

Abstract

Specimen collection is an important component of drug testing programs. A split specimen may be obtained by subdividing a single specimen or collecting two near-simultaneously-collected specimens. However, little information is available to validate that “near-simultaneously-collected” oral fluid specimens produce the same result. This report describes studies of healthy, male marijuana (MJ) users in which simultaneous oral fluid specimens (left and right sides of the mouth) were collected following supervised smoking of 1 MJ cigarette (Study #1, N = 10 subjects, collection times: 0-72 hrs.; Study #2, N = 5 subjects, 0-1.75 hrs.); and the oral consumption of 1 MJ cigarette in a “Brownie” (Study #3, oral MJ, N = 3, 0-72 hrs.) Each MJ cigarette contained 20-25 mg of THC. The two simultaneous specimens were collected with the Intercept™ DOA Oral Specimen Collection Device. Screening and confirmation for THC were performed with the Cannabinoids Intercept™ MICRO-PLATE Enzyme Immunoassay (EIA) (1.0 ng/mL cutoff) and by GC-MS-MS (0.5 ng/mL cutoff), respectively. Differences were evaluated for the left- and right-sided collections. For EIA, 165 of 169 results were in agreement (97.6%; 99 positives and 66 negatives). Two specimens displayed negative results on the left side and positive results on the right side, and two specimens displayed the opposite pattern. By GC-MS-MS, 162 of 169 results were in qualitative (positive/negative) agreement (95.9%; 93 positives and 69 negatives). The 7 discordant results were unevenly divided between the left and right sides (6 -/+ results and 1 +/- results). Average THC (± SEM) concentrations for the 7 discordant specimens from the left and right sides of the oral cavity were 0.4 (± 0.1) and 0.5 (± 0.1) ng/mL. Paired t-tests indicated no significant difference (p>0.05). These results indicated that two oral fluid specimens, near-simultaneously collected, by two Intercept™ Collection Devices produced the same result and would meet criteria to serve as a “split specimen” as defined in the proposed DHHS guidelines for Federal Workplace Drug Testing Programs.

Background

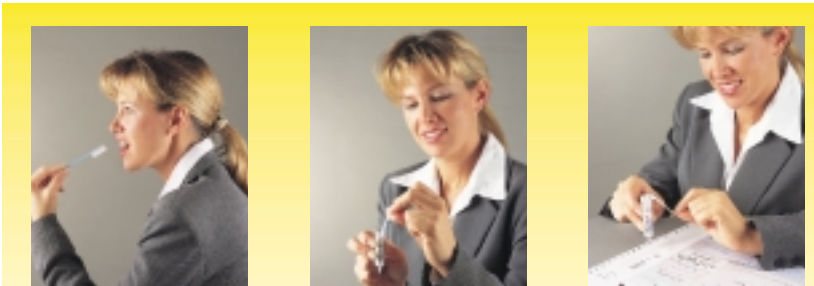
THC and metabolites appear in saliva shortly after use and, depending upon pH and rate of saliva flow, persist in saliva for as long as 14 hours⁽¹⁾ and may be used as an indication of current intoxication.⁽²⁾ In contrast, urine cannabinoid assays are useful only for the identification of cannabinoid use sometime in the past, with the urine detection period being highly variable and depending on time, dose, body weight, obesity, and habit.⁽²⁾ After smoking marijuana, saliva may contain THC-COOH and three metabolites: Δ⁹-THC, cannabidiol, and 11-hydroxy-Δ⁹-THC.⁽¹⁾ THC and its metabolites appear to be sequestered in the buccal cavity during smoking, rather than passing from the blood into saliva.⁽³⁾ The length of time following drug use for which a positive result may occur in saliva is dependent upon several factors including the frequency and amount of drug. The OTI Cannabinoids Intercept™ MICRO-PLATE EIA detects a variety of cannabinoids found in oral fluids.⁽¹⁾

Oral Fluid Collection



The Intercept™ DOA Oral Specimen Collection Device was developed for the purpose of collecting oral fluid for diagnostic testing. The collection device consists of a treated absorbent cotton fiber pad affixed to a nylon stick (Collection Pad) and a

preservative solution in a plastic container (Specimen Vial). The Collection Pad is impregnated with a mixture of common salts and gelatin which creates a hypertonic environment and an increased osmotic pressure wherever it contacts oral mucosal cells. The pad is placed in contact with the gingival mucosa (between the lower gum and cheek) which enhances the flow of mucosal transudate across the mucosal surfaces onto the absorptive cotton fibers of the pad. Following the collection period, the Collection Pad is placed into a vial containing a preservative solution which serves to inhibit the growth of oral micro-organisms recovered on the Collection Pad. The vial is sealed with a plastic cap and transported to a laboratory for processing and testing. Following processing, a fluid containing a mixture of saliva components and the preservative solution is recovered which is suitable for testing for the presence of cannabinoids in the OTI Cannabinoids Intercept™ MICRO-PLATE EIA manufactured by OraSure Technologies, Inc., Bethlehem, PA with GC/MS confirmation.



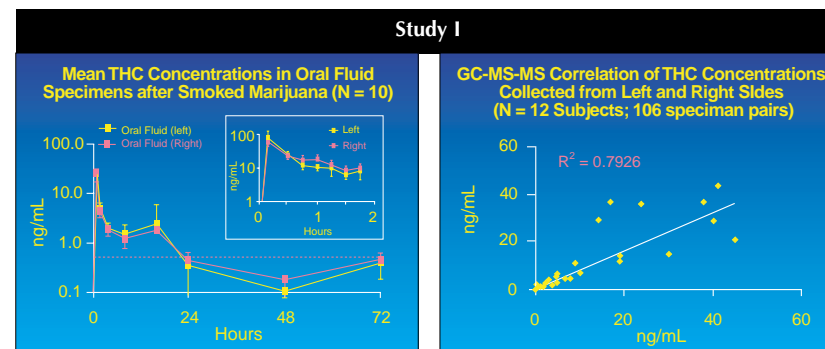
1. Peel open pad package far enough to allow easy removal of the Collection Pad.
2. Place pad between lower cheek and gum and gently rub back and forth until moist.
3. Keep the pad in place for 2 minutes (maximum 5 minutes) while timing.
4. Open vial in upright position.
5. Insert pad into the blue liquid at the bottom of the vial.
6. Break the pad handle by snapping it against the side of vial.
7. Replace the cap with a snap.
8. Place seal over top of vial and send sample to a laboratory for processing and testing.

Current Issues

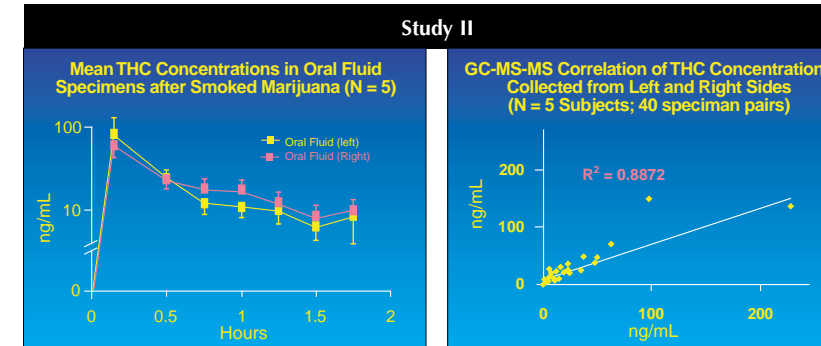
- A split specimen may be obtained by subdividing a single specimen or collecting two near-simultaneously-collected specimens.
- Data is needed to validate collection of simultaneous oral fluid specimens.
- Do split specimens produce equivalent results?

Marijuana Study Design

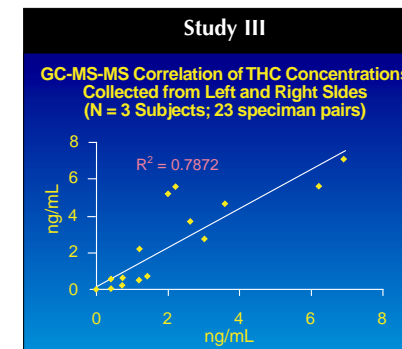
- Studies were performed in The Netherlands by OraSure with experienced marijuana users.
- Marijuana cigarettes were obtained from a coffee-house in Amsterdam; GC-MS analysis showed cigarettes contained 20-25 mg THC.
- Subjects were non-obese males (N = 18) with an average age of 22 years (±3, range 19-25).
- Studies were designed to simulate a social setting. Subjects were allowed to interact socially, drink soda, and eat pizza at times not interfering with the study.
- No exchange of marijuana was permitted.
- In all studies, simultaneous oral fluid collections were made from left and right sides of the mouth.
- Oral fluid specimens were collected with the Intercept™ DOA Oral Specimen Collection Device.
- Screening of oral fluid specimens was performed with the Cannabinoids Intercept™ MICRO-PLATE Enzyme Immunoassay (EIA) with a 1 ng/mL cutoff concentration.
- THC confirmation was performed by GC-MS-MS with a 0.5 ng/mL cutoff concentration.



Smoked 1 marijuana cigarette; N = 10 subjects; 5 casual users and 5 chronic users; collections of oral fluid and urine at 0, 1, 2, 4, 8, 16, 24, 48, and 72 hours



Smoked 1 marijuana cigarette with frequent collections; N = 5 subjects; collections of oral fluid and urine at 0, 15, 30, 45, 60, 75 and 90 minutes

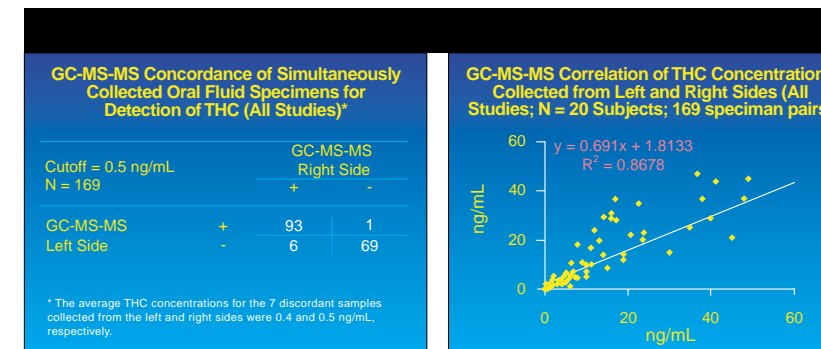


Oral ingestion of a cooked “Brownie” containing equivalent of 1 marijuana cigarette; N = 3 subjects; collections of oral fluid and urine at 0, 1, 2, 4, 16, 24, 48, and 72 hours

GC-MS-MS Correlation of THC Concentrations Collected from Left and Right Sides

- Mean THC concentrations (±SEM) across all specimens
 - Left side = 6.9 (±1.6) ng/mL
 - Right side = 6.6 (±1.2) ng/mL
- Paired t-tests of the sample means indicated no significant difference (p>0.05) between left- and right-side oral fluid specimens

All Studies			
EIA Concordance of Simultaneously Collected Oral Fluid Specimens for Detection of THC (All Studies)			
Cutoff = 1ng/mL N = 169		EIA, Right Side	
		+	-
EIA Left Side	+	99	2
	-	2	66



Summary

- There was 97.6% agreement in EIA results across all oral fluid specimens.
- There was 95.9% agreement in GC-MS-MS results across all oral fluid specimens.
- The correlation coefficient (r²) across all oral fluid specimens was 0.868 (highly significant).
- There were no significant differences (p>0.05) between sample means for left- and right-side specimens.

Conclusions

- Collection of two simultaneous oral fluid specimens by means of two Intercept™ Collection Devices produced the same analytical result in THC testing by EIA and GC-MS-MS.
- This collection procedure meets criteria as defined in the current proposed DHHS guidelines for Federal Workplace Drug Testing Programs.
- Split specimens produce equivalent results.

REFERENCES

1. Schramm, W., Smith, R.H., and Craig, P.A., “Drugs of Abuse in Saliva: A Review,” *Journal of Analytical Toxicology* 1992; 16:1-9.
2. Samyn, N., et al., “Analysis of Drugs of Abuse in Saliva,” *Forensic Science Review*, 11(1): 1999.
3. Inoue, T. and Seta, S., “Analysis of Drugs in Unconventional Samples,” *Forensic Science Review*, Vol. 4, No. 2, Dec. 1992.