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Capillary gas chromatographic assay of residual methenamine hippurate in equipment cleaning validation swabs[1]

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Abstract

A capillary gas chromatographic method is described for the determination of methenamine hippurate residue in swabs collected from manufacturing equipment surfaces. Any residual methenamine hippurate remaining on process equipment after cleaning is removed by swabbing with one wet polyester Absorbond™ swab (4″ × 4″) pre-moistened with water followed by a dry Absorbond swab. The residual methenamine hippurate is chromatographed on a 30 × 0.32 mm (i.d.) Supelcowax®-10 capillary column of 0.25-micron film thickness. The amount of residual methenamine hippurate is determined by comparing the ratio of methenamine hippurate peak area response to that of *p*-cresol (internal standard) obtained for the sample to a linear calibration curve obtained for a series of standard solutions. The method is demonstrated to be sufficiently linear, accurate, precise, sensitive and rugged for the determination of low levels of methenamine hippurate on equipment surfaces. Using this method, the mean recovery of methenamine hippurate from spiked Absorbond swab samples contained in high density polyethylene bottles was 105.2%, with a relative standard deviation (RSD) of ± 7.1% ($n = 25$). The mean recoveries of methenamine hippurate from spiked test plates for '180 Grit' Stainless Steel, Teflon and WARCO White (neoprene and PVC) gasket material were 77.2, 96.1 and 50.6%, with RSDs of ± 9.4 ($n = 25$), ± 4.3 ($n = 25$) and ± 36% ($n = 20$), respectively. Recovery correction factors have been incorporated into the method. The method was successfully applied to the assay of actual equipment cleaning validation swab samples. Stability studies demonstrate that methenamine hippurate is not very stable on the equipment surfaces or in the swabs. It is recommended that the surfaces be swabbed immediately after cleaning and the swabs analyzed within 24 h after sample collection. The results demonstrate that in order to fully validate the cleaning procedures, it is not only necessary to investigate the recovery of the drug from equipment surfaces and swabs but also that the stability of the drug on the surfaces and swabs be determined.

Keywords: Methenamine hippurate; Cleaning validation; Swab analysis; GC/FID

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1. Introduction

Methenamine hippurate is commercially available as a 1 g tablet dosage form marketed under the brand name of Hiprex®. It is indicated for use

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in cases where long-term therapy is required in the treatment of frequently recurring urinary tract infections [1]. The chemical name of methenamine hippurate is hexamethylenetetramine hippurate. It is prepared by refluxing one mole of hexamethylenetetramine with one mole of hippuric acid in methanol [2]. Methenamine and hippuric acid have a 1:1 stoichiometric relationship in methenaminamine hippurate salt. However, methenamine hippurate exhibits only one peak in the chromatogram when injected into the GC using the proposed chromatographic conditions and hence the peak is identified as methenamine hippurate. The chemical structure of methenamine hippurate is presented in Fig. 1. The important feature of this chemical structure relevant to this work is that methenamine does not have a strong chromophore and hence cannot be readily detected by UV/Visible detection techniques without prior derivatization. A variety of analytical methods including spectrophotometry using UV/Visible detection following derivatization of methenamine [3–5], capillary gas chromatography using a nitrogen-selective detector [6], gas chromatography using an open tubular packed steel column [7], HPLC [8] and proton nuclear magnetic resonance [9] have been cited in the literature. The tablet assay method included in the methenamine hippurate monograph in the current USP [10] is based on the titration of methenamine hippurate with 0.1 N sodium hydroxide. The titration and the spectrophotometric methods lack the sensitivity and/or specificity needed for the determination of low levels of methenamine hippurate on tablet manufacturing equipment cleaning validation swab samples. The main objective of this project was to develop a simpler, sensitive, repeatable, accurate, linear, and rugged cleaning validation method for the determination of residual methenamine hippurate in swabs collected by swabbing equipment surfaces.

Cleaning validation is a very critical issue faced by the pharmaceutical industry today. It is not a new issue, but in the recent years, it has become the subject of greater scrutiny on the part of the FDA. Cleaning validation is a requirement mandated by 1963 GMP regulations (Part 133.4) and by 1978 cGMP regulations (Section 211.6). The main objective of a thorough cleaning validation program is to prevent contamination or adulteration of drug products [11]. Visual inspection alone to ensure cleanliness and/or invalidated chemical residue analysis leading to the conclusion 'no residue detected' are things of the past and are no longer acceptable to the regulatory agencies [12]. Visual inspection of the equipment supported by chemical residue data obtained by using a validated analytical technique is required in order to ensure lack of cross-contamination between products [13,14].

The FDA has not published specific guidelines on the issue of setting acceptance specifications because of the wide variety of the products manufactured by the pharmaceutical industry using different kinds of equipment. One set of acceptance criteria is unlikely to apply to all products and types of equipment. Therefore, companies are expected to establish acceptance criteria based on logical and scientific rationale. Several acceptance criteria have been proposed in the literature [15–17]. One such criteria has been modified in order to develop a cleaning validation protocol using methenamine hippurate as the model compound. It is emphasized that this protocol is not a standard practice at Hoechst Marion Roussel. The residual acceptance criteria for methenamine hippurate was based on the rationale that not more than 1/10 000th of the labeled amount of active present in the dosage form should be available for transfer to a dose of the next product. The amount of the residue allowed on the total equipment train is termed a residual acceptance level (RAL). The RAL for a product's active(s) is based on the smallest batch size of any product which is produced on the equipment and the dose strength of the product for which the RAL is being established. The formula used for the calculation of the RAL value is shown in Eq. (1).

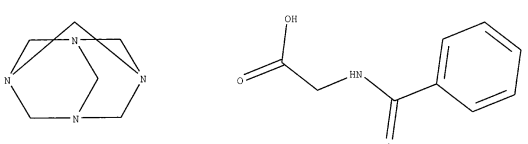

Fig. 1. Structure of methenamine hippurate.

Table 1
Swabbing pattern used for collecting methenamine hippurate residue from the actual equipment surfaces and from the spiked plates in the cleaning validation studies

1.	Use 4″ × 4″ Absorbond (Polyester) swabs. (*Note:* Vinyl, powder free, gloves must be worn to avoid interferences.)
2.	Place the swabs in water (swabbing solvent) contained in a suitable container ensuring that the swabs are completely immersed in the liquid.
3.	Fold the swab diagonally in half. Fold the swab again diagonally, splitting the 'right triangle'. The resulting swab is also a right triangle.
4.	Squeeze the excess swabbing solvent removing as much excess as possible. (Excess solvent dilutes the collected drug residue and could render artificially low results.)
5.	Hold the folded swab between the thumb and second finger using the first finger to apply pressure on the surface to be cleaned.
6.	Swab the surface in a horizontal manner ensuring that the total surface is wiped, starting from the outside towards the center. Overlapping the same surface is acceptable. Fold the exposed surface of the swab internally, resulting in a triangle one-half its original size. Expose a fresh swab surface and swab vertically, or 90° from the original direction.
7.	Repeat steps 3, 5 and 6 using a dry Absorbond swab, placing the resulting swab in the same container.
8.	Cap the sample container securely and label properly indicating drug substance, swab type, swab solvent, operator's name, the date and detailed swabbed location.

$$\mathrm{RAL} = \frac{D}{\mathrm{SF}} \times \text{Smallest batch size} \quad (1)$$

where D is the smallest dosage strength and SF is the safety factor (10 000). If the RAL is divided by the surface area of the entire equipment train used in the manufacture of the drug product, a concentration value in mass per unit surface area is obtained and is termed as specific residual cleaning level (SRCL). The formula used for the calculation of the SRCL is shown in Eq. (2).

$$\mathrm{SRCL} = \frac{\mathrm{RAL}}{\mathrm{SA}} \quad (2)$$

where SA is the surface area of the entire equipment train used in the manufacturing of the product.

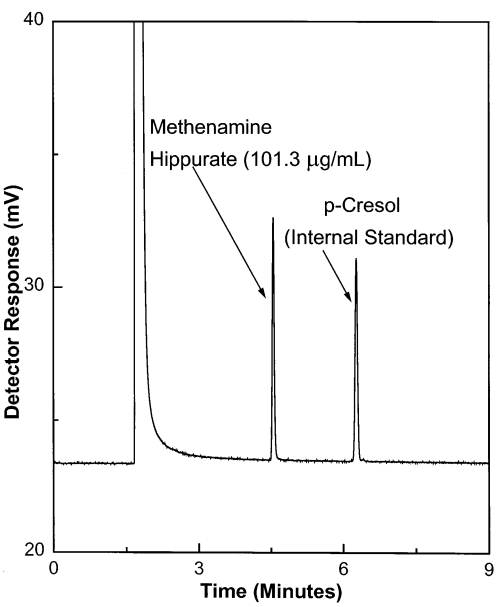

Fig. 2. Chromatogram obtained for a methenamine hippurate standard solution.

On the basis of the calculated SRCL value and the determined visual residual detection limit, the operating range required of the analytical technique is determined. In this article, a sensitive capillary gas chromatographic method using a flame-ionization detector is described for the determination of trace levels of methenamine hippurate in cleaning validation swab samples obtained from testing the equipment train. The results from recovery studies of methenamine hippurate from polyester swabs (Absorbond), stainless steel, Teflon and WARCO White (neoprene and PVC) gasket materials are presented. Also, results from the investigation of the stability of methenamine hippurate on stainless steel, undiluted swabs and swabs stored in the extraction solvent are discussed.

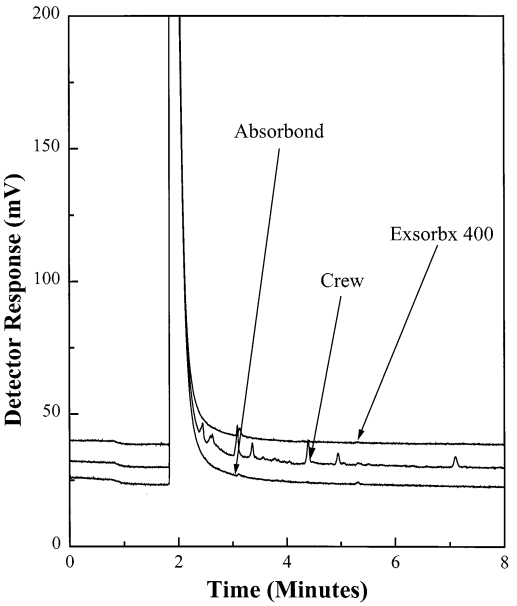

Fig. 3. Representative chromatograms for the blank extracts of Absorbond, Crew and Exsorbx 400 swabs.

2. Experimental

2.1. Apparatus

The gas chromatographs used in this study were Hewlett-Packard Model Numbers HP 5890 Series II and HP 6890, each equipped with an autoinjector and flame ionization detector (FID). The injector was capable of making split-mode injections.

2.2. Chemicals and materials

Methanol (Fischer Scientific, Pittsburgh, PA), methenamine and *p*-Cresol (Aldrich Chemical Company, Milwaukee, WI) were used as supplied. Methenamine Hippurate Analytical Reference Standard was obtained from United States Pharmacopeial Convention, Rockville, MD. All other chemicals used were of reagent grade. De-ionized water was used as the swabbing solvent. Absorbond (Texwipe 404), Exsorbx 400 (Berkshire) and Crew (Kimberly-Clark) swabs were all obtained from Baxter Products Division, McGaw Park, IL.

2.3. GC conditions

Suplecowax-10 (Supelco, Bellefonte, PA) capillary gas chromatography columns with dimensions of 30 × 0.32 mm (i.d.) and coated with 0.25 μm thick film were used. Helium was used as the carrier gas with nitrogen as the auxiliary gas. The flow rate of helium was 1.0 cm^3 min^{-1}. Temperature programming was used for the successful elution of all the peaks of interest. The temperature programming comprised of holding the oven temperature at 170°C for 8 min, ramping to 230°C at 30°C min^{-1} and finally holding at 230°C for 5 min. The injector and detector were set at a temperature of 250°C. The injector volume was 1 μl and the injector split ratio was 1:25.

2.4. Preparation of calibration standards

A methenamine hippurate stock solution was prepared by accurately weighing Methenamine Hippurate Reference Standard (100 mg) and transferring into a 100 ml volumetric flask. It was dissolved, made up to volume with methanol and mixed. Another stock solution was prepared by making a 10-fold dilution of this solution. An internal standard stock solution was prepared by pipetting 1.0 ml of *p*-cresol into a 100 ml volumetric flask. It was also dissolved, made up to volume

Table 2
Linearity of methenamine hippurate by regression analysis—[methenamine hippurate concentration vs. peak area ratio (methenamine hippurate peak area/*p*-cresol peak area)]

Methenamine hippurate concentration (μg ml^{-1})	Peak area ratio[a]
5.148	0.04965
10.29	0.10044
25.74	0.26054
51.48	0.52721
103.0	1.06446
205.9	2.15167
308.9	3.22743
Correlation coefficient:	1.00000
Slope [peak area ratio/(μg ml^{-1})]:	0.01048
y-Intercept (peak area ratio):	−0.008760

[a]Average of two replicates.

Table 3
Results obtained for the recovery of methenamine hippurate from spiked Absorbond swab samples

Micrograms methenamine hippurate recovered	Micrograms methenamine hippurate spiked	%Recovery
103	102	98.8
	101	97.8
	103	100.2
	102	98.5
	104	100.5
250	286	114.3
	281	112.3
	295	117.9
	215	86.0
	306	122.3
1000	1054	105.4
	1071	107.1
	1014	101.4
	1144	114.5
	980	98.0
2500	2648	105.9
	2722	108.9
	2627	105.1
	2626	105.0
	2647	105.9
4999	5168	103.4
	5371	107.4
	5428	108.6
	5184	103.7
	5064	101.3
	Mean:	105.2%
	RSD:	±7.1%
	n:	25

with methanol and mixed. A series of seven calibration standards were prepared by transferring appropriate aliquots of the methenamine hippurate and the internal standard stock solutions into separate 100 ml volumetric flasks. The flasks were diluted to volume with methanol. The flasks were shaken thoroughly. The concentrations of methenamine hippurate in these calibration standards were 5.000, 10.00, 25.00, 50.00, 100.0, 200.0 and 300.0 μg ml $^{-1}$, respectively. The concentration of the internal standard in all the standard solutions was 20.00 μg ml^{-1}.

2.5. Sample preparation

2.5.1. For recovery studies of methenamine hippurate from absorbond swabs, '180 grit' stainless steel, teflon and WARCO (neoprene and PVC) plates

The surfaces tested included '180 Grit' stainless steel, Teflon, and WARCO white (neoprene and PVC) gasket material. All surfaces tested were 10×10 cm plates prepared in-house. A spiking solution was prepared by dissolving ca. 1000 mg of methenamine hippurate into 100 ml of methanol. Using appropriate glass micro-syringes, aliquots ranging from 10 to 300 μl of the spiking solution were transferred onto four sets of five plates each. The spiked plates thus contained either ca. 100, 250, 1000, 2000 or 3000 μg of methenamine hippurate. The solutions on the test surfaces were allowed to air dry. The plates were then swabbed with an Absorbond swab which was previously moistened with water, wringing out the excess. This was followed by swabbing with a dry swab of the same kind. All the swabbings followed a prescribed wiping pattern (Table 1). The swabs for each sample were placed into high density polyethylene (HDPE) bottles. A 10.0 ml aliquot of the Internal Standard Solution was added to each bottle. Each bottle was capped and shaken vigorously for approximately one minute. The extract was collected using a 10 cc disposable syringe and transferred into an autosampler vial. In the study for the recovery of methenamine hippurate from Absorbond swabs, aliquots ranging from 10 μl to 500 μl of the spiking solution were deposited directly into separate HDPE bottles (five bottles per level) containing one Absorbond cleaning swab pre-moistened in water and one dry swab of the same kind. The same extraction procedure as described earlier was used.

2.5.2. For actual samples collected from the equipment train

A 10.0 ml aliquot of the Internal Standard Solution was pipetted directly on top of the sample swabs contained in HDPE bottles. Each bottle was capped and shaken vigorously for approximately one minute. The extract was collected us-

ing a 10 cc disposable syringe and transferred into an autosampler vial.

2.5.3. For stability studies

A series of '180 Grit' stainless steel plates were spiked with 2500 μg of methenamine hippurate, sets of which were allowed to sit undisturbed for up to seven days. Other sets of spiked plates were swabbed immediately, the swabs then placed in HDPE bottles, capped securely, without adding extraction solvent (Internal Standard Solution), and allowed to sit undisturbed. These were labeled as 'dry' swabs. The remaining sets were swabbed immediately, placed in HDPE bottles, the extraction solvent added and then, the bottles were capped securely. These were labeled as 'wet' swabs.

Table 4
Results obtained for the recovery of methenamine hippurate from spiked '180 Grit' stainless steel plates

Micrograms methenamine hippurate spiked	Micrograms methenamine hippurate recovered	%Recovery[a]
103	79	76.5
	79	76.5
	63	61.6
	74	71.7
	67	65.4
250	182	72.7
	184	73.7
	206	82.5
	190	76.1
	170	68.1
999	763	76.4
	732	73.2
	752	75.3
	786	78.7
	662	66.3
1998	1503	75.2
	1696	84.9
	1798	90.0
	1628	81.5
	1711	85.6
2997	2382	79.5
	2594	86.5
	2597	86.7
	2522	84.1
	2468	82.4
	Mean:	77.2%
	RSD:	±9.4%
	n:	25
	Recovery factor:	1.3

[a]Uncorrected for recovery factor used in the method.

2.6. Chromatographic procedure

A 1 μl aliquot of the samples and the calibration standard solutions was injected separately into the gas chromatograph with the aid of the autoinjector using the operating conditions described in Section 2.3. The amount of residual methenamine hippurate was determined by comparing the ratio of methenamine hippurate peak area response to that of *p*-cresol (internal standard) obtained for the sample to a linear calibration curve obtained from the calibration standard solutions.

3. Results and discussion

3.1. Development of the analytical method

The main objective of this project was to develop a sensitive, repeatable, accurate, linear, rugged and specific cleaning validation method for the assay of trace levels of residual methenamine hippurate collected by swabbing various manufacturing equipment surfaces. An initial attempt using gas chromatography was made using a DB-1, poly(dimethyl siloxane) film-coated capillary column. The methenamine hippurate peak exhibited some asymmetry most likely due to the polarity mismatch of the stationary phase. The peak symmetry was improved by using a more polar Supelcowax-10, poly(ethylene glycol) film-coated capillary column.

Throughout the development of the method, the detector and injector temperatures were set at 250°C. The effect of column temperature on the retention of methenamine hippurate was studied by varying the column temperature from 120 to 220°C in increments of 10°C. At the lower temperatures, the retention time of methenamine hippurate was relatively high and the peak was broad. Whereas, at the higher temperatures

methenamine hippurate eluted very close to the solvent front ($k' < 2$). The optimum temperature at which $k' > 2$ but < 10 was 170°C. Therefore, 170°C was chosen as the column temperature. In later studies, it was observed that the swab samples exhibited late-eluting peaks that would potentially interfere with the peaks of interest in the next chromatogram. It was therefore necessary, to ramp the column temperature up to 230°C after the elution of the peaks of interest. Further investigations demonstrated that two late-eluting peaks were due to the presence of residual detergent in the sample.

3.2. Choice of internal standard

A commonly used practice in improving the precision of an analytical methodology is to incorporate an internal standard into the standard and sample. Several compounds including 1-propanol, *o*-cresol, *p*-cresol, phenol, tetradecanol, xylene and 2,6-di-tert-butyl-4-methyl phenol were injected separately into the GC using the proposed chromatographic conditions. The best choice as an internal standard (IS) was *p*-cresol. A chromatogram obtained by analyzing a methenamine hippurate standard solution is presented in Fig. 2.

3.3. Determination of proper swabbing material

Two swabs of Absorbond, Exsorbx 400 and Crew types were placed in separate beakers containing water. The excess water was eliminated from the swabs by wringing and then the swabs were transferred into separate HDPE bottles, each containing a dry swab of the same kind. 10 ml of methanol was pipetted into each bottle. The bottles were manually shaken for ≈ 1 min. The extracts were transferred with the aid of disposable plastic syringes into GC vials. The swab extracts were injected into the GC using the chromatographic conditions from the proposed method. The chromatogram obtained from the Crew swabs exhibited several extraneous peaks, while the chromatograms obtained from Absorbond and Exsorbx 400 swabs were free of any extraneous peaks. The chromatograms obtained from the extracts of the three swab materials are presented in Fig. 3. Absorbond swabs were chosen for the intended purpose as they had the cleanest chromatogram.

Table 5
Results obtained for the recovery of methenamine hippurate from spiked Teflon plates

Micrograms methenamine hippurate spiked	Micrograms methenamine hippurate recovered	%Recovery[a]
103	105	102.1
	97	93.7
	100	97.4
	96	93.0
	100	97.4
250	227	91.0
	226	90.4
	223	89.3
	247	99.0
	261	104.4
999	966	96.7
	903	90.4
	992	99.3
	915	91.6
	961	96.2
1998	2013	100.8
	1922	96.2
	1803	90.2
	1979	99.0
	2027	101.4
2997	2818	94.0
	2928	97.7
	2929	97.7
	2821	94.1
	2964	98.9
	Mean:	96.1%
	RSD:	±4.3%
	n:	25
	Recovery factor:	1.1

[a]Uncorrected for recovery factor used in the method.

3.4. Assay validation

The validation of the method was carried out by determining the linearity, accuracy, repeatability, intermediate-precision and limit of quantitation of the method.

3.4.1. Linearity

A linearity study was performed in order to determine the linearity of the detector response for methenamine hippurate over a concentration range of 5.148 to 308.8 μg ml^{-1}. The resulting solutions were chromatographed using the GC conditions described in the method. A correlation coefficient (r) of 1.0000 was obtained from the linear regression analysis of the data (peak area ratio vs. concentration of methenamine hippurate). This demonstrates that the methenamine hippurate peak area response is linear over the concentration range examined. The results of the linearity study are tabulated in Table 2.

Table 6
Results obtained for the recovery of methenamine hippurate from spiked WARCO (neoprene and PVC) plates

Micrograms methenamine hippurate spiked	Micrograms methenamine hippurate recovered	%Recovery[a]
250	109	43.8
	187	74.9
	199	79.5
	173	69.4
	88	35.2
999	346	34.6
	573	57.3
	188	18.8
	624	62.4
	655	65.6
1998	1243	62.2
	873	43.7
	466	23.3
	560	28.0
	1312	65.7
2997	1085	36.2
	1285	42.9
	1124	37.5
	1862	62.1
	2036	67.9
	Mean:	50.6%
	RSD:	±36%
	n:	20
	Recovery factor:	2.0

[a]Uncorrected for recovery factor used in the method.

3.4.2. Accuracy

The accuracy of the method was determined by spiking the swabs, '180 Grit' stainless steel, Teflon and WARCO with known amounts of methenamine hippurate and analyzing the spiked samples by using the developed method. The accuracy was then calculated as the percent amount of methenamine hippurate recovered from the spiked samples. The range for the recovery studies was chosen by taking into consideration the visual limit of detection and SRCL for methenamine hippurate. The visual limit of detection for methenamine hippurate was determined to be approximately 100 μg 100 cm^{-2}. The SRCL was calculated based on the formula described in the introduction and was considerably higher than the visual limit of detection. This is understandable because methenamine hippurate is a high dose drug and is commercially available in a 1 g tablet dosage form. Recovery studies were carried out in the range of 100 to 5000 μg 100 cm^{-2}, thereby bracketing the visual limit of detection as well as the calculated SRCL level.

A mean recovery of 105.2%, with a relative standard deviation (RSD) of ±7.1% ($n = 25$) was obtained for the recovery of methenamine hippurate from the Absorbond swab material. The data are contained in Table 3. The data demonstrate that the method is sufficiently accurate and precise for the recovery of methenamine hippurate from Absorbond cleaning swabs. An overall mean recovery of 77.2% with an RSD of ±9.4% ($n = 25$) was obtained for the recovery of methenamine hippurate from '180 Grit' stainless steel plates. The data are contained in Table 4. In order to account for the low recovery of methenamine hippurate from the stainless steel plates, a recovery correction factor of 1.3 has been included in the method.

The recoveries of methenamine hippurate from Teflon and WARCO plates were 96.1 and 50.6%, with RSDs of 4.3% ($n = 25$) and 36% ($n = 20$), respectively. The recovery factors for Teflon and WARCO were calculated to be 1.1 and 2.0, respectively. The data are presented in Tables 5 and 6. The high RSD in the case of WARCO may be explained due to the fact that it is a highly porous material and also due to the variability in the

Table 7
Injection repeatability results obtained from five replicate injections of a methenamine hippurate calibration standard by using two different chromatographic systems (intermediate precision)

Replicate	GC Model: HP 5890 Series II		GC Model: HP 6890 Series	
	Methenamine hippurate concentration ($\mu g\ m^{-1}$)	Peak area ratio	Methenamine hippurate concentration ($\mu g\ m^{-1}$)	Peak area ratio
1	101.3	1.0091	99.20	1.0559
2		0.9790		1.0374
3		0.9935		1.0412
4		0.9738		1.0842
5		1.0054		1.0649
	Mean:	0.9922		1.0567
	RSD:	±1.6%		±1.8%
	n:	5		5

surface characteristics of the different WARCO plates. Two populations of WARCO test plates were identified within this study. Both sets of plates were obtained supposedly from the same stock of material and represented the same material as would be provided when a gasket is to be replaced. One set of plates exhibited high surface tension resulting in the beading of the spiking solution on the surface. The second set exhibited leveling of the spiking solution, showing no beading and resulting in much lower recoveries. After these two populations were identified and since both populations were to be expected within the manufacturing train, both types of randomly chosen plates were spiked. Replacing methanol as the swabbing solvent or Exsorbx 400 and Crew as the swabbing materials did not improve the recovery of methenamine hippurate from WARCO surfaces. This again indicates that the drug substance perhaps migrates into the porous material and can not be recovered by just swabbing the surface.

3.4.3. *Repeatability, intermediate precision and limit of quantitation*

The repeatability and intermediate precision of the method were investigated by making five consecutive injections of a standard solution using two different gas chromatographic systems. The

Table 8
Stability results obtained for methenamine hippurate spiked onto '180 Grit' stainless steel plates (stability of methenamine hippurate on dry plates)

	%Recovery of methenamine hippurate[a,b,c]			
Sample	Initial day	Day two	Day five	Day seven
1	91.9	34.1	14.7	11.6
2	86.4	34.5	15.3	11.8
3	83.3	32.2	11.8	4.8
Mean:	87.2%	33.6%	13.9%	9.4%
RSD:	±4.3%	±1.2%	±1.9%	±4.0%
n:	3	3	3	3

[a]Uncorrected for recovery factor used in the method.
[b]Sample stored dry on plates at ambient room temperature.
[c]2509 μg methenamine hippurate spiked per 100 cm^2.

Table 9
Stability results for methenamine hippurate in 'dry' swabs obtained by swabbing spiked '180 Grit' stainless steel plates

	%Recovery of methenamine hippurate[a,b,c]			
Sample	Initial day	Day two	Day five	Day seven
1	91.9	78.5	69.4	63.0
2	86.4	74.7	69.8	67.7
3	83.3	80.1	72.7	56.7
Mean:	87.2%	77.8%	70.6%	62.5%
RSD:	±4.3%	±3.6%	±2.5%	±8.8%
n:	3	3	3	3

[a]Uncorrected for recovery factor used in the method.
[b]Undiluted swab samples, after swabbing stored in HDPE bottles at ambient room temperature.
[c]2509 μg methenamine hippurate spiked per 100 cm^2.

Table 10
Stability results for methenamine hippurate in 'wet' swabs obtained by swabbing spiked '180 Grit' stainless steel plates

%Recovery of methenamine hippurate[a,b,c]				
Sample	Initial day	Day two	Day five	Day seven
1	91.9	71.9	70.4	69.8
2	86.4	74.7	66.1	72.3
3	83.3	70.5	68.7	66.0
Mean:	87.2%	72.4%	68.4%	69.4%
RSD:	±4.3%	±3.0%	±3.2%	±4.6%
n:	3	3	3	3

[a]Uncorrected for recovery factor used in the method.
[b]Swab samples, after swabbing, diluted with 10 ml of the internal standard solution in HDPE bottles and stored at ambient room temperature.
[c]2509 μg methenamine hippurate spiked per 100 cm^2.

mean and RSD were calculated for the peak area response ratios obtained from consecutive injections of the standard solution. The data from this study are tabulated in Table 7. The data suggest that the method is precise when methenamine hippurate standard solutions are analyzed using two different gas chromatographic systems.

The smallest level at which the recovery of methenamine hippurate was determined, 103 μg 100 cm^{-2} was defined as the limit of quantitation (LOQ). All the swab results lower than 103 μg 100 cm^{-2} were reported as less than 103 μg 100 cm^{-2} except in case of swabs taken from WARCO or similar gasket material, in which case the results were reported as less than 250 μg 100 cm^{-2}. The recovery at lower spiking levels for WARCO surfaces exhibited very high variability. Therefore, it was decided not to include a recovery at the ca. 103 μg level unlike the other surfaces. Both the LOQ levels were significantly lower than the calculated SRCL value of methenamine hippurate.

3.4.4. Stability of methenamine hippurate on stainless steel surface and in the swabs

In order to study the stability of methenamine hippurate on surfaces and in the swabs prior to analysis, at the initial day, Day-2, Day-5 and Day-7 time intervals, a set of three spiked stainless steel plates were swabbed and the extracted residue analyzed for methenamine hippurate using the proposed method. At the same time intervals, a set of three bottles containing 'dry' swabs which had been used to swab test plates, were extracted, the solutions then analyzed for methenamine hippurate. Similarly, set of three bottles containing 'wet' swabs, previously extracted, were also analyzed for methenamine hippurate. It was decided not to study the stability of methenamine hippurate on Teflon and WARCO surfaces because these surfaces represent less than 1% area of the entire equipment train and hence would not have a significant effect on the overall stability of the drug substance.

The spiked dry plate stability information is included in Table 8. The spiked plate stability study indicated the length of time the active, methenamine hippurate, can be left on the manufacturing equipment prior to initiation of the swabbing process. The results from the 'dry' swab stability study presented in Table 9, indicate the length of time the submitted swabs can set prior to analysis. The results from the 'wet' swab stability study are included in Table 10, indicate the length of time the swabs can be wetted and allowed to set during the analysis process. The results indicate that methenamine hippurate is not stable over long periods of time in the swabs in diluted or in undiluted form. The results also indicate that methenamine hippurate is not stable on the equipment surfaces as well. Therefore, it is recommended that the equipment be swabbed immediately after the completion of the cleaning process and the swabs be analyzed within 24 h thereafter.

3.4.5. Stability of methenamine hippurate standard solutions

A set of standard solutions were prepared and analyzed by following the procedure in the proposed method. These standard solutions were placed on a bench-top at ambient conditions away from direct sunlight. After 2, 5 and 7 days, these aged solutions were re-analyzed against freshly prepared standard solutions. The initial concentrations of methenamine hippurate in the standard solutions and the percent remaining in these solutions at Day-2, Day-5 and Day-7 time

Table 11
Stability results for a set of methenamine hippurate calibration standards

Standard	Methenamine hippurate concentration (μg ml^{-1})	%Remaining[a]		
	Initial day	Day two	Day five	Day seven
1	25.30	96.6	90.9	92.9
2	50.50	94.3	92.1	92.6
3	101.0	100.2	96.4	88.9
4	202.0	96.2	93.4	91.0
5	303.0	98.0	92.4	91.1
	Mean:	97.1%	93.0%	91.3%
	RSD:	±2.3%	±2.2%	±1.7%
	n:	5	5	5

[a]Standard flasks left on a desk-top away from sunlight at ambient conditions.

intervals are presented in Table 11. The data suggest that the standard solutions are not stable over long periods of time. After two days the average concentration of methenamine hippurate in these standard solutions reduced to 97.1% of its initial concentration. It is recommended that the standard solutions should be used within 24 h after preparation.

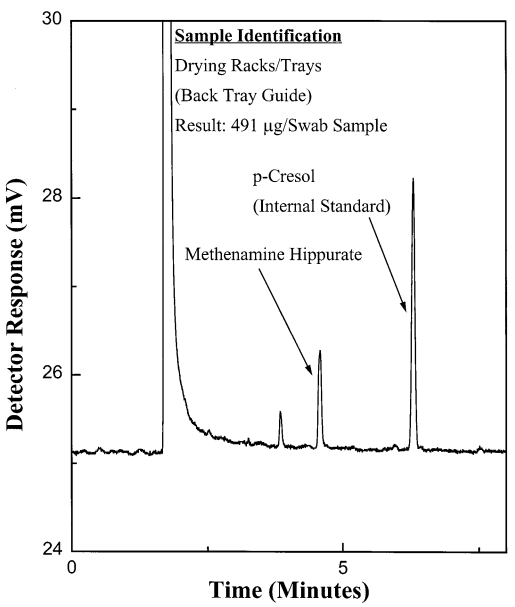

Fig. 4. Chromatogram obtained for a representative swab sample from the equipment train.

3.5. Assay of swab samples collected from different locations within the equipment train

Swab samples from different locations within the manufacturing equipment train were submitted to the laboratory for the analysis of residual methenamine hippurate. These samples were prepared and analyzed by the proposed method. A typical chromatogram obtained for a methenamine hippurate cleaning validation swab sample obtained from a location within the equipment train is presented in Fig. 4. The results obtained for these samples are presented in Table 12.

4. Conclusion

A sensitive capillary gas chromatographic cleaning validation method for the determination of trace levels of methenamine hippurate in swab samples collected by swabbing different equipment surfaces within the equipment train has been developed and found to be accurate and precise. A systematic protocol for setting the residual limit and validating the analytical method was utilized. This protocol can be easily adopted for ensuring the cleanliness of manufacturing equipment used in the manufacturing of a majority of the pharmaceutical dry products. This protocol emphasizes the need to demonstrate the stability of the drug substance on the equipment surfaces as well as on

Table 12
Results obtained for the determination of methenamine hippurate in actual swab samples collected from different locations within the equipment train

Equipment swabbed	Location swabbed	Area swabbed (cm^2)	Micrograms[a] methenamine hippurate detected
Drying racks	Left tray guide	269	408
Drying racks	Right tray guide	269	149
Blender	Inside wall	100	<103
Blender	Loading port gasket	586	248
Blender	Nitrogen port gasket	586	111
Blender	Discharge valve gasket	260	<103
Fitzmill	Grinding chamber	100	<103
Fitzmill	Hub of blades	305	<103
Mixer	Mixer bowl	100	<103
Mixer	Trunion	44	<103
Mixer	Shaft seal	102	112

[a]Results not normalized to 100 cm^2.

the swabs. In the absence of such stability data, it is possible to grossly under-estimate the amount of drug residue remaining on the equipment surfaces or on the swabs prior to analysis.

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