DATA CHEM
LABORATORIES, INC.

METHAMPHETAMINE ON COTTON GAUZE WIPES BY LC-MS-SIM

NIOSH 9111, ISSUE 1

BACKUP DATA REPORT

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Methamphetamine on Cotton Gauze Wipes by LC-MS-SIM

I.   Introduction

DataChem Laboratories in recent years has developed and verified according to

NIOSH method development criteria [1] two GC-MS methods for methamphetamine as

well as a number of other drugs. The first, NIOSH 9106 [2], has, in the back up data

report, an extensive discussion on the extent of illegal drug labs and the hazards they pose

to law enforcement and clean up personnel. Additionally there is background

information on illicit drugs and precursors. A study on the effectiveness of surface

wiping techniques is presented in the back up report for NIOSH 9109 [3]. Since these

subjects have been dealt with in considerable detail as part of these methods, they are not

discussed in this report.

The advantage of NIOSH 9106 and NIOSH 9109 is the wide variety of drugs that

can be addressed. The use of the mass spectrometer allows positive identification based

on the mass spectrum and retention time. However, the disadvantage is time consuming

sample preparation involving derivatization and cleanup steps.

For many applications, a rapid, simple way to quantify just methamphetamine is

needed. Liquid chromatography coupled to a mass spectrometer (LC-MS) has found

wider application to many analyses that previously could only have been accomplished

by gas chromatography with derivatization. Liquid chromatography with ultraviolet

detection (LC-UV) does not have the sensitivity to accommodate the requirements of

clients requesting methamphetamine analysis of contaminated surfaces without extensive

sample pre-treatment. [4,5] More sensitive liquid chromatography detectors are available

such as fluorescence and electrochemical detectors, but these detectors have either limited uses for drug metabolite [6] detection or require derivatization for optimum sensitivity. [5, 7, 8] The mass spectrometer in the selected ion mode (SIM) and the liquid chromatograph which allows direct injection of the drug sample desorbate without derivatization is a technique that is not only quick but sensitive as well.

In NIOSH 9110, only cotton gauze was evaluated for two reasons. First, the performance of cotton gauze in the other method evaluations was excellent. Second, commercially available synthetic media are sometimes discontinued by the manufacturer and therefore reliance on synthetic media could make the method obsolete in the future.

II. Reagents and Solutions

Reagents:

Reagent	Vendor	Purity
Acetic Acid	Aldrich	Reagent
Acetonitrile	Burdick & Jackson	HPLC residue free
d-Methamphetamine	Alltech	1 mg/mL in methanol
Methamphetamine $-D_{14}$	Cerilliant	1 mg/mL in methanol
Sulfuric Acid	Fisher	Reagent
Methanol	Burdick & Jackson	HPLC residue free
ASTM Type II water	DCL	

Solutions:

Desorption Solution: 0.2N Sulfuric acid.
    Add 22 mL concentrated Sulfuric acid to 4L deionized water.
Methamphetamine $-D_{14}$ Internal Standard.
    Dilute 1 mL of 1000 µg/mL stock to 10 mL in methanol.
Mobile Phase A: 0.1% Acetic acid, 5% Acetonitrile, balance ASTM Type II water.
Mobile Phase B: 0.1% Acetic acid, 95% Acetonitrile, balance ASTM Type II water.

III. Materials and Instrumentation

Cotton Gauze, 3" x 3" 12-ply or 4" x 4" 8-ply.
50 mL polypropylene centrifuge tubes with screw cap.
    VWR Cat. # 21008-169 or equivalent.
LC-MS capable of selected ion monitoring with autosampler and data collection system.
Vials and caps for autosampler.
Microliter syringes for making standard solutions and spikes.
Various glass volumetric flasks for standard and/or solution preparation.
Tumbler for centrifuge tubes, approximately 10-30 RPM.
Filters: Ion Chromatography Acrodisc®, 25 mm syringe filter with 0.45 μm Supor® (PES) membrane.
    (Pall number 4585T or equivalent).
Pasteur transfer pipettes.
A 4-L bottle with adjustable 10-50-mL dispenser for desorption solution.

IV. Sample Preparation

Cotton gauze wipes in 50 mL centrifuge tubes were desorbed by adding 50 μL of internal standard solution to the gauze wipe followed by the addition of 30 mL of 0.2 N Sulfuric acid. The tubes were capped and tumbled for at least 30 minutes. The extract was filtered through Pall 0.45 μm Ion Chromatography Acrodiscs into autosampler vials for analysis.

V. Analysis Conditions

Column: Zorbax Eclipse XDB-C18; 4.6 mm x 150 mm, 5 μm packing or equivalent.
Column temperature: 40 °C.
Mobile phase flow rate: 0.50 mL/min.
Gradient:

Time	%A	%B
1	100	0
10	0	100
15	0	100
17	100	0
25	100	0

Note: Other gradients may be substituted.

Injection Volume: 50 μL

Mass Spectrometer Conditions:

Ionization Mode:	API-ES (Atmospheric Pressure Ionization-Electro Spray)
Polarity:	Positive
SIM Parameters:	
Fragmentator:	100
Gain:	3.0 EMV
Actual Dwell:	294
SIM ions:	119 Methamphetamine confirmation ion
	150 Quantitation ion for Methamphetamine
	164 Ion for Methamphetamine $-D_{14}$

Spray Chamber:

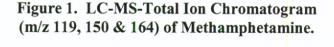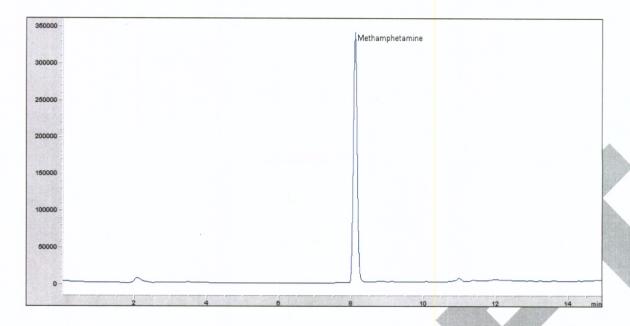|	
Gas Temperature:	200 °C.
Drying Gas:	12.0 L/min. nitrogen
Nebulizer Pressure:	50 psig

Note: The spray chamber parameters should be optimized for the particular instrument in use.

Methamphetamine and the Internal Standard essentially co-elute. Monitor m/z ions 119 and 150 for Methamphetamine quantitation and 164 for Methamphetamine – D_{14}. A typical chromatogram follows.

Figure 1. LC-MS-Total Ion Chromatogram
(m/z 119, 150 & 164) of Methamphetamine.

VI.   Limit of Detection (LOD) and Limit of Quantitation (LOQ)

There are no national health-based or feasibility-based surface contamination standards, criteria or guidelines for clandestine drug laboratory decontamination. However, several states have feasibility-based surface contamination limits. The most common limit is 0.1 µg of methamphetamine for a sample of 100 square centimeters of surface area wiped. Some jurisdictions require 1 square foot (929 square centimeters) to be wiped. In either case, the most common required sensitivity is 0.1 µg per sample for methamphetamine.

Table 1. State Methampheamine Surface Contamination Limits

There are no national health-based or feasibility-based surface contamination standards, criteria or guidelines for clandestine drug laboratory decontamination. However, several states have feasibility-based surface contamination limits.

State Surface Contamination Limit[9]*		Methamphetamine
0.5μ/100 cm^2		Colorado
1.0μ/ft^2	(Equivalent to 0.11 μ/100 cm^2)	Minnesota
0.1μ/100 cm^2		Alaska Arizona Arkansas California Idaho Montana North Carolina Tennessee Utah Washington
0.5 μ/ft^2	(Equivalent to 0.05 μ/100 cm^2)	Oregon

* State surface contamination limits are provided as an aid to those seeking additional information. NIOSH has not established health-based or feasibility-based airborne Recommended Exposure Limits (RELs) or surface contamination guidelines for clandestine drug laboratories and therefore inclusion of state surface contamination limits does not constitute endorsement by NIOSH. The National Alliance for Model State Drug Laws (NAMSDL) (http://www.natlalliance.org/) periodically summarizes state feasibility-based decontamination limits and proposed state legislative requirements and guidelines. However, state requirements and guidelines are subject to change and therefore the most recent state guidance should be obtained from directly from the state.

Two dilutions of the methamphetamine 1 mg/mL in methanol stock solution were prepared: one at 200 µg/mL and a second at 20 µg/mL. Cotton gauze wipes in 50 mL polypropylene centrifuge tubes were spiked with methamphetamine according to the following schedule and allowed to stand overnight:

Table 2. Media Spiked Standard Preparation.

Standard Concentration µg/mL	Spike Volume µL	Final Concentration µg/sample
200	500	100
200	100	20
200	25	5
200	5	1
20	25	0.5
20	5	0.1
20	2.5	0.05
20	1.2	0.024

The samples were desorbed and analyzed according to the procedure described above with the lowest four standards analyzed in duplicate. A normalized peak area was calculated by dividing the peak area of the methamphetamine peak by the area of the internal standard peak. The LOD and LOQ were determined using Burkart's method [10] with the normalized areas and methamphetamine final concentrations.

With the media spiked standards above and the instrument conditions previously described, the LOD calculated below the lowest standard. The Limit of Detection determined here, as well as any experimentally determined LOD, is a function of instrument performance, or lack thereof, e.g. a dirty source or degraded column. In previous gas chromatographic methods developed for methamphetamine, due to the needs of the end-users, limits of detection have needed to be at a minimum 0.1

µg/sample. As has been demonstrated here, this limit of detection is easily attainable.

Media spiked standards are recommended for routine analysis.

VII. Desorption Efficiency

Desorption efficiency had been determined for previous methamphetamine method developments but is repeated here because a different sample preparation procedure is used, namely a simple desorption as opposed to desorption followed by cleanup and derivatization.

As mentioned in the previous section, the end-user LOD requirement is typically 0.1 µg/sample. The experimentally determined LOD in the work conducted for this method calculated below the lowest standard. Spiking levels for the following desorption efficiency determination were made beginning at levels just above the client minimum LOQ instead of the LOQ calculated from the determination above. This provides data that covers concentrations at or above the action level for most users of methamphetamine data as opposed to a lower concentration that may or may not be always achievable. These chosen spiking levels correspond to a more conservative LOD than what had been previously determined.

At each level, six replicate samples were spiked, allowed to stand overnight then desorbed and analyzed. The LOQ used to calculate the spiking levels was 0.15 µg/sample. This corresponds to an LOD of 0.05 µg/sample. Spiking levels and recoveries are tabulated in the following tables:

Table 3. Desorption Efficiency Spiking Levels:

Spiking Level	Actual Target μg/sample
100 X LOQ	17.76
30 X LOQ	4.44
10 X LOQ	1.776
3 X LOQ	0.444

Table 4. Desorption Efficiency Results.

100 X LOQ				10 X LOQ			
	Result	Target	% Recovery		Result	Target	% Recovery
1	18.24	17.76	102.68	1	1.52	1.78	85.69
2	17.43	17.76	98.15	2	1.51	1.78	85.26
3	17.63	17.76	99.28	3	1.56	1.78	87.82
4	17.35	17.76	97.69	4	1.45	1.78	81.87
5	16.31	17.76	91.85	5	1.47	1.78	82.72
6	15.06	17.76	84.78	6	1.47	1.78	82.93
		average	95.74			average	84.38
		Std Dev	6.41			Std Dev	2.26
30 X LOQ				**3 X LOQ**			
1	4.43	4.44	99.83	1	0.36	0.44	82.07
2	3.87	4.44	87.26	2	0.37	0.44	83.76
3	3.83	4.44	86.24	3	0.44	0.44	99.91
4	3.92	4.44	88.28	4	0.45	0.44	100.74
5	3.92	4.44	88.20	5	0.43	0.44	97.36
6	3.86	4.44	86.92	6	0.38	0.44	84.62
		average	89.46			average	91.41
		Std Dev	5.14			Std Dev	8.79

VIII. Long-Term Storage

Since long term storage measures only the viability of analytes on a particular media over time, this determination was not repeated for this particular method; the reader is directed to the NIOSH 9106 Backup Data Report [2] for more detail. In summary, for methamphetamine normalized against methamphetamine $-D_{14}$, the long term storage study was accomplished as follows:

Media:	3 x 3 cotton gauze, 12 ply, two per sample prewetted with 3 mL isopropanol to simulate gauze dampened with alcohol for wipe sampling. Six replicates for each storage time.
Spiking Solutions:	Methamphetamine: 50.032 µg/mL Methamphetamine $-D_{14}$: 100 µg/mL
Spikes:	Methamphetamine: 60 µL or 3 µg/sample Methamphetamine $-D_{14}$: 50 µL
Storage:	One set of six stored at room temperature for seven days. Four additional sets of six stored at <6 °C for seven, fourteen, twenty-one and thirty days.

Results are tabulated in Table 4 below. No significant losses on storage were seen to occur.

Table 5. Long Term Storage Results Summary for Methamphetamine:

Storage Conditions		Ambient		Refrigerated		
Time	Day 0	Day 7	Day 7	Day 14	Day 21	Day 30
Percent Recovery	96.2	93.5	99.4	98.5	96.4	98.0

IX.   Method Precision and Accuracy

Method precision (S_r) was determined from the desorption efficiency data presented earlier. Using all data, method precision (S_r) was 0.06663. Accuracy was 20.7% and mean bias was -0.09753. See Appendix 1 for statistical results.

X.   Conclusions:

Methamphetamine determination by LC-MS has been found to be an acceptable method of analysis. It is fast, with simple sample preparation, and short analysis times. While other drugs and precursors were not considered for the purposes of this method, it in all likelihood could be easily expanded to include a number of other analytes of interest.

XI.   References:

[1]   Kennedy, E., Ph.D. *et al.* "Guidelines for Air Sampling and Analytical Method Development and Evaluation", U.S.Department of Health and Human Services, Public Health Services, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Physical Sciences and Engineering, May 1995; DHHS (NIOSH) Publication NO. 95-117.

[2]   Reynolds, J.M., Siso, M.C., and Perkins, J.B., "Backup Data Report for NIOSH 9106, Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Liquid-Liquid Extraction", prepared under NIOSH Contract 200-2001-0800, (Unpublished, 2004).

[3]   Reynolds, J.M., Siso, M.C., and Perkins, J.B., "Backup Data Report for NIOSH 9109, Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Solid Phase Extraction", prepared under NIOSH Contract 200-2001-0800, (Unpublished, 2004).

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[5]   al-Dirbashi, O.Y., Ikeda, K., Takahashi, M., Kuroda, N., Ikeda, S., and Nakashima, K., "Drugs of Abuse in a Non-conventional Sample; Detection of Methamphetamine and its Main Metabolite, Amphetamine in Abusers' Clothes by HPLC with UV and Fluorescence Detection", *Biomed. Chromatogr.*, 15(7): 457-463, (2001).

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[9]   NAMSLD 2007. State Controlled Substance(s) Environmental Issues Bill – Status Update, (http://www.natlalliance.org/) The National Alliance for Model State Drug Laws, Alexandra, Va. Access via the web on March 11, 2008.

[10] Burkart, J.A., "General Procedures for Limit of Detection Calculations in the Industrial Hygiene Chemistry Laboratory," *Applied Industrial Hygiene*, 1(3):153-155, (1986).

Appendix 1. Precision and Accuracy Calculation Tables for Methamphetamine.

TABLE 1. RECOVERY FROM SPIKED MEDIA

										Method:	LC-MS-SIM

Analyte: **Methamphetamine**   Method: LC-MS-SIM
Media: **Cotton Gauze**   Instrument: LC-MS
Int. Std.: **Methamphetamine-D14**   Column: C18
Derivatizing agent: none   Misc. :

Concentration Level

	3xLOQ		10xLOQ		30xLOQ		100xLOQ			
	Applied in ug/sample 0.25000		Applied in ug/sample 0.44400		Applied in ug/sample 1.77600		Applied in ug/sample 4.44000		Applied in ug/sample 17.76000		Applied in ug/sample 75.00000
Replicate	Found in ug/sample	Percent Recovery	Found in ug/sample	Percent Recovery	Found in ug/sample	Percent Recovery	Found in ug/sample	Percent Recovery	Found in ug/sample	Percent Recovery	Found in ug/sample	Percent Recovery
1			0.3644	82.07	1.5219	85.69	4.4324	99.83	18.2352	102.68		
2			0.3719	83.76	1.5143	85.26	3.8745	87.26	17.4321	98.15		
3			0.4436	99.91	1.5596	87.82	3.8292	86.24	17.6319	99.28		
4			0.4473	100.74	1.4540	81.87	3.9197	88.28	17.3492	97.69		
5			0.4323	97.36	1.4691	82.72	3.9159	88.20	16.3124	91.85		
6			0.3757	84.62	1.4728	82.93	3.8594	86.92	15.0569	84.78		
7			no data	no data	no data	no data	no data	no data	no data	no data		
average =			0.4059	91.41	1.4986	84.38	3.9719	89.46	17.0030	95.74		
std dev =			0.03904	8.7939	0.04009	2.2572	0.22822	5.1401	1.13860	6.4110		
CV i=			0.09620	ok	0.02675	ok	0.05746	ok	0.06696	ok		
Bias i =			-0.08589	ok	-0.15618	>10%	-0.10544	>10%	-0.04263	ok		
			N = 6		N = 6		N = 6		N = 6			

TABLE 2. CALCULATION of ACCURACY, OVERALL PRECISION, and MEAN BIAS   Method: LC-MS-SIM

Analyte: **Methamphetamine**   Instrument: LC-MS
Media: **Cotton Gauze**   Column: C18
Int. Std.: **Methamphetamine-D14**   Misc. :
Derivatizing agent: none   Range studied = 0.44400 to 17.76000 ug/sample

Section 1 — FINAL OVERALL VALUES

Options:	Pooled CV = Overall Sr	Caloulated, not using nomogram Accuracy	MEAN BIAS	Range of Bias From =	To =	Are both CVs and BIAS Poolable? dist. = 0.95 at a=0.05	dist. = 0.975 at a=0.025	CONCENTRATION LEVELS OMITTED for BARTLETT'S and F' TESTS (See Note)			
Option #1	0.06663	20.71	-0.09753	-0.15618	-0.04263	YES	YES	0	0	1xLOQ	300xLOQ
Option #2	0.07537	20.20	-0.07798	-0.10544	-0.04263	YES	YES	1xLOQ	1xLOQ	10xLOQ	300xLOQ
Option #3	0.05323	18.90	-0.10142	-0.15618	-0.04263	YES	YES	1xLOQ	1xLOQ	3xLOQ	300xLOQ
Option #4	0.06651	22.53	-0.11584	-0.15618	-0.08589	no	YES	1xLOQ	1xLOQ	100xLOQ	300xLOQ
Option #5	0.06941	20.91	-0.09490	-0.15618	-0.04263	no	YES	1xLOQ	1xLOQ	30xLOQ	300xLOQ
Option #6	0.04482	20.45	-0.13081	-0.15618	-0.10544	YES	YES	1xLOQ	3xLOQ	100xLOQ	300xLOQ
Option #7	0.05099	18.33	-0.09941	-0.15618	-0.04263	YES	YES	1xLOQ	3xLOQ	30xLOQ	300xLOQ

Note: If the degrees of freedom (df) given in th box below are 2, then the LOWEST level has also been omitted automatically.
Note: If the degrees of freedom (df) given in the box below are 1, then the LOWEST TWO levels have also been omitted automatically.

Section 2

	Homogeneity of individual group CVs.					Homogeneity of individual group biases.					
	Bartlett's Criteria for 4, 5, or 6 conc. levels			Pass Bartlett's?			F' Theoretical for 4, 5, or 6 conc. levels			PASS F' test? Is F' < theoretical?	
	Percentile of X^2 dist.		df, deg. of	Percentile of X^2 dist.					df, deg. of		
Options:	Chi sq'd	0.95	0.975	freedom	0.95	0.975	F =	at a=0.05	at a=0.025	freedom	at a=0.05	at a=0.025
Option #1	6.4767	7.81	9.35	3	YES	YES	2.12934	3.09839	3.85870	3	YES	YES
Option #2	1.3354	5.99	7.38	2	YES	YES	0.72263	3.68232	4.76505	2	YES	YES
Option #3	3.5107	5.99	7.38	2	YES	YES	3.14651	3.68232	4.76505	2	YES	YES
Option #4	6.3194	5.99	7.38	2	no	YES	1.07061	3.68232	4.76505	2	YES	YES
Option #5	6.0894	5.99	7.38	2	no	YES	2.50502	3.68232	4.76505	2	YES	YES
Option #6	2.3267	3.84	5.02	1	YES	YES	0.85119	4.96460	6.93673	1	YES	YES
Option #7	3.2394	3.84	5.02	1	YES	YES	3.94360	4.96460	6.93673	1	YES	YES