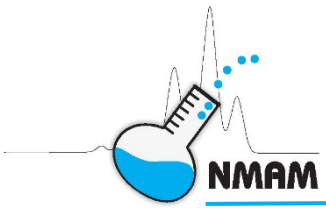


BACKUP DATA REPORT
NIOSH Method No. 9212

Title: Mitomycin C on Surfaces

Analyte: Mitomycin C

Author/developer: Clayton B'Hymer and Thomas H. Connor

Date: 10/16/2014

BACKUP DATA REPORT
NIOSH Method 9212
Mitomycin C on surfaces

Clayton B'Hymer and Thomas H. Connor
October 16, 2014

Substance: *Mitomycin C*
Exposure Limits: Not applicable

Chemicals Used for Evaluation:
1. Mitomycin C ([6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazireno [2',3':3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate, CAS no. 50-07-7) from the United States Pharmacopeia (Rockville, Maryland, USA), 98%, Lot no. K.
2. Porfiromycin ([1a*S*-(1aα,8β,8aα,8bα)]-6-Amino-8-[[(aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-1,5-dimethylazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indole-4,7-dione, CAS no. 801-52-5) from the National Cancer Institute, Lot no. 56410/3.

General

Synopsis

Mitomycin C is an alkylating agent and an antibiotic used as a chemotherapeutic agent because of its antitumor properties. Mitomycins are part of a family of aziridine containing natural products first isolated from the soil bacteria *Streptomyces caespitosus* and *Streptomyces lavendulae*. Mitomycin C is used occasionally in the intravenous treatment mode and used extensively in the topical treatment mode of cancer. It has also been used in instillation theory in the treatment of bladder tumors and has come into use of intraperitoneal chemotherapy procedures including Hyperthermic Intraperitoneal Chemotherapy (HIPEC) and Continuous Hyperthermic Peritoneal Perfusion (CHPP) therapy. Both of the latter chemotherapeutic procedures involve possible exposure to surgical staff by contamination of the operating room. The described procedure was developed for the detection and quantification of mitomycin C from contaminated surfaces typically encountered in an operating room. In summary, a 100 cm^2 template surface area is sampled by wetting the surface and then wiping the surface with either a Texwipe® swab or filter paper three times. The sampling media is placed in a polypropylene jar, extraction solvent and the porfiromycin internal standard are added and the jar swirled for 30 minutes. The extract solution is filtered and high-performance liquid chromatographic analysis is performed. Detection is by means of a tandem mass spectrometer (MS/MS).

Method Evaluation

This method was evaluated in the general areas recommended for typical method validation [1,2]. Furthermore, this method has been described by B'Hymer and Connor [3] and B'Hymer, *et al*. [4]. The key elements of method validation including accuracy, precision, linearity, specificity, robustness, stability and limit of detection have been investigated during this method's development. The accuracy and precision were determined by spiked matrix matched solutions and spiked surfaces. Recoveries were studied at up to six different concentration levels on two different HPLC-MS/MS systems which are described in detail later in this report. The other elements are also described in detail with their respective results within this report.

Sampling Aspects and Sample Preparation

Potential mitomycin C contamination was evaluated for three work surfaces using two separate wiping materials. The first wipe procedure evaluated for collecting and processing samples utilized a simple paper filter disk procedure used previously by NIOSH [5]. A 0.25 mL aliquot of the extraction solvent (20/45/35 (v/v/v) acetonitrile-isopropanol-water made 0.01 M in ammonium citrate, apparent pH 7.0) was used to wet a template area of 100 cm^2 on the desired surface. A filter paper disc (55 mm Whatman 42, GE Healthcare, Buckinghamshire, UK) was used to wipe the template area. This wiping procedure was conducted three times over the surface area. The three filters were then placed in a 125 mL polypropylene jar (Nalgene/Thermo Scientific Co., part number 2118-004, Rochester, NY, USA) and brought up to a total liquid volume of 9 mL with the extraction solvent. A 1.0 mL aliquot of the internal standard solution was added to the jar and it was swirled by means of an orbital shaker for 30 minutes to extract the mitomycin C. The approximate 10 mL volume of solvent was an optimum to cover the three pieces of filter paper in each jar. The second wipe procedure evaluated used polyester Texwipe® swabs (TX714A, Large Alpha Swab, ITW Texwipe, Kernersville, NC, USA). Similar to the filter paper, 0.25 mL aliquots of the extraction solvent were used and three swabs were used to wipe the template area. After wiping, the heads were removed from the swab shafts and placed in the 125-mL polypropylene jars. The swabs were treated by the same procedure as the filter paper. After the analytes were extracted into the solvent, the extracts were filtered using Millex 0.22 µm polyvinylidene difluoride filters (Millipore Corporation, Billerica, MA, USA) and placed in autosampler vials for analysis.

The recoveries for the full surface wiping procedure were evaluated by selecting three materials to represent surfaces likely to experience contamination in the workplace. The three materials selected were stainless steel, vinyl and Formica®. Stainless steel (Type 304 #3 satin finish, McMaster-Carr Supply, Cleveland, OH, USA) was used as a surrogate for biological safety cabinets, Formica® for counter tops and vinyl for floor tiles (Imperial Texture, Armstrong World Industries, Montreal, Quebec, Canada). Tiles were cut into 10 X 10 cm sections from sheets of stock materials, washed with methanol and air dried prior to use. Recovery studies from the surfaces were conducted by spiking the surfaces with known amounts of mitomycin C dissolved in 250 µL of acetonitrile. The acetonitrile was allowed to evaporate before wiping the surface for the recovery study. For all the recovery studies, both the spiked solutions and the spiked surfaces, equivalent mitomycin C levels of 0.1, 0.2, 2, 4, 10, 25 µg/100 cm^2 were prepared. Sample solutions prepared outside the range of the calibration curves [the 10 and 25 µg/100 cm^2 samples] were diluted 1 to 10 in extraction solvent before analysis.

Analytical Aspects

The chromatographic analysis was carried out using two liquid chromatography-tandem mass spectrometry systems. One was an Agilent Technologies model 1100 HPLC pumping system with autosampler (Palo Alto, California, USA) with an Agilent Technologies model 6430 triple quadrupole mass spectrometer used as the detector. The detector output and data were evaluated by Agilent's Mass Hunter software. The second system consisted of a Waters Corporation Aquity HPLC pumping system with autosampler (Milford, Massachusetts, USA) with a Waters Xevo TQ mass spectrometer used as the detector. The Waters system utilized their MassLynx software where all data were evaluated and the chromatographic peaks integrated. The type of analytical column used in this method was an Agilent Zorbax RX C18 (150 X 3mm, 3.5 µm particle size) along with a guard column consisting of a Phenomenex C18 SecurityGuard (4 X 2 mm). An SSI 2 micron precolumn filter was also found to be necessary for this procedure. Gradient elution with acetonitrile/water mobile phases both at 0.1% acetic acid was used. An example chromatogram is displayed in Figure 1. The chromatographic and mass spectrometric conditions were optimized for this method on each instrument and are described next.

Chromatographic Conditions

Mobile Phases: A = 10/90/0.1% (v/v/v) acetonitrile/water/acetic acid
B = 75/25/0.1% (v/v/v) acetonitrile/water/acetic acid

Flow Rate: 0.4 mL/min (during analysis time)

Gradient Program:	Time (minutes)	Mobile Phase Composition	Comments
	0 to 12	0 to 70% B	Gradient ramp
	12 to 13	70 to 100% B	Start post run
	13 to 16	100% B	Post run [Increase flow to 0.5 mL/min]
	16 to 18	0 to 100% A	Decrease flow to 0.4 mL/min
	18 to 24	100% A	Column re-equilibration

Injection Volume: 5 µL

Autosampler temperature held at 8 °C

Agilent Model 6430 Mass Spectrometric Conditions:

Ionization Source: Electrospray at 3500 Volts and positive scan mode, the nitrogen nebulizer gas at 35 psi

Drying Gas Flow: 10 L/min

Drying Gas temperature: 325°C

Multiple Reaction Mode (MRM)

Collision gas: Nitrogen

Quantification mass transitions: Mitomycin C = *m/z* 335 → 242, Porfiromycin (int. std.) = *m/z* 349 → 256

The following table lists the instrumental settings used for the Agilent 6430 mass spectrometer:

Analyte	Precursor Ion (*m/z*)	MS1 Resolution	Product Ion (*m/z*)	MS2 Resolution	Dwell Time (msec)	Fragmentor Voltage	Collision Energy	Cell Accelerator Voltage
Mitomycin C	335	unit	242	unit	200	97	8	4
Porfiromycin	349	unit	256	unit	200	97	6	4

Waters Corporation Xevo TQ Mass Spectrometric Conditions:

Ionization Source: Electrospray at 3.5 Kilovolts and positive scan mode

Desolvation gas: 650 L/hr

Drying Gas temperature: 350 °C

Multiple Reaction Mode (MRM)
Collision gas: Argon

Quantification mass transitions: Mitomycin C = *m/z* 335 → 242, Porfiromycin (int. std.) = *m/z* 349 → 256, mass span set at 1.0

The following table lists the instrumental settings used for the Waters Xevo TQ mass spectrometer:

Analyte	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell (s)	Cone (V)	Collison Energy (V)
Mitomycin C	335	242	0.100	17	15
Porfiromycin	349	256	0.100	17	16

Another important aspect of this method was the use of a 2 micron precolumn filter. It was found that a small amount of additional mobile phase dead volume was necessary to maintain good peak shape owing to the use of a high organic content and mismatch with the initial mobile phase conditions. The sample wiping solution's high organic content was necessary owing to the stability of target mitomycin C analyte. The stability problems will be addressed in the next section of this report.

RESULTS

Accuracy and Precision

Several recovery studies using two columns over several days demonstrated the accuracy and precision of this test method. These matrix matched samples were made to mimic matrices that would be found in the field. Blank surfaces were wetted and the surfaces sampled as in Steps 3 or 4 of the Sampling Section of the method. The solutions containing the three wipes (filter paper or swab heads) were then spiked with varying amounts of the analyte and carried through the analytical process. A primary recovery experiment using the optimized conditions with filter paper matrix matched solution fortified with mitomycin C was performed to demonstrate the general accuracy and precision of the HPLC conditions using the Agilent mass spectrometer. These data are presented in Table 1. Average recoveries ranged from 93 to 105% for the six fortified levels of mitomycin C investigated. A similar recovery study using the Texwipe® swab matrix matched solution fortified with mitomycin C was performed using the Waters Xevo mass spectrometer. These data are presented in Table 2. Average recoveries ranged from 90 to 107% for the four fortified levels of mitomycin C investigated. These results are well within the acceptable guidelines for the accuracy and precision of an analytical test method [2].

Table 1
Recovery of Mitomycin C Spikes Added to Filter Paper Matrix-Matched Extracts
(Analysis was performed using an Agilent Model 6430 Mass Spectrometer)

Surface	Equivalent Level (µg/100 cm^2)	Mean (n=10)	% Recovery	% RSD (n=10)
Stainless Steel	0.100	0.104	104	7.5
	0.200	0.202	100	3.3
	2.00	2.01	100	1.0
	4.00	4.08	102	2.7
	10.0	9.81	98	2.5
	25.0	23.2	93	5.1
Vinyl	0.100	0.105	105	3.0
	0.200	0.201	101	2.3
	2.00	1.99	100	3.1
	4.00	4.06	102	2.6
	10.0	9.82	98	2.7
	25.0	23.8	95	5.9
Formica®	0.100	0.103	103	3.3
	0.200	0.200	100	2.7
	2.00	2.02	101	3.0
	4.00	4.05	101	1.8
	10.0	9.66	97	2.0
	25.0	23.6	94	5.4

Notes: This recovery study was performed using two different Zorbax columns. RSD is relative standard deviation.

Table 2
Recovery of Mitomycin C Spikes Added to Texwipe® Swab Matrix-Matched Extracts
(Analysis was performed using a Waters Xevo TQ Mass Spectrometer)

Surface	Equivalent Level (µg/100 cm^2)	Mean (n=5)	% Recovery	% RSD (n=5)
Stainless Steel	0.100	0.090	90	1.3
	0.200	0.187	94	1.4
	2.00	2.02	101	4.1
	4.00	4.20	105	2.1
Vinyl	0.100	0.105	105	2.4
	0.200	0.200	100	1.3
	2.00	1.99	99	1.0
	4.00	3.96	99	1.1
Formica®	0.100	0.107	107	1.7
	0.200	0.214	107	0.8
	2.00	2.06	103	1.2
	4.00	4.21	105	0.8

The most important aspect of this method was to evaluate the most effective wipe sampling media to detect mitomycin C from various surfaces encountered in the operating room setting. The data for the surfaces tested after spiking the actual surface and wiping using either filter paper or the polyester

swabs are presented in Table 3. As can be seen in the table, stainless steel generally has higher recovery yields with some less than quantitative recoveries at the lower levels. There was little difference in using filter paper or swabs for extracting mitomycin C from stainless steel. Vinyl is a porous material and it generally had recovery yields of 50 to 60% for every spike level using either filter paper or swabs for the wipe procedure. Finally, the main quantitative advantage for using the swab over filter paper can be seen for the Formica® surface data (see Table 3). The swabs had recovery yields of 60 to 70% at the 0.100 and 0.200 µg/100 cm^2 levels while the filter paper had recoveries near 30%. The swabs also displayed greater precision at the lower mitomycin levels (see Table 3), although the recoveries were still less than quantitative at the two lower concentration levels studied.

Table 3

Recovery from Spiked Material Surfaces

Surface	Filter Paper Wipe				Texwipe® Swab			
	Level (µg/100 cm^2)	**Mean** (n=5)	**% Recovery**	**% RSD**	**Level** (µg/100 cm^2)	**Mean** (n=5)	**% Recovery**	**% RSD**
Stainless Steel	0.100	0.068	68	6.3	0.100	0.071	71	6.4
	0.200	0.124	62	10.8	0.200	0.122	61	5.6
	2.00	1.75	88	3.6	2.00	1.77	89	3.6
	4.00	3.67	92	3.8	4.00	3.76	94	2.5
	10.0	9.80	98	8.3	10.0	9.76	98	1.6
	25.0	23.9	96	3.1	25.0	24.8	99	2.6
Vinyl	0.100	0.058	58	7.9	0.100	0.062	62	6.2
	0.200	0.115	58	12.6	0.200	0.109	54	3.7
	2.00	1.12	56	7.9	2.00	1.32	66	4.3
	4.00	2.02	51	8.0	4.00	2.39	60	5.9
	10.0	6.25	63	3.4	10.0	5.32	53	8.2
	25.0	14.8	59	7.6	25.0	14.0	56	5.1
Formica®	0.100	0.030	30	47	0.100	0.073	73	7.9
	0.200	0.065	33	6.0	0.200	0.126	63	5.3
	2.00	1.53	77	8.5	2.00	1.74	87	1.2
	4.00	3.27	82	1.2	4.00	3.65	91	1.7
	10.0	9.05	91	2.0	10.0	9.26	93	2.9
	25.0	24.0	96	3.7	25.0	24.3	97	0.7

NOTE: Analysis was performed using an Agilent Model 6430 Mass Spectrometer.

Linearity, Range and LOD

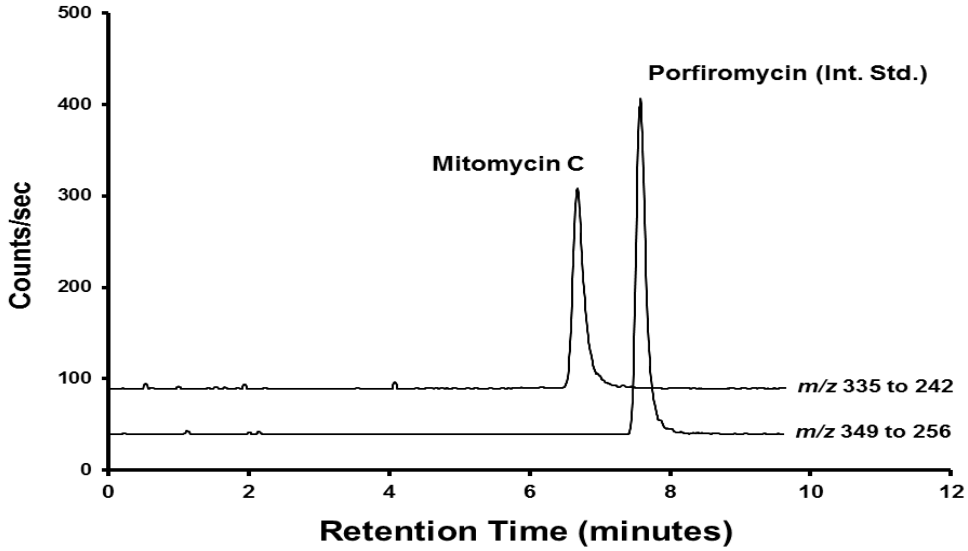
The linearity of this methodology was determined over the range of 0.002 to 0.5 µg/mL (0.02 to 5 µg/100 cm^2 spiked equivalent level). The calibration curves generated from the peak area ratio between mitomycin C and the internal standard showed correlation of determinations (r^2) of 0.99 or greater. As to the limit of detection (LOD), it should be noted that the actual LOD is dependent upon the performance of the chromatographic system and the detector at the time of an analysis. The lowest standard used was equivalent to 20 ng/100 cm^2 in the calibration curves and can be used as the LOD for wipe testing. If a column, chromatographic system or detector cannot detect the lowest standard level, corrective action would be required. During the development stage of this method, the instrumental LOD was determined in the traditional manner; three times the noise level of the chromatogram's baseline divided by the slope of a calibration curve using peak heights. This level was found to be approximately 2 ng/100 cm^2 equivalent (0.002 µg/100 cm^2) for the Agilent mass spectrometer. The Waters mass spectrometer was determined to have a lower LOD. Since the lowest standard is typically the one used as an operational LOD, both instruments were readily capable of meeting that level.

Specificity

The optimized chromatographic conditions developed for this procedure proved to be specific and have no interferences and enabled the accurate quantification of the target analyte. All non-fortified blank matrix samples chromatographed showed no interfering peaks for either mitomycin C or the internal standard. The chromatographic baselines displayed little drift during the gradient run and proved to be easily integrated for data analysis. An example chromatogram is displayed in Figure 1.

Figure 1

Chromatogram of a sample taken from a spiked matrix solution. The mitomycin C and porfiromycin solution concentrations are approximately 20 ng/mL.



Robustness

Two Agilent Zorbax RX C18 columns were used during the filter paper matrix matched recovery studies. Accuracy and precision did not appear to be affected; therefore, the method appears to be reproducible with any functioning Zorbax RX HPLC column. Two different mass spectrometric detectors both gave reasonable recovery results.

Stability

Mitomycin C exhibits antibiotic properties in addition to its antineoplastic one; it has been used in various pharmaceutical preparations including ophthalmic formulations. As such, its stability has been well established in the chemical literature. It can be easily hydrolyzed under acidic or basic conditions, but it is generally stable in a pH range between 7 and 8. Also, the use of aprotonic solvents or organic/aqueous solvents, such as mixtures of propylene glycol and water, which do not promote acid or base catalyzed hydrolysis of mitomycin C have been utilized and reported [6]. The acetonitrile-isopropanol-water system developed for the current method was found to be a good matrix for sample stability, and it was experimentally determined to be superior to the propylene glycol-water mixtures reported in patented pharmaceutical formulations [5]. Mitomycin C stability concerns also limited the amount of sample manipulation and preparation possible with this method. Early sample pre-concentration experiments including typical simple solvent evaporation demonstrated significant degradation of the analyte. Therefore, the method ultimately developed has been limited to simple filtration of the extraction solvent before HPLC-MS/MS analysis.

Mitomycin C solutions stored in the acetonitrile-isopropanol-water extraction solvent at room temperature in darkness and those held at 8 ºC in the autosampler were found to degrade only 3 to 4% after 8 days (n=3) (Table 4). The optimized extraction solvent also gave excellent recoveries during the surface wipe testing. Stock solutions of mitomycin C stored in 100% acetonitrile were found to only degrade 2 to 3% after 3 to 6 months of storage at -15 ºC. Mitomycin C did show some light sensitivity. Sample solutions (n=3) made in the 20/45/35 (v/v/v) acetonitrile-isopropanol-water 0.01 M ammonium citrate (pH 7) extraction solvent degraded 12% after 8 days exposed to window sunlight and the laboratory fluorescent lights (Table 4). From these experiments, it was deemed advisable to make an effort to minimize light exposure of mitomycin C solutions. Amber autosampler vials were used for all recovery studies while developing this method.

Table 4
Mitomycin C solution stability

Condition/Time	24 hours	72 hours	8 days
Clear Vials - Light	98.3	95.7	87.9
Darkness	97.5	96.2	95.6
Amber vials at 8ºC	98.2	96.6	95.9

Ruggedness

This method was originally developed for support of healthcare worker studies within the Biomonitoring and Health Assessment Branch (BHAB), Division of Applied Research and Technology (DART) at NIOSH. Reproducibility within the laboratory over the period of method validation was performed and these data are shown in Tables 1, 2 and 3. Laboratory-to-laboratory reproducibility of the analytical procedure was evaluated through the User Check process. An independent laboratory set up the method, spiked wipes and swabs at four different concentration levels, and analyzed them according to NIOSH Method 9212. The report of the User Check can be found in the Appendix. Acceptable values for precision and accuracy were obtained in the User Check process showing the method to be rugged and translatable to other laboratories.

References

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[4] B'Hymer C, Connor T, Stinson D, Pretty J [2015]. Validation of an HPLC-MS/MS and wipe procedure for mitomycin C contamination. J Chromatogr Sci *53*:619-624.

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Appendix

Review of User Check for NIOSH Method 9212 (Mitomycin C on surfaces)

User Check samples (swabs and wipes) were prepared by contract lab personnel blind to the analyst. The swab samples and the filter paper wipe samples were prepared separately, but at the same concentration levels. A total of 25 swab samples and 25 wipe samples were prepared. Five samples of each kind were left blank. Five fortified samples of each kind were prepared containing the analyte at each of the following levels: 5 ng, 10 ng, 50 ng, and 400 ng. The samples were then analyzed using the sample preparation procedures and analytical detection conditions found in draft NIOSH method 9212. These samples were all spiked directly on to the two-wipe media. No surfaces were analyzed.

Tables 1 (swabs) and 2 (wipes) show the data obtained from the User Check samples. Mitomycin C was not detected in any of the blank samples (data not shown), so no corrections were required. Summary Tables 3 (swabs) and 4 (wipes) show the average recoveries (at each level and total) and precision as calculated by relative standard deviation (RSD).

Table 1 (mitomycin C on swabs)

Spike Level (ng)	Recovery (ng)	Accuracy (%)
5	4.97	99.4
5	4.95	99.0
5	5.06	101.2
5	5.28	105.6
5	4.47	89.4
10	8.92	89.2
10	9.51	95.1
10	10.3	103.0
10	10.1	101.0
10	10.4	104.0
50	49.0	98.0
50	50.3	100.6
50	47.1	94.2
50	48.2	96.4
50	48.9	97.8
400	420	105.0
400	413	103.3
400	436	109.0
400	413	103.3
400	409	102.3

Table 2 (mitomycin C on filter paper)

Spike Level (ng)	Recovery (ng)	Accuracy (%)
5	5.22	104.4
5	5.26	105.2
5	5.10	102.0
5	5.35	107.0
5	5.02	100.4
10	8.94	89.4
10	9.76	97.6
10	9.63	96.3
10	9.76	97.6
10	9.37	93.7
50	46.4	92.8
50	48.0	96.0
50	48.2	96.4
50	49.7	99.4
50	48.5	97.0
400	413	103.3
400	374	93.5
400	412	103.0
400	427	106.8
400	420	105.0

Table 3 (Average recovery and relative standard deviation - swabs)

Spike level (ng)	Recovery (%)	RSD (%)
5	98.9	6.0
10	98.5	6.3
50	97.4	2.4
400	105	2.6
Overall	99.8	5.1

Table 4 (Average recovery and relative standard deviation – filter paper)

Spike level (ng)	Recovery (%)	RSD (%)
5	104	2.5
10	94.9	3.7
50	96.3	2.5
400	102	5.0
Overall	99.3	5.1

The accuracy at every concentration level for both swabs and wipes is within ± 5% of the true value, which is well within acceptable levels. The RSD for each individual level ranges between 2 and 7 percent. These precision values, as well as the overall precision values, are also well within acceptable limits. Two primary guidelines on method validation state that accuracy and precision be within 15% at each level and within 20% at the lowest level [1,2].

The contract lab reported no difficulties understanding the draft method nor in setting it up or analyzing the samples. The method has relatively few analytical steps, is quite straightforward, has been shown to give reasonable recoveries for different surfaces, and has adequate analytical precision and accuracy. It is recommended that the method, NIOSH 9212 (Mitomycin C on surfaces) be approved and accepted for inclusion in the NIOSH Manual of Analytical Methods.

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Research Chemist
April 5, 2018

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