

FAFOSS - Fast Automated Food Safety Screening

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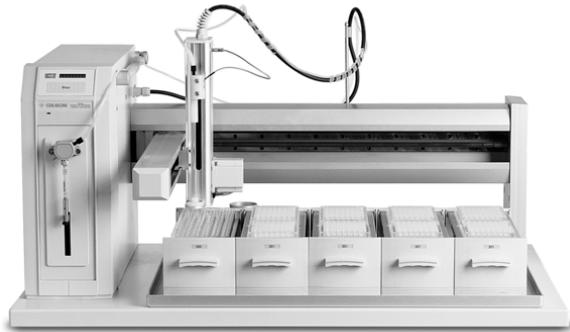


Background/Introduction

- Many current methods for measuring contaminants in foodstuffs tend to be slow and costly and often involve an initial screen by immunoassay followed up, where necessary, by analysis using conventional gas or liquid chromatography mass spectrometry (GC-MS and/or LC-MS).
- The initial testing can be expensive and the follow up testing is often characterised by high cost and delay.

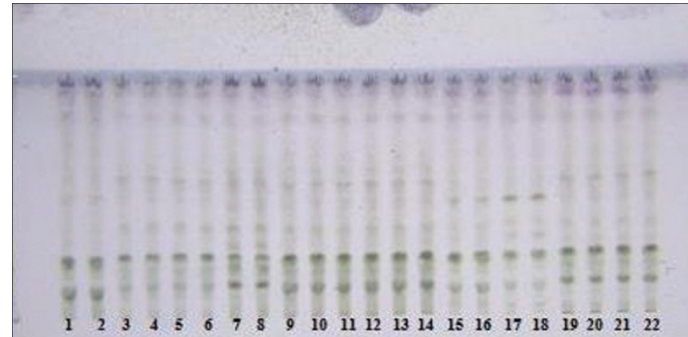
- The use of a novel HPTLC-MS system is being investigated for the quantitative analysis of samples that come from commodities which would be at risk of contamination or require positive release where no rapid techniques are currently available or only indicate presence or absence.
- The use of HPTLC-MS potentially removes the need for elaborate sample clean up and sample processing prior to analysis which can significantly extend the turnaround time.

Basic Strategy



- Automated QuEChERS (Quick Easy Cheap Effective Rugged Safe) Extraction Methodology
- Automated TLC Spotting

- Initial Examination of Plates (Visual/Fluorescence)



- "Positives" confirmation by TLC-MS



QuEChERS (Quick Easy Cheap Effective Rugged Safe) Extraction Methodology

- Manual QuEChERS procedure :
 - Weigh 10 g of sample
 - add 10 mL acetonitrile and internal standard
 - agitate intensively
 - add NaCl, MgSO₄ and buffering salts for phase-separation and pH-adjustment
 - agitate intensively and centrifuge ⇒ **Raw extract**
 - take an aliquot of the upper organic phase and subject it to dispersive SPE cleanup (d-SPE) by mixing it with MgSO₄ and a sorbent (e.g. PSA) to remove water and undesired co-extractives
 - agitate shortly and centrifuge (optionally add Analyte Protecting Agents) ⇒ **Final extract**
 - the final extract can be analyzed directly by GC- and / or LC-techniques
 - Both, extraction and cleanup can be scaled up or down as desired.

Feasibility Study

- Currently we have funding from TSB for a feasibility study to develop automated TLC and TLC-MS in which we are concentrating on the following contaminants:
 - Rhodamine B (in children's sweets),
 - Patulin (in Apple Juices and pulp)
 - DON in cereals
 - Sudan dyes as a food colorant.

Rhodamine B

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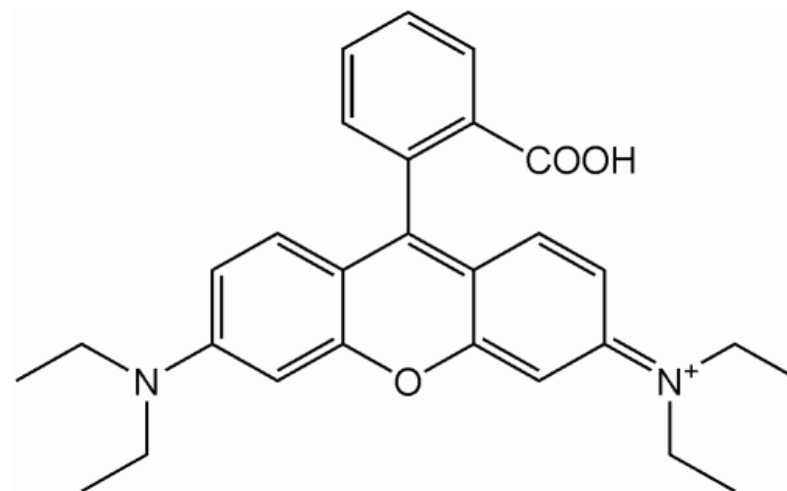
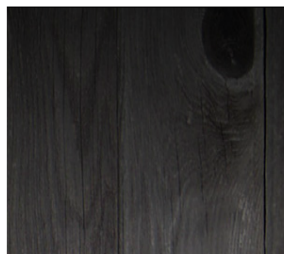


Illegal and potentially carcinogenic food dyes normally used by the sewage industry discovered in children's sweets

- Traces of Rhodamine B and Auramine found in sweets in West Yorkshire
- Asian sweets in Bradford and Kirklees were found to be contaminated
- The chemicals are usually used by the sewage industry to detect leaks
- They are banned for use in food production and are thought to cause cancer

By JOHN HALL

DIURNISHED: 05:34 17 February 2014 | UPDATED: 13:47 17 February 2014



Rhodamine B is a "Forbidden Substance"



Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

RHODAMINE B



General Information

| | |
|-------------------|--|
| Synonyms: | CI FOOD RED 15, D AND C RED No. 19 |
| Chemical Names: | [9-(o-CARBOXYPHENYL)-6-(DIETHYLAMINO)-3H-XANTHEN-3-YLIDENE] DIETHYLAMMONIUM CHLORIDE |
| CAS number: | 81-88-9 |
| Functional Class: | Food Additives COLOUR |

Evaluations

| | |
|------------------|---|
| Evaluation year: | 1964 |
| Meeting: | 28 |
| Specs Code: | W |
| Report: | NMRS 38/TRS 309-JECFA 8/24 |
| Specification: | WITHDRAWN (1984) |
| Previous Years: | 1964, NMRS 38/TRS 309-JECFA 8/24, FAS 66.25/NMRS 38B-JECFA 8/146. N |

Patulin

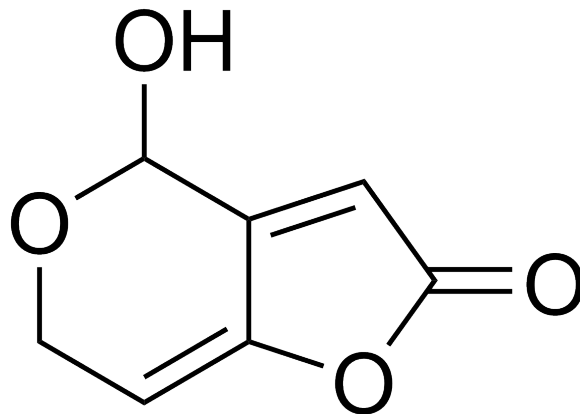


SupelMIP Patulin SPE columns protocol as per general procedure

- 4ml loaded (50:50 sample:water 2% acetic acid)
- 70% recovery
- EU legal limit (50ug/L) , loaded 10ug/L (10ng/ml in 2 ml)
- extraction if reconstituted into 0.25ml approx. 100ug/L

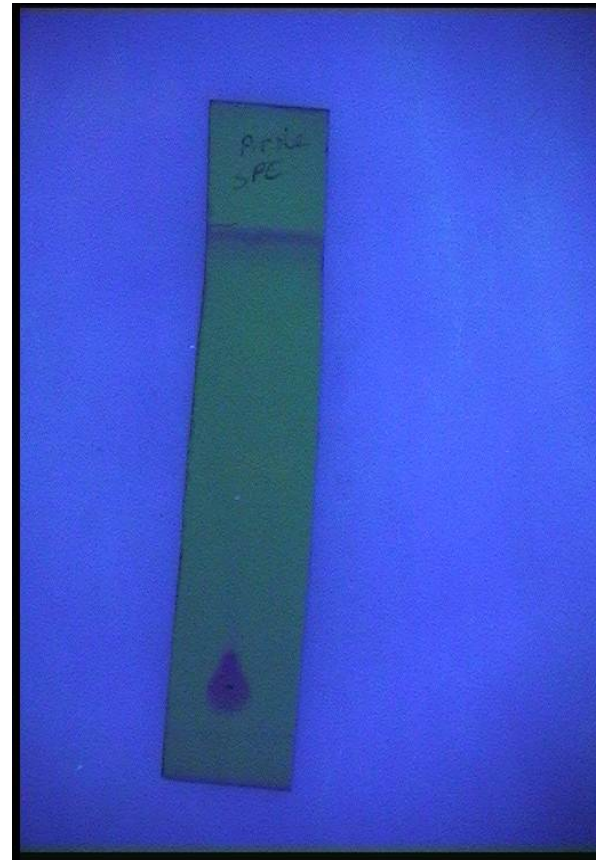
Patulin

- Patulin, 4-hydroxy-4*H*-furo[3,2*c*]pyran-2(6*H*)-one is a toxic mycotoxin secondary metabolite produced by a variety of molds, particularly *Aspergillus*, *Penicillium*, and *Byssochlamys*
- Patulin exhibits mutagenic and carcinogenic properties in several animal species and induces intestinal injuries, including epithelial cell degeneration, inflammation, ulceration, and haemorrhage



Patulin

World Health Organization recommends a maximum concentration of 50 µg/L of Patulin in apple juice



SPE columns protocol as per general procedure

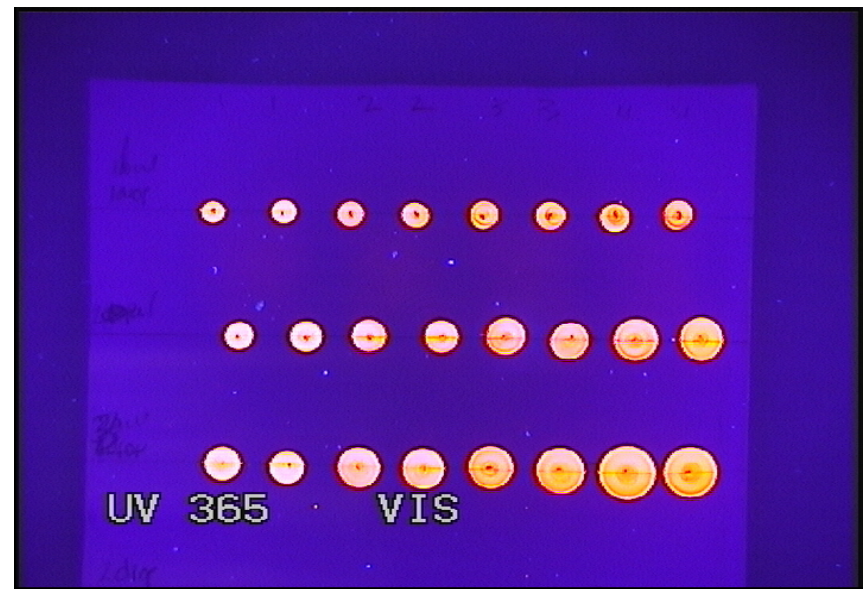
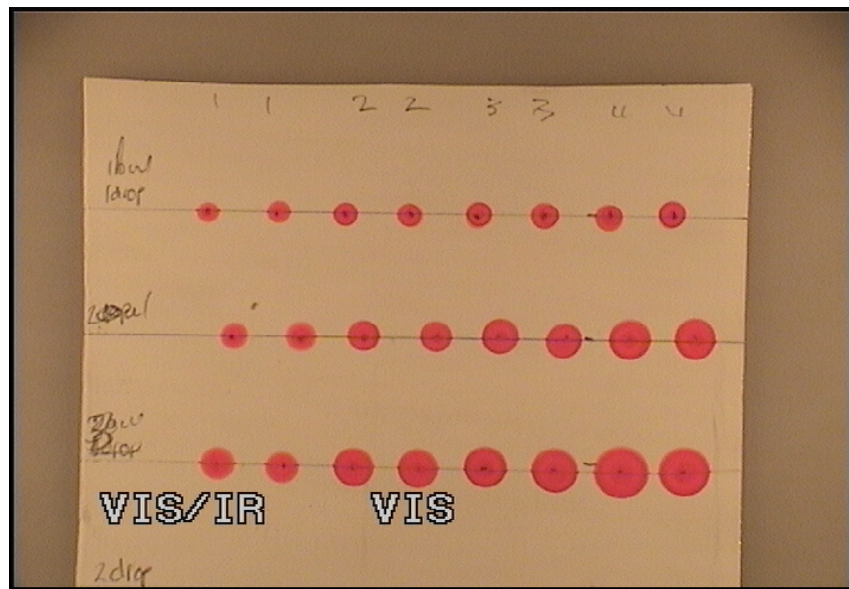
- Yet to be determined awaiting column delivery

Determination of spot volume

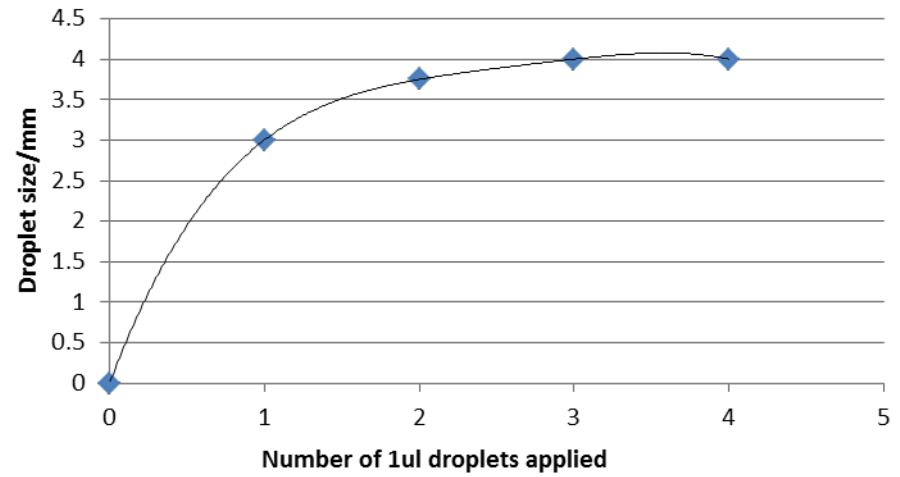
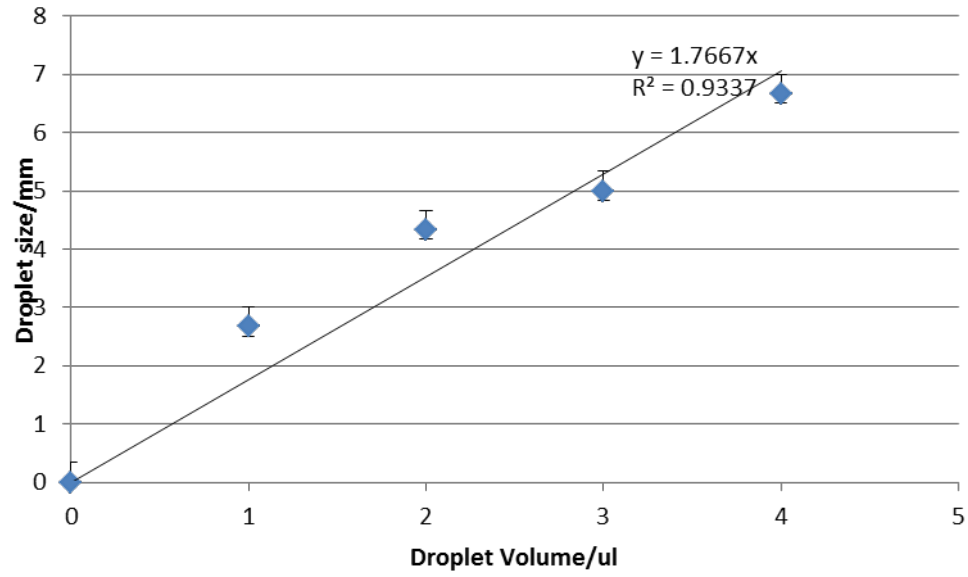
Rhodamine B (100ug/ml in MeOH) was chosen to investigate the optimal volume application upon the TLC plate to achieve the maximum size of 4mm for alignment with the Advion TLC-CMS reader head

- Volume application 1ul, 2ul, 3ul
- Repeat spot dosing 1, 2, 3, 4

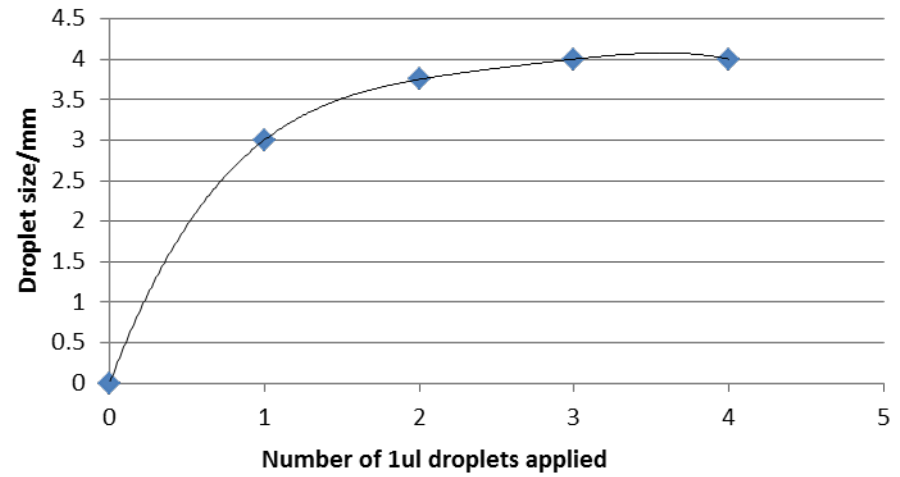
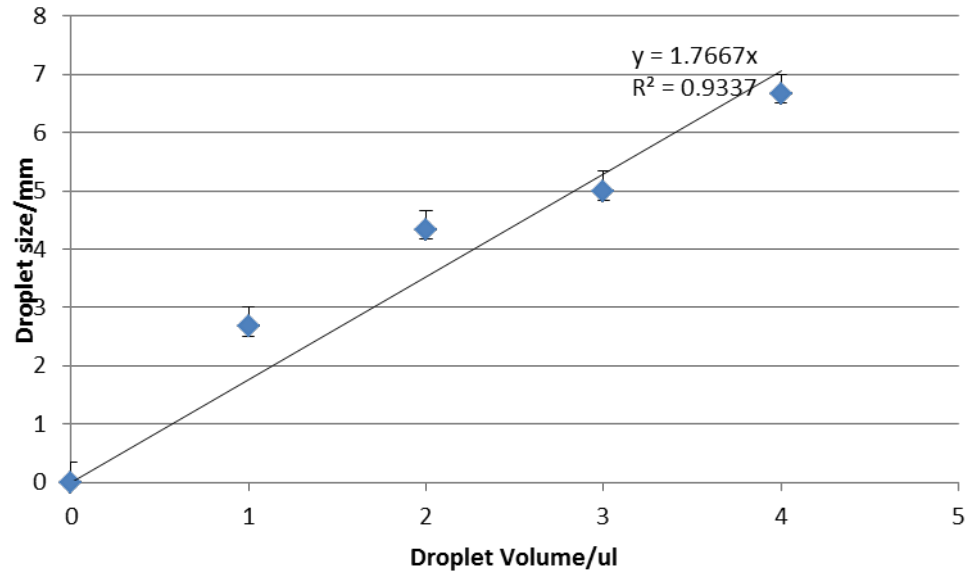
Results



Determination of optimal spot volume Results



Determination of optimal spot volume Results



Serial dilutions of Rhodamine B in MeOH to investigate the sensitivity of fluorescent detection

- Volume application 4X 100ug/ml, 10ug/ml, 100ng/ml, 10ng/ml, 1ng/ml

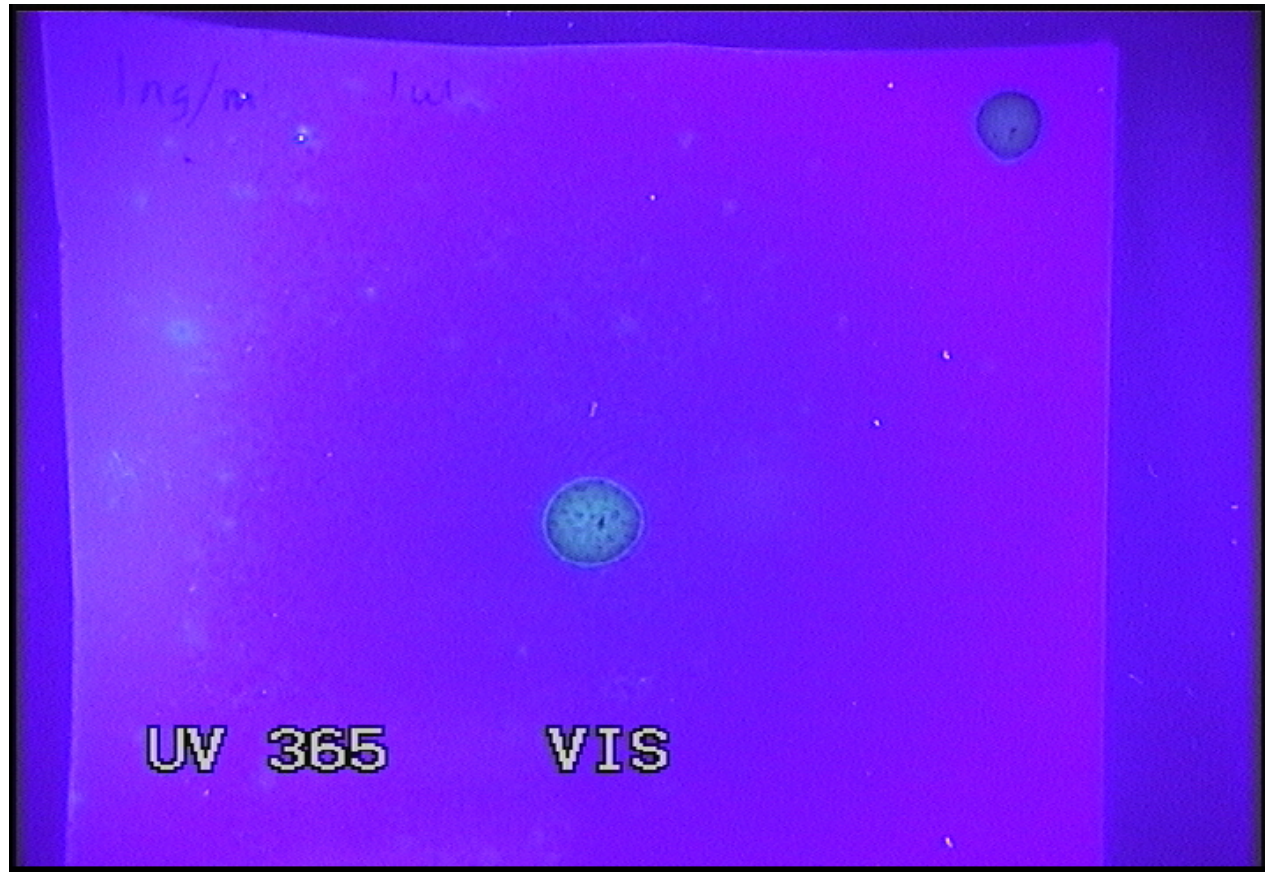
Sensitivity of Fluorescent Results



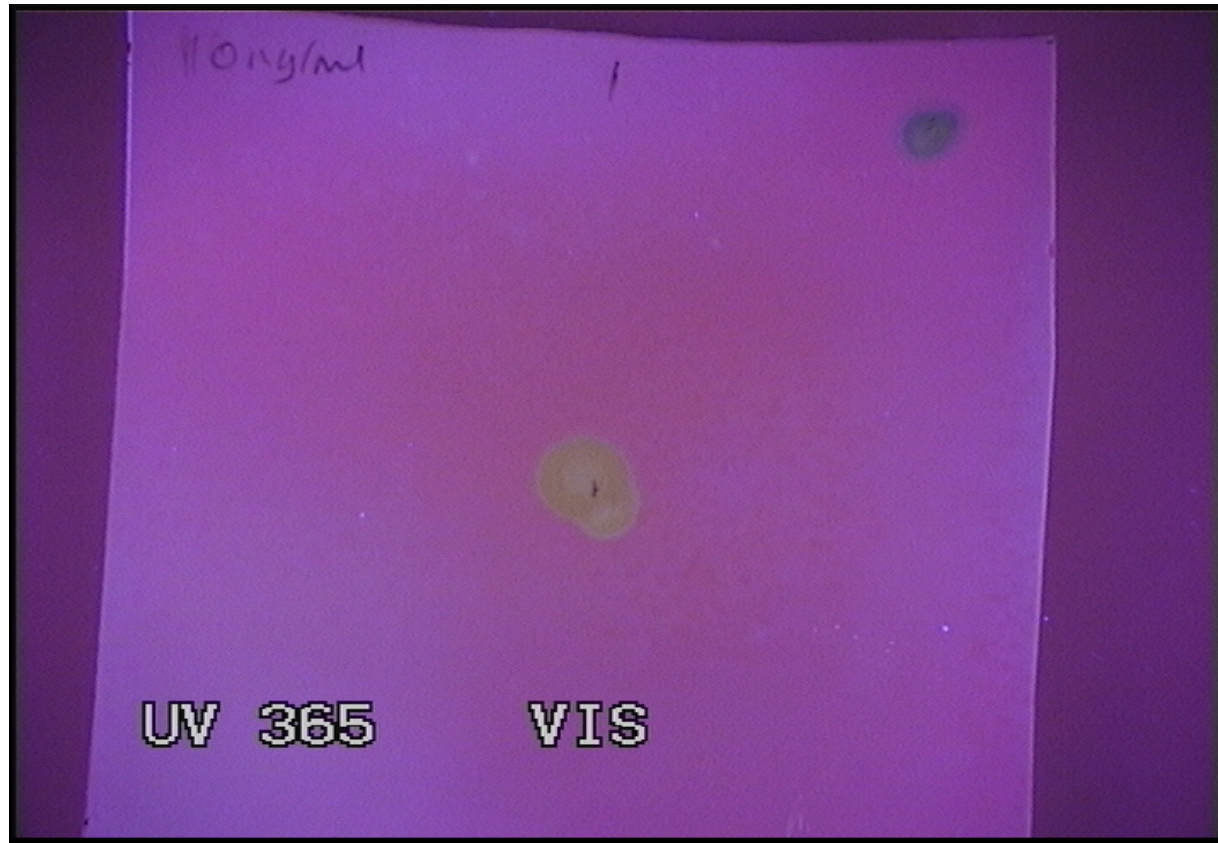
Patulin detection on TLC plate by derivatisation with 3-Methyl-2-benzothiazolinone hydrazone (MBTH)

- Volume application 4X 100ug/ml patulin
- MBTH (0.5% in 5% formic acid)
- heated 130° for 15 min

Patulin/MBTH detection Results



Patulin/MBTH detection Results

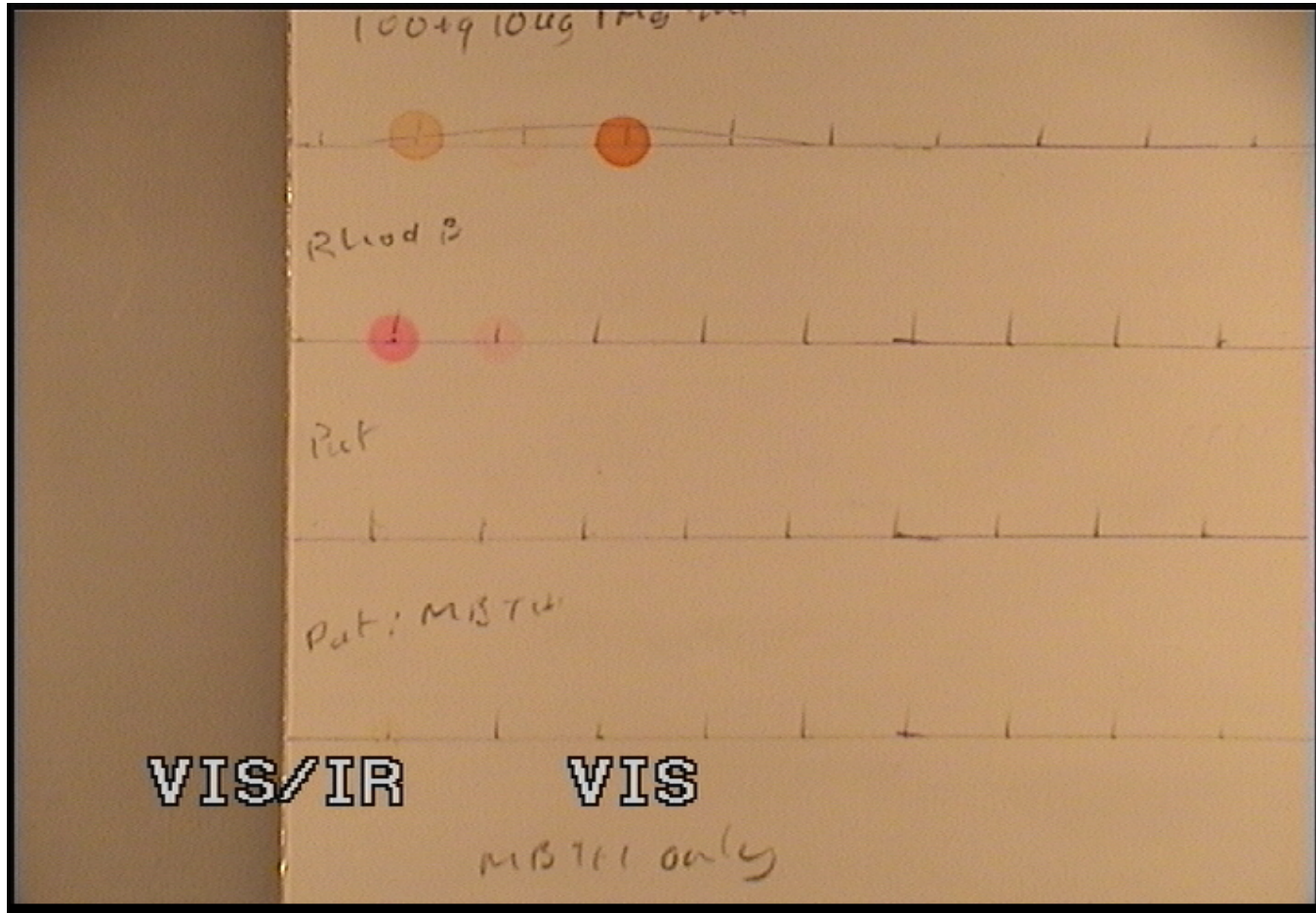


Optimum wavelength for fluorescent detection for Food Additives

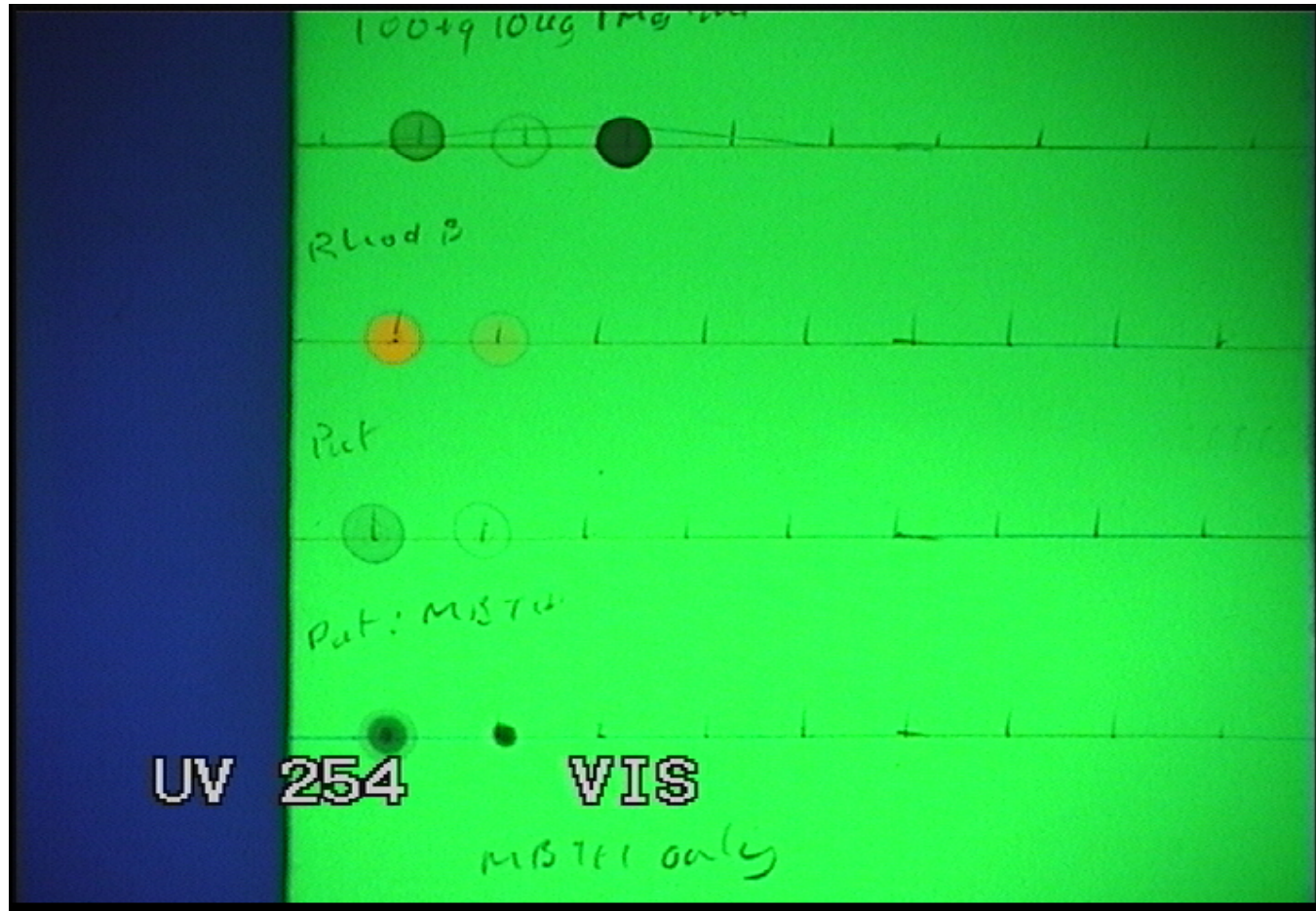
Varying concentrations of Patulin (with and without MBTH), Rhodamine B and Sudan1 were investigated at visible/IR, 254nm, 313nm and 365nm

- Volume application 4X 100ug/ml, 10ug/ml

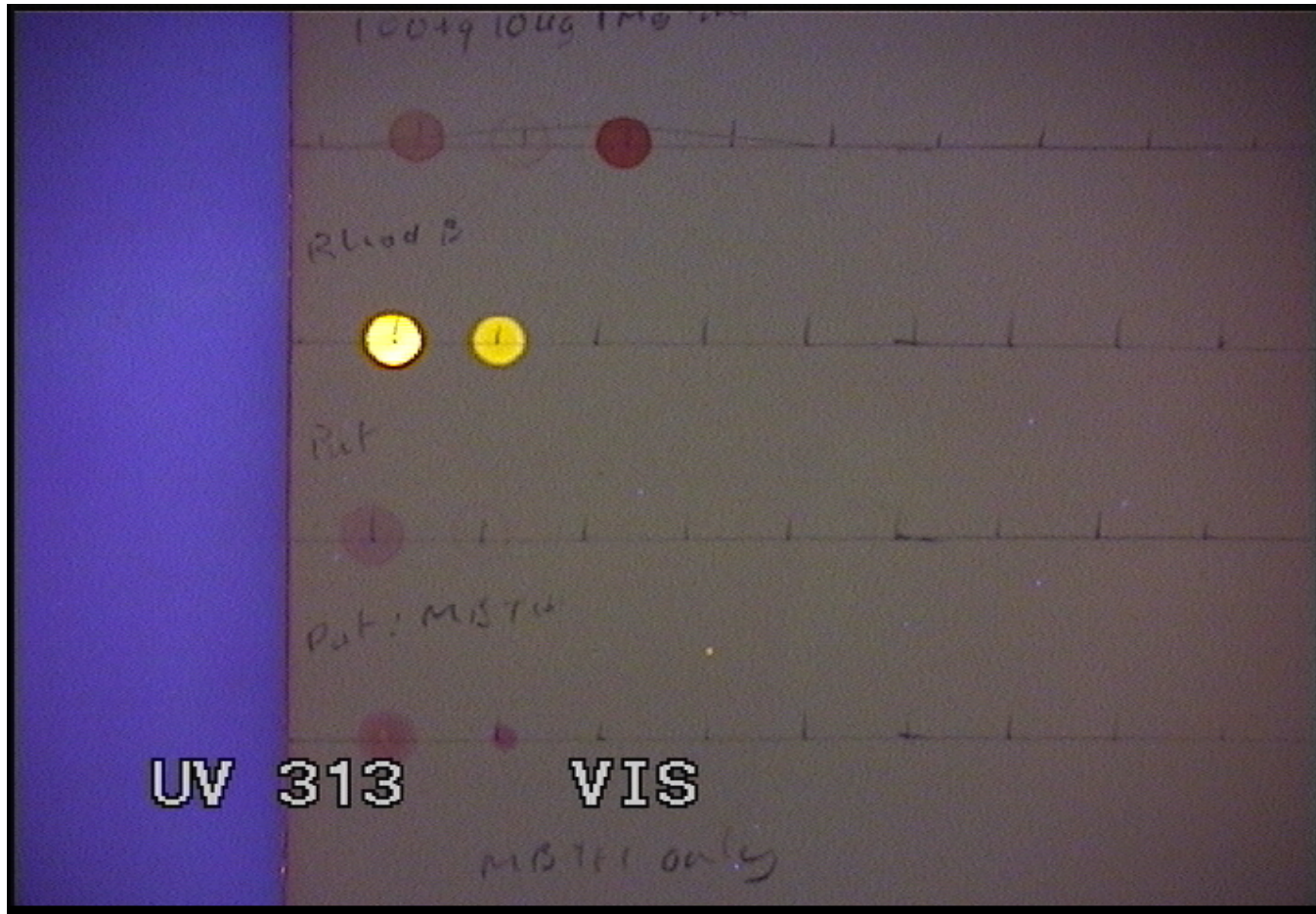
Optimum wavelength for fluorescent detection for Food Additives Results



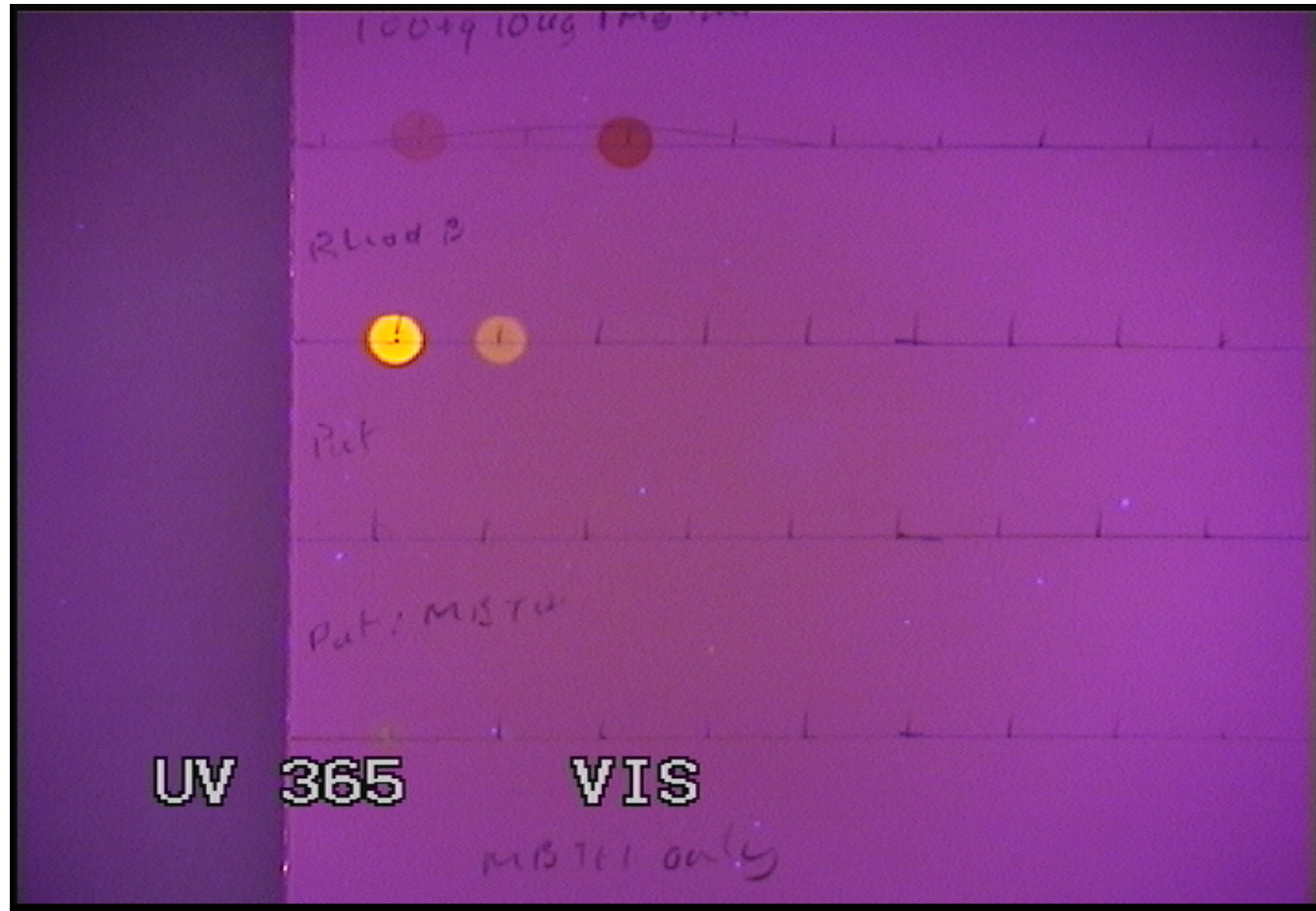
Optimum wavelength for fluorescent detection for Food Additives Results



Optimum wavelength for fluorescent detection for Food Additives Results



Optimum wavelength for fluorescent detection for Food Additives Results



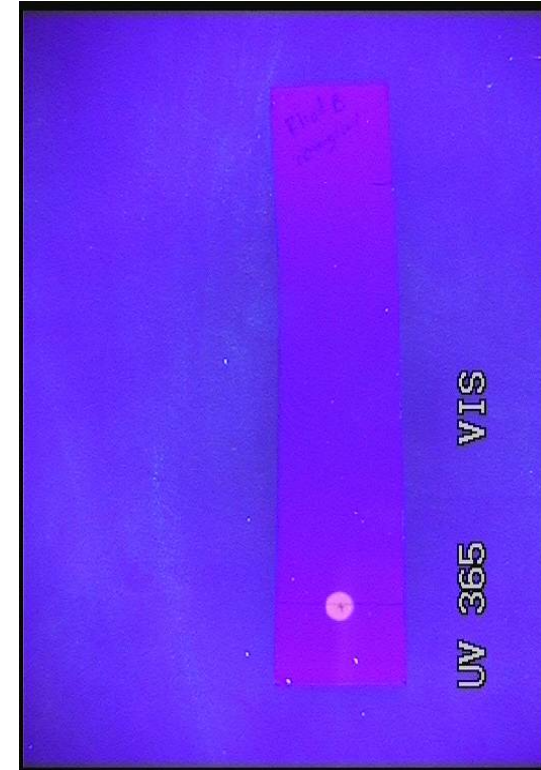
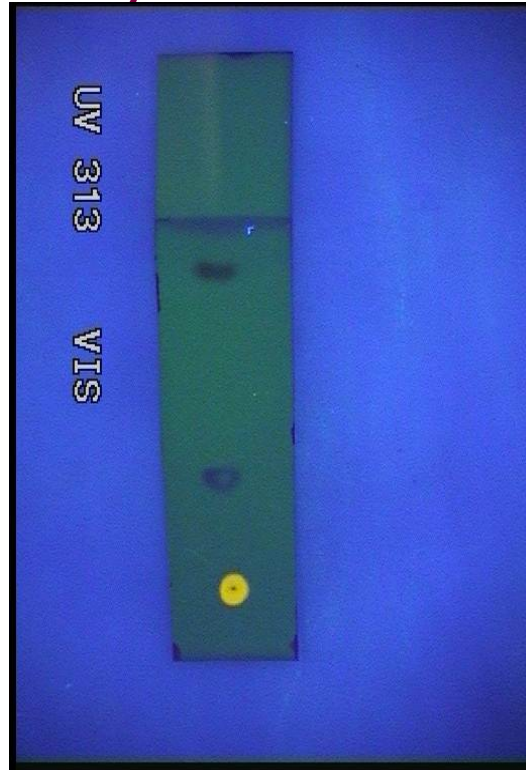
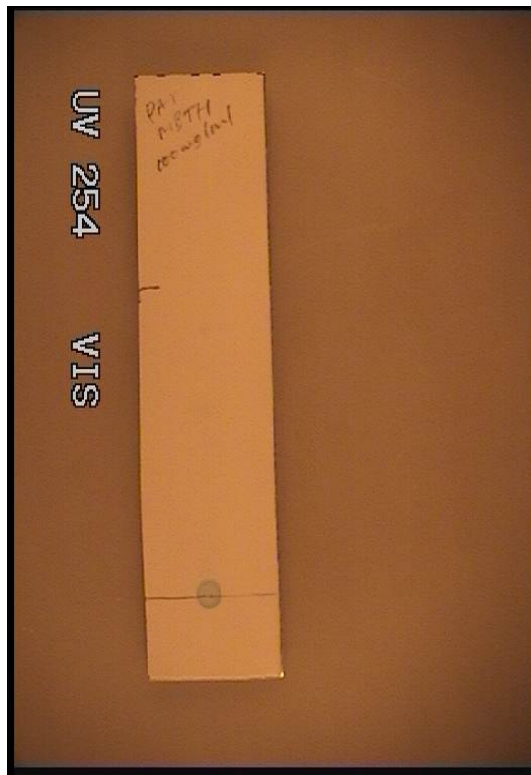
Patulin (with and without MBTH), Rhodamine B and Sudan1 were investigated at visible/IR, 254nm, 313nm and 365nm in various solvent systems

- Volume application 4X 100ug/ml, 10ug/ml
- Toluene:ethyl acetate:formic acid (6:3:1)
- Ethyl acetate:ethanol:water 7:3:2
- Ethyl acetate :toluene: formic acid (6:3:1)

TLC solvent systems for optimal food additive separation

Results

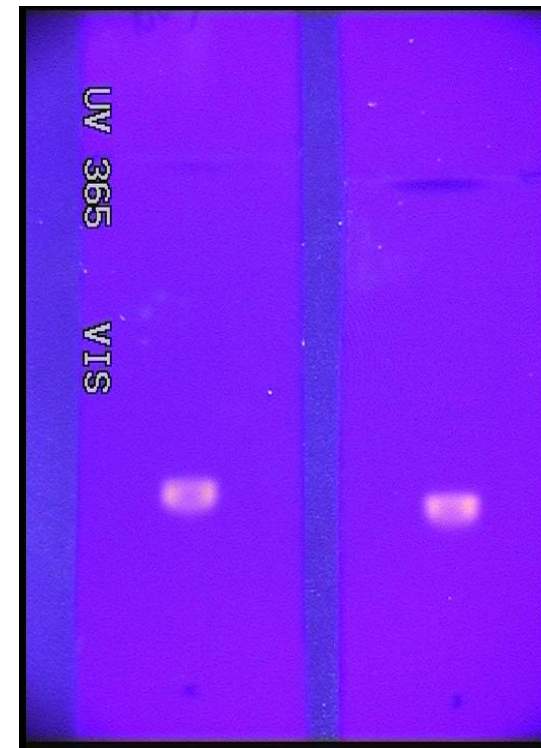
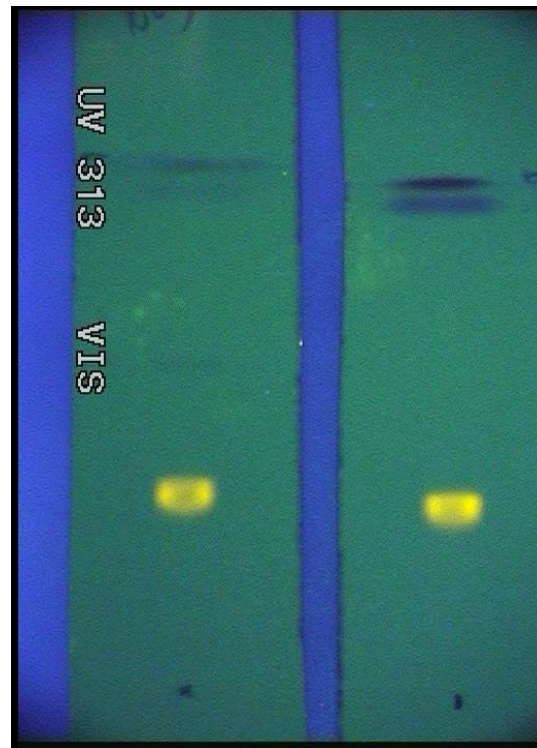
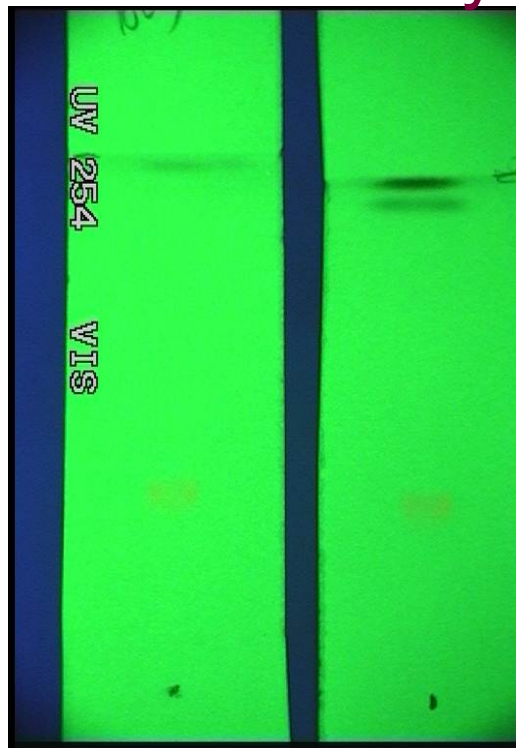
Toluene:ethyl acetate:formic acid



TLC solvent systems for optimal food additive separation

Results

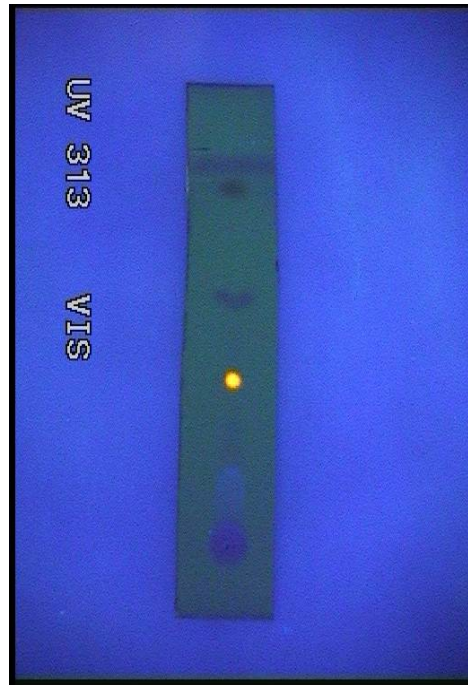
Ethyl acetate:ethanol:water 7:3:2



TLC solvent systems for optimal food additive separation (directly from apple juice)

Results

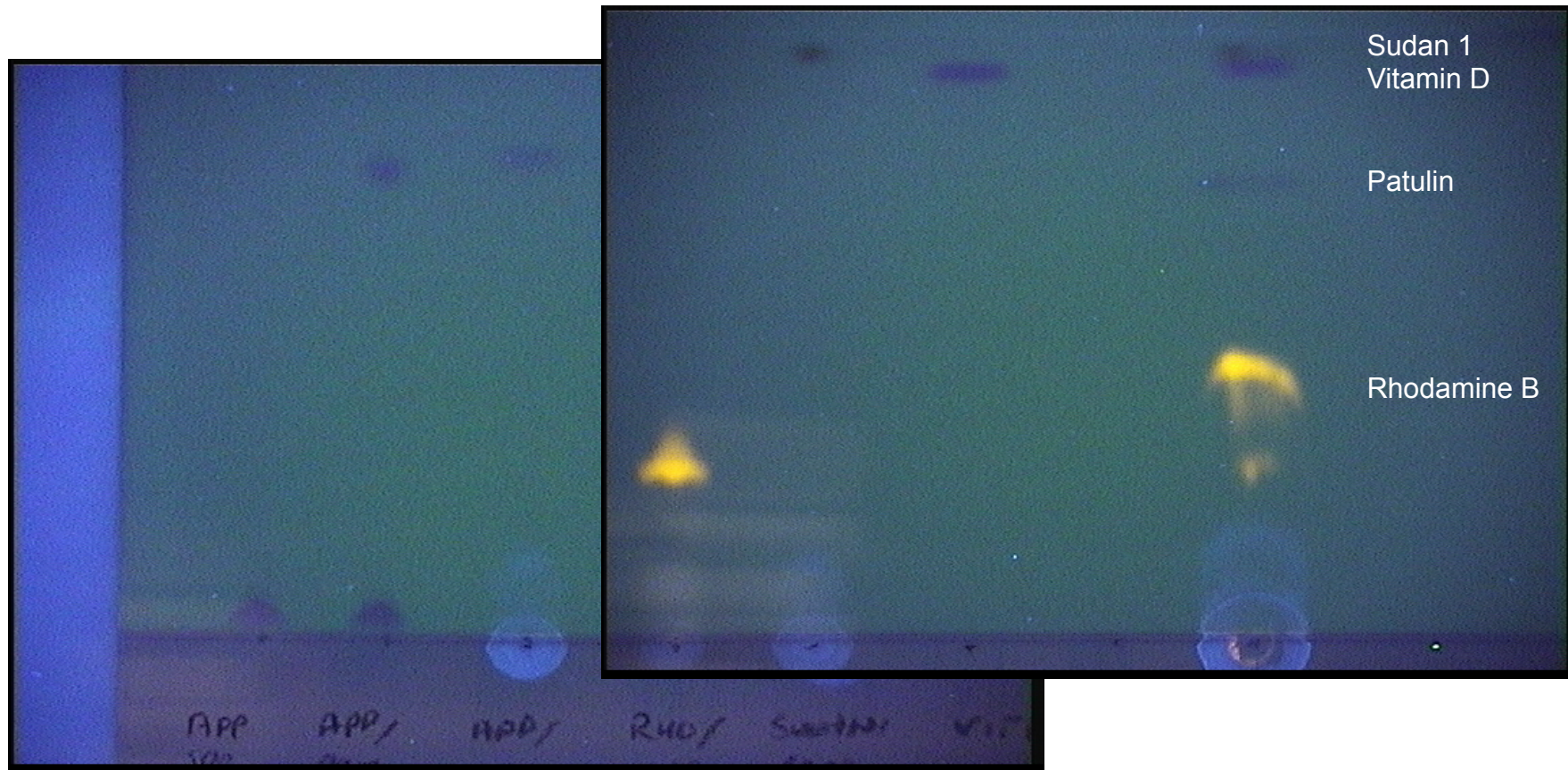
Ethyl acetate:toluene:formic acid 6:3:1



both SPE and directly from apple juice

Results

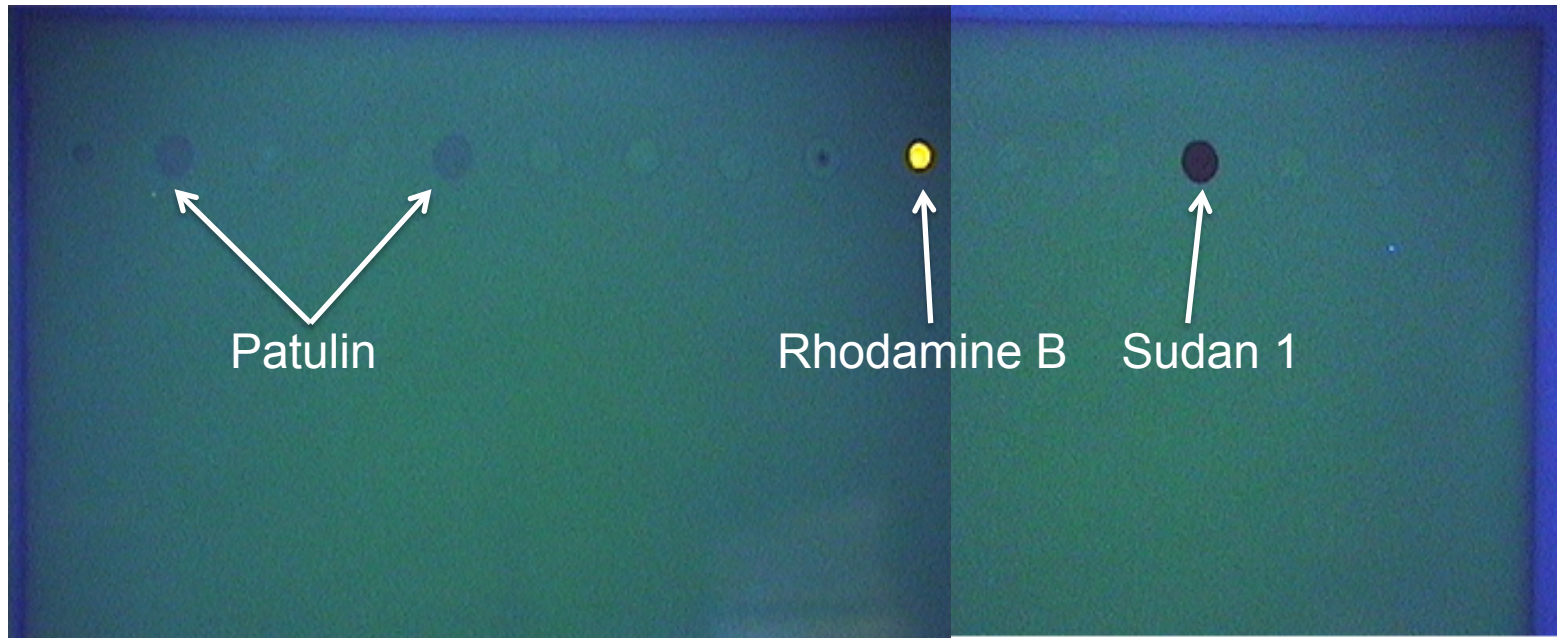
Ethyl acetate:toluene:formic acid 6:3:1



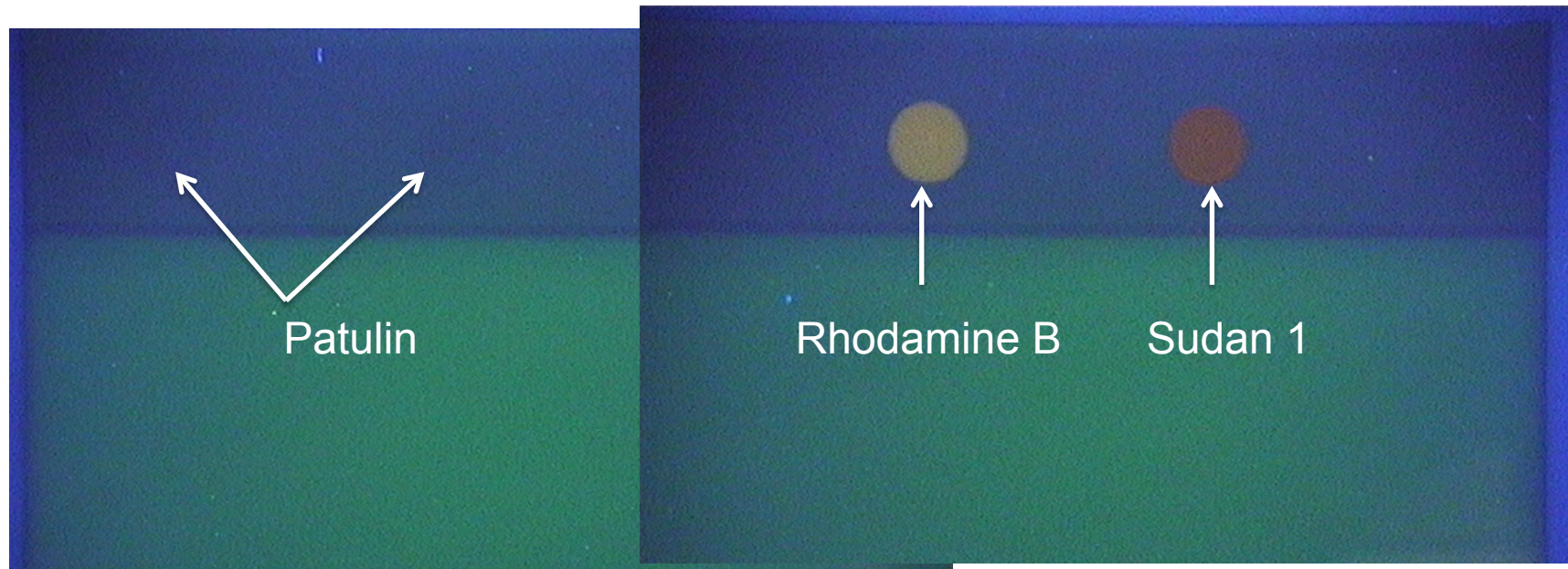
| | | | | | | |
|--------------|----------------|---------------------|--------------------|---------------------|-------------------|-----|
| Apple SPE | Patulin SPE | Patulin in apple | Rhod B in apple | Sudan 1 in apple | Vit D in apple | Mix |
|--------------|----------------|---------------------|--------------------|---------------------|-------------------|-----|

Work Package 1 Objectives: 01/01/14-28/02/14
Extraction protocol for chosen contaminants/additives

Work Package 3 Objectives: 01/03/14-30/04/14
Full TLC protocols for contaminants/additives

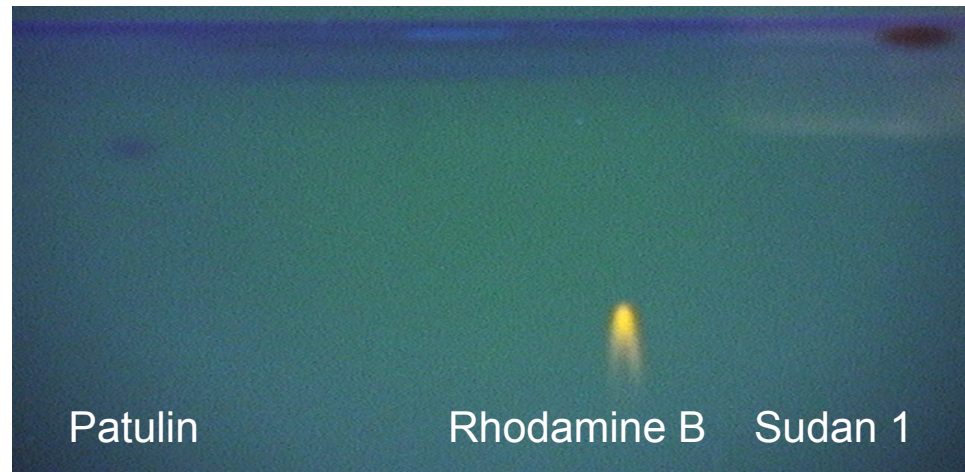


Merck TLC silica gel 60 F254 with concentrating zone plate spotted using a Gilson Automated spotter
Spotting volume: 500µl, 500µl, 500µl, 100µg/L Rhodamine B and 100µg/L Sudan 1, visualised at 313nm using a Foster and Freeman video spectral comparator.



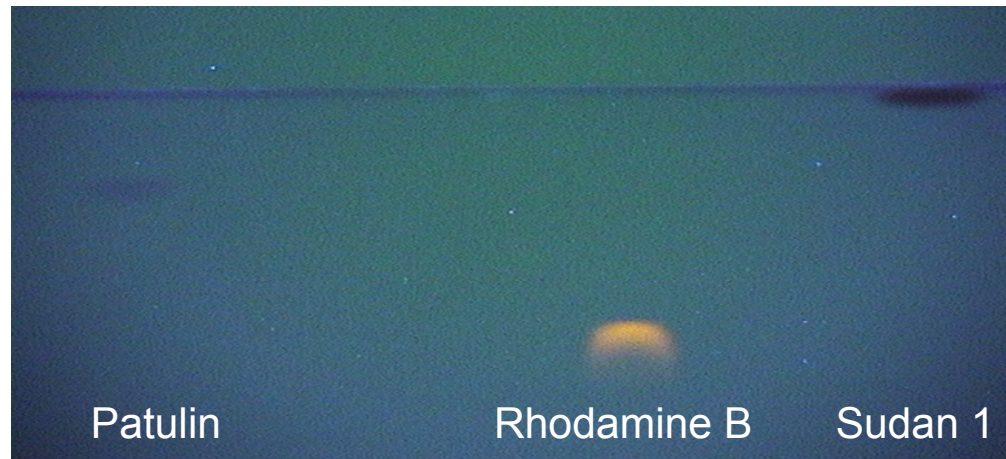
TLC analysis of Merck TLC silica gel 60 F254 plate spotted using a Gilson Automated spotter

Solvent :Ethyl acetate :toluene: formic acid (6:3:1)

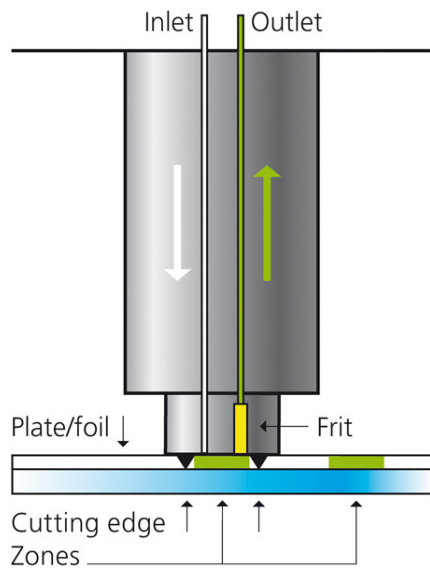


Solvent :Ethyl acetate :toluene: formic acid (6:3:1)

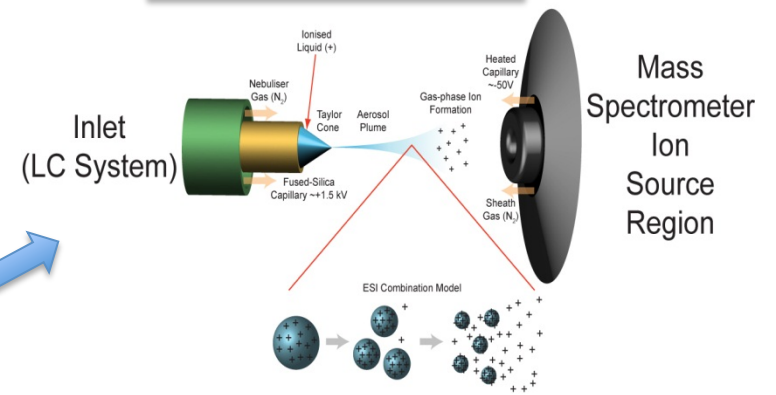
TLC analysis on Merck TLC silica gel 60 F254 with
concentrating zone plate spotted using a
Gilson Automated spotter



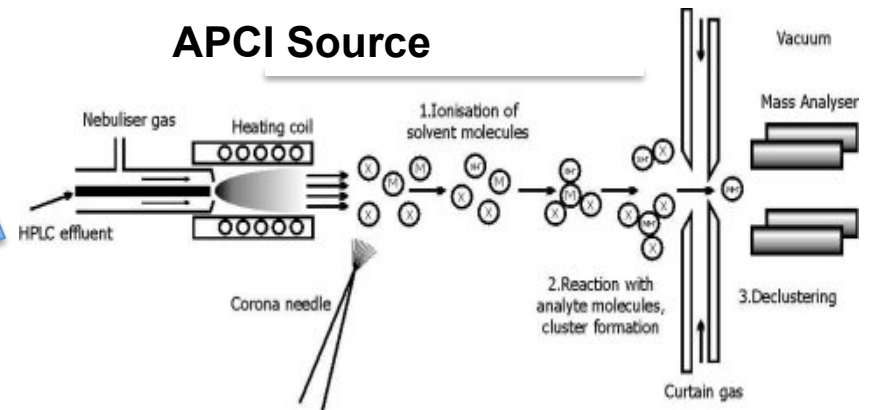
Confirmation by TLC-MS

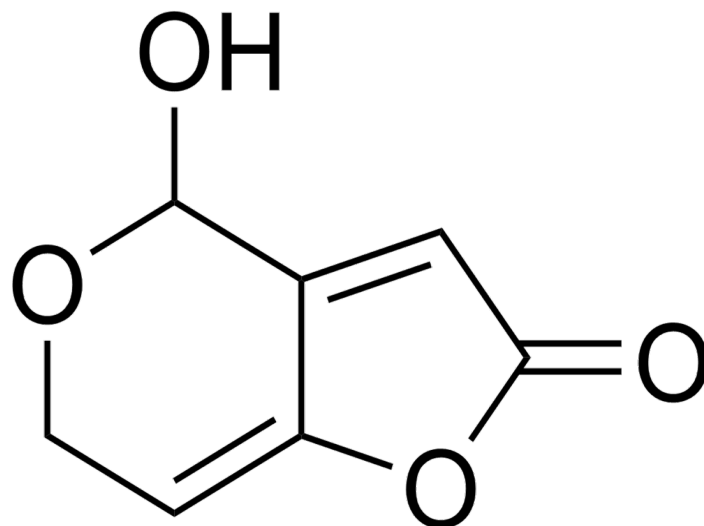


Electrospray Source



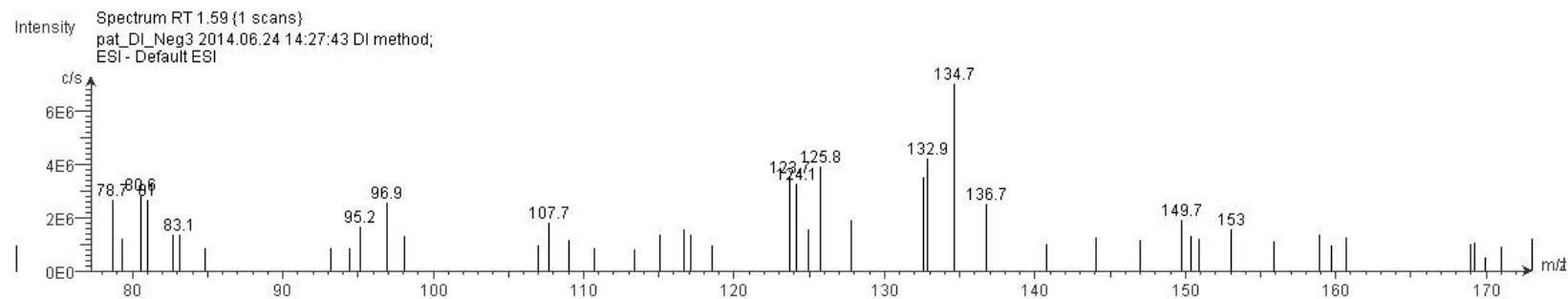
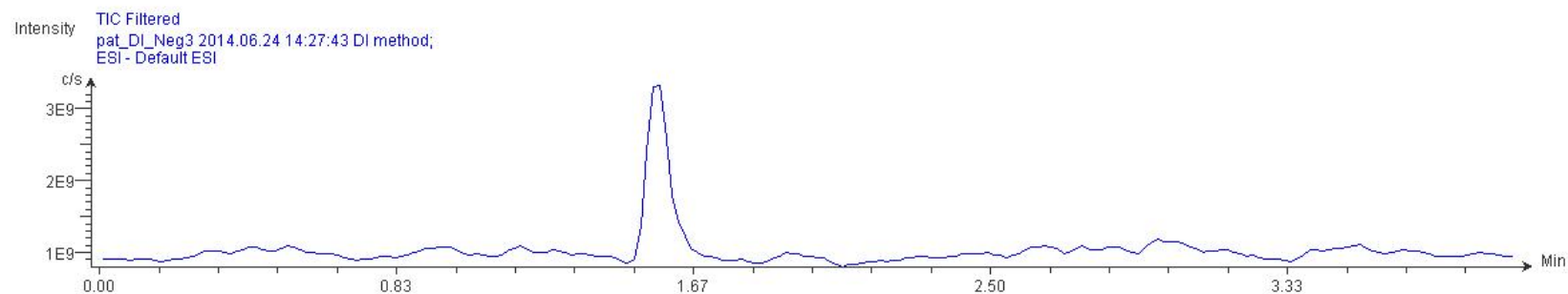
APCI Source





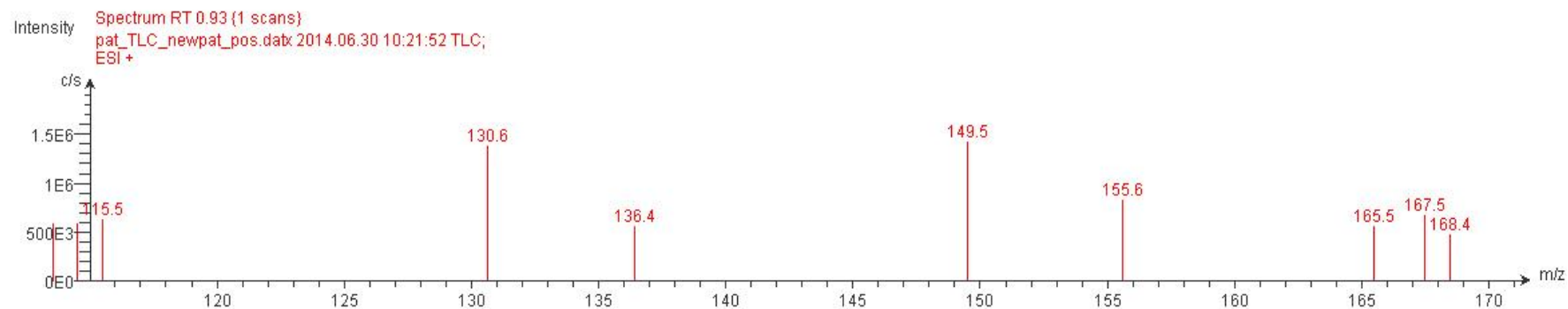
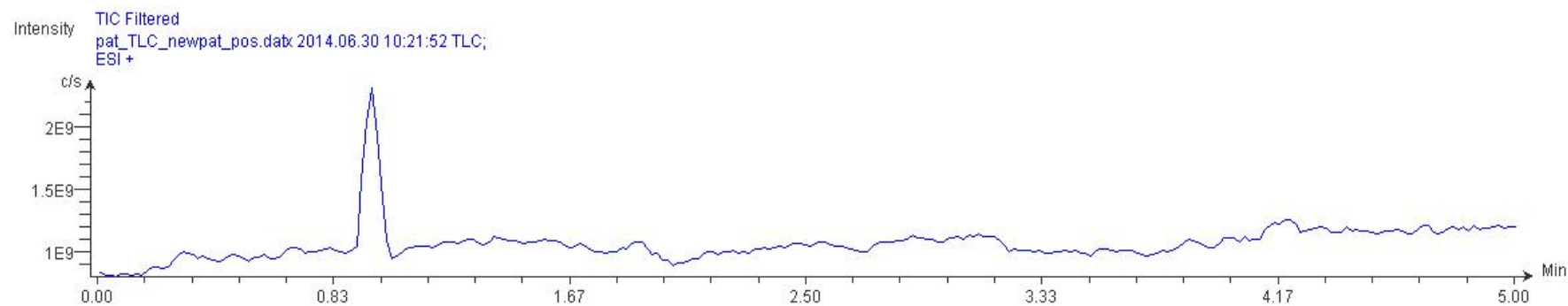
Analysed directly from TLC using the Advion Expression compact MS system fitted with a CAMAG TLC interface

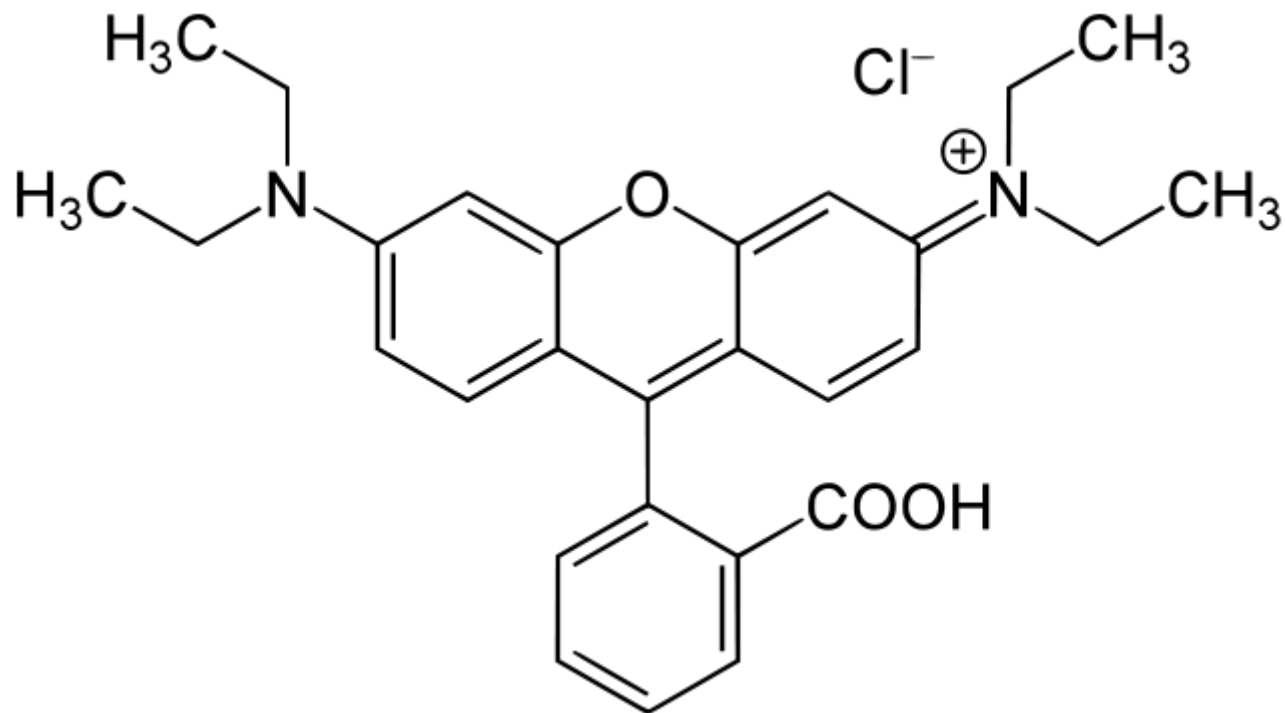
Solvent : 80% methanol 20% H₂O (0.1% formic acid)
Patulin M-H⁺ 153, M+H⁺



Analysed directly from TLC using the Advion Expression compact MS system fitted with a CAMAG TLC interface

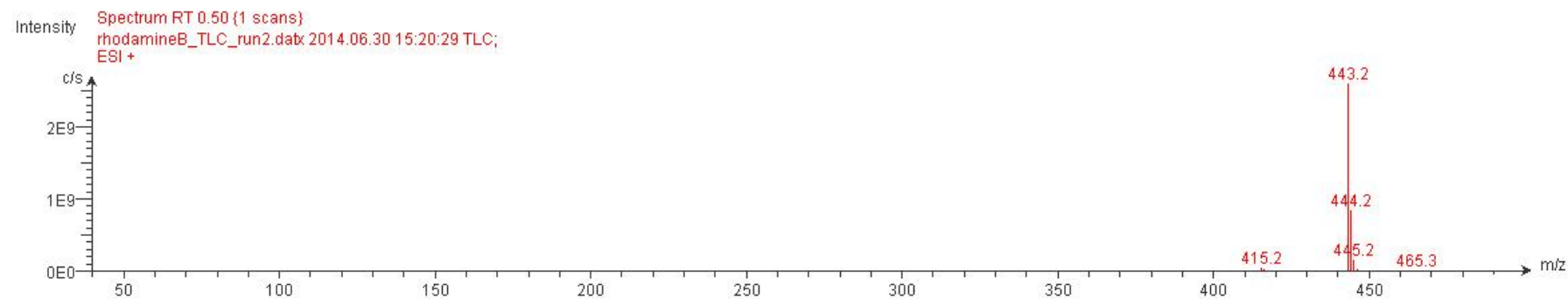
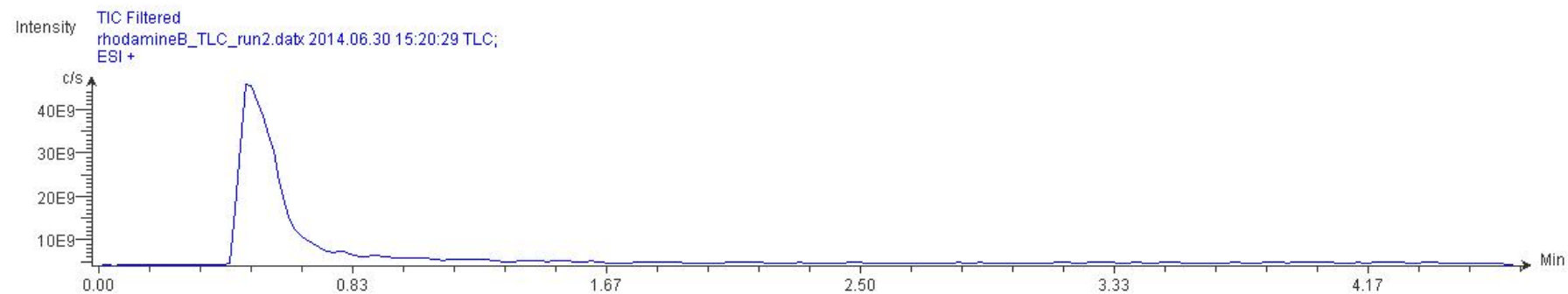
Solvent : 80% methanol 20% H₂O (0.1% formic acid)
Patulin M+H⁺ 155.6



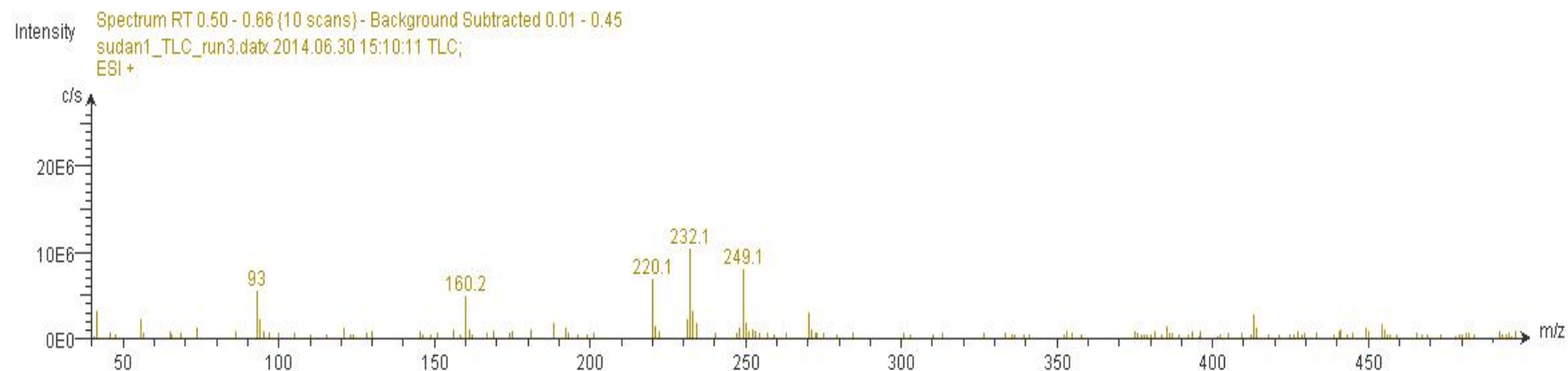
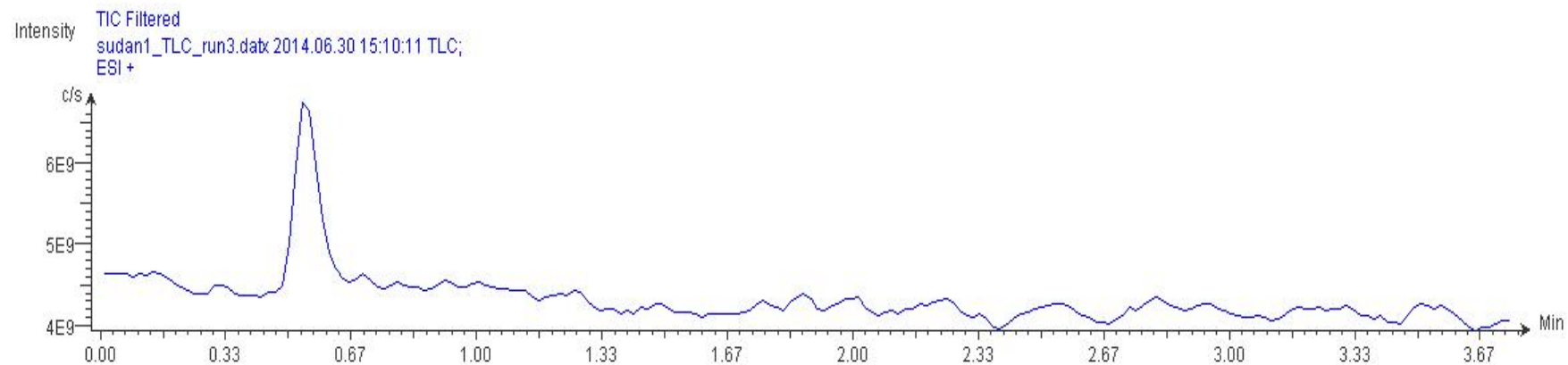


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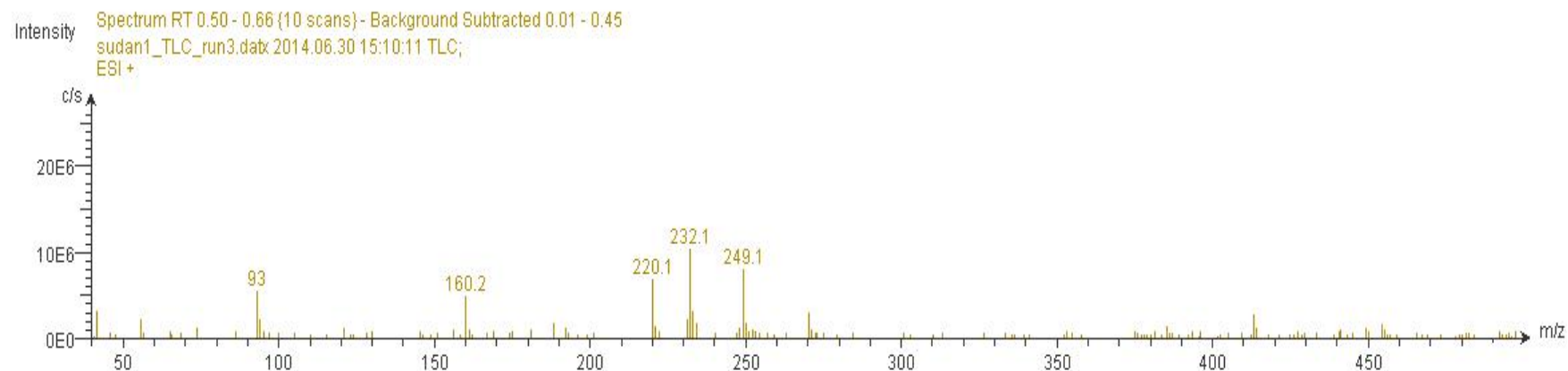
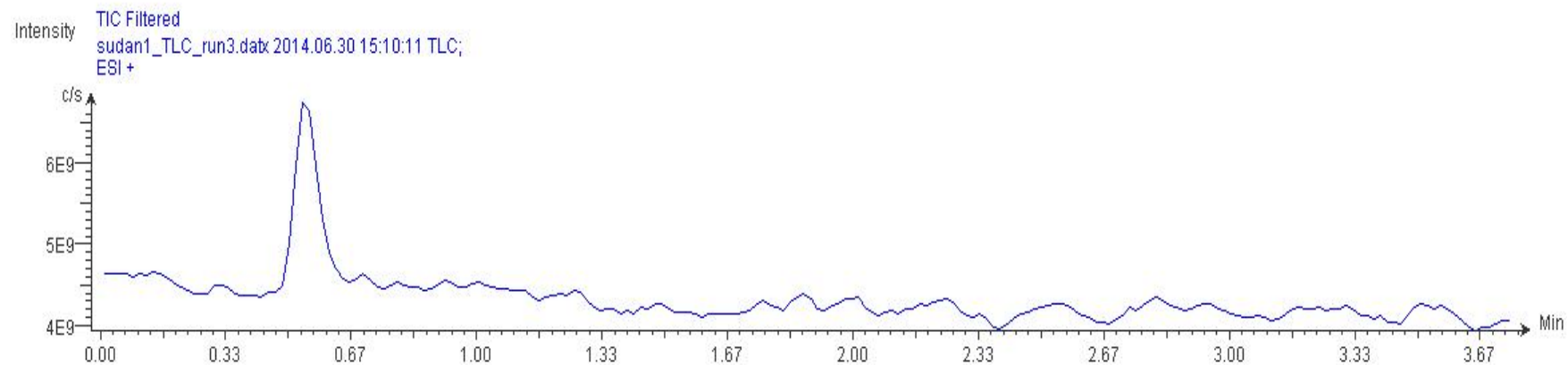
Solvent : 80% methanol 20% H2O (0.1% formic acid)
Rhodamine B M+H⁺-Cl⁻ 443.2, M+H⁺-ethyl 415.2



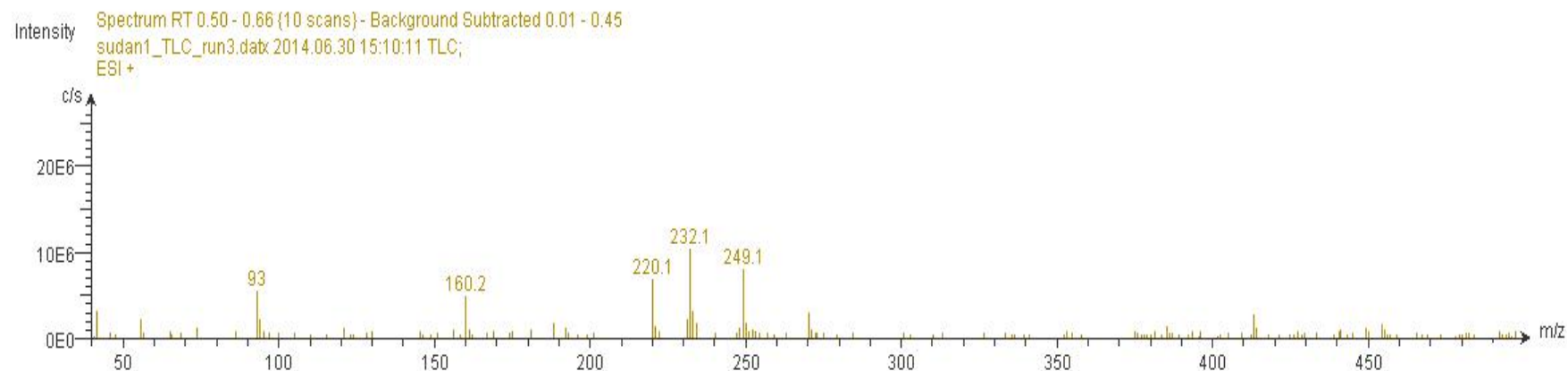
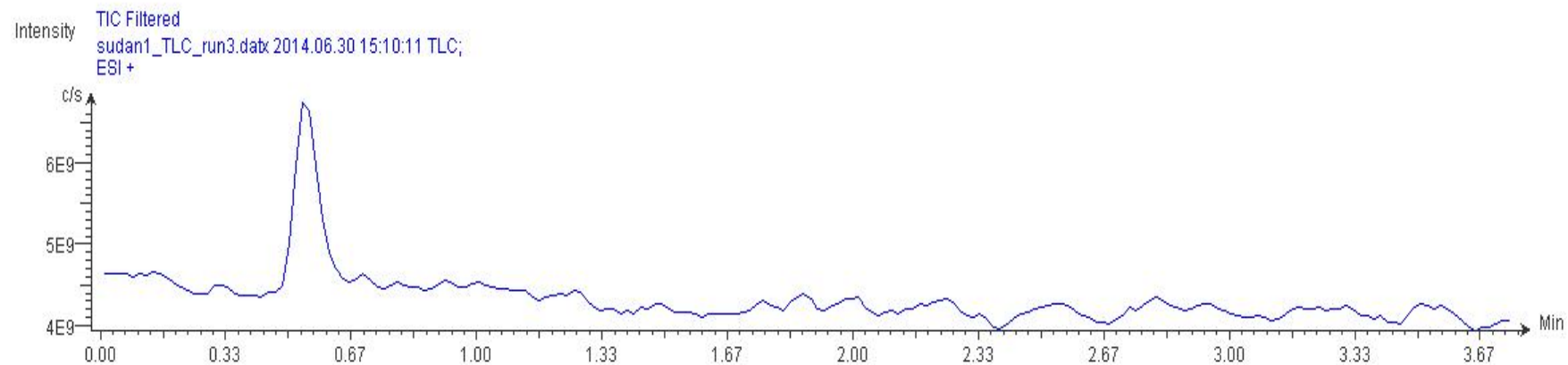
Solvent : 80% methanol 20% H₂O (0.1% formic acid)
Sudan 1 M+H⁺ 249.1, M+H⁺-OH 232.2 Mode APCI+



Solvent : 80% methanol 20% H₂O (0.1% formic acid)
Sudan 1 M+H⁺ 249.1, M+H⁺-OH 232.2 Mode APCI+



Solvent : 80% methanol 20% H₂O (0.1% formic acid)
Sudan 1 M+H⁺ 249.1, M+H⁺-OH 232.2 Mode APCI+



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- filters with transmission wavebands: 400-480, 400-540, 400-580, 450-580, 495-640, 520-640, 560-680, 620-740, 650-740nm and wide band 400-680nm.
- Incident white and infrared

Transmitted white and infrared

Incident ultraviolet, long wave

- 2 x 9W blacklight fluorescent lamps with peak wavelength at 365nm.

Transmitted ultraviolet, long wave

- 2 x 9W lamps, peak emission at 365nm.

Incident ultraviolet, medium wave

- 2 x 6W lamps, peak emission at 313nm, with safety interlocks.

Incident ultraviolet, short wave

- 2 x 6W lamps, peak emission at 254nm, with safety interlocks.

