DATA CHEM LABORATORIES, INC.

METHAMPHETAMINE and ILLICIT DRUGS, PRECURSORS, and ADULTERANTS on WIPES by LIQUID-LIQUID EXTRACTION

NIOSH 9106, Issue 1

Backup Data Report, Abridged Version

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August 30, 2005

Contract CDC-200-2001-0800

Submitted To:

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ABRIDGED BACKUP DATA REPORT FOR NIOSH 9106: METHAMPHETAMINE AND ILLICIT DRUGS, PRECURSORS, AND ADULTERANTS ON WIPES BY LIQUID-LIQUID EXTRACTION

I. INTRODUCTION

In December 2002 DataChem Laboratories (DCL) received a request from NIOSH to develop a method for determining methamphetamine on surfaces using gauze wipes. This method was to be used by NIOSH in a collaborative research project with the National Jewish Medical and Research Center (NJMRC) in a study of the contamination within clandestine drug laboratories and the hazards they present to first responders and occupants. [1] Three methods for analysis of drugs on wipes were subsequently developed. The first method used a liquid-liquid extraction cleanup procedure with derivatization by fluorinated acid anhydrides for analysis by GC-MS. [2] The second method used solid-phase extraction (SPE) cleanup with derivatization by a mixed silylation-acylation reagent for analysis by GC-MS. [3] The third method used LC-MS without derivatization and is still in the process of development. This Backup Data Report presents the evaluation results for the first method, the liquid-liquid extraction procedure, and only involves the use of cotton gauze wipes. It is an abridged version of a larger Backup Data Report for NIOSH 9106 that gives results for other sampling media and greater detail on the method development process. [4]

II. SCOPE AND OBJECTIVES

A. Introduction

This method was developed in accordance to the principles set forth in the NIOSH publication "Guidelines for Air Sampling and Analytical Method Development and Evaluation" [5]. The method had to meet the accuracy criterion requirement given therein that with a 95%

confidence a result must be within ±25% of the true value. Since the method was for surface wipe sampling and not air sampling, the procedures set forth in the guidelines had to be modified. No simulated vapor and aerosol sampling recovery study was performed. The precision and accuracies for NIOSH 9106 were therefore calculated from a desorption efficiency study and do not include sampling error.

However, a limited surface recovery study is reported in the Backup Data Report for NIOSH 9109. [6] Several surfaces and wipe methods were tested. Recovery rates vary greatly by surface material wiped, especially between porous rough surfaces compared to smooth non-porous surfaces and by wipe procedure used. The sampling recovery data were not used to compute measurement bias, overall precision and overall accuracy for the method for three reasons. First, surface recoveries vary greatly by surface material and only 6 surfaces were tested. Second, test surfaces were liquid spiked just prior to sampling and the sampling surface recovery test did not replicate recoveries of drug vapors and dusts deposited on surfaces for an extended period of time. Third, surface recovery is dependant upon the wipe procedure used and a comprehensive test of wipe procedures used or specified by various legal jurisdictions was not undertaken.

The studies performed and contained in this report cover the following areas:

1. Development of analytical procedures (extraction, derivatization, GC-MS conditions),
2. Selection of wipe media for evaluation,
3. Estimation of limits of detection (LODs) and quantitation (LOQs) for the method,
4. Evaluation of sampling media for long-term storage stability,
5. Evaluation of precision and accuracy for the method.

B. Analytical Techniques

Gas Chromatography with Mass Spectrometry (GC-MS) was used in order to provide unambiguous identification of the target analyte. Because of the poor chromatography of parent amphetamines in preliminary experiments, amphetamines were derivatized. Several derivatizing agents were tested for ease of handling, completeness of derivatization, and chromatographic characteristics. Two derivatizing agents were found to be acceptable for the liquid-liquid extraction procedure. These were pentafluoropropionic anhydride (PFPA) and chlorodifluoroacetic anhydride (CDFAA).

C. Wipe Media

Several media were screened. These included cotton gauze, AlphaWipe™, NU-GAUZE™, MIRASORB™, SOF-WICK™, and TOPPER™. The latter four materials were synthetic engineered fabric gauzes that have been discontinued by their manufacturer. Cotton gauze was found to be as good as or better than any of the synthetics tested and only the results for this material are given in this abridged report.

D. Target Analytes

The analytes studied were methamphetamine, the primary drug of clandestine manufacture in the U.S. at present, and other drugs of clandestine manufacture: amphetamine, ecstasy (MDMA), an ecstasy analog (MDEA), and phencyclidine (PCP). The method includes ephedrine and pseudoephedrine (precursors for methamphetamine), phenylpropanolamine (a precursor for amphetamine), phentermine (an anorexic used as an adulterant), and caffeine (an adulterant).

E. Surrogate and Internal Standards

Two kinds of internal standards were used. One kind was added to the final extract just prior to analysis by GC-MS. This internal standard was 4,4'-dibromooctafluorobiphenyl

(DBOFB). It was useful for monitoring GC-MS and autosampler performance in each sample and standard. It is a convenience but is not critical to the analysis.

The second kind of internal standards were deuterated analogs of the target analytes. These internal standards are critical for the success of the method. The preferred deuterated internal standards were methamphetamine-D_{14} and amphetamine-D_{11}. The more highly deuterated the compound the better. Deuterium labeling had to be in the side chain and not just in the aromatic portion of the compound. Steric hindrance around the amine was an important factor and the internal standard had to be similar to that of the target analyte. Primary amines gave best results when amphetamine-D_{11} was used and N-methyl secondary amines gave best results when methamphetamine-D_{14} was used. For MDEA, an N-ethyl secondary amine, another sterically hindered amine was required as the internal standard. N-propyl amphetamine, a sterically hindered secondary amine, was found to be an effective internal standard for MDEA.

F. Crystal Violet Visualization Reagent

At a certain stage in the sample preparation (after the eluates have been collected from the drying columns and just prior to concentration under a stream of nitrogen) crystal violet was added to make the dried residue more visible. The color of the eluates after the addition of crystal violet is violet. A good grade of crystal violet was obtained (95% purity or better) which gave no GC-MS interference.

The crystal violet is also a pH indicator for organic solutions. At acid pH the color is yellow. In the presence of a little residual isopropanol when the eluates are nearly evaporated to dryness, the crystal violet turns yellow. Just at dryness, as the excess hydrochloric acid evaporates, the dried residue turns green, then blue, and finally to purple. These color changes were not observed if methanol was used as the wipe solvent. But the color changes can be made to appear if a little isopropanol is added prior to drying.

The presence of anionic detergents and other contaminants affected the series of color changes, sometimes preventing color changes altogether.

If a viscous residue remained after nitrogen blow-down, a fresh aliquot of the desorbates was re-extracted using methylene chloride instead of hexane as the cleanup solvent. When viscous residues remained after evaporation under nitrogen, the derivatization reagent was not effective.

G. Gas Chromatographic and Mass Spectrometric Conditions

The conditions were chosen such that the initial temperature was below the boiling point of the solvent (primarily toluene) by 10-20 degrees Celsius so that Gröb splitless injection could be used. A simple temperature ramp of 10 °C/minute was adequate to separate all of the analytes in a reasonable period of time. This applied to either the CDFA or PFP derivatives. Only one type of GC column was tested: DB-5ms, a 5%phenylmethylsilicone column. Other columns might be just as good or perhaps better as far as peak shape goes for some derivatives and especially for the polar parent compounds.

Both scan mode (scanning from 20 to 470 AMU in about 0.2 to 0.3 seconds) or selected ion monitoring (SIM) mode were evaluated. The GC-MS conditions are given in NIOSH 9106.

III. ANALYTICAL METHOD

The liquid-liquid extraction procedure is described in NIOSH 9106 [2]. The details of the procedure are not repeated here for brevity.

All of the samples analyzed for the collaborative study between the National Jewish Medical and Research Center (NJMRC) and NIOSH [1] were processed by the liquid-liquid extraction procedure, NIOSH 9106. [2] Liquid-liquid extraction has advantages over the solid

phase extraction (SPE) technique (NIOSH 9109 [3]). Advantages include: cleaner chromatograms and much longer operating times for the mass spectrometer before cleaning is necessary. Cleaner chromatograms makes it easier to detect non-target analytes that may be unexpected and of interest. The major disadvantage of the liquid-liquid extraction procedure is the longer time it takes to prepare samples.

The major advantage of the SPE extraction procedure is much quicker sample preparation and it is easier to process a larger number of samples. The major disadvantages are that the mixed silylation-acylation reagents dirty the mass spectrometer source faster and the chromatograms are cluttered with silane by-product GC peaks, making it harder to spot non-target compounds. However, target compounds are easily sorted out from the noise through the use of reconstructed ion current profiles for quantitation since the noise does not share the same ions critical for quantification of the analytes.

IV. DETERMINATION OF LIMITS OF DETECTION AND QUANTITATION

A. Introduction and Objective

The objective of this study was to determine the limits of detection (LOD) and quantitation (LOQ) for the target analytes. 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 to be wiped. In either case, the most common required sensitivity is 0.1 µg per sample for methamphetamine. In addition, state surface

contamination standards for other drugs (ephedrine, pseudoepedrine, and Ecstasy (MDMA)) are also 0.1 µg per 100 square centimeters of surface area wiped or 0.1 µg per sample.[7]

TABLE 1. STATE MAXIMUM 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[7]*		Methamphetamine	Ephedrine	Pseudoepedrine	Ecstasy (MDMA)
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	Arizona Utah	Arizona Utah	Arizona Utah
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.

The LOD and LOQ are determined by a modification of NIOSH SOP 018 as described by Burkart [8]. The calibration curve was set up using duplicate spiked and extracted liquid standards for each concentration level, not duplicate injections of each standard. This is in accordance to the method, which also uses duplicate spiked liquid standards at each concentration level.

B. Reagents and Supplies

These are described in NIOSH 9106. Supplies that have specific lot numbers and/or concentrations unique to this study are given below.

a. Mixed analyte spiking solution (See Table 2.);

TABLE 2. MIXED ANALYTE SPIKING SOLUTION (1)

	ANALYTE	Source	Lot Number	Calculated concentration as free base in µg/mL
1	D-Amphetamine HCl	Alltech	413	50.00322
2	Caffeine	Chem Service	28-49C	50.01031
3	L-Ephedrine HCl	Alltech	1505	50.29991
4	MDEA HCl	Alltech	3506	47.63766
5	MDMA HCl	Alltech	6852	45.28192
6	D-Methamphetamine HCl	Alltech	389	50.03214
7	Phencyclidine HCl	Alltech	1293-33	50.07406
8	Phentermine HCl	Sigma	105F-0129	50.34771
9	(±)-Phenylpropanolamine HCl	Sigma	91F-0298	50.40394
10	Pseudoephedrine HCl	Sigma	32K-1358	50.28431

(1) The mixture was made up in methanol, HPLC grade, B&J lot CB331

b. Internal standard spiking solution (See Table 3.);

TABLE 3. INTERNAL STANDARD SPIKING SOLUTION (1)

	ANALYTE	Source	Lot Number	Calculated concentration as free base in µg/mL	Abbreviation in Following Tables
1	(±)-Amphetamine-D_{11}, HCl	Cerilliant	35129-58A	50.00	D_{11}-Amp
2	N-Propylamphetamine	Alltech	1604	83.099	D_{14}-Meth
3	(±)-Methamphetamine-D_{14},HCl	Cerilliant	30902-25G	100.00	N-PAmp

(1) The mixture was made up in methanol. About 2 µL of powdered crystal violet was added to about 10mL of the internal standard spiking solution to act as a visual reference as to which samples were spiked

c. Drying columns were prepared in blank 12-mL polypropylene (PP) columns (10mm i.d. x 75mm long barrel with a 16mm i.d. x 40mm long reservoir on top) fitted with fritted polyethylene discs. These were used instead of in 10-mL Eppendorf pipette tips.

C. Spiking Schedule and Derivatization Procedure

Liquid standards were prepared in duplicate as follows. Three milliliters of isopropanol were added to empty 50-mL PP centrifuge tubes. The isopropanol (IPA) was spiked with the mixed analyte spiking solution (Table 2) according to the following schedule.

TABLE 4. SPIKING SCHEDULE FOR PRECISION AND ACCURACY STUDY

		Amount applied per concentration level in microliters						
MEDIA	IPA mL	µL of mixed analyte spiking solution applied			µL of 1/10 dilution of mixed analyte spiking solution applied			
		300x LOQ Level	100x LOQ Level	30x LOQ Level	10x LOQ Level	3x LOQ Level	1x LOQ Level	0.5x LOQ Level
None (liquid only standards)	3	600	200	60	200	60	20	10

After the addition of the mixed analyte spiking solution, 50-µL of internal standard spiking solution was added to each tube. After spiking, 40mL of desorption solutions (0.2N aqueous sulfuric acid) were added to each tube. The resulting sample concentrations after spiking are given in the following table.

TABLE 5. CONCENTRATION OF ANALYTES AT EACH LEVEL

	ANALYTE	Calculated Concentration in µg/sample [1]						
		300x LOQ Level	100x LOQ Level	30x LOQ Level	10x LOQ Level	3x LOQ Level	1x LOQ Level	0.5x LOQ Level
1	D-Amphetamine	30.00193	10.00064	3.00019	1.00006	0.30002	0.10001	0.05000
2	Caffeine	30.00619	10.00206	3.00062	1.00021	0.30006	0.10002	0.05001
3	L-Ephedrine	30.17995	10.05998	3.01799	1.00600	0.30180	0.10060	0.05030
4	MDEA	28.58259	9.52753	2.85826	0.95275	0.28583	0.09528	0.04764
5	MDMA	27.16915	9.05638	2.71692	0.90564	0.27169	0.09056	0.04528
6	D-Methamphetamine	30.01928	10.00643	3.00193	1.00064	0.30019	0.10006	0.05003
7	Phencyclidine	30.04444	10.01481	3.00444	1.00148	0.30044	0.10015	0.05007
8	Phentermine	30.20862	10.06954	3.02086	1.00695	0.30209	0.10070	0.05035
9	Phenylpropanolamine	30.24236	10.08079	3.02424	1.00808	0.30242	0.10081	0.05040
10	Pseudoephedrine	30.17059	10.05686	3.01706	1.00569	0.30171	0.10057	0.05028

(1) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

The tubes were capped securely and tumbled for 2-3 hours (along with the cotton samples for the precision and accuracy evaluation study). After tumbling, 10 mL of the desorbates were processed according to NIOSH 9106 derivatizing with chlorodifluoroacetic anhydride in 14 mL collection tubes.

D. Results

The LOD and LOQ for each analyte, normalized against each internal applicable standard are summarized in the table 6.

TABLE 6. CALCULATED LIMITS OF DETECTION USING LIQUID STANDARDS IN SCAN MODE [1]

	ANALYTES	LOD, Concentration in µg/sample [2]		
		Int. Std: D_{11}-amp	Int. Std D_{14}-Meth	Int. Std N-PAmp
1	D-Amphetamine	0.0708	0.0585	---
2	Caffeine	0.2277 [3]	0.3755 [3]	---
3	L-Ephedrine	0.0891	0.0760	---
4	MDEA	---	---	0.0411 *
5	MDMA	0.0695	0.0540	---
6	D-Methamphetamine	0.0540	0.0366 *	---
7	Phencyclidine	0.1150	0.2647	---
8	Phentermine	0.0577	0.0502	---
9	Phenylpropanolamine	0.1692	0.1624	---
10	Pseudoephedrine	0.0845	0.0684	---

* Lowest standard was 0.05 µg/sample, therefore value was raised to 0.05 µg/sample.

(1) LOD calculated using the procedure of Burkart [8].

(2) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

(3) In the Precision and Accuracy study, the 0.3 µg/sample level was not detectable on any media. Therefore the level was raised to 1 µg/sample.

TABLE 7. CALCULATED LIMITS OF QUANTITATION USING LIQUID STANDARDS IN SCAN MODE [1]

	ANALYTES	LOQ, Concentration in µg/sample [2]		
		Int. Std: D_{11}-amp	Int. Std D_{14}-Meth	Int. Std N-PAmp
1	D-Amphetamine	0.2362	0.1949	---
2	Caffeine	0.7609	1.2525	---
3	L-Ephedrine	0.2974	0.2535	---
4	MDEA	---	---	0.1371
5	MDMA	0.2319	0.1802	---
6	D-Methamphetamine	0.1802	0.1220	---
7	Phencyclidine	0.3823	0.8858	---
8	Phentermine	0.1922	0.1672	---
9	Phenylpropanolamine	0.5614	0.5378	---
10	Pseudoephedrine	0.2818	0.2280	---

* Lowest standard was 0.05 µg/sample, therefore value was raised to 0.05 µg/sample.

(1) LOQ calculated using the procedure of Burkart [8].

(2) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

After the liquid standards were used for determining the LOD and LOQ in the scan mode of operation of the GC-MS, they were reanalyzed, after standing at room temperature for about 4 days, in the selection ion monitoring (SIM) mode.

TABLE 8. CALCULATED LIMITS OF DETECTION

USING LIQUID STANDARDS IN SELECTED ION MONITORING MODE [1]

	ANALYTES	LOD, Concentration in µg/sample [2]		
		Int. Std:	Int. Std	Int. Std
		D_{11}-amp	D_{14}-Meth	N-PAmp
1	D-Amphetamine	0.0480	0.0588	---
2	Caffeine	0.1728	0.1832	---
3	L-Ephedrine	0.1189	0.0931	---
4	MDEA	---	---	0.0713
5	MDMA	0.0565	0.0667	---
6	D-Methamphetamine	0.0401 *	0.0503	---
7	Phencyclidine	0.0650	0.0749	---
8	Phentermine	0.0261 *	0.0241 *	---
9	Phenylpropanolamine	Not analyzed due to breakdown on standing.		
10	Pseudoephedrine	0.0749	0.0873	---

* Lowest standard was 0.05 µg/sample, therefore value was raised to 0.05 µg/sample.

(1) LOD calculated using the procedure of Burkart [8].

(2) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

The results show that there appears to be excellent stability of the derivatives over several days at room temperature, except for phenylpropanolamine. Phenylpropanolamine almost completely disappeared. It is likely that breakdown consisted of hydrolysis of the ester group catalyzed by the proximity of the free proton on the primary amide group. For reliable quantification of this compound the samples need to be kept refrigerated until analysis and should be analyzed at least within the first 24-48 hours after warming to room temperature.

The results also show that the LODs for either scan or SIM mode of operation is adequate to meet the regulatory limits set for methamphetamine on surfaces.

TABLE 9. CALCULATED LIMITS OF QUANTITATION USING LIQUID STANDARDS IN SELECTED ION MONITORING MODE [1]

	ANALYTES	LOQ, Concentration in µg/sample [2]		
		Int. Std:	Int. Std	Int. Std
		D_{11}-amp	D_{14}-Meth	N-PAmp
1	D-Amphetamine	0.1599	0.1958	---
2	Caffeine	0.5762	0.6103	---
3	L-Ephedrine	0.3966	0.3103	---
4	MDEA	---	---	0.2375
5	MDMA	0.1883	0.2222	---
6	D-Methamphetamine	0.1338	0.1678	---
7	Phencyclidine	0.2168	0.2499	---
8	Phentermine	0.0869	0.0802	---
9	Phenylpropanolamine	Not measurable due to breakdown on standing at room temperature..		
10	Pseudoephedrine	0.2497	0.2906	---

* Lowest standard was 0.05 µg/sample, therefore value was raised to 0.05 µg/sample.

(1) LOQ calculated using the procedure of Burkart [8].

(2) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

V. EVALUATION OF LONG-TERM SAMPLE STORAGE STABILITY

A. Objective

The criterion for long-term stability is that the recoveries for samples stored under ambient conditions on day 7 should be within 10% of the recoveries determined for day zero. This is to ensure analyte stability on media during un-refrigerated shipment. To accomplish this the target analytes are spiked onto media and divided randomly into groups to be analyzed on different days. At least 6 replicates were stored at room temperature for 7 days. The others were stored at refrigerated temperatures for up to 30 days.

B. Reagents and Supplies

These are described in a previous section. Supplies that have specific lot numbers and/or concentrations unique to this study are given below.

a. Media (See Table 10). Storage stability was determined for all of the following media but only the results for cotton gauze will be given in this abridged Backup Data Report.

TABLE 10. MEDIA FOR LONG-TERM STABILITY TEST

	MEDIA	SIZE	PLY	Number per sample
1	AlphaWipe™	4"x 4"	1-ply, knit	2
2	Cotton gauze, Caring brand	3"x 3"	12-ply	2
3	MIRASORB™ Sponges (1)	4"x 4"	4-ply	1
4	NU GAUZE™ General Use Sponges (1)	4"x 4"	4-ply	1
5	SOF-WICK™ Dressing Sponges (1)	4"x 4"	6-ply	1

(1) Johnson & Johnson product.

b. Mixed analyte spiking solution (See Table 11.).

TABLE 11. MIXED ANALYTE SPIKING SOLUTION (1)

	ANALYTE	Source	Lot Number	Calculated concentration as free base in µg/mL
1	D-Amphetamine HCl	Alltech	413	50.00322
2	Caffeine	Chem Service	28-49C	50.01031
3	L-Ephedrine HCl	Alltech	1505	50.29991
4	MDEA HCl	Alltech	3506	47.66517
5	MDMA HCl	Alltech	6852	45.30759
6	D-Methamphetamine HCl	Alltech	389	50.03214
7	Phencyclidine HCl	Alltech	1293-33	50.06204
8	Phentermine HCl	Sigma	105F-0129	50.34771
9	(±)-Phenylpropanolamine HCl	Sigma	91F-0298	50.40394
10	Pseudoephedrine HCl	Sigma	32K-1358	50.28431

(1) The mixture was made up in methanol, HPLC grade, B&J lot CB331.

c. Internal standard spiking solution (See Table 12.);

TABLE 12. INTERNAL STANDARD SPIKING SOLUTION (1)

	ANALYTE	Source	Lot Number	Calculated concentration as free base in µg/mL
1	(±)-Amphetamine-D_{11}, HCl	Cerilliant	35129-58A	100.00
2	N-Propylamphetamine	Alltech	1604	201.393
3	(±)-Methamphetamine-D_{14},HCl	Cerilliant	30902-25G	100.00

(1) The mixture was made up in methanol.

d. Walk-in cooler maintained at <6 °C.

C. Procedure

Media were inserted into 50-mL PP centrifuge tubes. Two each of the cotton were used per sample. Just prior to spiking with the internal standard spiking solution, each wipe sample was pre-wetted with 3 mL of isopropanol. Adding this alcohol was to simulate the pre-wetting of media prior to sampling in the field. Each wipe was then spiked with 60 µL of internal standard spiking solution (which is equivalent to the 30x LOQ level of the precision and accuracy study) distributing aliquots of the 60 µL in several locations around the wipes. The tubes were then capped and stored in the dark for a designated period of time as outlined in Table 13.

TABLE 13. SCHEDULE FOR SPIKING AND DESORPTION

Storage Time (days)	Temperature	Number of Replicates
Zero	<6 °C	6
7	<6 °C	6
7	<6 °C	6
14	<6 °C	3
21	<6 °C	6
30	<6 °C	3

The process of desorption, extraction, derivatization, and analysis has been previously described, except 8 mL of methylene chloride was taken instead of the entire 10 mL for evaporation to dryness. The drying tubes were 12-mL instead of 14-mL test tubes.

In quantifying the data, the raw areas for each analyte was normalized against various internal standards to see which ones would give the best results.

D. Results

Results for day zero were incongruous with the other samples because they were prepared on a different day. Storage stability was calculated from an assumed 100% recovery for day zero. This is a more stringent test because it assumes that there is no matrix affect on day zero. Fortunately, the absolute recoveries on each subsequent day were high enough to make recovery on day zero a mute point.

For all analytes the storage stability criterion was met with at least one or more combinations of media and internal standard. The storage criterion was met for methamphetamine on all media regardless which internal standard was used. Cotton permitted the criterion to be met for all analytes regardless which internal standard was used, except for amphetamine and phenylpropanolamine, which required the use of D_{11}-amphetamine as the internal standard. MDEA, a sterically hindered amine, met the criterion for all media (except on AlphaWipes™) only when N-propylamphetamine was used as the internal standard.

In the tables below precisions for most of the analytes ranged from less than 1% to occasionally as high as 16%. Mostly the CVs were between 2 and 8% and averaged between 4 and 5%. Precisions on day zero for phenylpropanolamine ranged from 2 to 43% depending upon the media and recoveries ranged from 44 to 168%. Even so, omitting phenylpropanolamine, the CVs for day zero averaged between 5 and 6%. In order to simplify the presentation of the data, the CVs will be omitted from the following tables.

Recoveries for all analytes were dependant upon which internal standard the data was normalized to. This was a complicating issue for this and all subsequent studies. It multiplied the number of calculations that had to be made, and necessarily so in some cases, since recoveries for a few analytes were dependant upon use of a particular internal standard.

Recoveries are for data that is normalized to the internal standard that is closest in structure to the target analyte. The internal standards used for the following data were as follows:

1. Amphetamine and phenylpropanolamine (both primary amines) are normalized against D_{11}-amphetamine.

2. MDEA is normalized against N-propylamphetamine (a similarly hindered amine).

3. All other analytes, including methamphetamine, are normalized against D_{14}-methamphetamine.

4. Data is presented also for methamphetamine normalized against D_{11}-amphetamine to show comparison of results.

E. Analysis of Trends in Analyte Stability during Storage

The zero day set must be ignored since in most cases recoveries on day zero are much lower than for the following days. Only in a few cases was it high (ephedrine, phenylpropanolamine, and phencyclidine), and then only on certain media. The set was not prepared with the long-term storage stability study but taken from a theoretically identical set used in the precision and accuracy study. In congruities cannot be explained. But since recoveries were so high on subsequent days, trends can be analyzed in the absence of this set.

For methamphetamine the trend in recoveries tended to decline slightly over 30 days refrigerated for all media and internal standards except cotton and NU-GAUZE™. For NU-GAUZE™ it jumped up and then declined to about the 7-day level. For cotton it stayed about the

same or slightly increased. Recoveries at 7 days at room temperature were very good on all media and with every internal standard.

For amphetamine recoveries on all media tended to decline slightly except for cotton gauze which experienced a slight increase at day 30. Stability was very good on all media at 7 days at room temperature on all media.

For MDMA the results were similar to amphetamine, except on cotton, after a general increase in recovery, there was a decline by day 30. Still, the recoveries were all very nearly 100%. At room temperature for 7 days the recoveries were also good, except for NU-GAUZE™ which dropped slightly below 90%.

MDEA had a precipitous drop in recovery by day 30 for MIRASORB™ and SOF-WICK™. AlphaWipe™ recoveries were experiencing a steady decline. Cotton and NU GAUZE™ were good.

L-Ephedrine was experiencing a steady decline on SOF-WICK™.

For Pseudoephedrine, SOF-WICK™ barely got over 90% recovery on 2 days but dropped below on both day 30 refrigerated and day 7 at room temperature. On NU GAUZE™, recoveries dropped precipitously by day 21 and 30.

Phenylpropanolamine, for which recovery problems are normal, good recoveries were experienced and only dropped slightly below 90% on MIRASORB™ and NU GAUZE™. Recoveries by day 7 at room temperature were also good. This set of data shows that phenylpropanolamine can be analyzed successfully if analyzed promptly.

Phencyclidine did well on all media except on SOF-WICK™. Recoveries on NU GAUZE™ just barely dropped below 90% on day 30 refrigerated and day 7 at room temperature.

Phentermine did well on all media but recovery dropped on MIRASORB™ at day 30 refrigerated and day 7 at room temperature.

Caffeine did not fare well on AlphaWipe™ from the start. Recoveries were good for both refrigerated and room temperature storage on cotton gauze.

Table 14 lists the maximum time in days for which various analyses are stable on each media.

TABLE 14. SUMMARY OF STABILITY OF EACH ANALYTE ON EACH MEDIA (1)

			MEDIA				
	Analyte	Int Std (2)	Cotton	MIRASORB™	NU GAUZE™	SOF-WICK™	AlphaWipe™
1	Amphetamine	D_{11}-Amp	**30 days**	**30 days**	**30 days**	**30 days**	**30 days**
2	Caffeine	D_{11}-Amp	**30 days**	**30 days**	**30 days**	**30 days**	marginal (4)
3	L-ephedrine	D_{14}-Meth	**30 days**	30 days	30 days	Not OK	30 days
4	MDEA	N-PAmp	**30 days**	21 days	30 days	21 days	marginal (4)
5	MDMA	D_{11}-Amp	**30 days**	30 days	marginal (4)	30 days	30 days
6	Methamphetamine	D_{11}-Amp, D_{14}-Meth	**30 days**	**30 days**	**30 days**	**30 days**	**30 days**
7	Norephedrine (3)	D_{11}-Amp	**30 days**	marginal (4)	marginal (4)	30 days	30 days
8	Phencyclidine	D_{14}-Meth	**30 days**	30 days	marginal (4)	Not OK	30 days
9	Phentermine	D_{11}-Amp	**30 days**	**30 days**	**30 days**	**30 days**	**30 days**
10	Pseudoephedrine	D_{14}-Meth	**30 days**	marginal (4)	14 days	Not OK	30 days

(1) Acceptable recoveries are those that are 90% or better using one of the internal standards listed.

(2) Internal Standards: D11-Amp = D11-amphetamine, D14-Meth = D14-methamphetamine, N-PAmp = N-propylamphetamine

(3) Norephedrine = phenylpropanolamine

(4) Marginal = 85-90%.recoveries by day 30 refrigerated and/or day 7 at room temperature.

Table 15 gives a summary of stability in percent recovery for day 7 at room temperature and for 30 days under refrigeration at <6 °C for four media.

TABLE 15. PERCENT STORAGE STABILITY FOR 30 DAYS REFRIGERATED AND 7 DAYS AT ROOM TEMPERATURE [1]

	Compound	Amount spiked per sample [2]	Internal standard [3]	Percent Recovery [4] 3"x3" 12-ply Cotton gauze		4"x4" 4-ply MIRASORB™		4"x4" 4-ply NU GAUZE™		4"x4" AlphaWipe™	
				30 days	7 days	30 days	7 days	30 days	7 days	30 days	7 days
		µg/sample		<6 °C	22 °C	<6 °C	22 °C	<6 °C	22 °C	<6 °C	22 °C
1	Amphetamine	3.000	D_{11}-Amp	100.5	94.5	91.9	97.8	93.0	95.1	94.8	97.1
			D_{14}-Meth	99.7	87.9	89.2	93.9	88.6	88.5	87.4	89.4
2	Caffeine	3.001	D_{11}-Amp	99.3	98.8	103.8	106.8	96.9	96.1	87.4	87.8
			D_{14}-Meth	98.5	91.9	100.8	102.5	92.3	89.4	80.6	80.8
3	L-Ephedrine	3.018	D_{11}-Amp	95.6	97.2	100.5	104.8	101.9	101.3	101.9	100.9
			D_{14}-Meth	94.8	90.5	97.5	100.6	97.1	94.3	93.9	92.8
4	MDE	2.859	N-PAmp	98.9	102.1	83.6	109.9	93.5	95.6	83.6	85.4
5	MDMA	2.718	D_{11}-Amp	99.7	111.1	94.9	107.8	95.1	96.1	103.2	99.9
			D_{14}-Meth	98.9	103.2	92.2	103.4	90.6	89.4	95.2	91.8
6	Methamphetamine	3.002	D_{11}-Amp	98.7	100.6	93.3	102.0	93.6	103.1	101.3	101.4
			D_{14}-Meth	98.0	93.5	90.5	97.9	89.2	95.9	93.4	93.4
7	Phencyclidine	3.004	D_{11}-Amp	103.7	105.2	103.3	106.7	93.8	96.5	99.5	100.0
			D_{14}-Meth	102.9	97.7	100.2	102.3	89.4	89.8	91.8	92.1
8	Phentermine	3.021	D_{11}-Amp	102.0	101.5	85.2	87.1	95.4	96.9	94.0	94.8
			D_{14}-Meth	101.1	94.3	82.7	83.8	90.9	90.2	86.7	87.3
9	(±)-Norephedrine [5]	3.024	D_{11}-Amp	94.3	92.7	88.8	97.5	86.7	89.6	94.8	90.6
			D_{14}-Meth	93.6	86.2	86.2	93.5	82.6	83.4	87.5	83.5
10	Pseudoephedrine	3.017	D_{11}-Amp	100.4	97.9	101.2	87.2	85.3	98.8	112.2	106.5
			D_{14}-Meth	99.6	91.1	98.3	83.8	81.2	91.9	103.5	98.0

(1) Thirty samples were spiked for each media. Of the thirty samples for each media, six samples were analyzed immediately after preparation. Six were stored at room temperature (about 24 °C) for 7 days and then analyzed. Eighteen samples were stored at <6 °C. Of the 18 samples stored at <6 °C, six each were analyzed at 7 and 21 days and three each were analyzed at 14 and 30 days.

(2) Wipes were placed into 50-mL PP centrifuge tubes with 3-mL of isopropanol, and then spiked with analyte in 60 µL methanol.

(3) Recoveries vary slightly depending upon internal standard used.
Internal Standards: D11-Amp = D11-amphetamine, D14-Meth = D14-methamphetamine, N-PAmp = N-propylamphetamine.
Analysis was performed using the scan mode.

(4) For cotton gauze and AlphaWipes™ two wipes were used per sample. For MIRASORB™ and NU GAUZE™, one wipe was used per sample.

(5) (±)-Norephedrine = (±)-phenylpropanolamine.

F. Conclusions

Cotton appears to be the overall best media for all analytes tested. These data refute rumors that methamphetamine is not stable on cotton media. However, cellulose (taken to mean ground up wood fiber such as is used in tissue papers) is not included in this endorsement of cotton fibers.

All media tested are acceptable for the relatively simple phenethylamines, amphetamine, methamphetamine, and phentermine.

Phenylpropanolamine should be analyzed promptly, probably within 24 hours after derivatization. Vials in this study were amber. They should be routinely used.

The preferred internal standards appear to be those that have similar hindrance at the nitrogen group. D_{11}-Amphetamine, a primary amine, should be used with analytes that are primary amines. D_{14}-Methamphetamine, an N-methyl amine, should be used for analytes that are N-methyl amines. N-Propyl amphetamine or a similarly hindered amine should be used with MDEA. D_{14}-Methamphetamine is also useful for other amines.

These results apply to both analytical methods, NIOSH 9106 and NIOSH 9109, since storage stability is a function of the media and not of the method of determination.

VI. EVALUATION OF PRECISION AND ACCURACY WITH ISOPROPANOL AS THE WETTING SOLVENT

A. Objective

The Precision and Accuracy study determined whether the method can produce a result that is within ±25% of the true value with 95% confidence, which is the criterion for an acceptable method.

B. Scope and Limitations

In the "Guidelines for Air Sampling and Analytical Method Development and Evaluation" [5], the Precision and Accuracy evaluation presumes that both a desorption efficiency and a simulated sampling efficiency study will be performed. However, this method is not an air sampling method and no simulated sampling efficiency study can be clearly performed. Precision and accuracy have to be determined from what is essentially a desorption efficiency study on the wipe media. Therefore, the acceptable desorption efficiency will not be as low as 75% but between 90 to 110% which is the limit for the mean bias after correction for desorption efficiency.

A surface recovery study was made using Formica™, varnished hardwood paneling, a latex painted wall, an enameled appliance surface, and a vinyl veneered particle board book shelf. The results of this surface recovery study is reported in the Backup Data Report for NIOSH 9109 [6].

The following objectives were sought and met:

a. Overall precision: ≤10%;

b. Accuracy: ≤25%;

c. Mean bias: ≤±10%.

C. Reagents and Supplies

a. Media (See Table 16.);

TABLE 16. MEDIA FOR LONG-TERM STABILITY TEST

	MEDIA	SIZE	PLY	Lot Number
1	Cotton gauze [1]	3"x 3"	12-ply	1167807

(1) Caring brand

Other media were also tested but only results for cotton gauze are given in this abridged version for the Backup Data Report.

b. Mixed analyte spiking solution (See Table 17.);

TABLE 17. MIXED ANALYTE SPIKING SOLUTION (1)

	ANALYTE	Source	Lot Number	Calculated concentration as free base in µg/mL
1	D-Amphetamine HCl	Alltech	413	50.00322
2	Caffeine	Chem Service	28-49C	50.01031
3	L-Ephedrine HCl	Alltech	1505	50.29991
4	MDEA HCl	Alltech	3506	47.63766
5	MDMA HCl	Alltech	6852	45.28192
6	D-Methamphetamine HCl	Alltech	389	50.03214
7	Phencyclidine HCl	Alltech	1293-33	50.07406
8	Phentermine HCl	Sigma	105F-0129	50.34771
9	(±)-Phenylpropanolamine HCl	Sigma	91F-0298	50.40394
10	Pseudoephedrine HCl	Sigma	32K-1358	50.28431

(1) Mixed analyte spiking solution was made up in methanol, HPLC grade, B&J lot CB331.

c. Internal standard spiking solution (See Table 18.);

TABLE 18. INTERNAL STANDARD SPIKING SOLUTION (1)

	ANALYTE	Source	Lot Number	Calculated concentration as free base in µg/mL
1	(±)-Amphetamine-D_{11}, HCl	Cerilliant	35129-58A	50.00
2	N-Propylamphetamine	Alltech	1604	83.099
3	(±)-Methamphetamine-D_{14},HCl	Cerilliant	30902-25G	100.00

(1) The mixture was made up in methanol. About 2 µL of powdered crystal violet was added to about 10mL of the internal standard spiking solution to act as a visual reference as to which sample was spiked.

d. Drying columns were prepared in blank 12-mL PP columns (10mm i.d. x 75mm long barrel with a 16mm i.d. x 40mm long reservoir on top).

e. 50-mL PP centrifuge tubes.

D. Procedure

Cotton gauze was added to the PP centrifuge tubes. To each tube containing wipe media was added a volume of isopropanol (3 mL for cotton gauze), followed by an appropriate volume of mixed analyte spiking solution as given in table 19. Six replicates were prepared at each level for each wipe media. The preparation of the liquid standards is described in the section on the determination of the LOD and LOQ.

TABLE 19. SPIKING SCHEDULE FOR PRECISION AND ACCURACY STUDY

WIPE MEDIA	Number of wipes per tube	IPA mL	Amount applied per concentration level in microliters					
			µL of analyte spiking solution applied			µL of 1/10 dilution of analyte spiking solution applied		
			300x LOQ Level	100x LOQ Level	30x LOQ Level	10x LOQ Level	3x LOQ Level	1x LOQ Level
Cotton gauze	2	3	600	200	60	200	60	20

The final theoretical concentration of analytes on the wipe media at each concentration level is given in the table 20.

TABLE 20. CONCENTRATION OF ANALYTES AT EACH LEVEL

	ANALYTE	Calculated Concentration in µg/sample (1)					
		300x LOQ Level	100x LOQ Level	30x LOQ Level	10x LOQ Level	3x LOQ Level	1x LOQ Level
1	D-Amphetamine	30.00193	10.00064	3.00019	1.00006	0.30002	0.10001
2	Caffeine	30.00619	10.00206	3.00062	1.00021	0.30006	0.10002
3	L-Ephedrine	30.17995	10.05998	3.01799	1.00600	0.30180	0.10060
4	MDEA	28.58259	9.52753	2.85826	0.95275	0.28583	0.09528
5	MDMA	27.16915	9.05638	2.71692	0.90564	0.27169	0.09056
6	D-Methamphetamine	30.01928	10.00643	3.00193	1.00064	0.30019	0.10006
7	Phencyclidine	30.04444	10.01481	3.00444	1.00148	0.30044	0.10015
8	Phentermine	30.20862	10.06954	3.02086	1.00695	0.30209	0.10070
9	Phenylpropanolamine	30.24236	10.08079	3.02424	1.00808	0.30242	0.10081
10	Pseudoephedrine	30.17059	10.05686	3.01706	1.00569	0.30171	0.10057

(1) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

After spiking the samples, 50µL of internal standard spiking solution was added to each tube using a Hamilton repeating dispenser. The addition of internal standard was made by distributing several microliters at a time in several locations around the wipes. Following addition of internal standard solution, 40 mL of desorption solution (0.2N aqueous sulfuric acid) was added to each sample. The tubes were capped securely and tumbled for 2.5 to 5 hours. The samples were put into the walk-in cooler until the desorbates were desorbed and the desorbate extracted.

Two days later the samples were desorbed, extracted, derivatized, and analyzed as described in NIOSH 9106 using GC-MS in both the scan and SIM modes.

E. Analysis and Results

The samples were analyzed by GC-MS using the GC-MS conditions described NIOSH 9106. The recovery data for individual replicates are given in Tables 23 through 27.

Accuracy was calculated using a formula given by Dr. Eugene Kennedy of NIOSH rather than using the nomogram in the NIOSH Guidelines for Method Development and Evaluation manual. [9] The formula is as follows:

If the absolute value of the bias is less than Srt/1.645, the accuracy is

1.96 times the square root of the sum of bias squared and Srt squared;

$$1.96 \times \sqrt{((\text{bias})^2 + (\text{Srt})^2)}.$$

If the absolute value of the bias is equal to or greater than Srt/1.645, the accuracy is the absolute value of the bias plus the value Srt times 1.645;

$$|\text{bias}| + (\text{Srt} \times 1.645).$$

In calculating homogeneity of precision and bias, as many concentration levels were left in as possible. Bartlett's test was used to determine homogeneity of precision. The F' test (Eugene Kennedy, Ph.D. [9]) was used to determine homogeneity of bias. Only those concentration levels that passed BOTH the Bartlett's test and the F' test were used for calculating pooled CVs and average bias. Accuracy was then calculated from these. Where possible, the lowest concentration level was conserved, in order to report lower detection limits, and higher concentration levels having "inlier" CVs were omitted. This gives a more conservative estimate of the pooled CV as well. The concentration levels that were omitted are noted in Part B of Tables 23 through 27.

A second precision and accuracy study was conducted to test the effect of methanol as the gauze wetting solvent in place of isopropanol. This study is reported in section VIII.

Results are given for both scan and SIM modes of operation. Results are also given for two internal standards, amphetamine-D_{11} and methamphetamine-D_{14}. Results for a third internal standard, N-methylphenethylamine, are given in the un-abridged Backup Data Report. [4]

For MDEA, only results using N-propyl amphetamine as the internal standard are given since those results were the only viable ones.

TABLE 21. SUMMARY OF PRECISION AND ACCURACY EVALUATION ON COTTON GAUZE IN SCAN MODE [1]

	Compound	Internal Standard [2]	Range [3] µg/sample	Accuracy	Overall Precision $\hat{S}_{rT}$	Bias Average	Bias Range
1	(D)-Amphetamine	D_{11}-Amp	0.1-30	17.1	0.0670	-0.0613	-0.1048 - -0.0170
		D_{14}-Meth	0.1-30	13.4	0.0610	+0.0338	-0.0151 - +0.1056
2	Caffeine	D_{11}-Amp	1.0-30	20.0	0.0708	-0.0832	-0.1476 - -0.0542
		D_{14}-Meth	1.0-30	10.6	0.0636	-0.0014	- 0.0274 - +0.0381
3	(L)-Ephedrine	D_{11}-Amp	0.1-30	15.4	0.0627	+0.0510	-0.0148 - +0.1128
		D_{14}-Meth	0.3-30	17.8	0.0674	+0.0666	+0.0261 - +0.1660
4	MDEA	N-PAmp	0.3-29	15.7	0.0817	-0.0224	-0.0656 - +0.0657
5	MDMA	D_{11}-Amp	0.3-27	20.2	0.0778	-0.0739	-0.1011 - -0.0489
		D_{14}-Meth	0.3-27	16.6	0.0652	+0.0589	-0.0947 - +0.0036
6	(D)-Methamphetamine	D_{11}-Amp	0.1-30	14.7	0.0631	-0.0435	-0.0657 - -0.0060
		D_{14}-Meth	0.1-30	12.5	0.0546	-0.0348	-0.1144 - +0.0188
7	Phencyclidine	D_{11}-Amp	0.1-30	18.2	0.0690	-0.0683	-0.1257 - -0.0136
		D_{14}-Meth	0.3-3	13.4	0.0465	-0.0577	-0.0662 - -0.0493
8	Phentermine	D_{11}-Amp	0.1-30	15.2	0.0486	-0.0720	-0.1010 - +0.0291
		D_{14}-Meth	0.1-30	10.3	0.0509	+0.0190	-0.0395 - +0.0671
9	(±)-Norephedrine [4]	D_{11}-Amp	1-30	6.0	0.0328	+0.0061	-0.0070 - +0.0248
10	Pseudoephedrine	D_{11}-Amp	0.3-30	17.2	0.0571	-0.0783	-0.1273 - -0.0560
		D_{14}-Meth	0.3-30	14.9	0.0649	-0.0422	-0.0888 - +0.0395

(1) Data extracted from Appendix-I, this report. Values are for chlorodifluoroacetyl derivatives and analysis by GC/MS in scan mode (see NIOSH 9106 for conditions). Each sample consisted of a pair of 12 ply 3" x 3" cotton gauze pads. There were 6 replicate samples per concentration level.

(2) Internal Standards: D_{11}-Amp = Amphetamine-D_{11}
D_{14}-Met = Methamphetamine-D_{14}
N-PAmp = N-Propyl amphetamine

(3) Range over which the precision, accuracy, and bias were calculated. The range studied for all analytes was 0.1 to 30 µg/sample (1X LOQ to 300X LOQ).

(4) (±)-Norephedrine = (±)-phenylpropanolamine.

TABLE 22. SUMMARY OF PRECISION AND ACCURACY EVALUATION ON COTTON GAUZE IN SIM MODE [1]

Compound	Internal Standard [2]	Range [3] µg/sample	Accuracy	Overall Precision $\hat{S}_{rT}$	Bias Average	Bias Range
1 (D)-Amphetamine	D_{11}-Amp	0.1-30	14.3	0.0412	-0.0750	-0.1153 - -0.0351
	D_{14}-Meth	0.1-30	9.1	0.0508	-0.0074	-0.0500 - +0.0389
2 Caffeine	D_{11}-Amp	0.2-30	21.3	0.0578	-0.1182	-0.1949 - -0.0697
	D_{14}-Meth	0.2-30	14.4	0.0534	-0.0558	-0.1061 - -0.0170
3 (L)-Ephedrine	D_{11}-Amp	0.3-30	8.9	0.0421	-0.0199	-0.0423 - +0.0157
	D_{14}-Meth	0.3-30	20.5	0.0503	+0.1226	+0.0637 - +0.1883
4 MDEA	N-PAmp	0.3-29	10.3	0.0264	-0.0597	-0.0879 - -0.0095
5 MDMA	D_{11}-Amp	0.1-27	16.2	0.0503	-0.0750	-0.1423 - -0.0292
	D_{14}-Meth	0.1-0.9	15.4	0.0503 [4]	-0.0712	-0.1247 - +0.0032
6 (D)-Methamphetamine	D_{11}-Amp	0.1-10	16.5	0.0379	-0.1030	-0.1414 - -0.0660
	D_{14}-Meth	0.1-30	9.2	0.0351	-0.0343	-0.0767 - +0.0006
7 Phencyclidine	D_{11}-Amp	0.1-10	17.7	0.0428	-0.1068	-0.1303 - -0.0586
	D_{14}-Meth	0.1-3	11.3	0.0450	-0.0393	-0.0683 - -0.0205
8 Phentermine	D_{11}-Amp	0.1-30	12.8	0.0394	-0.0637	-0.0982 - -0.0433
	D_{14}-Meth	0.1-30	8.7	0.0495	-0.0051	-0.0375 - +0.0556
9 (±)-Norephedrine [5]		(5)	(5)	(5)	(5)	(5)
10 Pseudoephedrine	D_{11}-Amp	0.3-30	17.3	0.0402	-0.1073	-0.1496 - -0.0514
	D_{14}-Meth	0.3-30	11.5	0.0519	-0.0294	-0.0559 - +0.0532

(1) Data from Appendix-I, this report. Values are for chlorodifluoroacetyl derivatives and analysis by GC/MS in SIM mode (see NIOSH 9106 for conditions). Each sample consisted of a pair of 12 ply 3" x 3" cotton gauze pads. There were 6 replicate samples per concentration level. Norephedrine (phenylpropanolamine) was not evaluated in the SIM mode.

(2) Internal Standards: D_{11}-Amp = Amphetamine-D_{11}
D_{14}-Met = Methamphetamine-D_{14}
N-PAmp = N-Propyl amphetamine

(3) Range over which the precision, accuracy, and bias were calculated. The range studied for all analytes was 0.1 to 30 µg/sample (1X LOQ to 300X LOQ).

(4) The overall precision, $\hat{S}_{rT}$, is an estimate due to inlier precisions (<0.02) at several higher concentration levels.

(5) (±)-Norephedrine = (±)-phenylpropanolamine. No results are presented due to breakdown of derivative on standing un-refrigerated.

TABLE 23. MICROGRAMS RECOVERED ON COTTON (SCAN MODE, D_{11}-AMPHETAMINE)

PART A	MICROGRAMS PER SAMPLE RECOVERED in SCAN MODE								
SCAN MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{11}-Amphetamine								
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Phenyl-propanol-amine	Pseudo-ephedrine
Amount Applied =	30.00019	30.0062	30.1799	27.1692	30.0193	30.0444	30.2086	30.2424	30.1706
300x LOQ 1	28.017	25.476	29.609	24.393	27.251	28.668	28.530	28.940	25.470
300x LOQ 2	28.727	27.096	33.200	24.668	27.452	31.595	28.591	29.250	26.496
300x LOQ 3	30.086	27.836	35.348	24.683	27.656	32.307	29.448	30.962	28.088
300x LOQ 4	30.355	27.800	33.071	24.486	28.077	34.171	30.332	30.715	28.835
300x LOQ 5	29.735	30.432	39.657	29.074	30.248	41.641	29.827	30.356	28.594
300x LOQ 6	30.031	30.885	40.202	27.734	28.851	42.670	29.252	30.923	29.636
Average µg/sample =	29.492	28.254	35.181	25.840	28.256	35.175	29.330	30.191	27.853
CVi =	0.03111	0.07271	0.11699	0.07872	0.03995	0.16200	0.02390	0.02918	0.05623
Group Bias =	-0.01700	-0.05839	0.16571	-0.04894	-0.05874	0.17078	-0.02909	-0.00170	-0.07682
Average % Recovery =	**98.30**	**94.16**	**116.57**	**95.11**	**94.13**	**117.08**	**97.09**	**99.83**	**92.32**
Amount Applied =	10.0006	10.0021	10.06	9.0564	10.0064	10.0148	10.0695	10.0808	10.0569
100x LOQ 1	9.867	8.942	9.910	8.513	9.299	9.373	10.174	10.181	9.319
100x LOQ 2	9.736	8.923	9.889	7.689	8.490	9.244	10.126	10.235	9.080
100x LOQ 3	10.118	9.847	10.620	9.286	9.647	11.012	10.210	10.035	9.085
100x LOQ 4	9.927	9.724	11.307	9.117	9.747	10.211	10.139	10.583	9.622
100x LOQ 5	10.181	9.656	11.192	8.786	9.772	10.311	10.493	10.623	9.939
100x LOQ 6	10.010	8.845	10.498	8.177	9.139	9.123	10.114	10.330	9.562
Average µg/sample =	9.973	9.323	10.569	8.595	9.349	9.879	10.209	10.331	9.434
CVi =	0.01651	0.04983	0.05734	0.06958	0.05262	0.07589	0.01403	0.02241	0.03569
Group Bias =	-0.00275	-0.06791	0.05063	-0.05098	-0.06571	-0.01357	0.01389	0.02484	-0.06190
Average % Recovery =	**99.73**	**93.21**	**105.06**	**94.90**	**93.43**	**98.64**	**101.39**	**102.48**	**93.81**
Amount Applied =	3.0002	3.0006	3.018	2.7169	3.0019	3.0044	3.0209	3.0242	3.0171
30x LOQ 1	2.6059	2.4366	2.8452	2.4998	2.4369	2.4873	2.6903	3.1453	2.4569
30x LOQ 2	2.7374	2.7110	3.1123	2.5920	2.5926	2.6853	2.7737	3.5373	2.7531
30x LOQ 3	2.7234	2.6778	3.0234	2.4052	2.5070	2.6443	2.9517	3.6736	2.7683
30x LOQ 4	2.6834	2.5660	3.1266	2.3057	2.5176	2.6558	2.5744	3.4058	2.6980
30x LOQ 5	2.6671	2.3987	2.7851	2.4101	2.4187	2.7024	2.6113	3.3465	2.5025
30x LOQ 6	2.6974	2.5561	2.9466	2.4405	2.5546	2.5866	2.8207	3.1561	2.6185
Average µg/sample =	2.6858	2.5577	2.9732	2.4422	2.5046	2.6270	2.7370	3.3774	2.6329
CVi =	0.01742	0.04881	0.04706	0.03961	0.02671	0.03015	0.05138	0.06181	0.04958
Group Bias =	-0.10480	-0.14761	-0.01484	-0.10111	-0.16568	-0.12565	-0.09396	0.11679	-0.12733
Average % Recovery =	**89.52**	**85.24**	**98.52**	**89.89**	**83.43**	**87.44**	**90.60**	**111.68**	**87.27**
Amount Applied =	1.0001	1.0002	1.006	0.9056	1.0006	1.0015	1.007	1.0081	1.0057
10x LOQ 1	0.8958	1.0307	1.0161	0.8617	0.8169	0.9457	0.9289	0.9725	0.9246
10x LOQ 2	0.8218	1.0663	0.9832	0.8038	0.7955	0.9010	0.8790	0.9421	0.8722
10x LOQ 3	0.9069	0.8513	1.0311	0.8518	0.8647	0.9001	0.9274	1.0106	0.8702
10x LOQ 4	0.9316	0.9308	1.1273	0.8658	0.8510	0.9827	0.9645	none	1.0728
10x LOQ 5	0.9201	none	1.1560	0.8398	0.8604	0.9077	0.9398	1.0614	0.9961
10x LOQ 6	0.9004	0.8508	1.1182	0.7722	0.8012	0.9066	0.9388	1.0183	0.9602
Average µg/sample =	0.8961	0.9460	1.0720	0.8325	0.8316	0.9240	0.9297	1.0010	0.9494
CVi =	0.04320	0.10559	0.06587	0.04445	0.03704	0.03617	0.03032	0.04557	0.08210
Group Bias =	-0.10396	-0.05422	0.06559	-0.08074	-0.16892	-0.07740	-0.07669	-0.00704	-0.05602
Average % Recovery =	**89.60**	**94.58**	**106.56**	**91.93**	**83.11**	**92.26**	**92.33**	**99.30**	**94.40**
Amount Applied =	0.3		0.3018	0.2717	0.3002	0.3004	0.3021	0.3024	0.3017
3x LOQ 1	0.2707		0.3019	0.2835	0.2627	0.2962	0.2701	0.2789	0.2974
3x LOQ 2	0.2759		0.3332	0.2439	0.2415	0.2870	0.2844	0.3095	0.2630
3x LOQ 3	0.2707		0.3018	0.2086	0.2421	0.2447	0.2566	0.3192	0.2860
3x LOQ 4	0.2926		0.3153	0.2778	0.2351	0.2772	0.2925	0.3813	0.2959
3x LOQ 5	0.2446		0.3569	0.2574	0.2333	0.2537	0.2750	0.3885	0.2748
3x LOQ 6	0.2572		0.2757	0.2162	0.2406	0.2450	0.2509	0.3191	0.2677
Average µg/sample =	0.2686		0.3141	0.2479	0.2425	0.2673	0.2716	0.3328	0.2808
CVi =	0.06104		0.08973	0.12521	0.04333	0.08389	0.05864	0.12944	0.05173
Group Bias =	-0.10467		0.04087	-0.08757	-0.19202	-0.11032	-0.10097	0.10028	-0.06929
Average % Recovery =	**89.53**		**104.09**	**91.24**	**80.80**	**88.97**	**89.90**	**110.03**	**93.07**

TABLE 23. CONTINUED. MICROGRAMS RECOVERED ON COTTON (SCAN MODE, D_{11}-AMPHETAMINE)

PART A continued	MICROGRAMS PER SAMPLE RECOVERED in SCAN MODE								
SCAN MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{11}-Amphetamine								
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Phenyl-propanol-amine	Pseudo-ephedrine
Amount Applied =	0.1		0.1006	0.0906	0.1001	0.1001	0.1007	0.1008	0.1006
1x LOQ 1	0.0938		0.1122	0.1014	0.1103	0.1027	0.0945	0.1456	0.1150
1x LOQ 2	0.0841		0.1070	0.0940	0.0897	0.0938	0.0910	0.1695	0.1065
1x LOQ 3	0.0950		0.1066	0.0819	0.0893	0.0990	0.0849	0.1589	0.1217
1x LOQ 4	0.1155		0.1171	0.1160	0.1025	0.1133	0.0988	0.1585	0.1313
1x LOQ 5	0.1026		0.1114	0.0954	0.0986	0.0855	0.1024	0.1619	0.1127
1x LOQ 6	0.0974		0.1174	0.1089	0.1064	0.0977	0.0967	0.1849	0.1309
Average µg/sample =	0.0981		0.1120	0.0996	0.0995	0.0987	0.0947	0.1632	0.1197
CVi =	0.10671		0.04184	0.12056	0.08697	0.09382	0.06506	0.08047	0.08433
Group Bias =	-0.01940		0.11283	0.09978	-0.00597	-0.01479	-0.05937	0.61909	0.19007
Average % Recovery =	**98.06**		**111.28**	**109.98**	**99.40**	**98.52**	**94.06**	**161.91**	**119.01**

PART B	PRECISION AND ACCURACY RESULTS for SCAN MODE								
SCAN MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{11}-Amphetamine								
	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Phenyl-propanol-amine	Pseudo-ephedrine
OPTION #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1
Test Levels omitted and reason for omission	30x LOQ 100x LOQ inlier CVs	1x LOQ and 3x LOQ undetectable	NONE	1x LOQ CV>10%	3x LOQ 10x LOQ 30x LOQ all biases >10%	300x LOQ CV>10% bias>10%	100x LOQ inlier CV	1x LOQ 3x LOQ 30x LOQ all biases >10%	1x LOQ bias>10%
Degrees of freedom =	3	3	5	4	2	4	4	2	4
Accuracy =	**17.145**	**19.978**	**19.274**	**20.184**	**14.720**	**18.188**	**15.193**	**6.542**	**17.222**
Overall Precision =	**0.06698**	**0.07084**	**0.07453**	**0.07779**	**0.06306**	**0.06902**	**0.04858**	**0.03281**	**0.05711**
Chi^2 =	7.838	3.555	7.570	8.192	2.900	8.370	6.152	2.151	3.468
pass @ 0.95?	**no**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
pass @ 0.975?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
Mean bias =	**-0.06126**	**-0.08324**	**0.07013**	**-0.07387**	**-0.04347**	**-0.06834**	**-0.07202**	**0.00610**	**-0.07827**
from	-0.10467	-0.14761	-0.01484	-0.10111	-0.06571	-0.12565	-0.10097	-0.00704	-0.12733
to	-0.01700	-0.05422	0.16571	-0.04894	-0.00597	-0.01357	-0.02909	0.02484	-0.05602
F' =	2.25057	1.68768	2.84459	0.45055	0.85144	2.66852	1.29388	0.36106	1.04331
pass @ 0.05?	**YES**	**YES**	**no**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
pass @ 0.025?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
OPTION #2	Option #2		Option #2		Option #2			Option #2	
Test Levels omitted and reason for omission	1x LOQ, CV>10% 100x LOQ, inlier CV		300x LOQ CV>10% bias>10%		1x LOQ 3x LOQ, bias>10% 30x LOQ, bias>10%			1x LOQ & 3x LOQ, biases >10% 100x LOQ, ~inlier CV	
Degrees of freedom =	3		4		2			2	
Accuracy =	**15.075**		**15.412**		**16.973**			**11.678**	
Overall Precision =	**0.04142**		**0.06268**		**0.04373**			**0.04756**	
Chi^2 =	6.800		3.544		0.655			2.376	
pass @ 0.95?	**YES**		**YES**		**YES**			**YES**	
pass @ 0.975?	**YES**		**YES**		**YES**			**YES**	
Mean bias =	**-0.08261**		**0.05101**		**-0.09779**			**0.03855**	
from	-0.10480		-0.01484		-0.16892			-0.00704	
to	-0.01700		0.11283		-0.05874			0.11679	
F' =	2.93116		2.05181		4.27475			4.63507	
pass @ 0.05?	**YES**		**YES**		**no**			**no**	
pass @ 0.025?	**YES**		**YES**		**YES**			**YES**	

TABLE 24. MICROGRAMS RECOVERED ON COTTON (SCAN MODE, D_{14}-METHAMPHETAMINE)

PART A	MICROGRAMS PER SAMPLE RECOVERED								
SCAN MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine								
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Phenyl-propanol-amine	Pseudo-ephedrine
Amount Applied =	30.00019	30.0062	30.1799	27.1692	30.0193	30.0444	30.2086	30.2424	30.1706
300x LOQ 1	29.425	27.080	31.111	25.745	28.781	30.474	29.870	29.952	26.956
300x LOQ 2	31.356	29.839	36.027	27.120	30.249	35.005	31.135	31.205	29.083
300x LOQ 3	32.221	30.126	37.355	26.751	30.007	34.982	31.545	32.563	30.245
300x LOQ 4	33.680	31.234	36.873	27.563	31.600	38.598	33.538	33.208	32.081
300x LOQ 5	28.918	29.471	36.475	28.014	29.313	36.659	29.047	29.702	27.949
300x LOQ 6	30.808	31.564	39.341	28.408	29.773	40.277	30.081	31.490	30.435
Average µg/sample =	31.068	29.886	36.197	27.267	29.954	35.999	30.869	31.353	29.458
CVi =	0.05689	0.05341	0.07582	0.03500	0.03210	0.09464	0.05141	0.04427	0.06284
Group Bias =	0.03553	-0.00402	0.19937	0.00360	-0.00218	0.19820	0.02187	0.03673	-0.02362
Average % Recovery =	**103.55**	**99.60**	**119.94**	**100.36**	**99.78**	**119.82**	**102.19**	**103.67**	**97.64**
Amount Applied =	10.0006	10.0021	10.06	9.0564	10.0064	10.0148	10.0695	10.0808	10.0569
100x LOQ 1	11.040	10.093	11.164	9.555	10.481	10.626	11.390	12.750	10.445
100x LOQ 2	11.951	10.994	12.238	9.470	10.512	11.546	12.382	13.846	11.132
100x LOQ 3	10.584	10.362	11.107	9.695	10.122	11.538	10.676	11.800	9.517
100x LOQ 4	10.580	10.418	12.025	9.699	10.413	10.934	10.795	12.825	10.242
100x LOQ 5	10.761	10.272	11.814	9.288	10.361	10.950	11.075	12.804	10.491
100x LOQ 6	11.426	10.161	12.030	9.354	10.494	10.548	11.524	13.185	10.898
Average µg/sample =	11.057	10.383	11.730	9.510	10.397	11.024	11.307	12.868	10.454
CVi =	0.04913	0.03107	0.04089	0.01804	0.01405	0.03924	0.05487	0.05175	0.05377
Group Bias =	0.10561	0.03811	0.16596	0.05008	0.03905	0.10073	0.12291	0.27653	0.03949
Average % Recovery =	**110.56**	**103.81**	**116.60**	**105.01**	**103.90**	**110.07**	**112.29**	**127.65**	**103.95**
Amount Applied =	3.0002	3.0006	3.018	2.7169	3.0019	3.0044	3.0209	3.0242	3.0171
30x LOQ 1	3.0199	2.7604	2.9383	2.6069	2.8278	2.6338	3.1199	3.6079	2.5390
30x LOQ 2	3.1775	3.0859	3.2404	2.7140	3.0135	2.8568	3.2220	4.0558	2.8684
30x LOQ 3	3.2556	3.0423	3.1357	2.5031	2.9092	2.8065	3.4209	4.2019	2.8800
30x LOQ 4	3.1325	2.9306	3.2747	2.4115	2.9432	2.8400	3.0086	3.9281	2.8230
30x LOQ 5	3.0974	2.7215	2.8789	2.5139	2.8133	2.8767	3.2094	3.8423	2.5944
30x LOQ 6	3.2119	2.9707	3.1352	2.6115	3.0469	2.8192	3.3601	3.7136	2.7897
Average µg/sample =	3.1491	2.9186	3.1005	2.5602	2.9257	2.8055	3.2235	3.8916	2.7491
CVi =	0.02684	0.05083	0.05156	0.04133	0.03250	0.03130	0.04699	0.05620	0.05310
Group Bias =	0.04964	-0.02735	0.02735	-0.05770	-0.02541	-0.06622	0.06707	0.28680	-0.08882
Average % Recovery =	**104.96**	**97.27**	**102.73**	**94.23**	**97.46**	**93.38**	**106.71**	**128.68**	**91.12**
Amount Applied =	1.0001	1.0002	1.006	0.9056	1.0006	1.0015	1.007	1.0081	1.0057
10x LOQ 1	0.9512	1.0461	0.9290	0.7986	0.8659	0.9085	0.9882	1.0356	0.8541
10x LOQ 2	0.9485	1.1482	0.9763	0.8077	0.9187	0.9394	1.0165	1.0850	0.8740
10x LOQ 3	1.0736	0.9087	1.0494	0.8769	1.0245	0.9611	1.0994	1.1913	0.8932
10x LOQ 4	1.0957	1.0003	1.1412	0.8859	1.0016	1.0421	1.1362	none	1.0962
10x LOQ 5	1.0393	none	1.1248	0.8260	0.9718	0.9261	1.0632	1.1976	0.9777
10x LOQ 6	1.0286	0.8888	1.0999	0.7675	0.9152	0.9352	1.0743	1.1611	0.9527
Average µg/sample =	1.0228	0.9984	1.0534	0.8271	0.9496	0.9521	1.0630	1.1341	0.9413
CVi =	0.06003	0.10597	0.08100	0.05588	0.06310	0.04972	0.05078	0.06258	0.09489
Group Bias =	0.02275	-0.00179	0.04715	-0.08672	-0.05099	-0.04934	0.05563	0.12503	-0.06401
Average % Recovery =	**102.28**	**99.82**	**104.72**	**91.33**	**94.90**	**95.07**	**105.56**	**112.50**	**93.60**
Amount Applied =	0.3		0.3018	0.2717	0.3002	0.3004	0.3021	0.3024	0.3017
3x LOQ 1	0.2845		0.2856	0.2690	0.2751	0.2995	0.2843	0.2947	0.2830
3x LOQ 2	0.2977		0.3214	0.2382	0.2592	0.2975	0.3076	0.3351	0.2576
3x LOQ 3	0.3062		0.3059	0.2140	0.2733	0.2677	0.2900	0.3600	0.2915
3x LOQ 4	0.3245		0.3125	0.2764	0.2591	0.2947	0.3247	0.4242	0.2952
3x LOQ 5	0.2707		0.3528	0.2575	0.2580	0.2723	0.3058	0.4337	0.2758
3x LOQ 6	0.2893		0.2798	0.2207	0.2705	0.2671	0.2823	0.3587	0.2727
Average µg/sample =	0.2955		0.3097	0.2460	0.2659	0.2831	0.2991	0.3677	0.2793
CVi =	0.06304		0.08534	0.10461	0.02981	0.05519	0.05507	0.14431	0.04914
Group Bias =	-0.01512		0.02607	-0.09468	-0.11435	-0.05762	-0.00983	0.21595	-0.07426
Average % Recovery =	**98.49**		**102.61**	**90.53**	**88.57**	**94.24**	**99.02**	**121.60**	**92.57**

TABLE 24. CONTINUED. MICROGRAMS RECOVERED ON COTTON (SCAN MODE, D_{14}-METHAMPHETAMINE)

PART A continued	MICROGRAMS PER SAMPLE RECOVERED								
	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine								
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Phenyl-propanol-amine	Pseudo-ephedrine
Amount Applied =	0.1		0.1006	0.0906	0.1001	0.1001	0.1007	0.1008	0.1006
1x LOQ 1	0.0981		0.1221	0.1077	0.1172	0.1229	0.0988	0.1553	0.1232
1x LOQ 2	0.0870		0.1171	0.1006	0.0936	0.1141	0.0948	0.1826	0.1151
1x LOQ 3	0.1003		0.1176	0.0896	0.0941	0.1199	0.0886	0.1716	0.1304
1x LOQ 4	0.1153		0.1198	0.1156	0.1003	0.1270	0.0972	0.1628	0.1316
1x LOQ 5	0.1035		0.1167	0.0985	0.0986	0.1027	0.1032	0.1689	0.1168
1x LOQ 6	0.0984		0.1229	0.1115	0.1079	0.1148	0.0977	0.1951	0.1342
Average µg/sample =	0.1004		0.1194	0.1039	0.1020	0.1169	0.0967	0.1727	0.1252
CVi =	0.09131		0.02233	0.09166	0.08917	0.07268	0.05004	0.08254	0.06444
Group Bias =	0.00427		0.18655	0.14744	0.01885	0.16727	-0.03951	0.71333	0.24509
Average % Recovery =	**100.43**		**118.65**	**114.74**	**101.88**	**116.73**	**96.05**	**171.33**	**124.51**

PART B	PRECISION AND ACCURACY RESULTS								
SCAN MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine								
	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Phenyl-propanol-amine	Pseudo-ephedrine
OPTION #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1
Test Levels omitted and reason for omission	NONE	1x LOQ and 3x LOQ undetectable	1x LOQ 300x LOQ bias>10%	1x LOQ 100x LOQ, inlier CV	100x LOQ inlier CV	1x LOQ 100x LOQ 300x LOQ all biases >10%	100x LOQ bias>10%	1x LOQ bias>>10% 300x LOQ bias relatively low	1x LOQ bias>10%
Degrees of freedom =	5	3	3	3	4	2	4	3	4
Accuracy =	**13.658**	**12.474**	**17.751**	**16.611**	**12.464**	**13.428**	**10.656**	**37.613**	**14.906**
Overall Precision =	**0.06095**	**0.06363**	**0.06741**	**0.06519**	**0.05460**	**0.04654**	**0.05092**	**0.08855**	**0.06493**
Chi^2 =	6.558	6.644	3.277	7.143	9.738	1.469	0.124	7.323	3.068
pass @ 0.95?	**YES**	**YES**	**YES**	**YES**	**no**	**YES**	**YES**	**YES**	**YES**
pass @ 0.975?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
Mean bias =	**0.03378**	**0.00137**	**0.06663**	**-0.05888**	**-0.03482**	**-0.05773**	**0.01904**	**0.23047**	**-0.04224**
from	-0.01512	-0.02735	0.02607	-0.09468	-0.11435	-0.06622	-0.03951	0.12503	-0.08882
to	0.10561	0.03811	0.16596	0.00360	0.01885	-0.04934	0.06707	0.28680	0.03949
F' =	1.96437	0.68985	3.58959	1.96091	3.32688	0.07520	2.60315	2.09999	2.80846
pass @ 0.05?	**YES**	**YES**	**no**	**YES**	**no**	**YES**	**YES**	**YES**	**no**
pass @ 0.025?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
OPTION #2				Option #2					
Test Levels omitted				100x LOQ inlier CV					
Degrees of freedom =				3					
Accuracy =				**17.612**					
Overall Precision =				**0.07127**					
Chi^2 =				8.247					
pass @ 0.95?				**YES**					
pass @ 0.975?				**YES**					
Mean bias =				**-0.05888**					
from				-0.09468					
to				0.00360					
F' =				1.96091					
pass @ 0.05?				**YES**					
pass @ 0.025?				**YES**					

TABLE 25. MICROGRAMS RECOVERED ON COTTON (SIM MODE, D_{11}-AMPHETAMINE)

PART A	MICROGRAMS PER SAMPLE RECOVERED in SIM MODE							
SIM MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{11}-Amphetamine							
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Pseudo-ephedrine
Amount Applied =	30.00019	30.0062	30.1799	27.1692	30.0193	30.0444	30.2086	30.1706
300x LOQ 1	28.081	27.344	29.371	25.259	27.825	29.849	28.051	26.691
300x LOQ 2	28.813	28.718	31.563	25.534	28.080	31.628	28.748	27.186
300x LOQ 3	29.424	27.236	31.070	24.902	28.128	31.606	29.035	27.720
300x LOQ 4	30.143	27.723	29.127	24.001	28.227	31.221	30.209	28.046
300x LOQ 5	28.249	27.760	30.811	26.294	28.820	31.112	28.498	27.204
300x LOQ 6	28.979	28.715	31.990	25.621	27.995	31.860	28.864	26.965
Average µg/sample =	28.948	27.916	30.655	25.269	28.179	31.213	28.901	27.302
CVi =	0.02637	0.02340	0.03800	0.03058	0.01214	0.02317	0.02513	0.01823
Group Bias =	-0.03512	-0.06966	0.01574	-0.06995	-0.06130	0.03889	-0.04329	-0.09508
Average % Recovery =	**96.49**	**93.03**	**101.57**	**93.00**	**93.87**	**103.89**	**95.67**	**90.49**
Amount Applied =	10.0006	10.0021	10.06	9.0564	10.0064	10.0148	10.0695	10.0569
100x LOQ 1	9.997	9.672	10.644	8.670	9.635	9.692	10.455	8.967
100x LOQ 2	9.981	9.730	11.096	8.732	9.633	9.678	10.273	10.127
100x LOQ 3	10.286	9.839	11.258	8.572	9.631	9.829	10.521	10.088
100x LOQ 4	10.099	9.599	10.963	8.189	9.191	9.564	10.232	9.440
100x LOQ 5	10.078	9.508	10.907	8.388	9.380	9.705	10.343	9.401
100x LOQ 6	9.904	9.496	11.175	7.630	8.603	9.488	10.157	9.219
Average µg/sample =	10.057	9.641	11.007	8.363	9.346	9.659	10.330	9.540
CVi =	0.01316	0.01381	0.02003	0.04907	0.04344	0.01232	0.01335	0.04928
Group Bias =	0.00568	-0.03612	0.09418	-0.07651	-0.06604	-0.03551	0.02587	-0.05136
Average % Recovery =	**100.57**	**96.39**	**109.42**	**92.35**	**93.40**	**96.45**	**102.59**	**94.86**
Amount Applied =	3.0002	3.0006	3.018	2.7169	3.0019	3.0044	3.0209	3.0171
30x LOQ 1	2.7263	2.6138	2.9661	2.4231	2.6782	2.6886	2.8169	2.5000
30x LOQ 2	2.8794	2.4852	3.0867	2.4545	2.7207	2.7018	2.8897	2.6523
30x LOQ 3	2.7851	2.5377	2.9873	2.2834	2.6405	2.6094	2.8680	2.5944
30x LOQ 4	2.7601	2.5750	3.0119	2.2593	2.6103	2.6621	2.7639	2.5839
30x LOQ 5	2.8692	2.5291	2.8979	2.2719	2.6489	2.5856	2.9557	2.5074
30x LOQ 6	2.8115	2.4983	2.9204	2.2902	2.6202	2.6467	2.9203	2.5563
Average µg/sample =	2.8053	2.5399	2.9784	2.3304	2.6531	2.6490	2.8691	2.5657
CVi =	0.02157	0.01891	0.02274	0.03656	0.01535	0.01698	0.02432	0.02236
Group Bias =	-0.06497	-0.15356	-0.01313	-0.14226	-0.11619	-0.11829	-0.05024	-0.14960
Average % Recovery =	**93.50**	**84.64**	**98.69**	**85.77**	**88.38**	**88.17**	**94.98**	**85.04**
Amount Applied =	1.0001	1.0002	1.006	0.9056	1.0006	1.0015	1.007	1.0057
10x LOQ 1	0.8769	0.8183	0.9003	0.7595	0.8696	0.8622	0.9277	0.8211
10x LOQ 2	0.8864	0.8262	0.9054	0.7426	0.8628	0.8600	0.9112	0.8375
10x LOQ 3	0.9288	0.8286	0.9538	0.7646	0.9046	0.8924	0.9679	0.8741
10x LOQ 4	0.9547	0.8454	1.0508	0.7812	0.9093	0.9225	0.9992	0.9741
10x LOQ 5	0.9309	0.8428	0.9980	0.7720	0.8973	0.9015	0.9629	0.9027
10x LOQ 6	0.8975	0.7894	0.9726	0.7271	0.8530	0.8501	0.9378	0.8689
Average µg/sample =	0.9125	0.8251	0.9635	0.7578	0.8828	0.8815	0.9511	0.8797
CVi =	0.03309	0.02460	0.05937	0.02620	0.02705	0.03217	0.03342	0.06185
Group Bias =	-0.08753	-0.17505	-0.04226	-0.16321	-0.11780	-0.11985	-0.05545	-0.12524
Average % Recovery =	**91.25**	**82.49**	**95.77**	**83.68**	**88.22**	**88.01**	**94.45**	**87.48**
Amount Applied =	0.3	0.3001	0.3018	0.2717	0.3002	0.3004	0.3021	0.3017
3x LOQ 1	0.2796	0.2454	0.2869	0.2333	0.2696	0.2727	0.2796	0.2696
3x LOQ 2	0.2654	0.2614	0.2959	0.2339	0.2674	0.2677	0.2750	0.2659
3x LOQ 3	0.2641	0.2462	0.2850	0.2156	0.2520	0.2607	0.2742	0.2717
3x LOQ 4	0.2712	0.2171	0.2947	0.2207	0.2585	0.2546	0.2802	0.2786
3x LOQ 5	0.2597	0.2412	0.3049	0.2236	0.2502	0.2662	0.2656	0.2544
3x LOQ 6	0.2525	0.2382	0.2710	0.2087	0.2487	0.2459	0.2599	0.2616
Average µg/sample =	0.2654	0.2416	0.2897	0.2226	0.2577	0.2613	0.2724	0.2670
CVi =	0.03518	0.05969	0.04008	0.04442	0.03497	0.03739	0.02960	0.03145
Group Bias =	-0.11533	-0.19489	-0.03998	-0.18057	-0.14144	-0.13029	-0.09822	-0.11514
Average % Recovery =	**88.47**	**80.51**	**96.00**	**81.94**	**85.86**	**86.97**	**90.18**	**88.49**

TABLE 25. CONTINUED. MICROGRAMS RECOVERED ON COTTON (SIM MODE, D_{11}-AMPHETAMINE)

PART A continued	MICROGRAMS PER SAMPLE RECOVERED in SIM MODE							
SIM MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine							
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Pseudo-ephedrine
Amount Applied =	0.1	0.1	0.1006	0.0906	0.1001	0.1001	0.1007	0.1006
1x LOQ 1	0.0889	0.0857	0.1153	0.0909	0.0907	0.0978	0.0924	0.1088
1x LOQ 2	0.0880	0.1032	0.1160	0.0853	0.0851	0.0913	0.0895	0.1036
1x LOQ 3	0.0957	0.0886	0.1151	0.0866	0.0905	0.0930	0.0940	0.1080
1x LOQ 4	0.1046	0.0926	0.1176	0.0995	0.1002	0.1055	0.1058	0.1239
1x LOQ 5	0.0874	0.0926	0.1100	0.0837	0.0939	0.0882	0.0907	0.1084
1x LOQ 6	0.0923	0.0834	0.1111	0.0815	0.0957	0.0899	0.0888	0.1153
Average µg/sample =	0.0928	0.0910	0.1142	0.0879	0.0927	0.0943	0.0935	0.1113
CVi =	0.07070	0.07700	0.02600	0.07382	0.05571	0.06791	0.06739	0.06472
Group Bias =	-0.07189	-0.09002	0.13503	-0.02923	-0.07376	-0.05856	-0.07113	0.10704
Average % Recovery =	**92.81**	**91.00**	**113.50**	**97.08**	**92.62**	**94.14**	**92.89**	**110.70**

PART B	PRECISION AND ACCURACY RESULTS for SIM MODE							
SIM MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{11}-Amphetamine							
	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Pseudo-ephedrine
OPTION #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1
Test Levels omitted and reason for omission	100x LOQ inlier CV	1x LOQ 10x LOQ, bias>10% 30x LOQ, inlier CV	1x LOQ, bias>10% 100x LOQ, inlier CV	3x LOQ 10x LOQ all biases >10%	300x LOQ inlier CV	100x LOQ inlier CV 300x LOQ relatively high bias	100x LOQ inlier CV and relatively high bias	1x LOQ CV>10%
Degrees of freedom =	4	2	3	3	4	3	4	4
Accuracy =	**14.276**	**21.335**	**9.129**	**16.227**	**16.538**	**17.718**	**12.846**	**17.340**
Overall Precision =	**0.04121**	**0.05785**	**0.04211**	**0.05032**	**0.03789**	**0.04282**	**0.03939**	**0.04019**
Chi^2 =	8.892	5.399	3.997	4.324	7.631	8.327	8.060	9.415
pass @ 0.95?	**YES**	**YES**	**YES**	**YES**	**YES**	no	**YES**	**YES**
pass @ 0.975?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
Mean bias =	**-0.07497**	**-0.11819**	**-0.01991**	**-0.07949**	**-0.10305**	**-0.10675**	**-0.06367**	**-0.10729**
from	-0.11533	-0.19489	-0.04226	-0.14226	-0.14144	-0.13029	-0.09822	-0.14960
to	-0.03512	-0.06966	0.01574	-0.02923	-0.06604	-0.05856	-0.04329	-0.05136
F' =	1.57271	4.29082	1.06286	2.76738	2.00060	1.57385	0.88993	2.57164
pass @ 0.05?	**YES**	**no**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
pass @ 0.025?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
OPTION #2		Option #2		Option #2				
Test Levels omitted and reason for omission		1x LOQ 3x LOQ, bias>10% 100x LOQ, inlier CV		Omitting 1x LOQ 300x LOQ give smaller Chi^2.				
Degrees of freedom =		2		3				
Accuracy =		**16.967**		**20.646**				
Overall Precision =		**0.02244**		**0.04002**				
Chi^2 =		0.339		1.930				
pass @ 0.95?		**YES**		**YES**				
pass @ 0.975?		**YES**		**YES**				
Mean bias =		**-0.13276**		**-0.14064**				
from		-0.17505		-0.18057				
to		-0.06966		-0.07651				
F' =		4.50475		3.35165				
pass @ 0.05?		**no**		**no**				
pass @ 0.025?		**YES**		**YES**				

TABLE 26. MICROGRAMS RECOVERED ON COTTON (SIM MODE, D_{14}-METHAMPHETAMINE)

PART A	MICROGRAMS PER SAMPLE RECOVERED in SIM MODE							
SIM MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine							
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Pseudo-ephedrine
Amount Applied =	30.00019	30.0062	30.1799	27.1692	30.0193	30.0444	30.2086	30.1706
300x LOQ 1	29.153	28.420	30.436	26.297	29.124	31.133	29.083	27.710
300x LOQ 2	30.545	30.445	33.074	27.195	30.092	33.785	30.408	28.796
300x LOQ 3	31.456	29.229	32.931	26.821	30.454	34.174	30.980	29.599
300x LOQ 4	32.935	30.422	31.850	26.533	31.331	34.785	32.883	30.588
300x LOQ 5	28.677	28.199	31.055	26.693	29.415	31.485	28.902	27.635
300x LOQ 6	30.524	30.257	33.262	27.107	29.807	33.758	30.348	28.409
Average µg/sample =	30.548	29.495	32.101	26.774	30.037	33.186	30.434	28.789
CVi =	0.05065	0.03473	0.03662	0.01275	0.02631	0.04535	0.04757	0.03971
Group Bias =	0.01821	-0.01703	0.06366	-0.01453	0.00059	0.10458	0.00745	-0.04578
Average % Recovery =	**101.82**	**98.30**	**106.37**	**98.55**	**100.06**	**110.46**	**100.75**	**95.42**
Amount Applied =	10.0006	10.0021	10.06	9.0564	10.0064	10.0148	10.0695	10.0569
100x LOQ 1	10.643	10.292	11.981	9.2349	10.240	10.365	11.112	9.543
100x LOQ 2	10.660	10.386	12.501	9.3304	10.272	10.387	10.957	10.786
100x LOQ 3	11.179	10.691	12.898	9.3304	10.461	10.750	11.415	10.935
100x LOQ 4	11.503	10.932	13.166	9.3524	10.480	10.997	11.629	10.722
100x LOQ 5	11.155	10.525	12.740	9.3020	10.382	10.821	11.427	10.385
100x LOQ 6	12.066	11.564	14.310	9.3376	10.517	11.721	12.329	11.181
Average µg/sample =	11.201	10.732	12.933	9.3146	10.392	10.840	11.478	10.592
CVi =	0.04800	0.04350	0.06070	0.00455	0.01103	0.04596	0.04196	0.05442
Group Bias =	0.12003	0.07293	0.28554	0.02851	0.03851	0.08241	0.13987	0.05322
Average % Recovery =	**112.00**	**107.29**	**128.55**	**102.85**	**103.85**	**108.24**	**113.99**	**105.32**
Amount Applied =	3.0002	3.0006	3.018	2.7169	3.0019	3.0044	3.0209	3.0171
30x LOQ 1	2.8725	3.1115	3.3886	2.547	2.8025	2.8303	2.9694	2.6333
30x LOQ 2	3.1149	2.6857	3.6188	2.650	2.9248	2.9220	3.1274	2.8673
30x LOQ 3	3.1758	2.8891	3.6913	2.599	2.9946	2.9772	3.2710	2.9547
30x LOQ 4	3.1309	2.9159	3.7018	2.558	2.9445	3.0209	3.1363	2.9274
30x LOQ 5	3.1902	2.8085	3.4942	2.522	2.9284	2.8758	3.2871	2.7861
30x LOQ 6	3.2176	2.8548	3.6230	2.616	2.9829	3.0311	3.3425	2.9223
Average µg/sample =	3.1170	2.8776	3.5863	2.582	2.9296	2.9429	3.1890	2.8485
CVi =	0.04030	0.04876	0.03400	0.01854	0.02338	0.02746	0.04316	0.04257
Group Bias =	0.03893	-0.04100	0.18830	-0.04970	-0.02409	-0.02049	0.05564	-0.05586
Average % Recovery =	**103.89**	**95.90**	**118.83**	**95.03**	**97.59**	**97.95**	**105.56**	**94.41**
Amount Applied =	1.0001	1.0002	1.006	0.9056	1.0006	1.0015	1.007	1.0057
10x LOQ 1	0.9354	0.8591	1.0436	0.8077	0.9220	0.9180	0.9906	0.8754
10x LOQ 2	0.9570	0.8900	1.0622	0.7991	0.9263	0.9267	0.9849	0.9036
10x LOQ 3	1.0108	0.8995	1.1280	0.8293	0.9789	0.9693	1.0545	0.9505
10x LOQ 4	1.0508	0.9280	1.2572	0.8568	0.9956	1.0136	1.1010	1.0712
10x LOQ 5	1.0170	0.9184	1.1850	0.8406	0.9749	0.9831	1.0532	0.9854
10x LOQ 6	0.9912	0.8693	1.1674	0.8001	0.9374	0.9371	1.0370	0.9588
Average µg/sample =	0.9937	0.8941	1.1406	0.8223	0.9559	0.9580	1.0369	0.9575
CVi =	0.04233	0.03012	0.07014	0.02887	0.03253	0.03868	0.04211	0.07145
Group Bias =	-0.00636	-0.10613	0.13377	-0.09206	-0.04476	-0.04345	0.02971	-0.04793
Average % Recovery =	**99.36**	**89.39**	**113.38**	**90.79**	**95.52**	**95.65**	**102.97**	**95.21**
Amount Applied =	0.3	0.3001	0.3018	0.2717	0.3002	0.3004	0.3021	0.3017
3x LOQ 1	0.2832	0.2488	0.3114	0.2359	0.2717	0.2757	0.2832	0.2732
3x LOQ 2	0.2810	0.2761	0.3359	0.2467	0.2832	0.2829	0.2916	0.2813
3x LOQ 3	0.2873	0.2664	0.3319	0.2331	0.2750	0.2831	0.2989	0.2952
3x LOQ 4	0.2999	0.2380	0.3488	0.2424	0.2870	0.2807	0.3105	0.3073
3x LOQ 5	0.2824	0.2609	0.3556	0.2419	0.2730	0.2889	0.2894	0.2762
3x LOQ 6	0.2763	0.2590	0.3168	0.2269	0.2731	0.2683	0.2849	0.2856
Average µg/sample =	0.2850	0.2582	0.3334	0.2378	0.2772	0.2799	0.2931	0.2865
CVi =	0.02846	0.05173	0.05192	0.03042	0.02291	0.02541	0.03471	0.04468
Group Bias =	-0.05001	-0.13951	0.10471	-0.12468	-0.07670	-0.06827	-0.02980	-0.05051
Average % Recovery =	**95.00**	**86.05**	**110.47**	**87.53**	**92.33**	**93.17**	**97.02**	**94.95**

TABLE 26. CONTINUED. MICROGRAMS RECOVERED ON COTTON (SIM MODE, D_{14}-METHAMPHETAMINE)

PART A continued	MICROGRAMS PER SAMPLE RECOVERED in SIM MODE							
SIM MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine							
TEST LEVEL	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Pseudo-ephedrine
Amount Applied =	0.1	0.1	0.1006	0.0906	0.1001	0.1001	0.1007	0.1006
1x LOQ 1	0.0909	0.0876	0.1219	0.0929	0.0937	0.1000	0.0944	0.1116
1x LOQ 2	0.0925	0.1082	0.1261	0.0892	0.0913	0.0958	0.0942	0.1089
1x LOQ 3	0.0992	0.0915	0.1233	0.0895	0.0951	0.0963	0.0974	0.1121
1x LOQ 4	0.1094	0.0962	0.1270	0.1036	0.1062	0.1102	0.1106	0.1296
1x LOQ 5	0.0886	0.0943	0.1150	0.0849	0.0959	0.0894	0.0917	0.1102
1x LOQ 6	0.0969	0.0868	0.1201	0.0850	0.1022	0.0941	0.0932	0.1211
Average µg/sample =	0.0963	0.0941	0.1222	0.0909	0.0974	0.0976	0.0969	0.1156
CVi =	0.07820	0.08308	0.03581	0.07641	0.05788	0.07227	0.07181	0.07013
Group Bias =	-0.03756	-0.05919	0.21505	0.00316	-0.02663	-0.02511	-0.03753	0.14930
Average % Recovery =	**96.24**	**94.08**	**121.50**	**100.32**	**97.34**	**97.49**	**96.25**	**114.93**

PART B	PRECISION AND ACCURACY RESULTS for SIM MODE							
SIM MODE	UNITS = µg/sample (sample is desorbed in 40 mL 0.2N sulfuric acid) INT STD = D_{14}-Methamphetamine							
	Amphet-amine	Caffeine	Ephedrine	MDMA	Meth-amphet-amine	Phency-clidine	Phenter-mine	Pseudo-ephedrine
OPTION #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1
Test Levels omitted and reason for omission	100x LOQ bias>10%	3x LOQ, bias>10% 100x LOQ	1x LOQ 100x LOQ bias>>10%	30x LOQ 100x LOQ 300x LOQ all inlier CVs	100x LOQ inlier CV	100x LOQ 300x LOQ biases relatively larger	100x LOQ bias>10%	1x LOQ bias>10%
Degrees of freedom =	4	3	3	2	4	3	4	4
Accuracy =	**10.062**	**14.363**	**20.532**	**15.397**	**9.212**	**11.344**	**9.757**	**11.683**
Overall Precision =	**0.05081**	**0.05337**	**0.05028**	**0.05032**	**0.03514**	**0.04505**	**0.04952**	**0.05187**
Chi^2 =	5.396	6.111	3.230	5.889	6.697	7.035	3.085	2.344
pass @ 0.95?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
pass @ 0.975?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
Mean bias =	**-0.00736**	**-0.05584**	**0.12261**	**-0.07119**	**-0.03432**	**-0.03933**	**0.00509**	**-0.02937**
from	-0.05001	-0.10613	0.06366	-0.12468	-0.07670	-0.06827	-0.03753	-0.05586
to	0.03893	-0.01703	0.18830	0.00316	0.00059	-0.02049	0.05564	0.05322
F' =	1.88871	1.69127	2.89417	4.28815	1.62505	0.64100	2.15910	2.90807
pass @ 0.05?	**YES**	**YES**	**YES**	**no**	**YES**	**YES**	**YES**	**no**
pass @ 0.025?	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
OPTION #2				Option #2				
Test Levels omitted and reason for omission				1x LOQ 100x LOQ, inlier CV 300x LOQ, inlier CV				
Degrees of freedom =								
Accuracy =				**13.237**				
Overall Precision =				**0.02648**				
Chi^2 =				1.199				
pass @ 0.95?				**YES**				
pass @ 0.975?				**YES**				
Mean bias =				**-0.08881**				
from				-0.12468				
to				-0.04970				
F' =				1.96358				
pass @ 0.05?				**YES**				
pass @ 0.025?				**YES**				

TABLE 27. MICROGRAMS RECOVERED ON COTTON (SCAN MODE, N-PROPYL AMPHETAMINE)

PART A		MICROGRAMS PER SAMPLE RECOVERED					
		UNITS = µg/sample (sample = 40mL desorption volume) INT STD = N-Propyl Amphetamine					
		SCAN mode			**SIM mode**		
TEST LEVEL		MDEA			MDEA		
Amount Applied =		28.5826			28.5826		
300x LOQ	1	24.224			26.559		
300x LOQ	2	26.263			27.457		
300x LOQ	3	28.287			26.647		
300x LOQ	4	27.893			26.502		
300x LOQ	5	26.321			26.960		
300x LOQ	6	28.271			27.346		
Average µg/sample =		26.876			26.912		
CVi =		0.05927			0.01533		
Group Bias =		-0.05970			-0.05845		
Average % Recovery =		**94.03**			**94.15**		
Amount Applied =		9.5275			9.5275		
100x LOQ	1	9.802			9.425		
100x LOQ	2	9.725			9.537		
100x LOQ	3	10.807			9.616		
100x LOQ	4	10.396			9.633		
100x LOQ	5	10.165			9.322		
100x LOQ	6	10.026			9.091		
Average µg/sample =		10.153			9.437		
CVi =		0.03962			0.02190		
Group Bias =		0.06569			-0.00947		
Average % Recovery =		**106.57**			**99.05**		
Amount Applied =		2.8583			2.8583		
30x LOQ	1	2.5849			2.6497		
30x LOQ	2	2.7280			2.6829		
30x LOQ	3	2.6740			2.6765		
30x LOQ	4	2.4650			2.6513		
30x LOQ	5	2.8051			2.7446		
30x LOQ	6	2.7675			2.6637		
Average µg/sample =		2.6708			2.6781		
CVi =		0.04748			0.01313		
Group Bias =		-0.06560			-0.06303		
Average % Recovery =		**93.44**			**93.70**		
Amount Applied =		0.9528			0.9528		
10x LOQ	1	0.8784			0.8435		
10x LOQ	2	0.9201			0.8554		
10x LOQ	3	0.8630			0.8725		
10x LOQ	4	1.0549			0.9235		
10x LOQ	5	0.8521			0.9163		
10x LOQ	6	0.9348			0.8491		
Average µg/sample =		0.9172			0.8767		
CVi =		0.08153			0.03982		
Group Bias =		-0.03730			-0.07981		
Average % Recovery =		**96.27**			**92.02**		
Amount Applied =		0.2858			0.2858		
3x LOQ	1	0.2839			0.2551		
3x LOQ	2	0.3132			0.2702		
3x LOQ	3	0.2734			0.2513		
3x LOQ	4	0.3131			0.2682		
3x LOQ	5	0.2969			0.2660		
3x LOQ	6	0.2082			0.2534		
Average µg/sample =		0.2815			0.2607		
CVi =		0.13929			0.03198		
Group Bias =		-0.01531			-0.08791		
Average % Recovery =		**98.47**			**91.21**		

TABLE 27. CONTINUED. MICROGRAMS RECOVERED ON COTTON (SCAN MODE, N-PROPYL AMPHETAMINE)

PART A continued		MICROGRAMS PER SAMPLE RECOVERED					
		UNITS = µg/sample INT STD = N-Propyl Amphetamine					
		SCAN mode MDEA			SIM mode MDEA		
TEST LEVEL							
Amount Applied =		0.0953			0.0953		
1x LOQ	1	0.1223			0.1098		
1x LOQ	2	0.0781			0.0995		
1x LOQ	3	0.0771			0.1060		
1x LOQ	4	0.1069			0.1118		
1x LOQ	5	0.0996			0.0941		
1x LOQ	6	0.1619			0.1004		
Average µg/sample =		0.1076			0.1036		
CVi =		0.29462			0.06529		
Group Bias =		0.12988			0.08738		
Average % Recovery =		**112.99**			**108.74**		

PART B	PRECISION AND ACCURACY RESULTS					
	UNITS = µg/sample INT STD = N-Propyl Amphetamine					
	SCAN mode MDEA			SIM mode MDEA		
OPTION #1	Option #1			Option #1		
Test Levels omitted and reason for omission	1x LOQ CV>>10% bias>10%			1x LOQ CV>>10% bias>10%		
Degrees of freedom =	4			4		
Accuracy =	**16.609**			**10.323**		
Overall Precision =	**0.08171**			**0.02644**		
Chi^2 =	10.085			7.957		
pass @ 0.95?	**no**			**YES**		
pass @ 0.975?	**YES**			**YES**		
Mean bias =	**-0.02244**			**-0.05973**		
from	-0.06560			-0.08791		
to	0.06569			-0.00947		
F' =	2.05486			2.25971		
pass @ 0.05?	**YES**			**YES**		
pass @ 0.025?	**YES**			**YES**		
OPTION #2	Option #2					
Test Levels omitted and reason for omission	1x LOQ CV>>10% bias>10%					
Degrees of freedom =	5					
Accuracy =	**16.027**					
Overall Precision =	**0.08171**					
Chi^2 =	10.085					
pass @ 0.95?	**no**					
pass @ 0.975?	**YES**					
Mean bias =	**0.00294**					
from	-0.06560					
to	0.12988					
F' =	1.45409					
pass @ 0.05?	**YES**					
pass @ 0.025?	**YES**					

VII. PARTIAL EVALUATION OF PRECISION AND RECOVERY USING PENTAFLUOROPROPIONIC ANHYDRIDE

A. Objective and Scope

Chlorodifluoroacetic anhydride was chosen for complete evaluation because of milder reaction conditions, relative ease of manual interpretation of spectra, and less sensitivity to reaction times and temperatures. Never-the-less, there was concern that chlorodifluoroacetic anhydride may not be well received by analytical laboratories due to the established use and availability of pentafluoropropionic anhydride. Accordingly, an abbreviated evaluation of recoveries and precision using pentafluoropropionic anhydride was performed involving a single concentration level (approximately 30X LOQ), five analytes, and four media. Results were compared with chlorodifluoroacetic anhydride derivatization on aliquots from the same extracts.

B. Reagents and Supplies

Reagents and supplies were the same as those used in the Precision and Accuracy Study (Section VI). However, only five analytes were spiked (amphetamine, methamphetamine, ephedrine, pseudoephedrine, and phenylpropanolamine) and only four media were selected. Results are given in this abridged report only for cotton gauze.

C. Procedure

Single wipes were inserted into 50-mL PP centrifuge tubes. These were wetted with 3 mL of isopropanol and then spiked with a standard spiking solution containing only the five analytes. Seven replicates were prepared for each. Reference standards were prepared in triplicate by spiking the standard spiking solution into 50-mL PP centrifuge tubes containing only isopropanol. The samples and standards were stored overnight at 0-6 °C. On the following day the samples were spiked with an internal standard solution and then desorbed with 30 mL of 0.2N sulfuric acid (the desorption solution) and processed according to the method. The final

eluate from the drying columns was split into two 4.5 mL aliquots and separately evaporated to dryness. One set was derivatized with chlorodifluoroacetic anhydride at 65 °C for 15 minutes and the other with pentafluoropropionic anhydride at 90 °C for 15 minutes. Both sets were evaporated to dryness after derivatization and taken up in 1 mL of reconstitution solvent containing 4.0 µg/mL of 4,4'-dibromooctafluorobiphenyl. The solutions were transferred to GC vials and analyzed by GC-MS using the GC-MS conditions described in an earlier section.

The target analytes were spiked at the following concentrations:

Amphetamine	6.242 µg/sample	(approx. 60X LOQ)
Ephedrine	6.250 µg/sample	(approx. 60X LOQ)
Methamphetamine	6.290 µg/sample	(approx. 60X LOQ)
Phenylpropanolamine	6.250 µg/sample	(approx. 60X LOQ)
Pseudoephedrine	14.396 µg/sample	(approx. 160X LOQ)

However, since the eluates were split, they are equivalent to an original concentration one half as much, as follows:

Amphetamine	3.121 µg/sample	(approx. 30X LOQ)
Ephedrine	3.125 µg/sample	(approx. 30X LOQ)
Methamphetamine	3.150 µg/sample	(approx. 30X LOQ)
Phenylpropanolamine	3.125 µg/sample	(approx. 30X LOQ)
Pseudoephedrine	7.198 µg/sample	(approx. 80X LOQ)

D. Results

Table 28 gives recoveries and precisions for the pentafluoropropionyl derivatives and the chlorodifluoroacetyl derivatives of aliquots from the same sample extracts. Quantification was by single point calibration. This should be regarded as valid for practical purposes since all recoveries clustered around 100% and because both sets of data were treated identically.

Precision tends not to be significantly affected regardless of method of calibration, especially when recoveries are tightly clustered.

The internal standard used was 4,4'-dibromooctafluorobiphenyl. This was valid since all initial desorption volumes were identical (3 mL isopropanol plus 30 mL 0.2N sulfuric acid). Also, no hindered amines were involved such as MDEA.

TABLE 28. RECOVERY AND PRECISION FOR PENTAFLUOROPROPIONYL DERIVATIVES ON COTTON GAUZE

PERCENT RECOVERY FROM SPIKED MEDIA					
Pentafluoropropionyl derivatives					
	D-amphet-amine	phenylpro-panolamine	L-ephedrine bis-deriv.	D-metham-phetamine	pseudo-ephedrine
µg/sample =	6.24205	6.25052	6.25000	6.28989	14.39565
WIPE RT(min.) =	6.92	7.38	8.19	8.37	8.91
MEDIA m/z =	190	190	204	204	204
Cotton gauze-1	102.67	101.42	96.91	113.93	97.74
Cotton gauze-2	102.61	103.11	98.15	109.12	98.81
Cotton gauze-3	100.63	95.29	103.26	108.26	93.57
Cotton gauze-4	111.15	106.95	113.04	112.45	101.21
Cotton gauze-5	97.77	93.85	92.04	102.84	91.92
Cotton gauze-6	108.91	106.28	104.26	110.07	97.73
Cotton gauze-7	100.90	94.64	90.70	108.14	90.65
AVERAGE =	**103.52**	**100.22**	**99.76**	**109.26**	**95.95**
STD DEV =	4.78	5.59	7.76	3.56	3.92
%RSD =	**4.62**	**5.58**	**7.78**	**3.26**	**4.09**
Chlorodifluoroacetyl derivatives					
Cotton gauze-1	101.76	91.75	103.33	98.74	93.36
Cotton gauze-2	100.90	94.74	103.15	98.68	93.20
Cotton gauze-3	109.11	99.08	106.89	101.38	98.95
Cotton gauze-4	102.70	94.01	105.65	99.18	94.32
Cotton gauze-5	100.74	92.18	103.00	97.78	91.65
Cotton gauze-6	99.57	95.18	104.87	97.48	95.26
Cotton gauze-7	104.09	93.67	104.66	100.12	93.04
AVERAGE =	**102.69**	**94.37**	**104.51**	**99.05**	**94.25**
STD DEV =	3.18	2.42	1.45	1.35	2.35
%RSD =	**3.10**	**2.57**	**1.39**	**1.36**	**2.50**

All recoveries were within 90-110% except methamphetamine. All precisions were less than 8%.

The following observations were made. First of all, recoveries and precisions for both derivatizing reagents, for all five analytes, and all four media (only results for cotton are shown) were exceptional. From the data no significant difference between the two reagents can be found.

This would suggest that it is possible that pentafluoropropionic anhydride can be used in lieu of chlorodifluoroacetic anhydride. The level tested, 30X LOQ, is a mid range concentration.

Secondly, precision and recoveries at the 30X LOQ level were much better than in the Precision and Accuracy study in Section VI, in which recoveries at the 30X LOQ level were often relatively lower than those of the higher and lower concentration levels. This better performance is probably because the above study was conducted all on one day while that in section VI had the disadvantage of the analyses for the different concentration levels performed on different days. It is suspected that in the Precision and Accuracy Study in Section VI, if all analyses pertaining to the same media were done on the same day, the final precision and accuracy would probably have been even better than reported.

E. Typical pentafluoropropionic Anhydride (PFPA) Calibration Curve

A surface recovery study was performed and reported in the Backup Data Report for NIOSH 9109 [6]. More analytes were involved. It is worth showing some of the quadratic curve fit data and LODs possible with PFPA.

The calibration curves for that study covered a range from 0.025 to 6 µg/sample. Media standards were used (3" x 3" 12-ply cotton gauze, single wipes), wetting each with 1.5 mL of methanol. Desorption volume was 30 mL of desorption solution (0.2 normal sulfuric acid). The LOD and LOQs for the calibration curves and the r^2 values are given in Table 29.

TABLE 29. QUALITY OF CALIBRATION CURVES USING PFPA

	Compound	Range (µg/sample)	Quadratic Curve Fit, r^2	LOD (µg/sample)	LOQ (µg/sample)
1	Amphetamine	0.0250-6.004	0.9999	0.0165	0.0549
2	Cocaine	0.1513-6.051	0.9994	0.3787	1.120
3	Codeine	0.0500-6.004	0.9917	0.1748	0.5334
4	Ephedrine	0.0265-6.360	0.9995	0.0178	0.0591
5	Hydrocodone	0.0505-6.056	0.9949	0.1421	0.4450
6	MDEA	0.0238-5.717	0.9992	0.0593	0.1662
7	MDMA	0.0226-5.434	0.9990	0.0380	0.1264
8	Methamphetamine	0.0252-6.039	0.9999	0.0131	0.0437
9	Phencyclidine	0.0254-6.089	0.9999	0.0284	0.0945
10	Phentermine	0.0252-6.042	0.9990	0.0182	0.0606
11	Phenylpropanolamine	0.0270-6.485	0.9994	0.0268	0.0891
12	Pseudoephedrine	0.0251-6.034	0.9996	0.0224	0.0746

Two analytes, codeine and hydrocodone, had r^2 values less than the acceptable 0.995. It is suspected that the results for these two, and for cocaine, would have been better using isotopic or chemically similar analogs for their internal standards. For MDEA the internal standard used was N-propylamphetamine. For all the other analytes methamphetamine-D_{14} was acceptable.

Other than for cocaine and the two opiates, the LODs are very low.

F. Conclusions

It is possible if not probable that pentafluoropropionic anhydride is just as good as chlorodifluoroacetic anhydride for use as a derivatization reagent for most amphetamine like substances. At least the data show that pentafluoropropionic anhydride is not worse as a derivatizing agent, and may be a better derivatizing agent for a variety of amines than chlorodifluoroacetic anhydride. In addition, if improved sensitivity is needed, pentafluoropropionyl derivatives may be of value due to sharper GC peaks.

Both reagents may have unique advantages. Never-the-less, chlorodifluoroacetic anhydride was adequate for those compounds being evaluated in the precision and accuracy

study of Section V Part B. Increasing the temperature for derivatization with chlorodifluoroacetic anhydride might help to remove any distinctions. Accordingly the temperature used with CDFAA in all Precision and Accuracy, LOD, and long-term storage stability studies was raised to 70 °C from the 65 °C used in the preliminary method development studies.

VIII. EVALUATION OF PRECISION AND ACCURACY WITH METHANOL AS THE GAUZE WETTING SOLVENT

A. Objective

In a surface sampling recovery study (reported in the Backup Data Report for NIOSH 9109, [6]), it was shown that methanol was a better wetting solvent than isopropanol for surface wipe sampling. If methanol is to be included as an acceptable solvent for this method, it becomes necessary to show that methanol does not affect the precision and accuracy of the method. Accordingly, precision and accuracy were evaluated by spiking 24 blank cotton gauze wipes wetted with methanol in 50-mL PP centrifuge tubes, six at each of four different concentration levels. The results are reported in this section.

B. Scope

Only the lowest four concentration levels were evaluated, since this region includes the action level set by several states and it is more critical to determine the sensitivity, recovery, and reproducibility in this region than at higher concentration levels, where the precision and recoveries are usually better anyway. The commonly set action level is at the 1X LOQ level (0.1 µg/sample, where a sample usually covers 100 cm^2). Only the cotton gauze wipes were evaluated, since it is assumed that the effects of methanol, if any, will be independent of the media. This assumption is not necessarily true since methanol might gradually solvolyze the

polyesters of synthetic wipes more rapidly than isopropanol, if at all. Cotton is the preferred medium in any case. The same analytes and internal standards were used as in the previous evaluations of precision and accuracy. Only the effect of methanol on the liquid-liquid extraction cleanup procedure was evaluated. It was deemed unnecessary to test its affect on the solid phase cleanup procedure of NIOSH 9109 since methanol is used in one of the rinse steps anyway.

C. Discussion of Possible Effects of Methanol on NIOSH 9106

For the wipe recovery data it was assumed that whichever method was used (NIOSH 9106 or 9109) it would be inconsequential since sampling techniques were being evaluated and should be independent of the analytical technique. This may be true to a point. In NIOSH 9106, the methanol, for example gets diluted by the acidic desorption solution, and from there a portion of it can get extracted by the methylene chloride extract. If so, it should evaporate more readily than isopropanol, which would also be present in the final methylene chloride extract if isopropanol is used as the wetting solvent. Indeed, not only does methanol evaporate more readily than isopropanol, it evaporates more readily than methylene chloride which has a lower vapor pressure. Apparently it is forming an azeotrope with methylene chloride, whereas isopropanol apparently does not, or at least not to a significant degree. It is hard to conceive how one alcohol compared to another would have any serious affect upon the recoveries, as long as the residues are completely dried before the derivatization reagents are added. The only two things that might happen are a small change in the partition constant for the analytes between the aqueous base and methylene chloride, and the possibility of a change in the volatility of the amphetamine salts during evaporation under nitrogen, such as azeotroping or co-distillation. A study on of the effect on the NIOSH 9106 procedure was therefore warranted.

D. Analytes, Sampling Media, and Internal Standard

Samples were made using 3"x3" 12-ply non-sterile Accolade™ brand cotton gauze. It was U.S.P type VII, lot number 60305009 (reference number 908293). It was made in China for Banta Health Care Ltd. Neehah, WI 54956 and Rialto CA, 02376. The cotton was very bright white, and appears to have been the bleached variety (the precision and accuracy study in section VI using isopropanol was performed on an unbleached variety of cotton gauze). The change in types of cotton was necessitated because an order for the Caring brand previously used had not arrived yet.

The same mixed analyte spiking solution was used as was used in the precision and accuracy study (section VI) using isopropanol as the wetting solvent. The mixed analyte spiking solution volumes and concentrations used are the same as the four lowest concentration levels in that study. The resulting concentrations are given in Table 30. The internal standard spiking solution contained only methamphetamine-D_{14} at 100 µg/mL for this study.

E. Procedure

Liquid and media standards and blanks were the same as those prepared in the section for the surface wipe recovery study given in the Backup Data Report for NIOSH 9109 [6], and were prepared by spiking over a range of from 0.025 µg through 6 µg of analytes. The 50-mL PP centrifuge tubes containing the samples, blanks, and standards were capped and stored overnight.

The samples were spiked the next day with 60 µL of internal standard spiking solution and 30 mL of 0.2 normal aqueous sulfuric acid, capped, and tumbled for 2 hours. Subsequent cleanup, derivatization, and analysis were conducted using NIOSH 9106, using chlorodifluoroacetic anhydride. The chlorodifluoroacetic anhydride had a brownish oily residue on the Teflon bottle cap liner and the reagent was slightly discolored.

TABLE 30. CONCENTRATION OF ANALYTES AT EACH LEVEL

	ANALYTE	Calculated Concentration in µg/sample (1)					
				30x LOQ Level	10x LOQ Level	3x LOQ Level	1x LOQ Level
1	D-Amphetamine			3.00019	1.00006	0.30002	0.10001
2	L-Ephedrine			3.01799	1.00600	0.30180	0.10060
3	MDEA			2.85826	0.95275	0.28583	0.09528
4	MDMA			2.71692	0.90564	0.27169	0.09056
5	D-Methamphetamine			3.00193	1.00064	0.30019	0.10006
6	Phencyclidine			3.00444	1.00148	0.30044	0.10015
7	Phentermine			3.02086	1.00695	0.30209	0.10070
8	Phenylpropanolamine			3.02424	1.00808	0.30242	0.10081
9	Pseudoephedrine			3.01706	1.00569	0.30171	0.10057

(1) The number of significant figures was kept large until the final calculations to avoid cumulative rounding off errors.

F. Analysis and Results

The samples were analyzed by GC-MS using the SIM mode of analysis as described in NIOSH 9106. There was much more chromatographic noise than usual making it difficult to get good results for some of the analytes, and there was suspicion that the chlorodifluoroacetic anhydride derivatization reagent had become contaminated or had degraded somehow. There may have been some correlation with the brownish oily residue on the cap liner. The recoveries at the lower concentration levels for the ephedrine type compounds were very poor. On the other hand, recoveries for amphetamine, methamphetamine, MDMA, and phencyclidine were very good. It is unclear whether the low recoveries were due to methanol or to the contaminated reagent. But because the ephedrine type compounds have been very sensitive in the past to various types of contamination such as detergents and so on; it is strongly believed that the poor results for these types of compounds should not be used to argue against the use of methanol. At least for the other analytes, amphetamine, methamphetamine, MDMA, and PCP, the data show that methanol does not cause a problem in the NIOSH 9106 procedure.

It was observed that when methanol was used as the wetting solvent, the crystal violet does NOT go through a color change as the methylene chloride extracts are evaporated to

dryness. The color remains a blue to blue-violet through all stages of drying. In subsequent trials the color changes were restored by adding 100 µL of isopropanol to the methylene chloride extract prior to evaporation. Apparently crystal violet needs to be in an alcoholic solution in order for the color changes to occur. In pure methanol the color changes do occur, but apparently the residual methanol in the extracts evaporates so fast (through an azeotrope) that no alcohol remains. The evaporation rate of isopropanol is slow enough such that some remains after the methylene chloride is evaporated making it possible for the crystal violet to develop it color changes as the pH changes. However, it is not necessary to add isopropanol or even the crystal violet for the success of the method. It is just a convenience for the operator to follow the drying process and to better judge when to take the samples out of the drying operation.

Accuracy was calculated as previously described in Section VI.

A criterion for the overall bias is that the bias must be less than ±10% (±0.10).

In calculating homogeneity of precision and bias, as many concentration levels were left in as possible. Bartlett's test was used to determine homogeneity of precision. The F' test (Eugene Kennedy, Ph.D. [9]) was used to determine homogeneity of bias. Only those concentration levels that passes BOTH the Bartlett's test and the F' test were used for calculating pooled CVs and average bias. Accuracy was then calculated from these. Where possible, the lowest concentration level was conserved, in order to report lower detection limits, and higher concentration levels having "inlier" CVs were omitted. This gives a more conservative estimate of the pooled CV as well.

The complete recovery data for each of the replicates in this study is given in Table 33 and the precision and accuracy data and concentration levels that were omitted to obtain these values are given in Table 34 at the end of this section.

A summary of the precision and accuracy for methanol as a gauze wetting solvent is given in Table 31.

TABLE 31. PRECISION AND ACCURACY ON COTTON GAUZE USING METHANOL AS THE WETTING SOLVENT [1]

Compound	Internal Standard [2]	Applicable Range [3] µg/sample	Accuracy	Overall Precision $\hat{S}_{rT}$	Bias Average	Bias Range
(D)-Amphetamine	**D_{14}-Meth**	**0.3-30**	**15.9**	**0.0565**	**-0.0664**	**+0.0531 to +0.0801**
(L)-Ephedrine	D_{14}-Meth	0.1-30	43.5 [5]	0.1003 [5]	-0.2703 [5]	-0.3657 - -0.1128
MDEA	No N-propylamphetamine included as the internal standard so no results are possible.					
MDMA	**D_{14}-Meth**	**0.1-27**	**11.4**	**0.0317**	**-0.0619**	**-0.0960 to -0.0109**
(D)-Methamphetamine	**D_{14}-Meth**	**0.1-30**	**8.5**	**0.0337**	**+0.0297**	**+0.0112 to +0.4114**
Phencyclidine	**D_{14}-Meth**	**0.3-30**	**10.8**	**0.0577**	**+0.0130**	**-0.0487 to +0.0801**
Phentermine	D_{14}-Meth	**0.3-30**	**24.4 [6]**	**0.0483**	**+0.1642 [6]**	**+0.1245 to +0.2035**
(±)-Norephedrine [4]	D_{14}-Meth	0.1-30	45.6 [5]	0.1692 [5]	-0.1772 [5]	-0.2357 to -0.0557
Pseudoephedrine	D_{14}-Meth	0.1-30	41.5 [5]	0.1253 [5]	-0.2083 [5]	-0.3715 to -0.0953

Bold values are those that meet the accuracy criterion.

(1) Values are for the chlorodifluoroacetyl derivatives and analysis by GC-MS in scan mode. Each sample consisted of a pair of 3" x 3" 12-ply cotton gauze pads. There were 6 replicate samples per concentration level and four concentration levels evaluated from approximately 0.1 to 3 µg/sample.

(2) Internal Standard: D_{14}-Meth = Methamphetamine-D_{14}

(3) Range used for calculation of precision, accuracy, and bias. The entire range studied for all analytes was approximately 0.1 to 3 µg/sample (1X LOQ to 30X LOQ).

(4) (±)-Norephedrine = (±)-phenylpropanolamine.

(5) Accuracies, overall precision, and mean bias were all high and unacceptable.

(6) Recovery for the 1X LOQ level was 173%, which was very high. This point not included in calculations. At the other three concentration levels recoveries were slightly high for phentermine. The precisions at these levels were good.

In Table 32 the precision and accuracy for methanol and isopropanol as gauze wetting solvents are compared.

TABLE 32. COMPARISON OF PRECISION AND ACCURACY VALUES WITH METHANOL AND ISOPROPANOL AS THE WETTING SOLVENTS [1]

Compound	Wetting Solvent	Internal Standard[2]	Applicable Range [3] µg/sample	Accuracy	Overall Precision $\hat{S}_{rT}$	Bias	
						Average	Range
Amphetamine	Methanol	D_{14}-Meth	0.3-3	15.9	0.0565	-0.0664	+0.0531 to +0.0801
Amphetamine	Isopropanol	D_{14}-Meth	0.1-30	9.1	0.0508	-0.0074	-0.0500 to +0.0389
MDMA	Methanol	D_{14}-Meth	0.1-2.7	11.4	0.0317	-0.0619	-0.0960 to -0.0109
MDMA	Isopropanol	D_{14}-Meth	0.1-27	15.4	0.0503	-0.0712	-0.1247 to +0.0032
Methamphetamine	Methanol	D_{14}-Meth	0.1-3	8.5	0.0337	+0.0297	+0.0112 to +0.4114
Methamphetamine	Isopropanol	D_{14}-Meth	0.1-30	9.2	0.0351	-0.0343	-0.0767 to +0.0006
Phencyclidine	Methanol	D_{14}-Meth	0.3-3	10.8	0.0577	+0.0130	-0.0487 to +0.0801
Phencyclidine	Isopropanol	D_{14}-Meth	0.1-30	11.3	0.0450	-0.0393	-0.0683 to -0.0205
Phentermine	Methanol	D_{14}-Meth	0.3-3	24.4 [4]	0.0483	+0.1642 [6]	+0.1245 to +0.2035
Phentermine	Isopropanol	D_{14}-Meth	0.1-30	8.7	0.0495	-0.0051	-0.0375 to +0.0556

(1) Values for methanol are from Table 31. Values for isopropanol are from Table 22. Both data are for SIM mode operation and for cotton gauze.

(2) Internal Standard: D_{14}-Meth = Methamphetamine-D_{14}

(3) Range used for calculation of precision, accuracy, and bias.

(4) Recoveries were inexplicably high for phentermine. The precisions at each level were good.

G. Discussion of Results

Recoveries for the ephedrine type compounds (phenylpropanolamine, ephedrine, and pseudoephedrine) were unusually low and the precisions were large at the low concentration levels. These problems may appear to be due to methanol but are more likely due to the aged and contaminated derivatizing reagent. The nature of the contamination is unknown but it was a brownish color and appeared to be coming from the cap. The bottle was also almost empty and being an older bottle of reagent, it may have succumbed to atmospheric moisture, which conceivably could have weakened the reagent's ability to derivatize the hydroxyl groups in the ephedrine type compounds. Amides are more stable once formed. Table 32 does not include the ephedrine compounds due to the poor results.

Results for MDEA are not presented because an appropriate internal standard (e.g., N-propyl amphetamine) was not included.

Phentermine barely passes. Recoveries were unusually high for this compound, for unknown reasons. The 1X LOQ level had to be omitted due to very high recovery.

The results were very good in Table 31 and compare very favorably in Table 32 for the other analytes having no alpha-hydroxyl groups, especially the more important analytes, methamphetamine and MDMA, for which the methanol data was slightly better than the isopropanol data.

H. Conclusions

While the results are inconclusive for the ephedrine type compounds (phenylpropanolamine, ephedrine, and pseudoephedrine), methanol appears to be an acceptable substitute for isopropanol as far as the analytical procedure in NIOSH 9106 is concerned.

Chlorodifluoroacetic anhydride should not be used if it is discolored. It may need to be stored refrigerated. No problems with chlorodifluoroacetic anhydride have been observed as long as the solution was colorless and clear. A different cap liner may be necessary than the one supplied by Aldrich. Aldrich supplies pentafluoropropionic anhydride in a glass ampoule but chlorodifluoroacetic anhydride was supplied in a bottle with some kind of elastomer cap liner. Pentafluoropropionic anhydride appears to be more stable on standing than chlorodifluoroacetic anhydride since no discoloration has yet been observed in this reagent after opening the ampoule and transferring the contents to the same kind of bottle used for chlorodifluoroacetic anhydride and standing at room temperature for several months. However a Teflon™ disc was added to the face of the cap liner. It is strongly suspected that the chlorodifluoroacetic anhydride may have reacted with the cap liner or the cap material itself causing the discoloration.

If the color changes of crystal violet during evaporation are desired, 100 µL of isopropanol needs to be added to the methylene chloride extract just prior to the first nitrogen blow-down step.

I. Recovery, Precision, and Accuracy Data for Methanol Wetted Cotton Gauze

Table 33 gives the individual recoveries for each analyte at the four levels tested. Table 34 gives the final accuracy, overall precision, and mean bias determinations for each of the analytes. Several options are presented which differ by which concentration levels had to be omitted to get the Bartlett's and the F' tests to pass.

TABLE 33. PRECISION AND ACCURACY STUDY FOR METHANOL AS A WETTING SOLVENT

Methanol wetting study, using Accolade brand cotton and CDFAA deriv. agent									
Internal Standard =		D_{14}-Methamphetamine							
		µg/SAMPLE RECOVERED				µg/SAMPLE RECOVERED			
Test Level	Replicate	**Amphet-amine**	**Ephedrine**	**MDMA**	**Methamphet-amine**	**PCP**	**Phentermine**	**Phenylpro-panolamine**	**Pseudo-ephedrine**
30x LOQ	1	3.005	3.300	2.827	3.090	3.087	3.104	3.177	3.234
30x LOQ	2	3.177	2.519	2.686	3.151	3.062	3.453	2.598	2.510
30x LOQ	3	3.036	2.263	2.532	3.089	2.739	3.386	2.551	2.490
30x LOQ	4	3.256	2.408	2.671	3.269	3.073	3.479	2.909	2.534
30x LOQ	5	3.300	2.593	2.651	3.133	2.948	3.480	2.686	2.650
30x LOQ	6	3.183	2.983	2.757	3.161	3.070	3.478	3.215	2.959
Average µg/sample =		3.160	2.678	2.687	3.149	2.996	3.397	2.856	2.730
CVi =		0.03719	0.14535	0.03730	0.02099	0.04538	0.04356	0.10181	0.11084
Group Bias =		0.05312	-0.11279	-0.01086	0.04891	-0.00266	0.12447	-0.05570	-0.09531
Average % Recovery =		**105.31**	**88.72**	**98.91**	**104.89**	**99.73**	**112.45**	**94.43**	**90.47**
10x LOQ	1	none	0.681	0.835	1.092	none	none	0.821	0.740
10x LOQ	2	1.073	0.599	0.806	1.014	0.992	1.177	0.550	0.647
10x LOQ	3	1.067	0.665	0.798	1.011	0.924	1.162	0.876	0.772
10x LOQ	4	1.100	0.668	0.824	1.038	0.996	1.209	0.827	0.768
10x LOQ	5	1.047	0.705	0.814	1.020	0.946	1.183	0.770	0.762
10x LOQ	6	1.043	0.759	0.836	0.977	0.906	1.134	0.932	0.851
Average µg/sample =		1.066	0.680	0.819	1.025	0.953	1.173	0.796	0.756
CVi =		0.02154	0.07734	0.01903	0.03730	0.04203	0.02339	0.16626	0.08684
Group Bias =		0.06605	-0.32452	-0.09602	0.02439	-0.04873	0.16482	-0.21056	-0.24788
Average % Recovery =		**106.61**	**67.55**	**90.40**	**102.44**	**95.13**	**116.48**	**78.94**	**75.21**
3x LOQ	1	0.3439	0.1686	0.2559	0.3203	0.3663	0.3988	0.1873	0.1631
3x LOQ	2	0.3023	0.1899	0.2419	0.2903	0.3218	0.3375	0.2550	0.1948
3x LOQ	3	0.3255	0.2105	0.2450	0.2997	0.2961	0.3436	0.2545	0.2203
3x LOQ	4	0.2807	0.1811	0.2442	0.2938	0.3096	0.3507	0.1842	0.1430
3x LOQ	5	0.3513	0.2130	0.2604	0.3087	0.3385	0.3765	0.2789	0.2198
3x LOQ	6	0.3406	0.1855	0.2527	0.3086	0.3148	0.3742	0.2269	0.1968
Average µg/sample =		0.3241	0.1914	0.2500	0.3036	0.3245	0.3636	0.2311	0.1896
CVi =		0.08468	0.09031	0.02964	0.03662	0.07640	0.06469	0.16800	0.16351
Group Bias =		0.08010	-0.36569	-0.07978	0.01124	0.08012	0.20346	-0.23573	-0.37146
Average % Recovery =		**108.01**	**63.43**	**92.02**	**101.12**	**108.01**	**120.35**	**76.43**	**62.85**
1x LOQ	1	0.1416	0.0654	0.0801	0.1033	0.1549	0.1786	0.0833	0.0826
1x LOQ	2	0.1301	0.0770	0.0856	0.1006	0.1221	0.1706	0.0976	0.1114
1x LOQ	3	0.0851	0.0742	0.0898	0.1084	0.1308	0.1691	0.0560	0.0851
1x LOQ	4	0.1447	0.0791	0.0850	0.1066	0.1403	0.1839	0.1001	0.0868
1x LOQ	5	0.1385	0.0691	0.0860	0.1041	0.1407	0.1703	0.0646	0.0844
1x LOQ	6	0.1036	0.0710	0.0837	0.0980	0.1003	none	0.0781	0.0815
Average µg/sample =		0.1239	0.0726	0.0850	0.1035	0.1315	0.1745	0.0800	0.0886
CVi =		0.19483	0.07042	0.03722	0.03684	0.14314	0.03705	0.21961	0.12758
Group Bias =		0.23925	-0.27800	-0.06107	0.03434	0.31322	0.73295	-0.20691	-0.11868
Average % Recovery =		**123.93**	**72.20**	**93.89**	**103.43**	**131.32**	**173.29**	**79.31**	**88.13**

TABLE 34. PRECISION AND ACCURACY STUDY FOR METHANOL AS A WETTING SOLVENT

	Chlorodifluoroacetic anhydride							
	Amphet-amine	**Ephedrine**	**MDMA**	**Methamphet-amine**	**PCP**	**Phentermine**	**Phenylpro-panolamine**	**Pseudo-ephedrine**
OPTION #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1	Option #1
Test Levels omitted =	NONE	NONE	NONE	NONE	NONE	NONE	NONE	NONE
Degrees of freedom =	3	3	3	3	3	3	3	3
Accuracy =	**29.424**	**43.521**	**11.406**	**8.508**	**23.699**	**36.915**	**45.550**	**41.452**
Overall Precision =	**0.1111**	**0.1003**	**0.0317**	**0.0337**	**0.0886**	**0.0460**	**0.1692**	**0.1253**
Chi^2 =	19.839	3.263	2.383	1.828	8.741	3.998	2.550	1.941
pass @ 0.95?	no	YES	YES	YES	no	YES	YES	YES
pass @ 0.975?	no	YES	YES	YES	YES	YES	YES	YES
Mean bias =	**0.1115**	**-0.2703**	**-0.0619**	**0.0297**	**0.0913**	**0.2935**	**-0.1772**	**-0.2083**
from	0.0531	-0.3657	-0.0960	0.0112	-0.0487	0.1245	-0.2357	-0.3715
to	0.2393	-0.1128	-0.0109	0.0489	0.3132	0.7329	-0.0557	-0.0953
F' =	2.1941	8.3821	2.4013	0.4114	10.3807	73.8830	1.9385	8.2456
pass @ 0.05?	YES	no	YES	YES	no	no	YES	no
pass @ 0.025?	YES	no	YES	YES	no	no	YES	no
OPTION #2	**Option #2**	Option #2			**Option #2**	**Option #2**	Option #2	Option #2
Test Levels omitted =	1x LOQ	1x LOQ			1x LOQ	1x LOQ	1x LOQ	1x LOQ
Reason for omission =	CV, bias>10%	bias>>10%			CV, bias>10%	bias>>10%	CV, bias>10%	CV, bias>10%
Degrees of freedom =	2	2			2	2	2	2
Accuracy =	**15.932**	**44.602**			**10.786**	**24.360**	**41.176**	**44.316**
Overall Precision =	**0.0565**	**0.1084**			**0.0577**	**0.0483**	**0.1486**	**0.1246**
Chi^2 =	7.140	2.088			1.925	3.545	1.312	1.890
pass @ 0.95?	no	YES			**YES**	**YES**	YES	YES
pass @ 0.975?	**YES**	YES			**YES**	**YES**	YES	YES
Mean bias =	**0.0664**	**-0.2677**			**0.0130**	**0.1642**	**-0.1673**	**-0.2382**
from	0.0531	-0.3657			-0.0487	0.1245	-0.2357	-0.3715
to	0.0801	-0.1128			0.0801	0.2035	-0.0557	-0.0953
F' =	0.1512	9.7801			3.3201	1.3815	3.1560	9.5318
pass @ 0.05?	**YES**	no			**YES**	**YES**	YES	no
pass @ 0.025?	**YES**	no			**YES**	**YES**	YES	no
OPTION #3		Option #3					Option #3	Option #3
Test Levels omitted		1x LOQ bias>>10% 3x LOQ bias>>10%					1x LOQ CV, bias>10% 3x LOQ CV, bias>10%	1x LOQ CV, bias>10% 3x LOQ bias>>10%
Degrees of freedom =		2					2	2
Accuracy =		**40.851**					**43.663**	**33.442**
Overall Precision =		**0.1034**					**0.1695**	**0.1097**
Chi^2 =		3.063					2.487	0.665
pass @ 0.95?		YES					YES	YES
pass @ 0.975?		YES					YES	YES
Mean bias =		**-0.2384**					**-0.1577**	**-0.1540**
from		-0.3245					-0.2106	-0.2479
to		-0.1128					-0.0557	-0.0953
F' =		6.7138					2.0656	3.1805
pass @ 0.05?		no					YES	YES
pass @ 0.025?		no					YES	YES

IX. OVERALL CONCLUSIONS:

The method passes the accuracy and the long-term sample storage stability criteria for NIOSH analytical methods for all of the analytes evaluated if paired properly with an appropriate internal standard and the correct wipe media is used.

No synthetic gauze was better than cotton gauze, and due to its universal availability, it is the preferable wipe material. The data show that cotton, contrary to some reports, is an acceptable media for methamphetamine. This endorsement does not necessarily extend to "cellulose" (meaning wood fiber cellulose) wipe media.

Methanol is an acceptable substitute for isopropanol as a wipe solvent.

GC-MS in either the scan mode or SIM mode is able to attain the required limit of detection for methamphetamine (0.1 µg/sample). Additional sensitivity is possible in the SIM mode. The scan mode is essential for unknown identification.

Chlorodifluoroacetic anhydride (CDFAA) is acceptable as the derivatization reagent and is effective under milder conditions. Pentafluoropropionic anhydride (PFPA) was comparable to CDFAA when compared at one concentration level. However, calibration curves and actual analyses using PFPA indicate performance at least equal to that of CDFAA. Additional sensitivity may be possible with PFPA due to sharper peak shapes for its derivatives. PFPA may be more stable in storage than (CDFAA), but both reagents should be kept tightly sealed from moisture during storage. Derivatization efficiency with CDFAA or PFPA is related to steric hindrance and structure of the analyte. The best results are obtained using isotopic analogs as internal standards for the analytes of interest.

This analytical method may be applicable to a wide variety of other basic (nitrogen containing) illicit drugs and amphetamine like substances on a variety of wipe media using isotopic analogs of the target analyte as internal standards.

This method is amenable to the analysis of non-alkaline bulk samples (e.g. cloth) and air samples (using acidified glass fiber filters).

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