SEMI-QUANTITATIVE SEPARATION OF CANNABINOIDS USING RP-TLC PLATES

BY ROBERT KERR AND ROBERT COTTA
SORBENT TECHNOLOGIES
NORCROSS, GEORGIA, USA

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 AND METHODS

- **Water (Chromatographic Quality)**
- **Methanol (Chromatographic Quality)**
- **Fast Blue BB Salt. From Sigma-Aldrich and other suppliers.**
  - https://www.sigmaaldrich.com/catalog/product/sigma/f3378?lang=en&region=US
- **Potassium Hydroxide**
- **Sorbtech Rocket**
  - https://www.sorbtech.com/chromatography/thin-layer-chromatography/tlc-accessories/tlc-rocket/
- **RP TLC Plates**
  - **Plates**
    - https://www.sorbtech.com/chromatography/thin-layer-chromatography/normal-phase/c18-w-hptlc-plates/
    - Sorbtech Part Number: 2715126

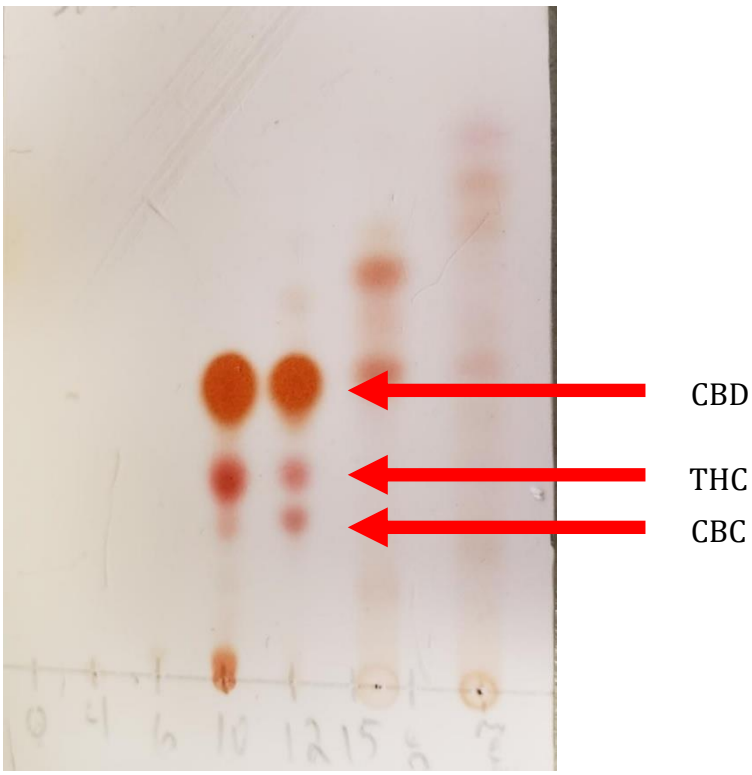
- **Sorbtech Plate Cutter**
  - https://www.sorbtech.com/chromatography/thin-layer-chromatography/tlc-accessories/tlc-plate-cutter/
- **Micropipettes**
  - https://www.sorbtech.com/chromatography/thin-layer-chromatography/tlc-accessories/glass-capillaries/
- **TLC Plate Sprayer**
  - Sorbtech Reference Part Number: 814101. Glass laboratory sprayer with rubber bulb
- **Heat Gun**
- **20mL Liquid Scintillation Vials**
- **Plastic Transfer Pipettes**

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 Water. Shake until dissolved.
4. Weigh about 100mg of Fast Blue BB into a 20mL vial and add 20mL Water. Shake until dissolved. Note: This solution MUST be prepared fresh every day.
5. Spot 1µl to 5µl of sample solution on a TLC plate (2.54cm x 7.5cm) approximately 1 to 1.2cm from the bottom of the plate
6. Dry the spot with a heat gun
7. Add ~0.5 to 1mL of Developing Solvent to the well of the Sorbtech Rocket base.
8. Place the TLC plate into the well of the Sorbtech Rocket base and put the top on the Sorbtech Rocket.
9. Let the TLC plate develop – a few minutes.
10. When development has completed, remove the TLC plate from the Sorbtech Rocket and dry the TLC Plate with a heat gun.
11. Once the TLC plate has cooled down, put the TLC plate in a fume hood and spray the TLC plate with ~1mL of the KOH solution. Do not spray until the solution is dripping off.
12. Then spray the TLC plate with ~1mL of the Fast Blue BB solution.

RESULTS

Characteristic colors and Rf factors for Cannabinoids are illustrated below



Substance	Rf	Color
CBDA	0.68	Red
CBG	0.59	Orange-Brown
CBD	0.58	Red-brown
CBN	0.48	Purple
Δ9-THC	0.44	Red
Δ8-THC	0.43	Red
THCA	0.40	Red
CBC	0.37	Purple

Note that the detection limit is ~20-50ng deposed on the plate.

Concentration of Sample	mg/mL	1	5	10	10	10
Concentration of Sample	µg/mL	1	5	10	10	10
Volume deposed on TLC Plate	µl	1	1	1	1	5
Sample Weight on Plate	µg	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.

https://www.alchimiaweb.com/blogen/make-cannabinoid-analysis/

https://www.omicsonline.org/open-access/microscopical-and-chemical-study-of-cannabis-sativa-2157-7145.1000210.php?aid=23170&view=mobile

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

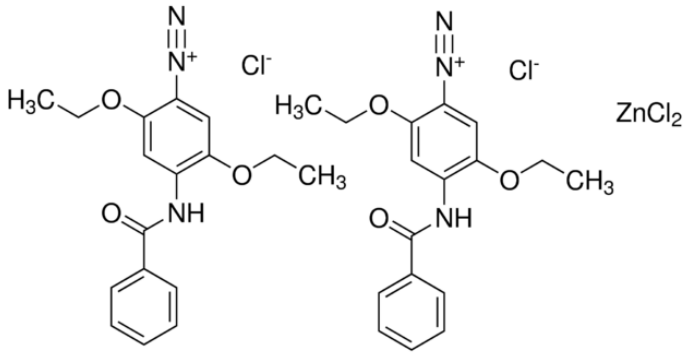
Fast Blue BB Salt hemi(zinc chloride) salt

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