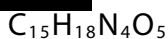


# Mitomycin C on Surfaces

9212



MW: 334.33

CAS: 50-07-7

**METHOD:** 9212, Issue 1      **EVALUATION:** FULL      **Issue 1:** 30 May 2020  
**OSHA:** None established      **PROPERTIES:** Solid; purple colored crystal; MP >360 °C [1]  
**NIOSH REL:** None established

**SYNONYMS:** (not all inclusive) Mutamycin; Ametycine; [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.

SAMPLING		MEASUREMENT	
<b>SAMPLER:</b>	Polyester swab or filter paper wipes	<b>TECHNIQUE:</b>	HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY
<b>SURFACE AREA:</b>	100 cm <sup>2</sup>	<b>ANALYTE:</b>	Mitomycin C
<b>PRESERVATIVE:</b>	None	<b>HPLC - COLUMN:</b>	C18 (150 x 3 mm, 3.5 μm) equipped with same type guard column (4 x 2 mm) and 2-micron precolumn filter
<b>SHIPMENT:</b>	Ship frozen using dry ice	<b>MOBILE PHASES:</b>	A = 10/90/0.1% (v/v/v) acetonitrile/water/acetic acid B = 75/25/0.1% (v/v/v) acetonitrile/water/acetic acid
<b>SAMPLE STABILITY:</b>	>8 days (see Evaluation Section for discussion)	<b>FLOW RATE:</b>	0.4 mL/min (0.5 mL/min post run)
<b>FIELD BLANKS:</b>	2 to 10 per set	<b>GRADIENT:</b>	Time (min)      Mobile Phase Composition
			0 to 12      0 to 70% B
			12 to 13      70 to 100% B
			3 to 16      100% B Flow increased to 0.5 mL/min
			16 to 18      100 to 0% B (re-equilibration, 0.4 mL/min)
			18 to 24      0% B (re-equilibration)
		<b>INJECTION VOLUME:</b>	5 uL
		<b>IONIZATION SOURCE:</b>	Electrospray at 3500 Volts and positive scan mode, nebulizer gas at approximately 10 L/min flow
		<b>DETECTOR(MS/MS):</b>	Dwell time=100-200 msec Collision gas: Nitrogen or Argon
		<b>MULTIPLE REACTION MODE:</b>	Quantification mass transitions: Mitomycin C = m/z 335 → 242, Porfiromycin = 349 → 256
		<b>TOTAL RUN TIME:</b>	Approximately 24 minute cycle time
		<b>CALIBRATION:</b>	Standards from Mitomycin C solutions with internal standard
		<b>QUALITY CONTROL:</b>	At least one level of spiked sample prepared from a separately weighed stock solution
		<b>RANGE:</b>	20 to 5000 ng/100 cm <sup>2</sup>
		<b>ESTIMATED LOD:</b>	LOD is dependent upon column and instrumentation. Instrumental LOD is equivalent to approximately 2 ng/100 cm <sup>2</sup> ; by lowest standard level LOD is equivalent to 20 ng/100 cm <sup>2</sup>
		<b>PRECISION (<math>\bar{S}_r</math>):</b>	See Table 1
ACCURACY			
<b>RANGE STUDIED:</b>	0.1 to 25 μg/100 cm <sup>2</sup> (See Tables 1 and 2).		
<b>BIAS:</b>	None established.		
<b>OVERALL PRECISION (<math>\hat{S}_{rT}</math>):</b>	See Tables 1 and 2.		
<b>ACCURACY:</b>	Full recovery (90 to 107%) was established by spiked matrix-matched solutions at up to 6 different concentration levels and using 3 surfaces (See Tables 1 and 2). The precision by relative standard deviation was no larger than 7.5% at any concentration level (See Tables 1 and 2).		

**APPLICABILITY:** Mitomycin C is an antineoplastic drug often used in the operating room setting. This method measures the quantity of the target analyte from surface contamination. The method was tested on three "hard" surfaces likely to be found in operating rooms. (See Table 1.)

**INTERFERENCES:** None found or identified.

**OTHER METHODS:** This method is from the one described by B'Hymer [2,3]. There are numerous literature methods for this compound, but standardized methods from governmental or other agencies are lacking.

**REAGENTS:**

1. Mitomycin C (CAS no. 50-07-7) reference standard 0.10 mg/mL stock solution in acetonitrile. Store in a freezer at typical temperatures (-10 to -20 °C)
2. Porfiromycin (CAS no. 801-52-5) reference standard 0.05 mg/mL stock solution in acetonitrile. Store in a freezer at typical temperatures (-10 to -20 °C)
3. Porfiromycin spiking solution made 0.25 µg/mL in acetonitrile. Store in a freezer at typical temperatures (-10 to -20 °C)
4. Acetonitrile, HPLC grade or better\*
5. Acetic acid, glacial, HPLC grade or better\*
6. Ammonium hydroxide, ACS grade or better\*
7. Citric acid, monohydrate, ACS grade or better
8. Isopropanol, HPLC grade or better\*
9. Water, HPLC grade or better
10. Autosampler needle rinse solution, 50/50% (v/v) acetonitrile/water
11. Extraction solvent 20/45/35% (v/v/v) acetonitrile /isopropanol/water. Mix the components and then add enough citric acid monohydrate to make 0.01 M. Adjust the apparent pH of the mixture to 7.0 by the addition of ammonium hydroxide.
12. Mobile phase A (10/90/0.1%, v/v/v) acetonitrile/water/acetic acid, filtered
13. Mobile phase B (75/25/0.1%, v/v/v) acetonitrile/water/acetic acid, filtered
14. Nitrogen, UHP or better

\*See SPECIAL PRECAUTIONS.

**EQUIPMENT:**

1. High-performance liquid chromatograph (HPLC) equipped with a tandem mass spectrometric detector with data collection system
2. HPLC column (C18, 150 x 3 mm, 3.5 µm particle size)
3. HPLC guard column (C18, 4.0 x 2.0 mm)
4. Autosampler capable of maintaining 8 °C
5. Pre-column filter, 2 µm
6. Pipettor with disposable tips in the 1000 µL volume delivery range
7. Chemical orbital shaker
8. pH meter
9. Filter paper disc, 55 mm, Grade 42
10. Polyester swab, 13 mm head width
11. Filter discs, polyvinylidene difluoride (PVDF), 0.22 µm
12. Polypropylene jar, 125 mL wide mouth
13. Polypropylene tubes, 15 mL
14. Metal spatula
15. Volumetric flasks, glass: 10, 50, 100 and 250 mL
16. Autosampler vials, amber glass, with caps and septa
17. Disposable glass pipets
18. Sintered glass filtering apparatus and 1-L side-armed Erlenmeyer flask
19. Filters, glass microfiber, size to fit filtering apparatus, 0.7 µm
20. Analytical balance, to ± 0.00001 g
21. Template: 10 cm x 10 cm hole. Template made of relatively rigid disposable cardstock (highly recommended) or sheet of PTFE.
22. Wire cutters or other device capable of snipping the heads off the swabs.
23. Gloves, chemotherapy-safe, powderless
24. Waste containers, chemotherapy approved

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**SPECIAL PRECAUTIONS:** Acetic acid, acetonitrile and isopropanol are flammable; handle with care and use in a chemical fume hood. Handle all chemicals using the required safety precautions. Reagents with manufacturer expiration dates should be observed. Mitomycin C and porfiromycin are carcinogens [4] and should be handled and disposed of properly [5,6].

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**SAMPLING:**

1. Identify the surface area to be sampled. Wearing a clean pair of powderless, chemotherapy-safe gloves, place a 10 x 10 cm (100 cm<sup>2</sup>) template on the surface and secure with tape. If the template cannot be placed on the targeted surface, measure 100 cm<sup>2</sup> and outline with tape. For irregular shaped objects, measure the length and width in order to calculate and record the surface area.  
NOTE: Mitomycin C does show some light sensitivity (see Stability under Evaluation of Method section), so reasonable steps to minimize light exposure should be taken.
2. Apply 250 µL of extraction solvent to the area inside the template by use of an automatic pipet, moving it over the surface in order to spread the solvent as much as is possible. The surface can be wiped immediately. Reapply an aliquot of extraction solvent within the template for each wiping instance.  
NOTE: If the surface to be sampled is not amenable to the application of the extraction solvent (such as a wall or doorknob/handle or other surfaces where the solvent would run off), the solvent may be applied to the swabs or filter paper and used to wipe. Apply 250 µL of the extraction solvent to each of the three swabs or wipes in this case.
3. If using the polyester swab procedure:
  - a. Apply even pressure with the swab and wipe using an overlapping S-pattern in the horizontal direction within the template. Invert the swab exposing the unused side, then wipe the same surface using an overlapping S-pattern in the vertical direction. Ensure that you have wiped the entire area inside the template. Cut, snip, or break the swab at the indentation above the swabbing material and drop into the 125-mL wide-mouth jar.  
NOTE: The wide-mouth jar should be pre-labelled.
  - b. Repeat the process two more times and combine all three swab wipes into the wide-mouth jar.  
NOTE: Gloves and the template are not to be changed during these repeated samplings of the same area.
  - c. Record the time, date, location and condition of the area that was sampled along with the name of the analyst performing the sampling. Photographs are useful when evaluating surface results.
4. If using the alternative filter paper procedure:
  - a. Grasp the opposite edges of the filter paper disc producing a flat area with which to wipe the surface. Apply even pressure with the paper disc and wipe using an overlapping S-pattern in the horizontal direction within the template. Then wipe the same surface with the same side of the paper disc using an overlapping S-pattern in the vertical direction. Ensure that you have wiped the entire area inside the template. Use caution not to touch the area being sampled with your gloved hand while collecting the sample. Place the filter paper in the 125-mL wide-mouth jar.  
NOTE: The wide-mouth jar should be pre-labelled.
  - b. Repeat the process two more times and combine all three filter wipes into the wide-mouth jar.  
NOTE: Gloves and the template are not to be changed during these repeated samplings of the same area.
  - c. Record the time, date, location and condition of the area that was sampled along with the name of the analyst performing the sampling. Photographs are useful when evaluating surface results.
5. Remove gloves and discard into trace/residual chemotherapy waste containers, often known as the yellow containers in hospital settings (or follow applicable guidelines [5,6]). Wear a new pair of clean gloves for each new sample.
6. Use a new disposable template for each new sample. The used template can be discarded into trace/residual chemotherapy waste containers, often known as the yellow containers in hospital settings (or follow applicable guidelines [5,6]). The use of disposable templates is recommended as it is very difficult to properly clean reusable templates, especially in a field setting
7. Wash hands thoroughly after sampling is completed.

8. To collect field blanks, remove unexposed wipes (3) or swabs (3) from their packaging and place into pre-labelled 125-mL wide-mouth jars. Add 750  $\mu$ L of extraction solvent to the field blanks to mimic the volume used in the sampling procedure. If desired, the jars may be secured with a sealing film or tape before shipment.
9. Ship the wide-mouth jars in a packing container that is designed for dry-ice storage and transport. Ship express for delivery to the laboratory.
10. Upon receipt in the laboratory, inventory, and store the samples in a freezer at typical temperatures (-10 to -20 °C or colder) until the time of analysis.

#### **SAMPLE PREPARATION:**

11. Remove the sample jars from storage and add extraction solvent to make 9.0 mL of volume [0.75 mL was used to wipe the surface, so 8.25 mL would be added assuming minimum evaporation from the jar].
12. Add 1.0 mL of the porfiromycin internal standard spiking solution to the sample jar.  
NOTE: At this point, the swab heads or filter paper discs should be covered in solvent.
13. Tightly cap the jar and place on the orbital shaker for swirling. Swirl for 30 minutes at 100 rpm.
14. Remove and filter an aliquot of the sample through the 0.22  $\mu$ m PVDF filter disc into a 15-mL polypropylene tube.
15. Transfer approximately 1 mL of each filtered sample solution into an amber HPLC autosampler vial. Protect from light sources.

#### **CALIBRATION AND QUALITY CONTROL:**

16. Prepare a mitomycin C dilute standard solution by diluting 5.0 mL of the stock solution to 100 mL with acetonitrile (approximately 5  $\mu$ g/mL mitomycin C concentration).
17. Mitomycin C standard solutions. The 5  $\mu$ g/mL concentration solution is diluted in extraction solvent to make 2, 5, 10, 50, 100, 200, 400 and 500 ng/mL mitomycin C solutions.
18. Transfer 1.0 mL of each mitomycin C solution from step 17 into separate HPLC autosampler vials.
19. Add 0.100 mL of the internal standard spiking solution to each vial. These standards are equivalent to the extract solutions which would result for wiping surfaces prepared at levels of 20 to 5000 ng/100 cm<sup>2</sup> mitomycin C.
20. Prepare at least one blank sample of the extraction solvent without analyte spikes or internal standard and at least one extraction solvent blank with 0.100 mL internal standard added.
21. Prepare at least one quality control (QC) standard of mitomycin C using separately prepared stock solutions. A 50 ng/mL equivalent spike level is suggested, and more than one level can be used if desired. Run enough QC samples to be greater than 5% of the sample set. QC values should normally be within  $\pm$ 20% of the spiked values. If not, the batch is considered out of control, the batch data discarded, and corrective actions taken before more specimens are analyzed.

#### **MEASUREMENT:**

22. Set the high-performance liquid chromatograph according to the manufacturer's recommendations and to the conditions listed on the first page of this method. Following each injection, a needle rinse with 50/50% (v/v) acetonitrile/water is required to eliminate sample carry-over by the autosampler.
23. Set the mass spectrometric detector to multiple reaction mode (MRM) according to the manufacturer's recommendations and the conditions listed on the first page of this method. Example conditions are summarized below and should be optimized for a specific instrument for mitomycin C and porfiromycin:

## MS/MS conditions using positive electrospray ionization and nitrogen as the collision gas

Analyte	Parent ion (m/z)	MS1 Resolution	Daughter ion (m/z)	MS2 Resolution	Dwell (ms)	Fragmentor (V)	Collision Energy (V)	Cell Voltage (V)
Mitomycin C	335	unit	242	unit	200	97	8	4
Porfiromycin	349	unit	256	unit	200	97	6	4

## MS/MS conditions using positive electrospray ionization and argon as the collision gas (mass span at 1.0)

Analyte	Parent ion (m/z)	Daughter ion (m/z)	Dwell (ms)	Cone (V)	Collision Energy (V)
Mitomycin C	335	242	100	17	15
Porfiromycin	349	256	100	17	16

NOTE: Differences in detector parameters and their optimal values reflect use of different models of MS detectors. Models used can be found in Tables 1 and 2 and the method backup data report [2].

24. Inject 5  $\mu\text{L}$  of each sample extract, field blank, standard, QC standard, and blank. A sample chromatogram is shown in Figure 1.
25. Measure the peak areas of the mitomycin C analyte and that for the internal standard in the chromatograms. Divide the peak area of the analytes by the peak area from the internal standard (IS).
26. Prepare calibration curves of the peak area mitomycin C/Area IS versus the concentration of the standards for the analyte. The standard curve should have a coefficient of determination ( $r^2$ ) of equal to or greater than 0.98 to be acceptable for use. Furthermore, when each standard is plugged back into the calibration equation, the value should be within  $\pm 20\%$  of the expected.

**CALCULATIONS:**

27. Determine the concentration of the mitomycin C in the extracts from the surface area (10.0 mL solution for the 100  $\text{cm}^2$  wiped surface area) from the curves obtained in step 26. The results can be expressed as  $\text{ng}/100 \text{ cm}^2$  mitomycin C.

**EVALUATION OF METHOD:**

This method was evaluated and described in detail by B'Hymer [2,3]. A general summary of this previously published information is given below.

**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. 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 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 accuracy and precision numbers fall well within parameters established by guidelines suggested by the US Food and Drug Administration [7].

The second recovery study was performed from surfaces with mitomycin C and these data are presented in Table 3. Both filter paper and Texwipe® polyester swabs were evaluated. Some general conclusions can be drawn from these data. Recoveries from the vinyl surface were generally lower owing to the porous nature of the surface. The two types of wipes worked similarly on stainless steel and showed less than quantitative recoveries at the two lowest concentrations tested. The polyester swab gave higher recoveries and greater precision than that of the filter paper when used on the Formica surface at the lower concentrations. Similar to the stainless steel, recoveries at the two lowest concentrations still average less than 75%.

The two high concentration points (10 and 25 µg/100 cm<sup>2</sup>) studied for both recovery studies were outside the standard calibration curve range. For these levels, a 1 to 10 dilution of the filtered extract solution was performed to demonstrate that the method was still accurate at higher levels of mitomycin C surface contamination.

**Linearity.** All calibration curves used during the development of this method were linear, had correlation of determinations ( $r^2$ ) of 0.99 or greater, and had y-intercepts near zero. The concentration range was equivalent to 20 to 5000 ng/100 cm<sup>2</sup> mitomycin C surface contamination. Calibration curves were run at the beginning and end of all sample batch runs; calibration curve slope drift was found to be minimal during this method's development.

**Specificity.** The optimized chromatographic conditions developed for this method, along with the tandem mass spectrometric detector, proved to be specific and have no major interferences. The mass transition ion selected for mitomycin C had the greatest response and the predominant daughter ion was chosen.

**Robustness.** Two Zorbax Rx-C18 HPLC columns of different manufacturing lots were used during the recovery studies. Accuracy and precision did not appear to be affected; therefore, the method appears to be reproducible with any functioning Zorbax Rx column. Two HPLC-MS/MS systems were investigated for use with this method and found to give reasonable recovery results.

**Stability.** Mitomycin C is a pharmaceutical product, and as such, its stability in solution 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. The acetonitrile-isopropanol-water ammonium citrate 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 [2,8]. Mitomycin C solutions stored in the acetonitrile-isopropanol-water extraction solvent at room temperature in darkness and those held at 8 °C in the autosampler were found to degrade only 3 to 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 degrade only 2 to 3% after 3 to 6 months of storage at -15 °C. Mitomycin C did show some light sensitivity. Sample solutions (n=3) made in the 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. While no sample holding time studies were performed, two pieces of information would seem to be relevant in this matter. The first is that solid mitomycin C is known to be stable for at least one year when stored frozen [9-11]. The second is that solutions of mitomycin C in the extraction solvent have been shown to be stable for greater than 8 days at 8 °C [2]. Collected samples are dissolved in the extraction solvent on the surface of the filter paper or swab and then frozen. One can safely state that the samples would be expected to be stable for at least 8 days and since they are frozen and more like their solid state, it would be expected that they would be stable for much longer timeframes.

**Range.** This method should be considered accurate for the estimation of mitomycin C surface contamination within the standard curve range. Field samples at higher levels can be diluted to a concentration within that range for analysis.

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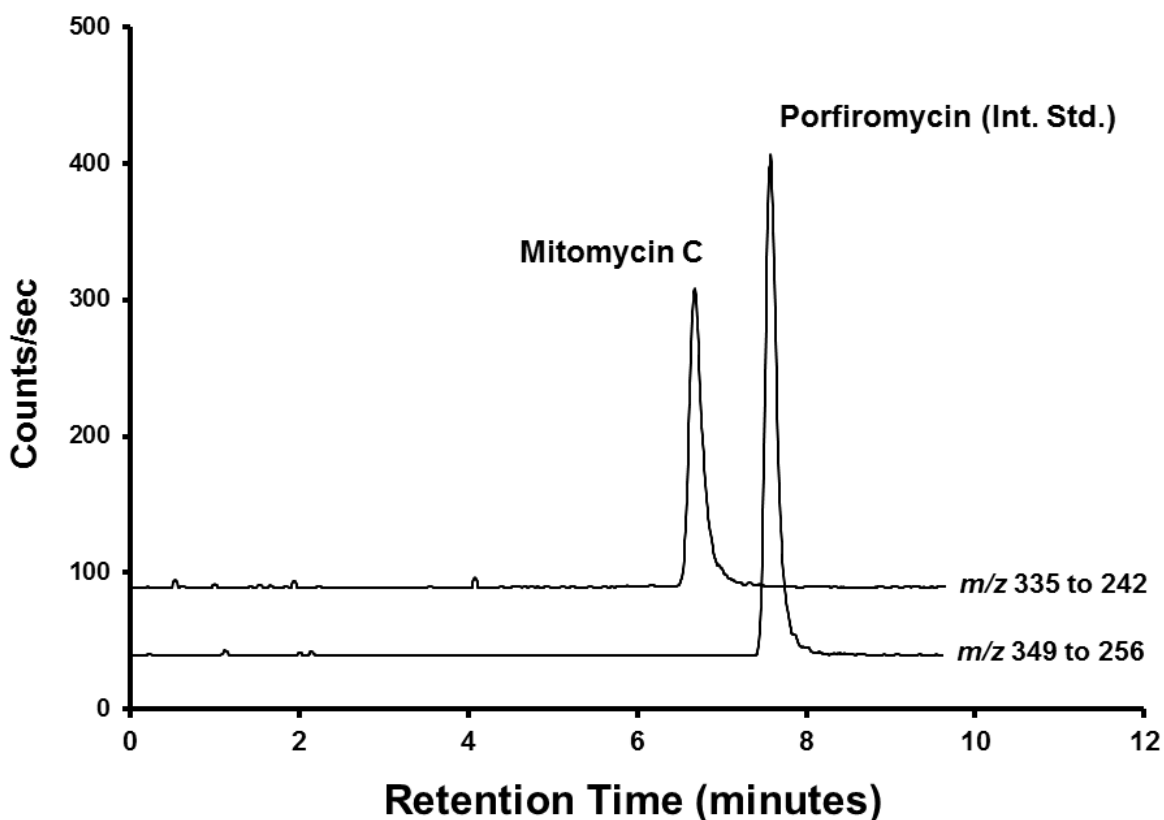
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**FIGURE 1**

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





**Table 1**

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

<b>Surface</b>	<b>Equivalent Level</b> ( $\mu\text{g}/100\text{ cm}^2$ )	<b>Mean</b> (n=10)	<b>% Recovery</b>	<b>% RSD</b> (n=10)
<b>Stainless Steel</b>	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
<b>Vinyl</b>	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
<b>Formica<sup>®</sup></b>	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)

<b>Surface</b>	<b>Equivalent Level</b> ( $\mu\text{g}/100\text{ cm}^2$ )	<b>Mean</b> (n=5)	<b>% Recovery</b>	<b>% RSD</b> (n=5)
<b>Stainless Steel</b>	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
<b>Vinyl</b>	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
<b>Formica®</b>	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

**Table 3**  
**Recovery from Spiked Material Surfaces**

Surface	Filter Paper Wipe				Polyester 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
<b>Stainless Steel</b>	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
<b>Vinyl</b>	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
<b>Formica®</b>	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: These data were collected using the Agilent HPLC-MS/MS system.