

## Validation of an HPLC-MS/MS and Wipe Procedure for Mitomycin C Contamination

Clayton B'Hymer\*, Thomas Connor, Derek Stinson and Jack Pretty

U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Applied Research and Technology, Taft Laboratory C-23, 4676 Columbia Parkway, Cincinnati, OH 45226, USA

\*Author to whom correspondence should be addressed. Email: cbhymer@cdc.gov

Received 4 April 2014; revised 16 June 2014

**A high-performance liquid chromatography–tandem mass spectrometric (HPLC-MS/MS) method was developed for the determination of mitomycin C, an anticancer drug, from contamination on various surfaces. Mitomycin C is often used in various forms of intraperitoneal chemotherapy, and operating room healthcare worker exposure to this drug is possible. The surface testing method consisted of a wiping procedure utilizing a solution of 20/45/35 (v/v/v) of acetonitrile–isopropanol–water made 0.01 M in ammonium citrate (apparent pH 7.0). The wipe solutions were analyzed by means of HPLC-MS/MS using a reversed-phase gradient system and electrospray ionization in positive ion mode with a triple-quadrupole MS detector. Accuracy and precision of this method were demonstrated by a series of recovery studies of both spiked solutions and extracted wipes from various surfaces (stainless steel, vinyl and Formica®) spiked with known levels of mitomycin C. Recoveries of spiked solutions containing the analyte demonstrate mean recoveries (accuracy) ranged from 93 to 105%. Precision as measured by the relative standard deviation (% RSD) of multiple samples ( $n = 10$ ) at each concentration level demonstrated values of 7.5% or less. The recoveries from spiked surfaces varied from 30 to 99%. The limit of detection for this methodology is  $\sim 2$  ng/100 cm<sup>2</sup> equivalent surface area, and the limit of quantitation is  $\sim 6$  ng/100 cm<sup>2</sup>.**

### Introduction

Since their introduction in the 1940s, antineoplastic drugs have been extensively used to treat various types of cancer. Mitomycins are a family of aziridine containing natural products first isolated from the soil bacteria *Streptomyces caespitosus* and *Streptomyces lavendulae* (1–4). Mitomycin C (see Figure 1) is used as a chemotherapeutic agent because of its antitumor properties, and it also exhibits antibiotic activity. Mitomycin C exhibits DNA crosslinking capability and is an alkylating agent. It has been used occasionally in the intravenous treatment mode and used extensively in the topical treatment mode of cancer. Mitomycin C has been utilized in the treatment of bladder tumors by means of instillation therapy (5, 6). In recent years, different types of intraperitoneal chemotherapy procedures have come into use for cancer treatment (7–9) whereby solutions of antineoplastic drugs are infused into the human body by surgical means. Hyperthermic intraperitoneal chemotherapy (HIPEC) and continuous hyperthermic peritoneal perfusion (CHPP) have gained acceptance in the USA and other countries owing to the improved survival rates in the treatment of some forms of cancer. These treatment procedures specifically involve preheating a large volume (up to 3 L) of antineoplastic drug solution to  $\sim 42^\circ\text{C}$ , introducing the solution into the peritoneal

cavity and then siphoning the solution out of the patient after a prescribed time. Mitomycin C has become a preferred drug for these types of procedures.

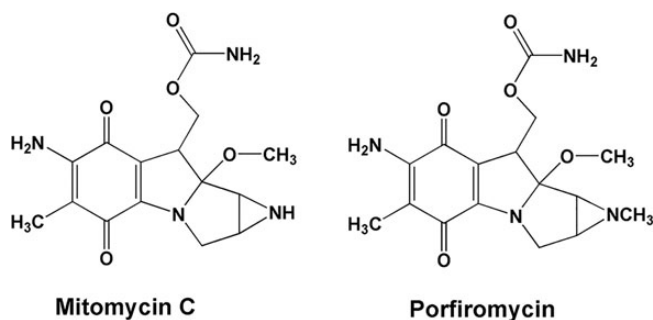
Researchers have become interested in minimizing the exposure to antineoplastic drugs used by healthcare workers in general, and many have become focused upon operating room exposure (10, 11). Many antineoplastic drugs, mitomycin C included, can be hazardous to health. Antineoplastic drugs have been shown to exhibit a number of acute and chronic toxicological effects including carcinogenicity, mutagenicity and teratogenicity (12). The determination of mitomycin C contamination in operating room environments has been studied in the past (13, 14), and it was of interest to this laboratory. Both surface contamination and possible exposure through air in personnel breathing zones from aerosols were considered.

Although many analytical techniques can be used to monitor antineoplastic drugs in the healthcare environment, high-performance liquid chromatography (HPLC) utilizing mass spectrometric (MS) detection has been demonstrated to be versatile and of great utility (15). Mitomycin C has been analyzed by HPLC by multiple researchers over recent years (13, 16, 17). Gerhard *et al.* (16) developed a reversed-phase HPLC system using UV detection to assay mitomycin C in human plasma. Velpandian *et al.* (18) used an HPLC–UV assay method to monitor the concentration of mitomycin C ophthalmic formulations. In this study, HPLC-MS/MS was the chosen technique owing to its high sensitivity and the higher degree of specificity, the ability of an analytical technique to accurately measure the analyte in the presence of all potential sample matrix components without interference. Mitomycin C also has the problem of having a narrow range of stable conditions when in solution (18–20); thus, a procedure utilizing a simple dilution and without extensive sample pretreatment was necessary. Furthermore, the developed method conforms to the validation requirements of the National Institute for Occupational Safety and Health (NIOSH) as well as the general accepted requirements of method validation (21, 22).

### Experimental

#### Instrumentation and reagents

The chromatographic analysis was conducted using an Agilent Technologies model 1100 liquid chromatograph (Palo Alto, CA, USA) with a pump (model G1312A), degasser (model G1379A) and cooled autosampler (model G1329A), and it was equipped with an Agilent Model 6430 triple quadrupole mass spectrometer (MS/MS) with an electrospray ionization (ESI) interface. The general chromatographic and MS conditions are summarized in Table 1. It should be noted that a post run of 100% mobile



**Figure 1.** Chemical structures of mitomycin C and porfiromycin.

phase B at the higher 0.5 mL/min flow rate was included to remove any well-retained sample matrix components from the column before the system was re-equilibrated to initial conditions. The MS/MS was run in multiple reaction monitoring (MRM).

Mitomycin C reference standard was obtained from the United States Pharmacopeia (Rockville, MD, USA). Porfiromycin (the methyl analog of mitomycin C, see Figure 1) was donated by the National Cancer Institute for use as an internal standard. Acetonitrile (HPLC grade), isopropanol (HPLC, GC and pesticide residue grade) and water (HPLC grade) were obtained from Burdick & Jackson (Honeywell International, Inc., Muskegon, MI, USA). The sample extraction solvent was buffered with ammonium citrate prepared using citric acid monohydrate (Sigma-Aldrich, St. Louis, MO, USA) and ammonium hydroxide (Fisher Scientific, Pittsburgh, PA, USA).

## Methods

### General sample preparation

Potential mitomycin C contamination was evaluated for three work surfaces using two separate wiping materials. The first wipe procedure evaluated for performing and processing samples was a modification of the one described by Larson *et al.* (23) and Pretty *et al.* (15). 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<sup>2</sup> on the desired surface. A filter paper disc (GE Healthcare, Buckinghamshire, UK; 55 mm Whatman 42) 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., Rochester, NY, USA; part number 2118-004) 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 min 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<sup>®</sup> swabs (ITW Texwipe, Kernersville, NC, USA; TX714A, Large Alpha Swab); 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 solvent samples were filtered using polyvinylidene difluoride (PVDF) Millex<sup>®</sup> 0.22 µm filters (Millipore

**Table 1**

Instrumental Parameters of the Method

#### HPLC conditions

High-performance liquid chromatograph: Agilent Model 1100 pump and autoinjector (8°C sample vial cooling)

Column: Agilent Zorbax Rx C18, 3.5 µm, 250 × 3.0 (ID) mm

Guard column: Phenomenex C18 SecurityGuard 4 × 2 mm (AQ-18-AJO-7510)

Column flow: 0.4 mL/min (during chromatographic analysis)

Injection size: 5 µL

Data acquisition time: 12 min

#### Mobile phase

A = 10/90/0.1% acetonitrile–water–acetic acid

B = 75/25/0.1% acetonitrile–water–acetic acid

#### Gradient program

Run time (min)	Mobile phase composition	Comments
0–12	0–70% B	Gradient
12–13	70–100% B	Start post run
13–16	100% B (flow at 0.5 mL/min)	Post run

Note: Re-equilibration back to 0% B was 8 min.

#### MS/MS conditions

Mass spectrometer (MS/MS): Agilent Model 6430 with electrospray ionization source in positive ion mode

Electrospray voltage: 3,500 V

Nebulizer gas pressure: 35 psi

Drying gas: nitrogen

Drying gas flow: 10 L/min

Drying gas temperature: 325°C

Fragmentor voltage: 97 V for all analytes

Collision gas: nitrogen

Collision gas flow rate: 0.06 L/min (factory default)

Collision energy: 8 V for all mitomycin C, 6 V for porfiromycin (IS, internal standard)

Dwell time: 200 ms

Mass transitions: *m/z* 335–242 for mitomycin C, *m/z* 349–256 for porfiromycin (IS)

Corporation, Billerica, MA, USA) and placed in autosampler vials for analysis.

To evaluate recoveries for the full surface wiping procedure, the three materials selected to represent surfaces likely to experience contamination in the workplaces were stainless steel, vinyl and Formica<sup>®</sup>. Stainless steel (Type 304 #3 satin finish, McMaster-Carr Supply, Cleveland, OH, USA) was used as a surrogate for biological safety cabinets, Formica<sup>®</sup> for counter tops and vinyl for floor tiles (Imperial Texture; Armstrong World Industries, Montreal, Quebec, Canada). Tiles were cut into 10 × 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<sup>2</sup> were prepared. Sample solutions prepared outside the range of the calibration curves (the 10 and 25 µg/100 cm<sup>2</sup> samples) were diluted 1:10 in extraction solvent before analysis.

### Standard sample preparation

Stock solutions of mitomycin C and porfiromycin were prepared in acetonitrile. Calibration standard samples were prepared at 0, 2, 5, 10, 50, 100, 200, 400 and 500 ng/mL concentrations in the extraction solvent described previously. This corresponded to equivalent wiped levels of 0, 0.02, 0.05, 0.1, 0.5, 2 and 5 µg of

mitomycin C per 100 cm<sup>2</sup> surface area. The porfiromycin internal standard solution used for spiking was prepared in acetonitrile at a concentration of 0.2 µg/mL. A 1.0-mL aliquot of each standard solution and 100 µL of the internal standard solution were added to each autosampler vial for analysis.

### Calculations

All analyte quantification for the validation of this test method was based on the peak area ratio of mitomycin C to porfiromycin. The standard calibration curves used for the recovery and extraction studies were linear within the standard sample concentration range; correlation coefficients were 0.98 or greater and the *y*-intercepts approached zero for all calibration curves generated with this chromatographic system. Calibration curves were generated at the beginning, middle and end of the analysis for each sample set to verify the lack of any significant signal drift during the use of this method. Sample quantification was calculated using the bracketed standard curves, the preceding and following curves were used and the two assay values were averaged.

The limit of detection (LOD) was calculated in the traditional manner (21, 22), using three times the noise level of the chromatogram's baseline divided by the slope of the calibration curve. Because instrument noise is a function of peak height, the average baseline level of height noise was determined for each batch run in chromatograms at the retention time window for each analyte from non-spiked samples. The slope from the calibration curve using peak heights of the standards was used as the divisor for the LOD calculation. It should be stressed that peak heights were used only for the estimation of the LOD of this method; peak area ratios were used for the quantification of mitomycin C during the recovery studies of the method's validation.

## Results

### Precision and accuracy

A recovery experiment using the optimized HPLC-MS/MS assay conditions with filter paper wipe solutions fortified with mitomycin C (i.e., the analyte standards were prepared in matrix-matched extraction solvent) was performed to demonstrate the accuracy and precision of the HPLC-MS/MS conditions. These data are presented in Table II: average recovery levels were between 93 and 105% (*n* = 10) with precision measured by percent relative standard deviations (RSDs) of 7.5% or less. The recovery levels and precision of spiked solutions were acceptable for this type of method and no significant method bias was determined from these recovery data when using the internal standard.

### Wipe recovery

The most important aspect of this methodology was to determine the most effective wipe procedure to detect mitomycin C from various surfaces encountered in an operating room setting. The data for the surfaces tested using both filter paper and the polyester swabs are presented in Table III. As can be seen in the table, stainless steel generally has higher recovery yields with some problems 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 only had recovery yields of 50–60% 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 III). The swabs had recovery yields of 60–70% at the 0.100 and 0.200 µg/cm<sup>2</sup> levels while the filter paper had recoveries near 30%. The swabs also displayed greater precision at the lower mitomycin levels (see Table III).

### Linearity and LOD

The linearity of this methodology was determined over the range of 0.002–0.5 µg/mL (0.02–5 µg/100 cm<sup>2</sup> spiked equivalent level). The calibration curves generated from the peak area ratio between mitomycin C and the internal standard showed correlation coefficients (*r*<sup>2</sup>) of 0.98 or greater. The LOD was determined to be ~2 ng/100 cm<sup>2</sup> equivalent (0.002 µg/100 cm<sup>2</sup>). The LOD was calculated in the traditional manner (16, 17) using three times the noise level of the chromatogram's baseline divided by the slope of a calibration curve using peak heights. The limit of quantitation (LOQ) may be estimated to be approximately three times the LOD. The lowest standard used was equivalent to 20 ng/100 cm<sup>2</sup> in the calibration curves.

## Discussion

### Chromatography and detection

The optimized chromatographic conditions developed for the HPLC-MS/MS method were demonstrated to be specific and have no obvious interferences; mitomycin C and the porfiromycin internal standard peaks were both easily and accurately quantifiable in the chromatograms generated (see Figure 2). Generally, tandem mass spectrometric detection gives a high degree of specificity to an HPLC method. Method specificity, one of the more critical elements of a method validation, was

**Table II**

Recovery of Mitomycin C Spikes Added to Matrix-Matched Extracts (Prepared by Wiping Blank Material Surfaces with Filter Paper)

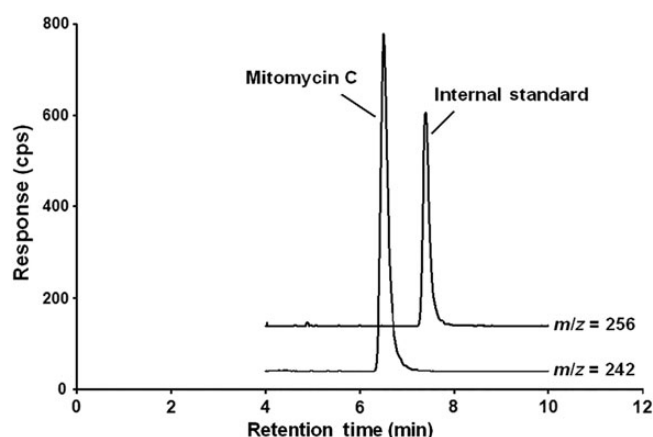
Surface	Equivalent level (µg/100 cm <sup>2</sup> )	Mean ( <i>n</i> = 10)	% Recovery	% RSD ( <i>n</i> = 10)
Stainless steel	0.100	0.104	104	7.5
	0.200	0.202	100	3.3
	2.00	2.01	100	1.0
	4.00	4.08	102	2.7
	10.0	9.81	98	2.5
	25.0	23.2	93	5.1
Vinyl	0.100	0.105	105	3.0
	0.200	0.201	101	2.3
	2.00	1.99	100	3.1
	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

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

**Table III**

Recovery from Spiked Material Surfaces

Surface	Filter paper wipe				Texwipe <sup>®</sup> swab			
	Level ( $\mu\text{g}/100\text{ cm}^2$ )	Mean ( $n = 5$ )	% Recovery	% RSD	Level ( $\mu\text{g}/100\text{ cm}^2$ )	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
Vinyl	25.0	23.9	96	3.1	25.0	24.8	99	2.6
	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
Formica <sup>®</sup>	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
	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

**Figure 2.** A chromatogram of mitomycin C (40 ng/mL) and the porfiromycin internal standard (20 ng/mL) using the conditions described dissolved in a filter paper matrix-matched extraction solvent.

demonstrated during this research by the analysis of non-fortified wipe samples from each surface (stainless steel, vinyl and Formica<sup>®</sup>) and each sample wipe material (filter paper and polyester swab) to verify the absence of any extraneous peaks present near the retention time of the analyte peaks. A typical chromatogram is presented in Figure 2, which shows a fortified stainless steel surface analysis. Possible sample carryover was eliminated by the use of a 50/50 (v/v) acetonitrile–water rinse of the autosampler needle and loop. No interference peaks were detected in any of the blank wipe solutions analyzed during this method's validation.

The mobile phase consisted of 0.1% (v/v) acetic acid; acetic acid was found to give the best signal response for mitomycin C using the mass spectrometer. Ammonium acetate and formic acid were tested early during method development, but neither of these alternative buffer or acid systems gave an  $m/z$  response comparable to the optimized mobile phase ultimately used. A post-run column purge step was added to insure that no artifact peaks from other surface residues would interfere with the target analytes. Ion suppression was not detected in this procedure,

but some high bias, ~5%, was noticed in an early plate wipe recovery study when an internal standard was not present. The use of isotopically labeled analogs is typically ideal for HPLC-ESI-MS, but the methyl analog of mitomycin C, porfiromycin, was used in this method. Both mitomycin C and porfiromycin are natural extracts of soil bacteria, as mentioned in the "Introduction" section. Porfiromycin internal standard was the most economical alternative choice because deuterated reference compounds were not available for obvious economic and chemical reasons. The good recovery results obtained throughout this study (see Tables II and III) demonstrate porfiromycin as a valid choice for an internal standard.

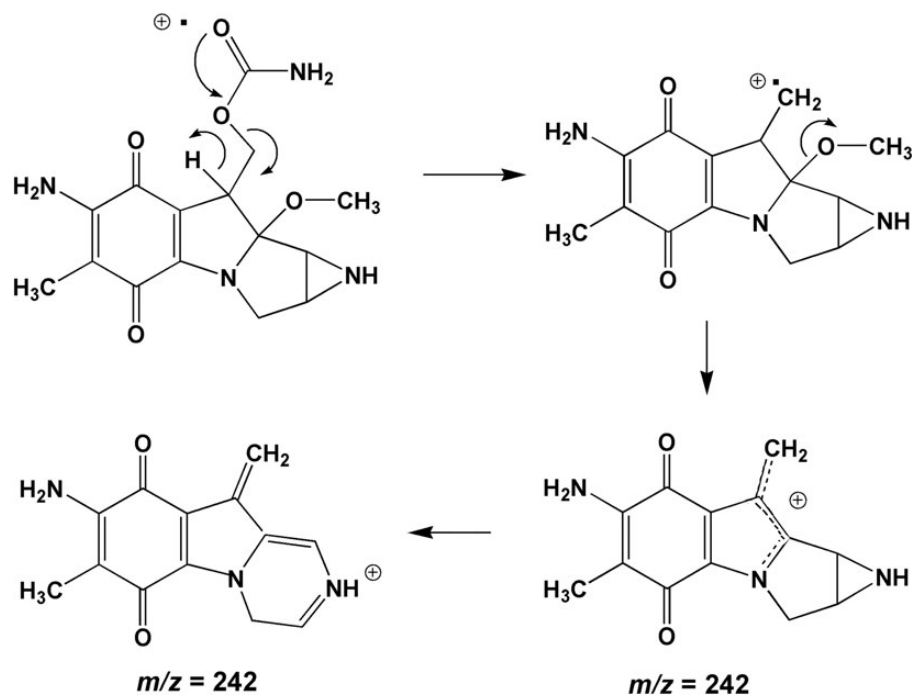
#### Mass spectra of mitomycin C

The MS analysis and ionization of mitomycin C and other similar antibiotics have been described in the literature by Van Lear (24). The fragmentation scheme and ion structures proposed by Van Lear (24) are outlined in Figure 3. The mitomycin C precursor ion under positive ion mode of the electrospray source in collision-induced dissociation (CID) eventually forms a daughter ion at  $m/z$  242. The mass transition monitored for quantification for mitomycin C in this study was  $m/z$  335–242. Porfiromycin undergoes a similar transition and  $m/z$  349–256 was monitored for its quantification. These daughter ions were the major ions and were monitored for maximum analysis sensitivity. The only other significant ion noted was  $m/z$  272 for mitomycin C, which was from the sodium adduct. This ion was minimized by the use of acetic acid in the mobile phase.

#### Mitomycin C stability and the sample extraction solvent

Mitomycin C exhibits antibiotic properties in addition to its antineoplastic one; it has been used in various pharmaceutical preparations including ophthalmic formulations (1, 18). As such, its stability has been well established. It can be easily hydrolyzed under acidic or basic conditions, but it is generally stable in a pH range between 7 and 8. Furthermore, the uses of aprotic solvents or organic/aqueous solvents, such as mixtures of





**Figure 3.** Daughter ion formation of mitomycin C as described by Van Lear (24).

propylene glycol and water, which do not promote acid- or base-catalyzed hydrolysis of mitomycin C, have been utilized and reported (18). The wipe/extraction solvent employed previously in studies by both Larson *et al.* (23) and Pretty *et al.* (15) consisting of acetonitrile–methanol–water at pH 6 was not suitable for optimum mitomycin C stability. 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 (18). 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 held at 8°C in the autosampler were found to degrade only 3 and 4% after 8 days ( $n = 3$ ); 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 and 3% after 3–6 months of storage at  $-15^{\circ}\text{C}$ . Mitomycin C did demonstrate light sensitivity for degradation; 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. 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.

#### Other aspects and future work

Again, the most important aspect of this method was to determine the most effective wipe procedure to detect mitomycin C from various surfaces encountered in an operating room setting. The swab wiping procedure was found to have a number of advantages. The swabs displayed precision at the lower mitomycin levels (see Table III) over the filter paper wipes as was noted previously. In actual use, the swabs tended to cause less physical hand fatigue for the analyst performing during the wiping of surfaces. Another advantage was the swab would not crumble or tear during the wiping procedure. Finally, the swab wipe procedure did allow for the use of alternative extraction vessels. In a limited test experiment, it was found that swabs could be extracted in 15-mL polypropylene tubes with shaking just as efficiently as with the 125-mL jar swirling procedure. This might be more desirable should the 125-mL jars not be available or if they presented a shipping problem.

Future work in this laboratory will include the application of this validated method to surgical operating rooms utilizing mitomycin C intraperitoneal treatment. This is part of multiple larger and more comprehensive occupational safety studies and is beyond the scope of this reported work. The focus of this work is the description and validation of the HPLC-MS/MS method along with the data evaluating the use of filter paper and polyester swabs for wipe testing. This method will eventually be issued in the NIOSH manual of analytical methods for widespread distribution.

#### Conclusion

An analytical method to measure the levels of mitomycin C contamination of various typical surfaces was developed and fully validated. The use of polyester swabs was found to be more

effective for wiping Formica® surfaces over the more traditional approach of using pieces of filter paper. In actual use, the swabs also caused less fatigue for the analyst performing the wiping procedure. The HPLC-MS/MS method was demonstrated to be accurate and precise. The LOD of the method was estimated to be ~2 ng/100 cm<sup>2</sup> equivalent for mitomycin C, and linearity was established at solution levels of 2–500 ng/mL.

## Acknowledgments

The authors would like to thank Dennis Lynch, Kenneth Cheever and Jeanette Krause for their help and assistance with the preparation and editing of this manuscript. Mention of company names and/or products does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH). The findings and conclusions in this report have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

## References

- Wakaki, S., Marumo, H., Tomioka, K., Shimizu, M., Kato, E., Kamada, H., *et al.*; Purification and isolation study on gancidins; *Journal of Antibiotics*, (1958); 11: 150–155.
- Hata, T., Sano, Y., Sugawara, R., Matsumae, A., Kanamori, K., Shima, T., *et al.*; Mitomycin, a new antibiotic from Streptomyces; *Journal of Antibiotics*, (1956); 9: 141–146.
- Claridge, C.A., Bush, J.A., Doyle, T.W., Nettleton, D.E., Moseley, J.E., Kimball, D., *et al.*; New mitomycin analogs produced by directed biosynthesis; *Journal of Antibiotics*, (1986); 39: 437–446.
- Danshiitsoodol, N., de Pinho, C.A., Matoba, Y., Kumagai, T., Sugiyama, M.; The mitomycin C (MMC)-binding protein from MMC-producing microorganisms protects from the lethal effect of bleomycin: crystallographic analysis to elucidate the binding mode of the antibiotic to the protein; *Journal of Molecular Biology*, (2006); 360: 398–408.
- Mishina, T., Oda, K., Murata, S., Ooe, H., Mori, Y.; Mitomycin C bladder instillation therapy for bladder tumors; *Journal of Urology*, (1975); 114: 217–219.
- Washburn, D.J.; Intravesical antineoplastic therapy following transurethral resection of bladder tumors: nursing implications from the operating room to discharge; *Clinical Journal of Oncology Nursing*, (2007); 11: 553–559.
- Begossi, G., Gonzalez-Moreno, S., Ortega-Perez, G., Fon, L.J., Sugarbaker, P.H.; Cytoreduction and intraperitoneal chemotherapy for the management of peritoneal carcinomatosis, sarcomatosis and mesothelioma; *European Journal of Surgical Oncology*, (2002); 28: 80–87.
- Gonzalez-Moreno, S., Gonzalez-Bayon, L., Ortega-Perez, G.; Hyperthermic intraperitoneal chemotherapy: methodology and safety considerations; *Surgical Oncology Clinics of North America*, (2012); 21: 543–557.
- Sugarbaker, P.H., Mora, J.T., Carmignani, P., Stuart, O.A., Yoo, D.; Update on chemotherapeutic agents utilized for perioperative intraperitoneal chemotherapy; *Oncologist*, (2005); 10: 112–122.
- Connor, T.H., van Balen, P., Sessink, P.J.M.; Monitoring for hazardous drugs in the operating room; *Annals of Surgical Oncology*, (2003); 10: 821.
- Stuart, O.A., Welch, L.S., Sugarbaker, P.H.; Operating room environmental safety with heated intraperitoneal chemotherapy; *Annals of Surgical Oncology*, (2003); 10: 822–823.
- NIOSH alert: Preventing occupational exposure to antineoplastic and other hazardous drugs in healthcare settings. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Department of Health and Human Services, DHHS (NIOSH) Publication No. 2004–165, Cincinnati, Ohio, USA, 2004.
- Schmid, K., Boettchre, M.I., Pelz, J.O.W., Meyer, T., Korin, G., Angerer, J., *et al.*; Investigations on safety of hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) with mitomycin C; *European Journal of Surgical Oncology*, (2006); 32: 1222–1225.
- Stuart, O.A., Stephens, A.D., Welch, L., Sugarbaker, P.H.; Safety monitoring of the coliseum technique for heated intraoperative intraperitoneal chemotherapy with mitomycin C; *Annals of Surgical Oncology*, (2002); 10: 186–191.
- Petty, J.R., Connor, T.H., Spasojevic, I., Kurtz, K.S., McLaurin, J.L., B'Hymer, C., *et al.*; Sampling and mass spectrometric analytical methods for five antineoplastic drugs in the healthcare environment; *Journal of Oncology Pharmacy Practice*, (2011); 18: 23–36.
- Gerhard, J., Biederbick, W., Woschke, U., Theisohn, M., Klaus, W.; Sensitive and convenient high-performance liquid chromatographic method for the determination of mitomycin C in human plasma; *Journal of Chromatography B*, (1997); 698: 261–267.
- Ploemakers, H.H.J.L., Vanoort, M.J.M., Vanoot, W.J.; Improved HPLC-ECD analysis of mitomycin-C, porfiromycin, VP-16–213 and VM-26 by implantation of software filters; *Anticancer Research*, (1987); 7: 1315–1319.
- Velpandian, T., Saluja, V., Ravi, A.K., Kumari, S.S., Mathur, R., Biswas, N.R., *et al.*; Evaluation of the stability of extemporaneously prepared ophthalmic formulation of mitomycin C; *Journal of Ocular Pharmacology and Therapeutics*, (2005); 21: 217–222.
- Paborji, M., Bogardus, J.B., Agharkar, S.N., Coppola, W.P.; Stable solutions of mitomycin C. United States Patent 5216011, publication date 06/01/1993 assignee. Bristol-Myers Squibb Co. (New York).
- Underberg, W.J., Lingeman, H.; Aspects of chemical stability of mitomycin and porfiromycin in acidic solution; *Journal of Pharmaceutical Sciences*, (1983); 72: 549–553.
- Green, J.M.; A practical guide to analytical method validation; *Analytical Chemistry*, (1996); 68:305A–309A.
- Guidance for industry: bioanalytical method validation, US Food and Drug Administration, Rockville, Maryland USA, (2001).
- Larson, R.R., Khazaeli, M.B., Dillon, H.K.; Monitoring method for surface contamination caused by selected antineoplastic agents; *American Journal of Health-System Pharmacy*, (2002); 59: 270–277.
- Van Lear, G.E.; Mass spectrometric studies of antibiotics. I. Mass spectra of mitomycin antibiotics; *Tetrahedron*, (1970); 26: 2587–2597.