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Short communication

Determination of methenamine, methenamine mandelate and methenamine hippurate in pharmaceutical preparations using ion-exchange HPLC

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Abstract

An ion-exchange column high-performance liquid chromatography (HPLC) method has been developed for the determination of methenamine in methenamine and methenamine hippurate pharmaceutical preparations. The HPLC method uses a Zorbax SCX-300 column with acetonitrile–0.1 M sodium perchlorate monohydrate (pH 5.8) (70:30, v/v) as the mobile phase at the flow rate of 1 mL/min. UV-detection was at 212 nm. The linear concentration plots for methenamine were linear over the concentration range of 0.25–50 mM for methenamine and methenamine mandelate standards. The intra-day RSD precision was <1.25%, and for inter-day, <1.85%. The peaks for mandelic acid, hippuric acid and the other ingredients from placebo tablets do not interfere with the analysis for methenamine. The accuracy of this method was shown to be 99–101% by measuring the recovery of methenamine from spiked placebo tablets. The assay of methenamine from methenamine hippurate tablets and from a urinary antiseptic tablet containing methenamine were in the range of 98–102%. This HPLC method is a fast, simple and straightforward method for the analysis of methenamine in pharmaceutical preparations.

Keywords: Methenamine; Methenamine mandelate; Methenamine hippurate; High-performance liquid chromatography; UV-detection; Ion-exchange; Validation

1. Introduction

Methenamine (hexamethylenetetramine) and its salts (methenamine mandelate and methenamine hippurate (Fig. 1) are often used for the treatment of recurring urinary tract infections [1] and in the prophylaxis of urinary tract infections in patients with sterile urine after the eradication of urinary tract infections by other antibiotics [1]. Methenamine hippurate and methenamine mandelate both have a 1:1 stoichiometric relationship as their respective salts. The antiseptic property for methenamine and its salts is attributed to its slow hydrolysis in acidic urine to ammonia and the non-specific antibacterial, formaldehyde which most likely acts by denaturation of the antibacterial protein [2]. The few reported methods for the determination of methenamine include titration with sodium tetraphenylborate by ion-selective electrode [3], capillary gas-chromatography [4], spectrophotometry using UV–vis detection following derivatization of methenamine with 2-hydrazinobenzothiazole [5], first derivative spectrophotometry of methenamine [6], gas-chromatography using an open tubular packed steel column [7] and proton magnetic resonance [8]. The USP 28-NF23 monographs for the analysis of methenamine, methenamine mandelate and methenamine hippurate dosage forms involve three very different non-selective multi-step methods [9]. Methenamine tablets are analyzed spectrophotometrically at 570 nm following methenamine's reaction (formaldehyde) with hot sulfuric acid and chromotropic acid. Methenamine hippurate tablets are titrated with sodium hydroxide to measure the amount of hippuric acid from which the methenamine is calculated differentially and methenamine mandelate tablets involve potentiometric titration with silver nitrate standard solution and silver billet electrode.

Therefore, the main purpose of this study was to develop a simple high-performance liquid chromatography (HPLC) method for the direct analysis of methenamine and its salts in pharmaceutical preparations. To the best of our knowledge, this

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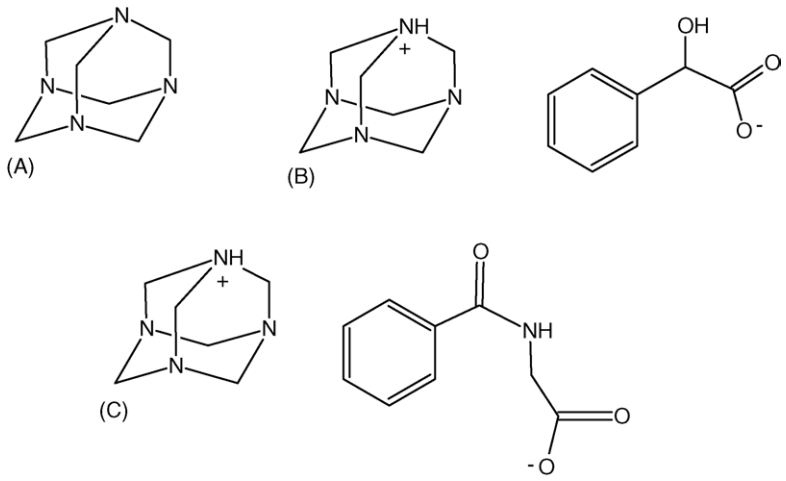

Fig. 1. Structures for methenamine (A), methenamine mandelate (B), and methenamine hippurate (C).

paper is the first ion-exchange HPLC method with UV-detection for the analysis of methenamine and its salts. This method was validated for linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ).

2. Experimental

2.1. Materials and reagents

HPLC grade acetonitrile was obtained from Burdick & Johnson (Muskegon, MI), and sodium perchlorate monohydrate from Aldrich (Milwaukee, WI). De-ionized water was polished by passing through a demineralizer cartridge (catalog #26303-234, Barnstead, Dubuque, IA) and filtered through a 0.22 μm Gelman filter (catalog #66602, PALL Gelman Sciences, Ann Arbor, MI). Methenamine reference standard (#1409003 lot G) was purchased from United States Pharmacopeia (Rockville, MD). Methenamine mandelate, mandelic acid, hippuric acid and tartrazine were obtained from Sigma Chemical (St. Louis, MO). Povidone K30 was from BASF (Mount Olive, NJ), saccharin sodium from Spectrum Chemical (Gardena, CA), and magnesium stearate from Mallinckrodt (Hazelwood, MO). Methenamine hippurate tablets (Hiprex®) labeled to contain 1 g of methenamine hippurate and urinary antiseptic tablets (Urimax®) labeled to contain 81.6 mg methenamine were obtained commercially.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a Hewlett-Packard HP 1090 complete with autosampler and diode-array detector. Peak areas were processed using HP Chemstation software Rev. Ao7.01 (Agilent, CA). pH meter (EA920) was from Orion Research (Beverly, MA)

The chromatographic system included a Zorbax SCX-300 column (150 mm × 4.6 mm i.d., 5 μm, Agilent, CA) and a mobile phase of acetonitrile–sodium perchlorate monohydrate (pH 5.8; 0.1 M) (70:30, v/v). The mobile phase was sparged for 15 min with helium and pumped at a flow rate of 1 mL/min. Void volume was 1.6 mL. The chromatography was performed at ambient temperature and the eluent was monitored at wavelength of 212 nm. The injection volume was 10 μL.

2.3. Standard solution preparation

A standard solution of methenamine USP was prepared by dissolving methenamine USP reference standard in mobile phase to give a final concentration of 4.57 mg/mL methenamine. This solution was used to prepare a working solution for the linear concentration plots and for the analysis of methenamine mandelate and methenamine hippurate tablets. A solution of 0.816 mg/mL methenamine USP reference solution was used for the analysis of urinary antiseptic tablets.

Methenamine mandelate stock solution: 0.1 g of methenamine mandelate was accurately weighed and quantitatively transferred into 10 mL volumetric flask. Approximately 7 mL of mobile phase was added and the mixture was sonicated for 5 min. The flask was filled to volume with mobile phase and mixed well to give a final solution of 4.39 mg/mL methenamine.

2.4. Commercial tablet sample preparation

Methenamine hippurate tablets: Ten tablets were weighed and crushed to 'fine' uniform particle size powder. After calculating the average tablet weight, a composite of the powder equivalent to the average of one tablet (approximately 439 mg methenamine) was accurately weighed and quantitatively transferred into a 100 mL volumetric flask. Approximately 70 mL of mobile phase was added and the mixture was sonicated for 5 min. The flask was filled to volume with mobile phase and mixed well. These preparations were repeated six times. Prior to injection, all samples were filtered through a 0.2 μm nylon syringe filter (catalog #42213-NN, Chromacol).

Urinary antiseptic tablets: 10 tablets were weighed and crushed to 'fine' uniform particle size powder. After calculating the average tablet weight, a composite equivalent to the

average of one tablet (approximately 82 mg methenamine) was accurately weighed and quantitatively transferred into a 100 mL volumetric flask. Approximately 70 mL of mobile phase was added and the mixture was sonicated for 5 min. The flask was filled to volume with mobile phase and mixed well. These preparations were repeated six times. Prior to injection, all samples were filtered through a 0.2 μm nylon syringe filter (catalog #42213-NN, Chromacol).

The mg methenamine/tablet recovered and the percent recovery of methenamine was calculated as follows:

$$\text{mg methenamine/tablet recovered} = \frac{\text{peak area methenamine sample}}{\text{peak area methenamine standard}} \times \text{concentration standard solution (mg/mL)}$$

$$\%\,\text{recovery} = \frac{\text{mg methenamine recovered/tablet}}{\text{mg methenamine labeled/tablet}}$$

3. Results and discussion

3.1. Method development

The development of a HPLC method for the determination of methenamine was challenging because of its highly hydrophilic properties and weak UV absorptivity at low wavelengths. The reversed-phase HPLC methods evaluated initially included C18, C8, phenyl and cyano columns, with unsatisfactory retention times and results. An ion-pair reagent, heptanesulfonic acid, was added in an attempt to improve the retention time of methenamine, but again, no significant improvement of retention times for methenamine was observed. Thus, we turned to a silica-based cation-exchange column and found that adjusting the concentration and type of counter-ion and pH produced the desired results. Perchlorate (0.1 M sodium perchlorate) was found to be the most effective counter-ion to elute the methenamine from the ion-exchange column. The addition of acetonitrile to the mobile phase and adjusting pH of the perchloric solution to pH 5.8 optimized column performance and retention time. Thus, a mobile phase consisting of acetonitrile–perchloric solution (pH 5.8; 0.1 M) (70:30) gave the optimized results (the tailing factor for methenamine was 2.5) with a retention time of about 5 min. The resolution factor for methenamine mandelate standard was 2.7. This mobile phase was used to analyze the commercial samples.

3.2. Standard solution stability

Methenamine and methenamine mandelate standard solutions were stable in mobile phase for at least 48 h at room temperature. The methenamine percent recovery of these 48 h standards were shown to be 99.78 and 100.32% for methenamine and methenamine mandelate, respectively, when compared to freshly prepared standard solutions.

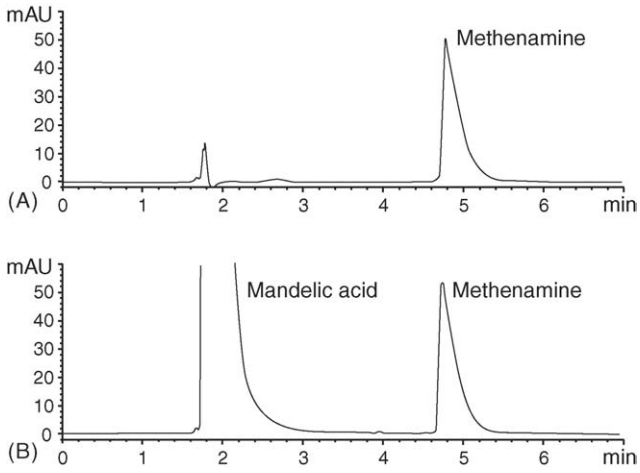

Fig. 2. Chromatograms for (A) methenamine USP reference standard and (B) methenamine mandelate using Zorbax SCX-300 column and a mobile phase of acetonitrile–sodium perchlorate monohydrate (pH 5.8; 0.1 M) (70:30, v/v) at 212 nm and flow rate of 1 mL/min.

3.3. Specificity

The specificity of the method is shown in Fig. 2 for the standard solutions of methenamine (1.68 mg/mL methenamine) (Fig. 2A) and methenamine mandelate (1.68 mg/mL methenamine) (Fig. 2B). The chromatogram of methenamine hippurate tablets (1.68 mg/mL methenamine) is shown in Fig. 3A, urinary antiseptic tablets (0.82 mg/mL) in Fig. 3B, and their respective placebo tablets in Fig. 3C and D, respectively. The Urimax® placebo tablets were available. However, the methenamine hippurate placebo tablets could not be obtained from the manufacturer, and thus they were produced in our laboratory according to the ingredients listed by manufacturer (tartrazine, magnesium stearate, povidone, saccharin sodium) [1]. Since the actual quantity of these excipients in the methenamine hippurate tablets were unknown, these placebo tablets were formulated using the maximum percent of these excipients (0.1% tartarzine, 0.8% magnesium stearate, 8% povidone and 0.8% saccharin sodium) expected to be used in the formulation to ensure the specificity of this method. Under the experimental conditions investigated (Figs. 2 and 3), the retention time for methenamine was 4.8 and 2.0 min for both mandelic acid and hippuric acid. The results with the placebos (Fig. 3C and D) exhibited no interference between hippuric acid, mandelic acid and other tablet constituents from the methenamine peak, thus confirming the specificity of this method.

Forced degradation studies for methenamine and methenamine mandelate standards (Fig. 4) were performed to provide an indication of the stability properties and specificity of the procedure. A 30 mM solution of methenamine or methenamine mandelate were prepared in 0.1 M HCl, 0.1 M NaOH, and in DI water. The solutions were heated in a temperature-controlled oven (Fisher Scientific, Pittsburgh, PA) at 60 °C for 60 min, after which 200 μL of these solutions were neutralized with 800 μL perchloric acid solution prior to HPLC analysis. Under the acidic conditions as expected, the methenamine

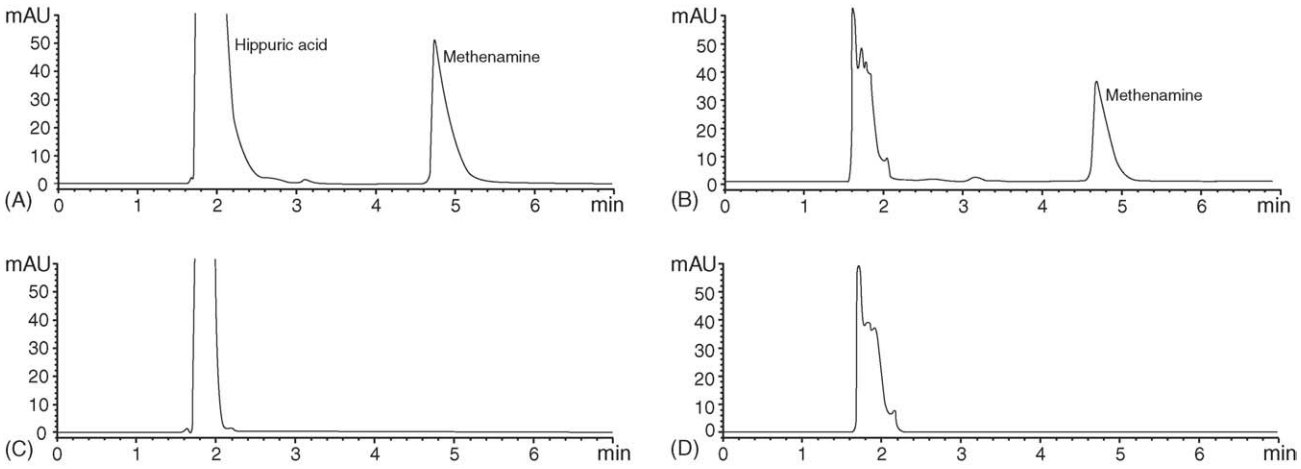

Fig. 3. Chromatograms for (A) methenamine hippurate tablets (Hiprex®), (B) urinary antiseptic tablets (Urimax®), (C) methenamine hippurate placebo tablets and (D) urinary antiseptic placebo tablets using Zorbax SCX-300 column and a mobile phase of acetonitrile–sodium perchlorate monohydrate (pH 5.8; 0.1 M) (70:30, v/v) at 212 nm and flow rate of 1 mL/min.

had decomposed to ammonia (ammonium ions) and formaldehyde (<2 min) (Fig. 4A and D), leaving no residual peaks to interfere with the methenamine peak. The alkaline solutions of methenamine and methenamine mandelate exhibited a loss of methenamine (Fig. 4B and E), whereas the water solutions of methenamine and methenamine mandelate exhibited minimal loss (not >10%) (Fig. 4C and F).

Forced degradation studies similar to the previously described conditions were also performed on methenamine hippurate (Fig. 5) and urinary antiseptic tablets (Fig. 6), and their respective placebo tablets. No peaks interfering with the retention time for methenamine were observed in any of these stress studies performed on the tablets including the placebo tablets.

3.4. Linearity

Linearity concentration curves for methenamine and methenamine mandelate were obtained by diluting the standard solutions to 0.035–7.010 mg/mL methenamine in mobile phase in order to show that the methenamine concentration was linear over the concentration range. The peak area versus concentration curve was linear over the examined concentration

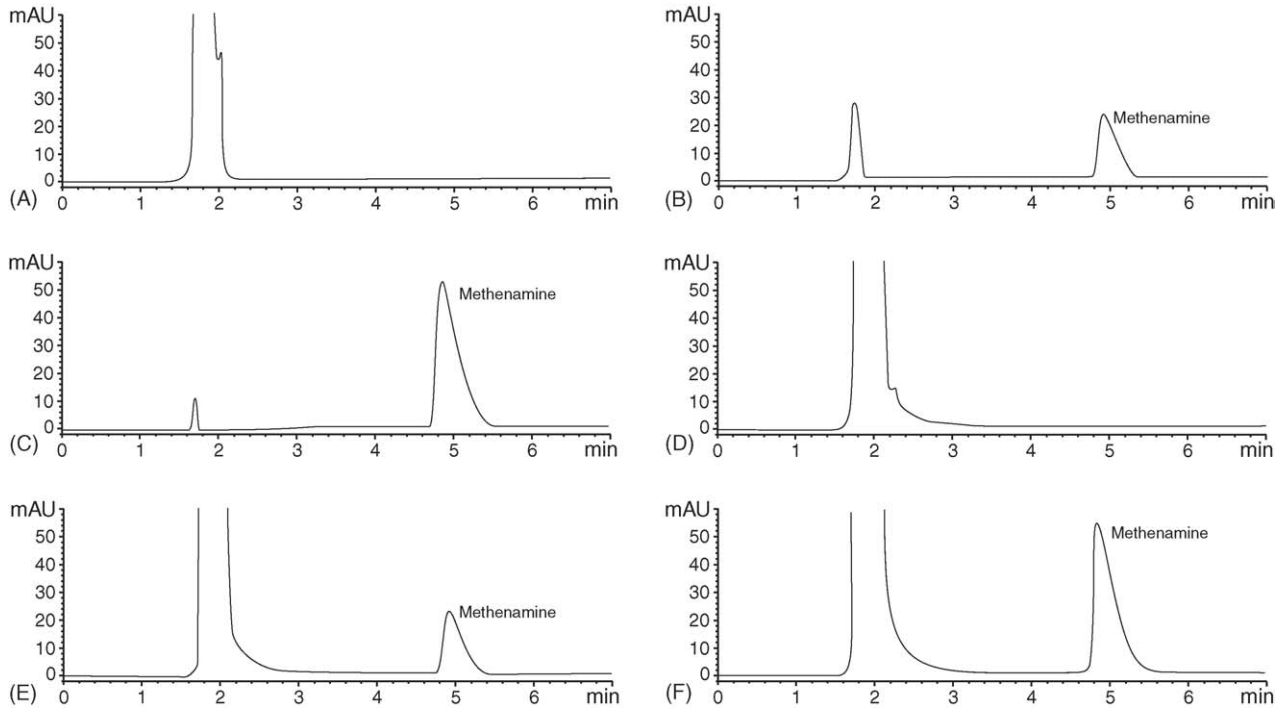

Fig. 4. Chromatograms for methenamine USP reference standard in 0.1 M HCl (A), 0.1 M NaOH (B), water at 60 °C (C), methenamine mandelate in 0.1 M HCl (D), 0.1 M NaOH (E), and water at 60 °C (F) for 60 min using Zorbax SCX-300 column and a mobile phase of acetonitrile–sodium perchlorate monohydrate (pH 5.8; 0.1 M) (70:30, v/v) at 212 nm and flow rate of 1 mL/min.

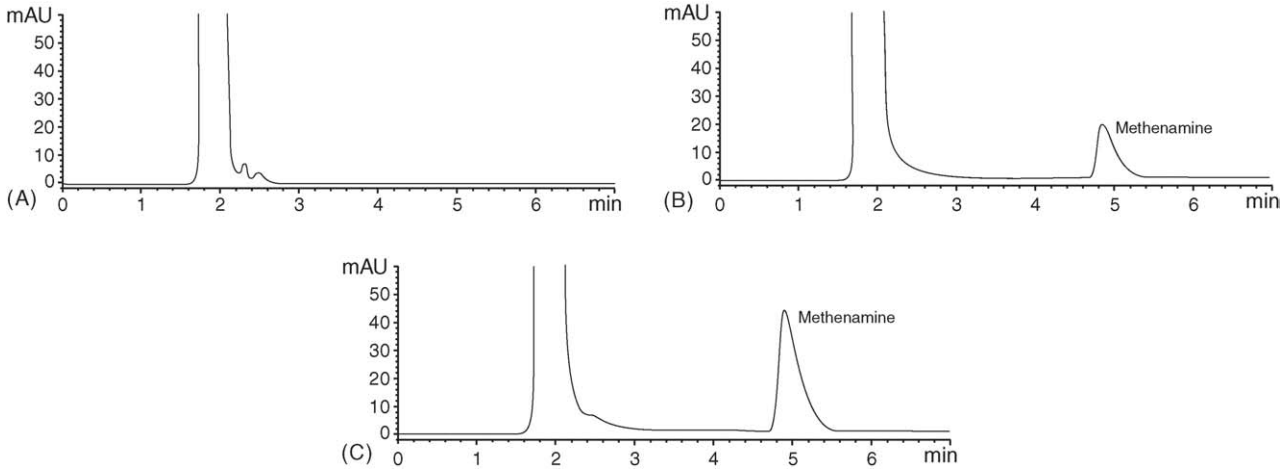

Fig. 5. Chromatograms for methenamine hippurate tablets (Hiprex®) in 0.1 M HCl (A), 0.1 M NaOH (B), and water at 60 °C (C) for 60 min using Zorbax SCX-300 column and a mobile phase of acetonitrile–sodium perchlorate monohydrate (pH 5.8; 0.1 M) (70:30, v/v) at 212 nm and flow rate of 1 mL/min.

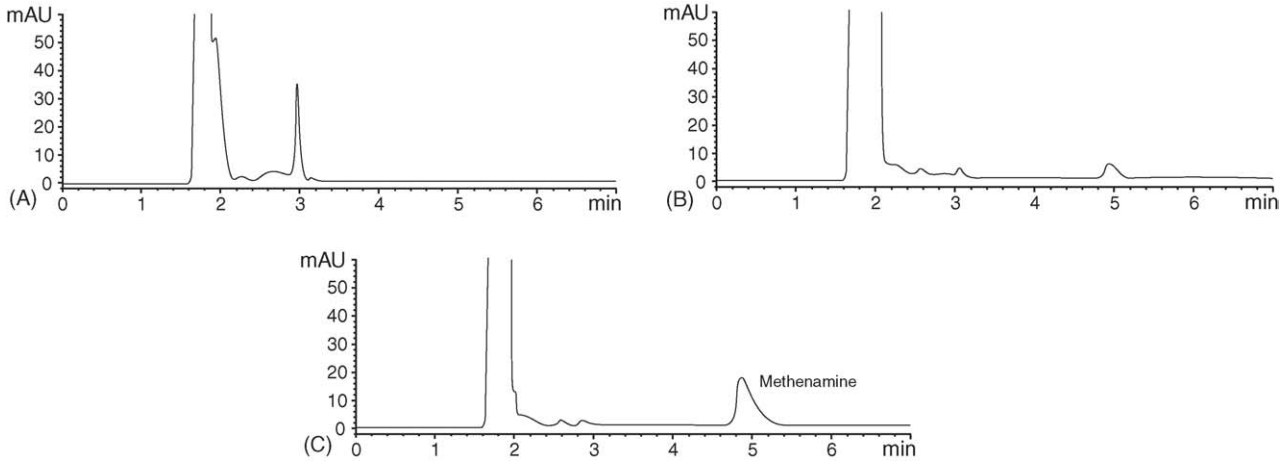

Fig. 6. Chromatograms for urinary antiseptic tablets (Urimax®) in 0.1 M HCl (A), 0.1 M NaOH (B), and water at 60 °C (C) for 60 min using Zorbax SCX-300 column and a mobile phase of acetonitrile–sodium perchlorate monohydrate (pH 5.8; 0.1 M) (70:30, v/v) at 212 nm and flow rate of 1 mL/min.

range (r^2 = 0.9999, 1.0000 for methenamine and methenamine mandelate, respectively) (Table 1). The intercept values for the two compounds were not significantly different from zero.

3.5. Precision

The overall precision of the method was evaluated by analyzing six consecutive injections of methenamine and methenamine mandelate standard solutions at 0.070 (0.5 mM), 0.701 (5 mM) and 7.010 mg/mL (50 mM) methenamine representing the low, middle and high concentration of the linearity concentration range. The intra-day precision (%RSD, n = 6), and inter-day precision (days 1 and 5, %RSD, n = 12) are shown in Table 2.

3.6. Limit of detection and quantification

The LOD was 0.02 mg/mL (0.14 mM) for methenamine at a (S/N) ratio of 3, and the LOQ was 0.03 mg/mL (0.21 mM) for methenamine at RSD of 6.54% (%RSD ≤ 10, n = 6).

3.7. Accuracy

The accuracy of the method for methenamine was determined by spiking the urinary antiseptic placebo tablet and methenamine hippurate placebo tablet with 60, 80, 100, 120 and 140% of the labeled amount of methenamine in each tablet. The result of the accuracy study is shown in Table 3 with a mean percentage recovery of 99.88% for methenamine

Table 1
Linearity curve

	Slope (mAu/(mg mL))	RSD slope	Intercept (mAu)	RSD intercept	r^2
Methenamine	100.45	0.0199	1.6979	0.0028	0.9999
Methenamine mandelate	96.232	0.0043	1.7314	0.0087	1.0000

Table 2
Intra-day and inter-day (days 1 and 5) precision for methenamine and methenamine mandelate at three concentrations (low, medium and high)

Sample solutions	Concentration[a]	Mean peak area ± S.D. (%RSD) (intra-day, $n = 6$)	Mean peak area ± S.D. (%RSD) (inter-day, $n = 12$)
Methenamine	0.070 (0.5)	49.4 ± 0.5 (1.04)	49.2 ± 0.7 (1.45)
	0.701 (5.0)	507.7 ± 1.4 (0.28)	513.2 ± 6.1 (1.17)
	7.010 (50)	5024.0 ± 9.0 (0.18)	5061.7 ± 68.3 (1.25)
Methenamine mandelate	0.070 (0.5)	49.6 ± 0.6 (1.24)	49.7 ± 0.9 (1.83)
	0.701 (5.0)	505.3 ± 3.2 (0.64)	509.1 ± 4.0 (0.79)
	7.010 (50)	5035.5 ± 12.6 (0.25)	5056.2 ± 23.6 (0.47)

[a] In mg/mL values in parenthesis are given in mM.

Table 3
Accuracy of the assay for methenamine by spiking methenamine reference standard into methenamine hippurate placebo tablets and urinary antiseptic placebo tablets

Sample	Percentage of methenamine added	Amount added (mg)	Mean amount found (mg)	Recovery (%)	%RSD
Methenamine hippurate placebo tablet					
1	60 ($n = 3$)	263.40	262.10	99.51	0.69
2	80 ($n = 6$)	351.20	351.78	100.17	0.58
3	100 ($n = 6$)	439.00	438.89	99.97	0.45
4	120 ($n = 6$)	526.80	524.89	99.64	0.47
5	140 ($n = 3$)	614.60	615.25	100.11	0.39
Urinary antiseptic placebo tablet					
1	60 ($n = 3$)	48.96	48.66	99.39	0.75
2	80 ($n = 6$)	65.28	64.89	99.40	0.68
3	100 ($n = 6$)	81.60	80.98	99.24	0.54
4	120 ($n = 6$)	97.92	98.25	100.33	0.44
5	140 ($n = 3$)	114.24	114.67	100.38	0.46

hippurate, and 99.75% for the urinary antiseptic tablets, and %RSD < 1%.

3.8. Accuracy

The accuracy of the method for methenamine was determined by spiking the urinary antiseptic placebo tablet and methenamine hippurate placebo tablet with 60, 80, 100, 120 and 140% of the labeled amount of methenamine in each tablet. The result of the accuracy study is shown in Table 3 with a mean percentage recovery of 99.88% for methenamine hippurate, and 99.75% for the urinary antiseptic tablets, and %RSD < 1%.

3.9. Assay of methenamine from commercial tablets

This HPLC method was then applied to the recovery of methenamine from several commercial products (Table 4). The percent recovery of methenamine from methenamine hippurate tablets (Hiprex®) was calculated as 98.8% (RSD = 0.41%, $n = 6$) and from the urinary antiseptic tablets (Urimax®) as 102.4% (RSD = 0.90%, $n = 6$) (Table 4).

Table 4
Recovery of methenamine from methenamine hippurate tablets (Hiprex®) and urinary antiseptic tablets (Urimax®)

Sample	Mean amount found (mg) ($n = 6$)	Mean percent recovery ($n = 6$)	%RSD
Methenamine hippurate tablets	433.78	98.83	0.41
Urinary antiseptic tablets	83.65	102.51	0.90

4. Conclusions

This paper describes an ion-exchange HPLC method for the determination of methenamine methenamine hippurate tablets (Hiprex®) and a urinary antiseptic tablet (Urimax®). The method was shown to be specific, accurate, precise, and suitable for the analysis of methenamine in these pharmaceutical formulations. The results of this study show the methenamine peak response to be precise and linear over the range of 0.035–7.01 mg/mL. The percent recovery of methenamine from methenamine hippurate tablets and urinary antiseptic tablets was shown to be in the range of 98–102% (RSD < 2%). This method appears to be reliable and convenient for the direct analysis of methenamine in pharmaceutical preparations.

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