

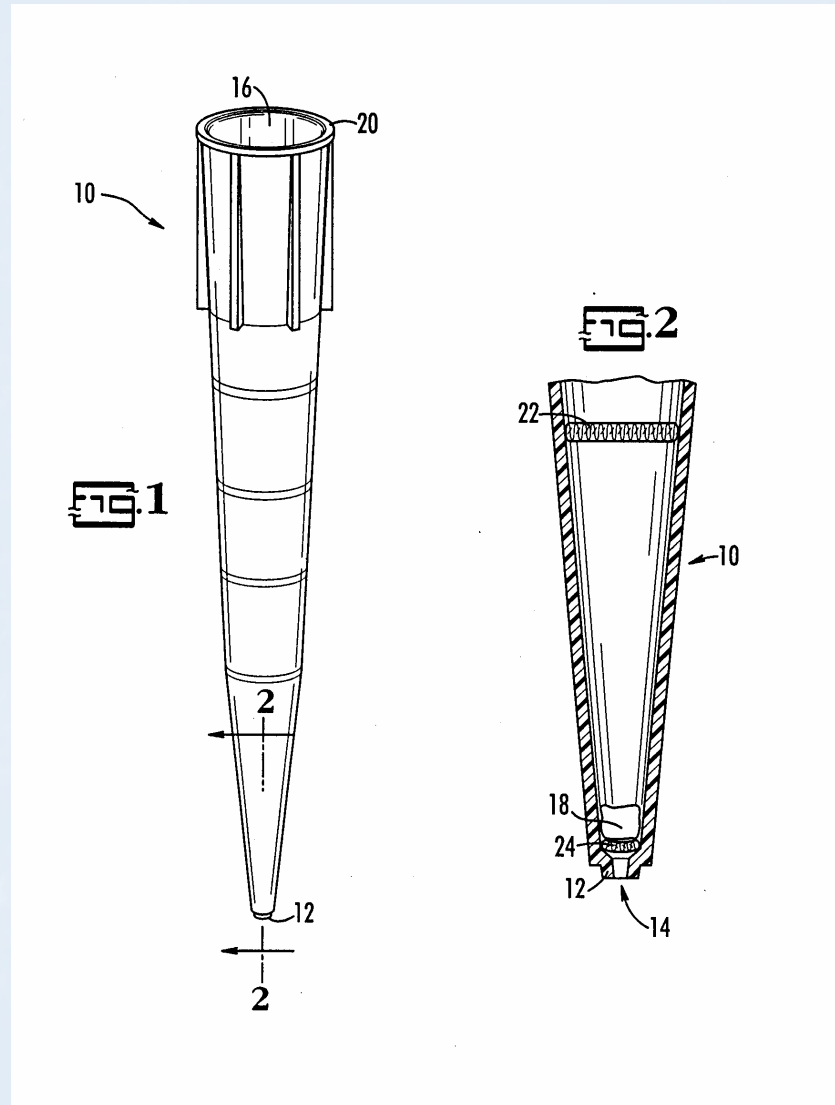
Rapid Analysis of THC and Metabolites Using Disposable Pipette Extraction

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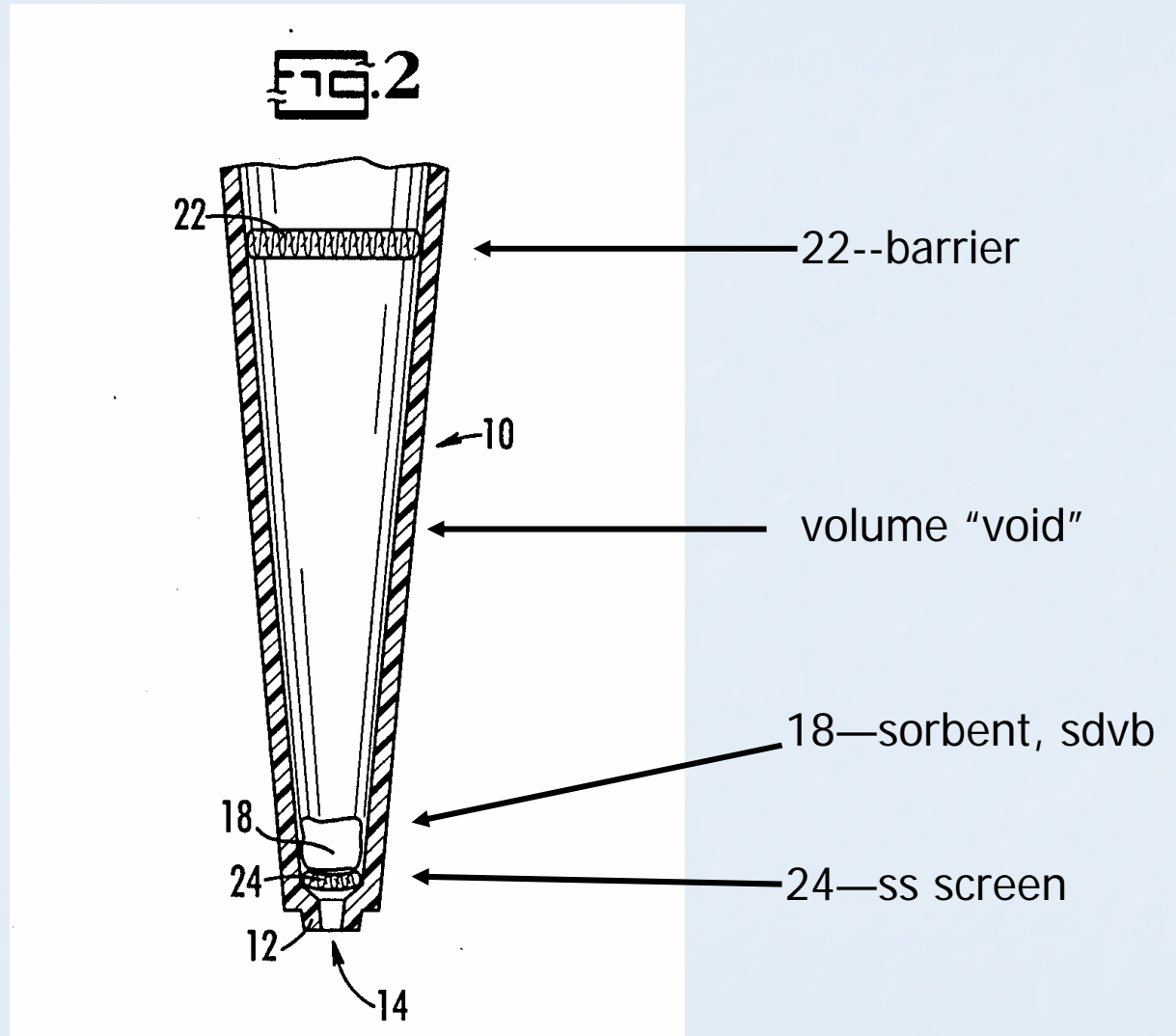
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What is DPX?



Brewer, US patent No. 6,566,145 B2

What is DPX?



Brewer, US patent 6,566,145 B2

DPX demo



dpxDye Demo

DPX advantages

- Extractions are rapid
- Extraction efficiencies based on equil. time
 - easily controlled
 - efficiencies not dictated by flow rates
- Readily automated (or semi-automated)
- Use less sorbent—less solvent required
- Minimal solvent waste generated

Extraction Goals (GC/MS)

- Analysis of THC at 1 ng/mL with 3 ions
- Analysis of COOH-THC at 5 ng/mL with 3 ions
- One derivatization/one chromatogram per sample
- TMS derivative
- Minimal solvent and glassware usage (min. waste)
- Minimal time for extraction

Chromatographic conditions

- GC column: DB-5 ms, 30m, 250um ID, 0.2um
- Oven temp: 80 °C hold 1 min; ramp 40 C°/min to 200 °C; ramp 10 C°/min to 240 °C, hold 5 min; ramp 10 C°/min to 300 °C, hold 5 min (total GC run of 23 min).
- Column flow set at constant flow at 2mL/min (He)
- Selected ion monitoring (5973 MSD, Agilent Technologies) :
 - THC : 386, 371, 303, 389 (is)—50 ms
 - COOH-THC: 473, 371, 488, 476 (is)—50 ms

Preliminary Studies

(moderate results)

- Addition of 0.1 M HCl to sample solution:
 - Improves recoveries of COOH-THC
 - Increases interferences of THC analysis
- Elution with hexanes/ethyl acetate:
 - Improves “cleanliness” of THC analysis (with acid*)
 - Decreases recoveries of COOH-THC (more polar)

Preliminary Studies

(good results)

- Addition of water to sample solution:
 - Improves “cleanliness” of chromatograms
 - Good recoveries for THC and COOH-THC
- Elution with acetonitrile/ethyl acetate:
 - Improves recoveries of COOH-THC
 - “Clean” chromatograms for THC (with water*)

DPX method (cont'd)

Initial Preparation of samples (time consuming)

1. Pipette 1 mL samples, add internal standard, vortex
2. Add 1.5 mL acetonitrile, vortex mix
3. Centrifuge, transfer supernatant with Pasteur pipette
4. Add 2 mL DI water

DPX method

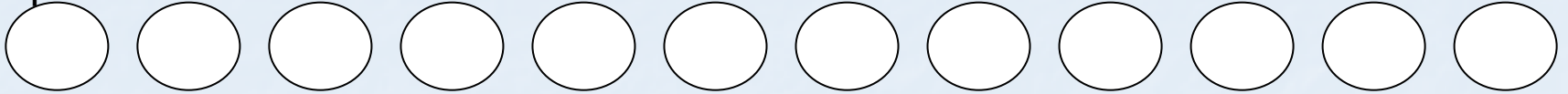
DPX preparation

5. Pipette 0.8 mL 33% acetonitrile solution for cond/wash
6. Pipette 0.4 mL 50/50 acetonitrile/ethyl acetate for elution
7. Place (labeled) sample tubes in test tube rack with cond/wash tubes, elution tubes and conical tubes (vials) in corresponding positions

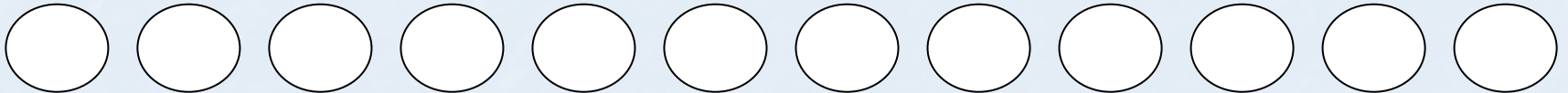
Test tube rack with test tube solutions for DPX

1 2 3 4 5 6 7 8 9 10 11 12

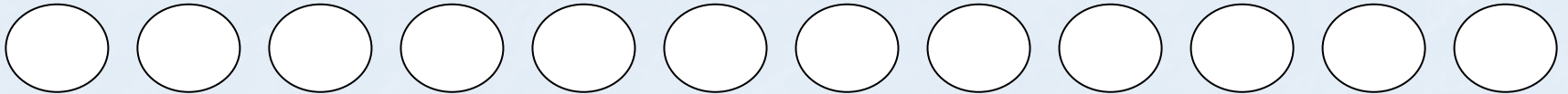
samples



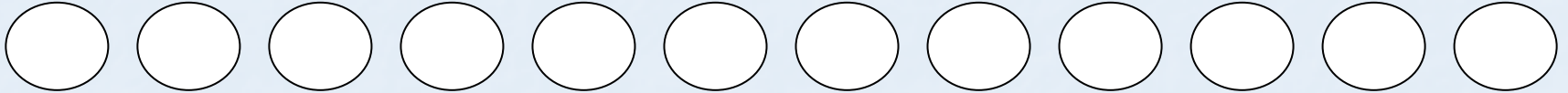
condition/wash solutions



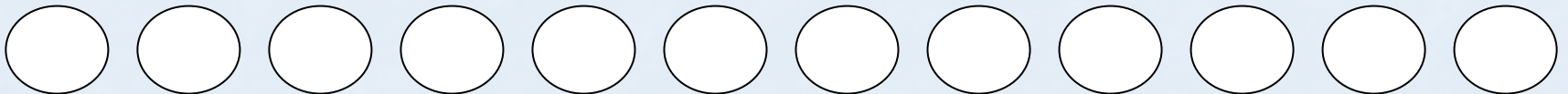
elution solvent



conical tubes



GC vials (labeled*)



Match sample labels to final tube/vial labels—track samples and maintain chain-of-custody.

Manual extractions with multiple syringe devices or semi-autosampler (12 (or 24) simultaneously).

DPX method (cont'd)

DPX extraction (12 simultaneously with semi-auto)

8. Draw in conditioning/wash solution and mix with air, dispense back into tubes
9. Draw in sample solution and mix with air
10. Wait 30 s, dispense back into tubes
11. Draw in wash solution and mix with air, dispense back into tubes
12. Draw in elution solvent, wait 10 s, dispense into labeled tubes
13. Transfer upper layer to silanized conical GC vials

DPX method (cont'd)

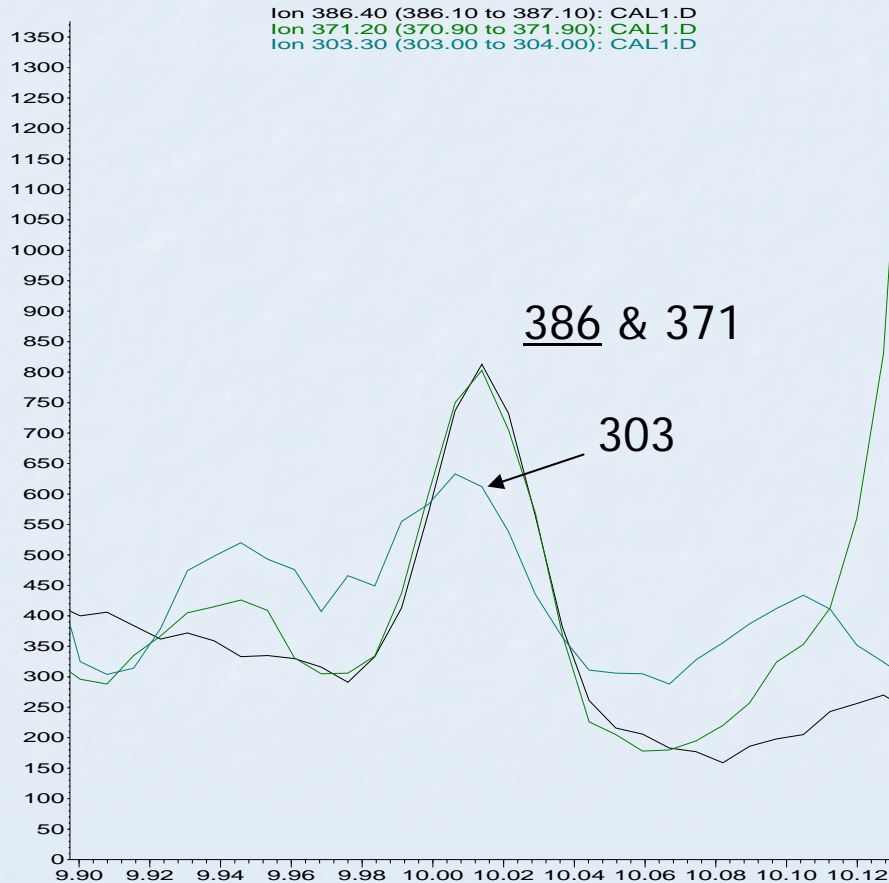
Derivatization (time consuming)

16. Dry solvent with N₂ and heat at 40 °C in labeled GC vial
17. Add 20 uL BSTFA and 20 uL ethyl acetate
18. Heat at 90 °C for 30 min
19. Cool, place on autosampler, and shoot into GC/MS
20. TAKE A BREAK!

GC/MS chromatograms

1ng/mL THC

Abundance



Very clean extracts

One elution step used

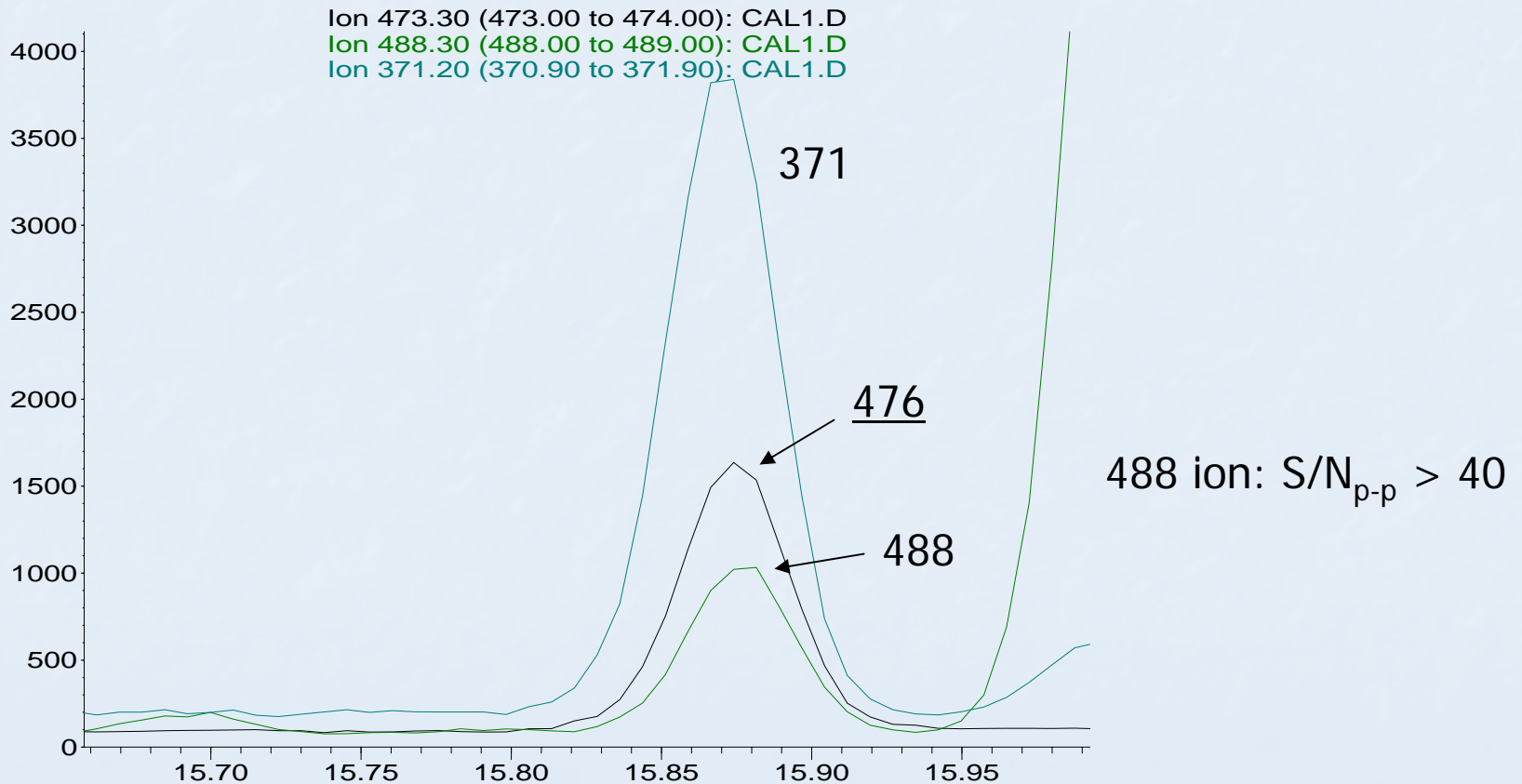
2nd elution improves sensitivity

Time-->

GC/MS chromatograms

5ng/mL COOH-THC

Abundance



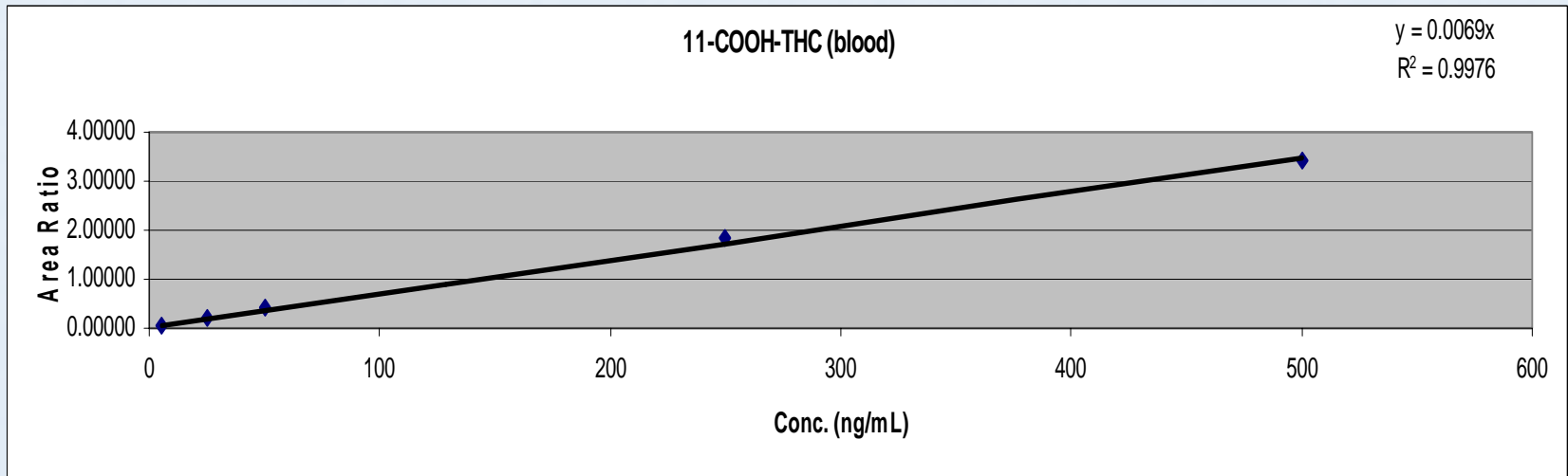
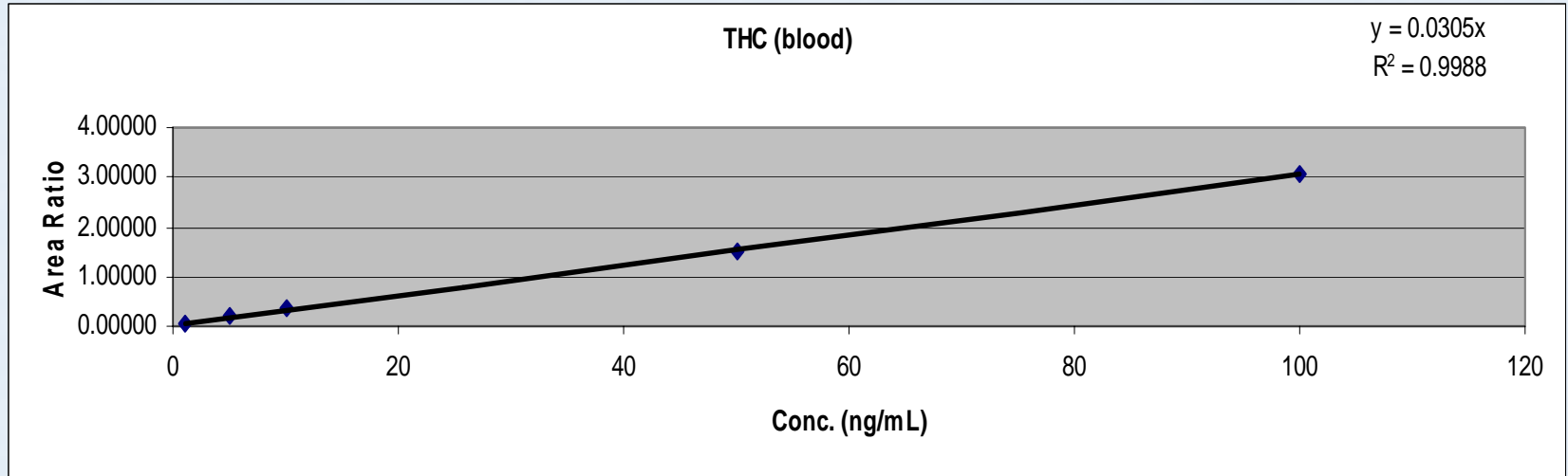
Time-->

Statistical data

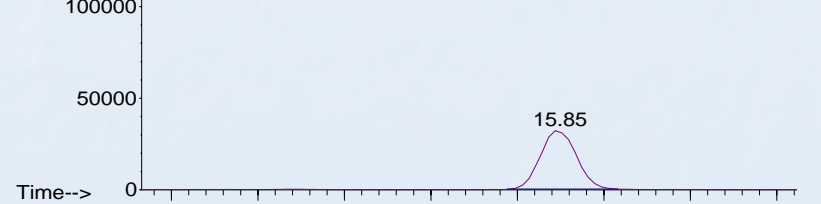
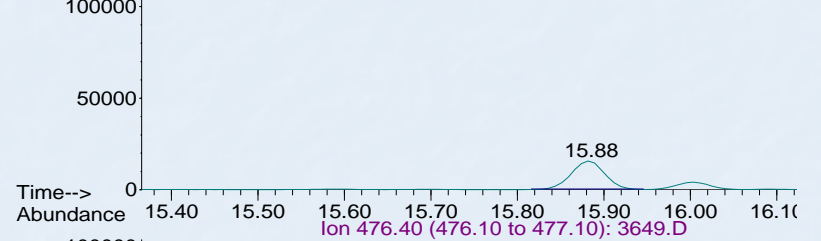
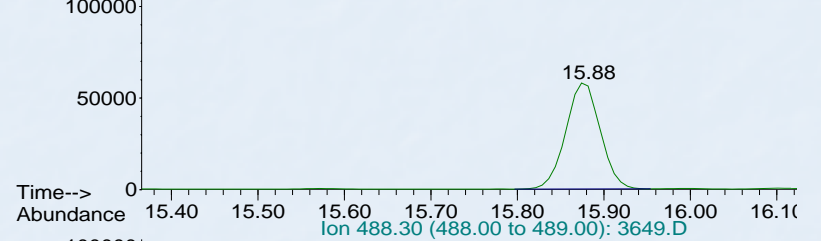
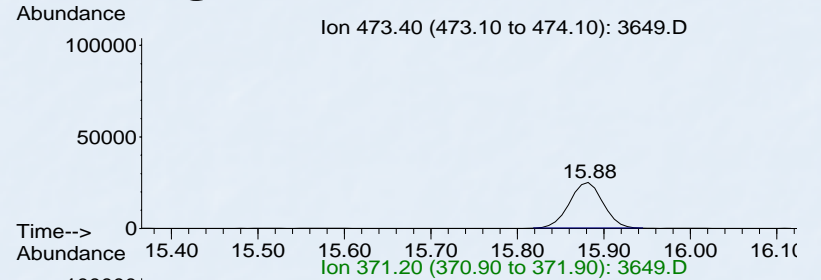
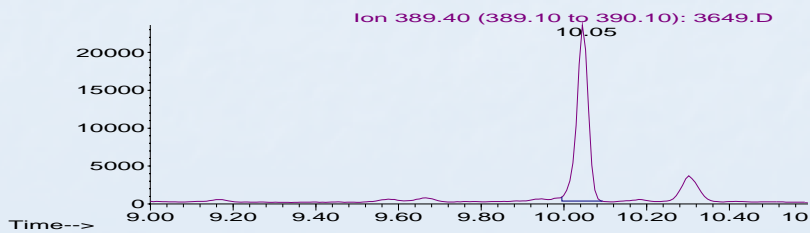
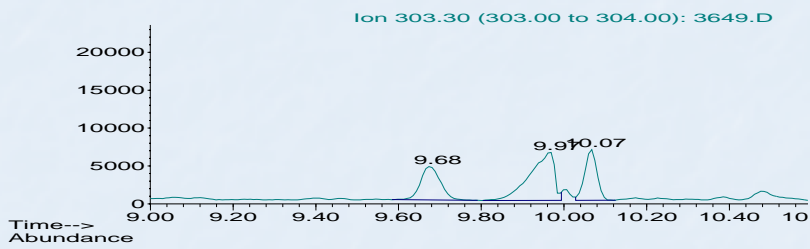
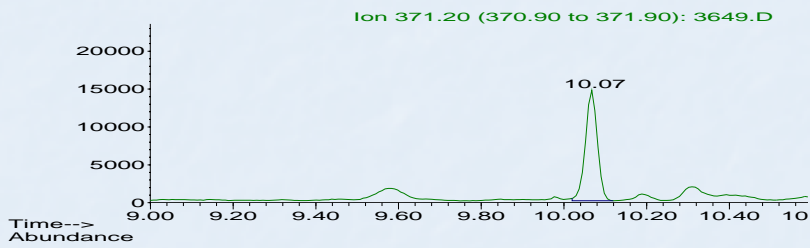
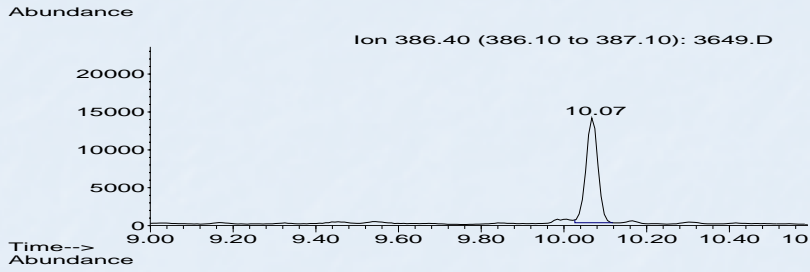
	THC	COOH
LOD (ng/mL)	1	<5
Recov. Post ppt.-1 EI	~ 50%	~ 40%
Recov. Post ppt.-2 EI	70-80%	50-60%
C.V. (lowest cal.)	10.1%	4.7%

Protein precipitation results in greatest losses of recoveries.

Calibration Data



Case Study



Conclusions

- Achieved 1 ng/mL LOD for THC and less than 5 ng/mL for COOH-THC
- Rapid extractions using 1 conditioning-wash solution and 1 elution solvent
- Combined GC/MS of THC and COOH-THC in a single analysis
- Great reproducibility with clean extracts
- Very reliable method—minimize “repeats”

Acknowledgments

- DPX Labs, LLC, for supplying DPX tips and semi-autosampler for study

Visit www.DPXLabs.com for more information.

Questions?