ml.
2. Prepare about 20mL of Developing Solvent in a 20mL vial. 20% Water: 80% Methanol (v/v) [4mL Water + 16mL Methanol].
3. Weigh about 1g of Potassium Hydroxide pellets into a 20mL vial and add 20mL Methanol. Shake until dissolved.
4. Weigh about 50mg of Fast Blue BB into a 20mL vial and add 10mL Water. Shake until dissolved. Note: This solution MUST be prepared fresh every day or stored in the refrigerator when not in use.
5. Prior to use, cut the 4 cm x 8cm TLC plate in half so the plates are 2 cm x 8cm
6. Spot 1 μ l to 5 μ l of sample solution on a TLC plate (2 cm x 8 cm) approximately 1 to 1.2cm from the bottom of the plate.
7. Dry the spot with a heat gun or air dry.
8. Add ~0.5 to 1mL of Developing Solvent to the well of the Sorbtech Rocket base.
9. Place the TLC plate into the well of the Sorbtech Rocket base and put the top on the Sorbtech Rocket.
10. Let the TLC plate develop – a few minutes.
11. When development has completed, remove the TLC plate from the Sorbtech Rocket and dry the TLC Plate with a heat gun or allow to air dry.
12. Once the TLC plate has cooled down, put the TLC plate in a fume hood and spray the TLC plate with enough Fast Blue BB solution to fully wet the plate. Do not overspray the TLC plate because this step will need to be repeated.
13. Spray the TLC plate with the KOH solution until spots appear to no longer darken.

RESULTS

Characteristic colors and Retention Factors (R_f) for Cannabinoids are illustrated below



Substance	R_f	Color
CBDA	0.68	Red
CBG	0.59	Orange-Brown
CBD	0.58	Red-brown
CBN	0.48	Purple
Δ^9 -THC	0.44	Red
Δ^8 -THC	0.43	Red
THCA	0.40	Red
CBC	0.37	Purple

Note that the detection limit is ~20-50ng applied to the plate.

Concentration of Sample	mg/mL	1	5	10	10	10
Concentration of Sample	μ g/mL	1	5	10	10	10
Volume deposited on TLC Plate	μ l	1	1	1	1	5
Sample Weight on Plate	μ g	1	5	10	10	50
%THC in Sample	%	1.0%	1.0%	1.0%	0.3%	0.3%
Amount of THC on Plate	ng	10.00	50.00	100.00	30.00	150.00
THC Detectable?		No	Yes	Yes	No	Yes

REFERENCES

There are MANY descriptions of this procedure. Here are a few of them.

Zampa, F & Furlan, G & Bellizia, M & Juliano, G & Ripani, L. (2014). New forensic perspective for fast blue B: From cannabinoid reagent in toxicology to latent fingerprint developer in drug cases. Journal of Forensic Identification. 64. 523-535.

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J Pharm Sci. 1979 Aug;68(8):976-8. Simple field test for marijuana. Lau-Cam CA, Pizzitola V.. PMID: 480177 DOI: 10.1002/jps.2600680815

Evaluating the selectivity of colorimetric test (Fast Blue BB salt) for the cannabinoids identification in marijuana street samples by UV-Vis, TLC, ESI(+)FT-ICR MS and ESI(+)MS/MS. Nayara A. dos Santos, Lindamara M.Souza, Eloilson Domingos, Hildegardo S. França, Valdemar Lacerda Jr., Adilson, Beatriz, Boniek G.Vaz, Rayza R.T.Rodrigues, Verônica V. Carvalhod, Bianca B. MerloeRicardo M.Kuster, Wanderson Romãoa.
<https://doi.org/10.1016/j.forc.2016.07.001>

APPENDIX A: FAST BLUE BB SALT

Common Name

Fast Blue BB Salt hemi(zinc chloride) salt

Synonyms

4-Amino-2,5-diethoxybenzanilide diazotated zinc double salt

4-Benzoylamino-2,5-diethoxybenzenediazonium chloride hemi (zinc chloride) salt

Identification Information

CAS Number -----5486-84-0

Linear Formula -----C₁₇H₁₈N₃O₃Cl · 1/2 ZnCl₂

Molecular Weight -----415.94

Colour Index Number -----37175

Beilstein/REAXYS Number -----1845288

EC Number -----226-817-4

MDL number -----MFCD00074765

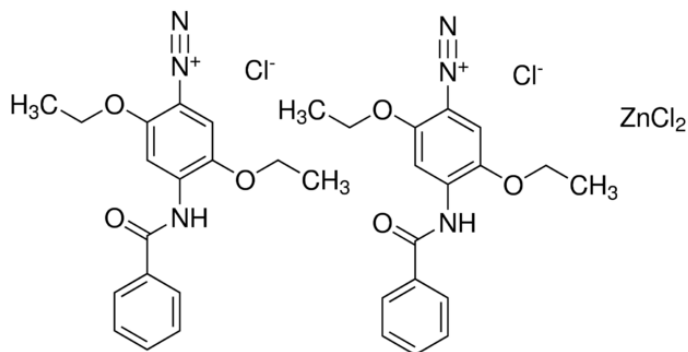
PubChem Substance ID -----24894840

S-A Part Numbers

F3378-1G

F3378-5G

F3378-25G



APPENDIX 2: REACTION MECHANISM OF FAST BLUE BB WITH THC

