



Identification of THC-COOH in oral fluid using LC-MS/MS: Essential analyte in workplace testing



Cynthia Coulter, Margaux Garnier, Christine Moore
Immunalysis Corporation, Pomona, CA, U.S.A.

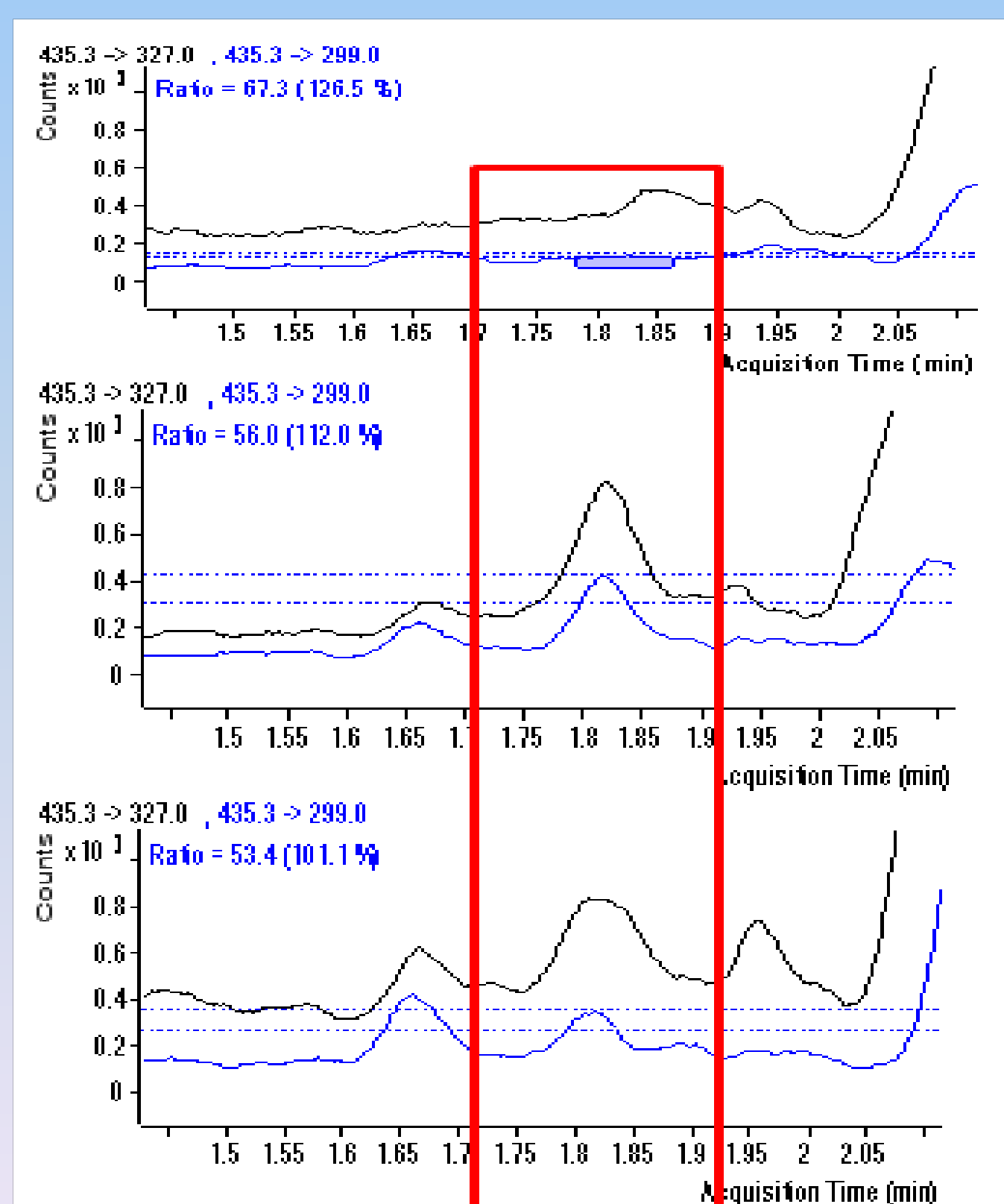
Abstract

• The detection of the marijuana metabolite THC-COOH in oral fluid (OF) minimizes the possibility of identifying individuals from testing positively for marijuana after passive exposure to smoke. Its determination requires instrumentation which is extremely sensitive since the concentrations present are very low; compared to the parent drug, THC.

Objectives

• Our objective was to develop a procedure for analysis using LC-MS/MS and apply the method to authentic specimens.

Calibrator and authentic oral fluid specimen



Negative
Extracted Sample

Extracted Calibrator
THC-COOH
20pg/mL

Extracted
Authentic Specimen
23pg/mL

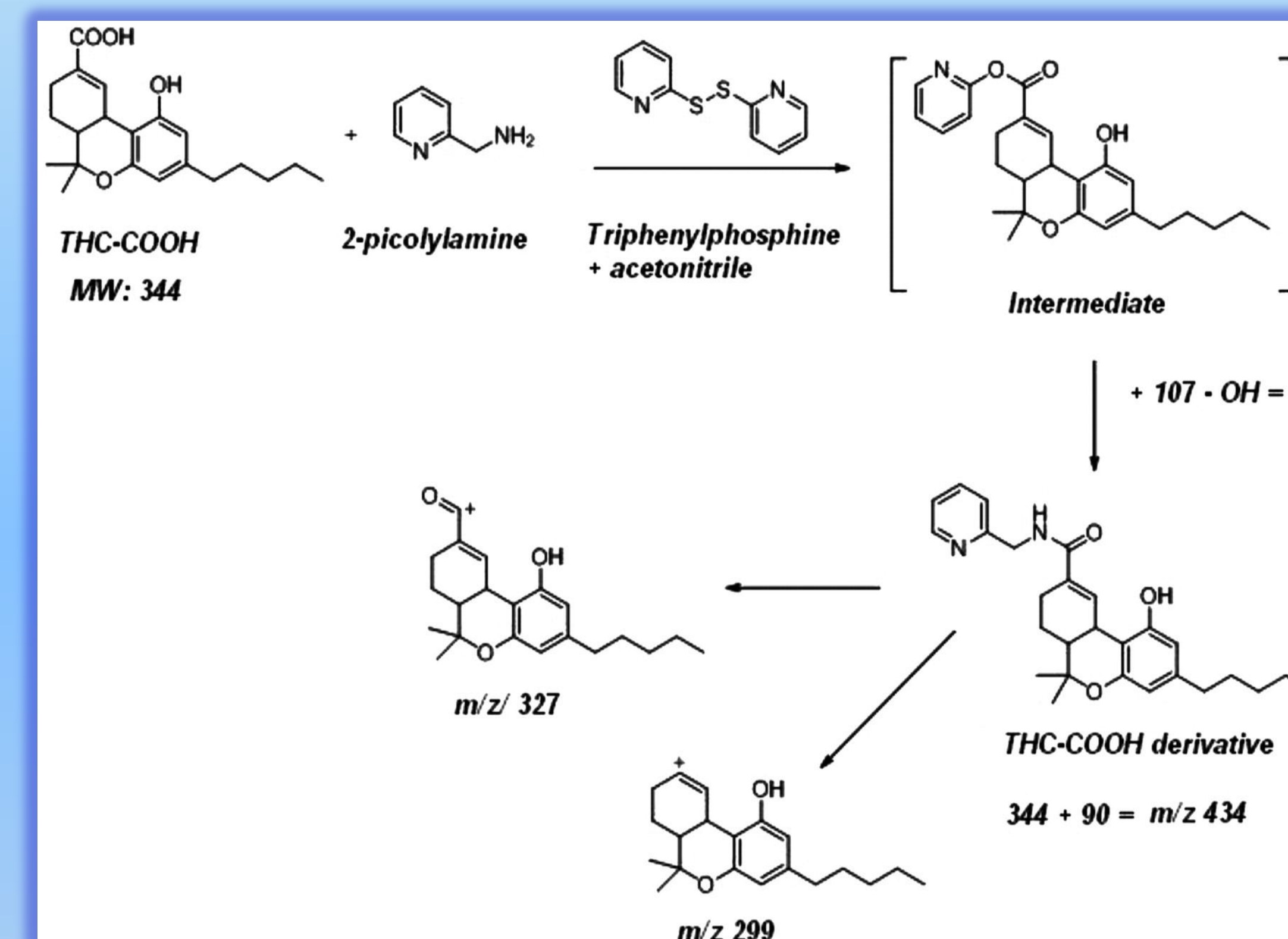
Methods

- To achieve the requisite sensitivity for THC-COOH an Agilent Technologies 6490 LC-MS/MS instrument, base hydrolysis and extraction of the drug from OF, and derivatization were employed.
- Samples were hydrolyzed and extracted according to a fully validated and previously published method.
- Once samples were eluted and evaporated to dryness TPP(10mM); DPDS(10mM) and 2-PA(10µg) were added to the vial capped and heated for 15 min at 60°C. The derivative was allowed to cool and evaporated to dryness then reconstituted in ACN: deionized water (50:50; 25µL).
- Extracts were run in (+) ESI with Jet Stream technology with a 20uL injection on a Stable Bond C-18 (2.1 x 50mm x 1.8µm) column. Mobile phase A consisted of ammonium formate (pH 6.4) with B as 0.5% formic acid in ACN.
- The pump program held B at 70% for 2 minutes; by 4 minutes B was held at 90% with a stop time of 7 minutes. The flow rate was held constant at 0.2 mL/min.
- All voltages; gas flows; and temperatures were optimized for the highest level of sensitivity and reproducibility.
- Transitions for THC-COOH and THC were **435.3>327.0**; 435.3-299.0 and **315.3>193.3**; 315.3-123.3 respectively. Qualifying transitions for THC-COOH 2-PA were 41.6%-62.4% and THC were 47%-71%.

Results

- The procedure was developed and fully validated. The limit of quantitation for THC-COOH was 10pg/mL and linearity was established up to 1000pg/mL. The limit of quantitation for THC was 1ng/mL and linearity was established up to 100ng/mL.
- Six replicate analyses over 5 days (n=30) at 12pg/mL and 75pg/ml resulted in an imprecision of 6.5% and 1.9% for THC-COOH and replicates at 12ng/ml and 75ng/ml resulted in an imprecision of 4.0% and 4.8% for THC. Authentic oral fluid specimens as well as proficiency samples were analyzed for both THC and THC-COOH.
- In the case of 2 authentic oral fluid specimens THC levels were at 1.2 and 2.1ng/mL with corresponding THC-COOH levels at 14 and 23pg/mL; illustrating the need for very sensitive methods. Other samples ranged in THC concentrations from 11 - 3440ng/mL with corresponding THC-COOH levels 34 - 1651pg/mL.
- There appeared to be some correlation between the THC and THC-COOH concentrations in authentic oral fluid samples, although more samples need to be analyzed.
- All proficiency samples reported for THC and THC-COOH were within 10% of the target value.

Fragmentation pattern for derivatized THC-COOH



Conclusion

- An analytical procedure for the simultaneous determination of THC and THC-COOH in oral fluid with the required sensitivity needed to analyze THC-COOH in oral fluid has been developed using LC-MS/MS instrumentation.
- The adapted use of the 2-picolyamine (2-PA) derivative increased the sensitivity of THC-COOH by 20-fold and had no impairing effects on THC.
- The method when applied to authentic specimens provided a possible correlation between the two compounds THC and THC-COOH.



Oral Fluid

SOFT, Boston, 2012