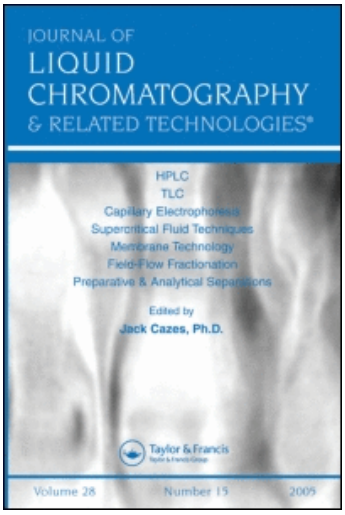

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A Simple and Quantitative Method for Analysis of Methoxyamine by Capillary Zone Electrophoresis

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A Simple and Quantitative Method for Analysis of Methoxyamine by Capillary Zone Electrophoresis

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Abstract: Methoxyamine (MX) is a potential new chemotherapeutic agent. In structure, MX lacks a chromophore for spectrophotometric detection. Derivatization of MX with chromophoric 4-(diethylamino)benzaldehyde (DEAB) was performed and the resultant MX derivative ($DBMOH^+$) can be analyzed by capillary zone electrophoresis (CZE) with ultraviolet (UV) detection at 200 nm. The limit of detection ($SD/S = 3.3$) of the method for MX was 2.50 μM with a 2 s injection at the pressure of 3.45 kPa. The linear calibration range for MX was 5.00–500 μM using *N,N*-dimethyl-*p*-toluidine (DMPT) as the internal standard. The intra- and inter-assay precision and accuracy of the method were ≤4% and within 98.4–104%, respectively. This method may be used for the quantitative determination of MX in pharmaceutical preparations.

Keywords: Methoxyamine, Capillary zone electrophoresis

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INTRODUCTION

Methoxyamine (MX, CH_3ONH_2) is a small organic molecule, which has been recently discovered to be an effective inhibitor of cellular DNA base excision repair (BER) pathway.[1,2] MX synergizes with the cytotoxic effect of an alkylating agent whose DNA damage is normally repaired by BER, and induces tumor cell death.[3–5] The investigation of MX as a new chemotherapeutic agent is currently sponsored by the National Cancer Institute. To further study this potential new anticancer agent, quantitative methods are needed for the determination of MX in pharmaceutical products and in biological fluids.

The measurements of MX have been reported by polarography,[6] spectrophotometry,[7] and liquid chromatography.[8] However, none of these methods can be used for quantitative analysis of MX in pharmaceutical preparations or plasma due to the lack of selectivity and specificity. Recently, a tandem mass spectrometric method for MX was developed by Xu's group.[9] In this method, MX was first derivatized by 4-(diethylamino) benzaldehyde and collected with an on-line solid-phase extraction column, and the quantitation was done by electrospray ionization mass spectrometry. The method was proven useful for quantitation of MX in plasma.

In the present study, a rapid and cost effective capillary zone electrophoresis (CZE) method for MX has been developed and validated. This method has made use of the advantages of 4-(diethylamino)benzaldehyde as a derivatizing agent, CZE as an efficient separation technique, and ultraviolet (UV) detector as a means for on-line detection. This paper is the first report on the quantitative analysis of MX by capillary electrophoresis.

EXPERIMENTAL

Materials

4-(Diethylamino)benzaldehyde (DEAB), *N,N*-dimethyl-*p*-toluidine (DMPT), phosphoric acid (99.999%), formic acid, and sodium hydroxide (semiconductor grade, 99.99%) were purchased from Aldrich (Milwaukee, WI, USA). Methoxyamine hydrochloride was from Sigma (St. Louis, MO, USA). Glacial acetic acid (HPLC grade) was from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical reagent grade. Deionized water was obtained from a NANOpure system (Barnstead, Dubuque, IA, USA) and was used to prepare aqueous solutions.

A stock solution of phosphoric acid (0.5000 M) was prepared by diluting the concentrated phosphoric acid (14.61 M) with deionized water. Sodium hydroxide solution, 1.000 M, was prepared by dissolving an appropriate amount of sodium hydroxide in a known volume of deionized water. Phosphate buffer, 50.00 mM, (pH 2.5) was prepared by dilution of the

0.5000 M phosphoric acid solution with deionized water and adjustment of pH to the desired value with 1.000 M sodium hydroxide solution. Phosphate buffer, 15.00 mM, (pH 2.5) was prepared by dilution of 50.00 mM phosphate buffer (pH 2.5) with deionized water, which was filtered through a 0.45 μm filter and used as the separation buffer. Sodium hydroxide solution of 0.1000 M was prepared by dilution of 1.000 M sodium hydroxide solution with deionized water.

A stock solution of MX (1.000 M) was prepared by dissolving an appropriate amount of methoxyamine hydrochloride in a known volume of deionized water. A set of working solutions of MX (0.500, 1.00, 2.50, 5.00, 10.0, 25.0, 50.0 mM) was prepared from the stock solution by serial dilution with deionized water. A stock solution of DEAB (1.000 M) was prepared by dissolving an appropriate amount of 4-(diethylamino)benzaldehyde in 66.7% acetic acid. A working solution of DEAB (100.0 mM) was prepared by dilution of the stock solution with 33.3% of formic acid. A working solution of DMPT (10.0 mM) solution was prepared by dissolving an appropriate amount of *N,N*-dimethyl-*p*-toluidine in 0.333% of acetic acid and used as the internal standard solution.

Instrumentation

Capillary zone electrophoresis (CZE) experiments were carried out using a Beckman P/ACE 2200 system (Fullerton, CA, USA), which was controlled by an IBM PC with System Gold software (Version 8.1). The on-line UV detector was set at a wavelength of 200 nm and the separation was performed at a constant temperature of 25°C.

An open tubular fused-silica capillary (100 μm i.d., 354 μm o.d.) was purchased from Polymicro Technologies (Phoenix, AZ, USA). A length of 47 cm of the uncoated capillary was used for separation (i.e., a length of 40 cm from the inlet to the window of detection). The capillary was mounted in a P/ACE cartridge where the separation temperature was controlled by a liquid-circulating thermostat. A new capillary was conditioned sequentially by rinsing (2 min) and parking (10 min) with 0.10 M NaOH, deionized water and the separation buffer, respectively. Prior to daily use, the capillary was rinsed with 0.10 M NaOH, deionized water and the separation buffer for 2 min each.

Samples were introduced into the capillary by pressure injection at 3.45 kPa for 2 sec. Separations were carried out in the separation buffer (15 mM phosphate buffer at pH 2.5) at an applied potential of 20 kV with a normal polarity (the cathode was placed at the outlet of the capillary), which produced a current of 66.0 μA. Between runs, the capillary was rinsed with 0.10 M NaOH, deionized water, and the separation buffer for 0.5, 1, and 2 min, respectively.

An HP 8453 UV-visible spectrophotometer (Hewlett-Packard, Wilmington, DE, USA) and an HP PC with ChemStation software were used for taking the UV spectra of MX, DEAB, DMPT, and protonated 4-(diethylamino)benzaldehyde *o*-methyloxime ($DBMOH^+$).

Quantitation

The standard solutions of MX at the concentrations of 5.00, 10.0, 25.0, 50.0, 100, 250, and 500 μM were prepared by mixing equal volumes (40.0 μL) each, individual MX working solution, the derivatizing agent DEAB (100 mM), and the internal standard DMPT (10.0 mM), and a dilution to a total volume of 4.00 mL with the separation buffer. The relative peak area ratios of the MX derivative ($DBMOH^+$) to the internal standard (DMPT) (Y-axis values) were plotted against the MX concentrations (X-axis values) for the regression equation. The MX concentration in an unknown sample could be determined by the regression equation after obtaining the relative peak area ratio of the unknown to the internal standard from the electropherogram.

RESULTS AND DISCUSSION

Chemical Derivatization of Methoxyamine

The reaction mechanism of MX and DEAB was previously postulated by Xu's group.[9,10] Under an acidic condition, MX condenses with DEAB to form the $DBMOH^+$ (Figure 1). If the pH value of solution increases, the derivatization product $DBMOH^+$ can be converted to 4-(diethylamino)benzaldehyde-*o*-methyloxime (DBMO). In this work, the quantitation took place in the acidic solution; therefore, $DBMOH^+$ was the analyte of interest.

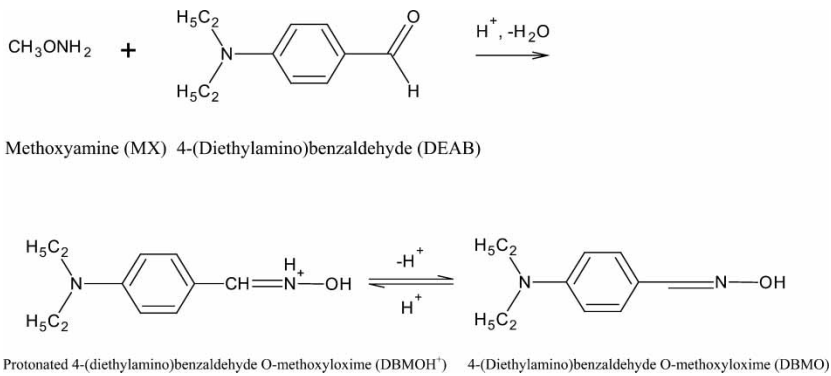

Figure 1. The derivatization reaction of MX with DEAB.

Selection of Wavelength

The absorption spectra of MX, DMPT, DEAB, and $DBMOH^+$ are displayed in Figure 2. As shown in Figure 2, the derivatizing agent DEAB and the product $DBMOH^+$ reached maximum absorbance at 200 nm; whereas MX did not absorb within the range of 190–400 nm and the internal standard DMPT showed adequate absorbance at 200 nm. Hence, the wavelength of 200 nm was chosen as the detection wavelength for the CZE method.

Effect of pH

The pH of the separation buffer affects not only the dissociation of the silanol groups (Si-OH) on the inner surface of the fused-silica capillary, but also the protonation of the analytes. At the low pH values ($\leq$3.0), due to the conversion of the charged SiO^- to the uncharged SiOH, the electroosmotic flow (EOF) is suppressed. As the results, the migration times of the internal

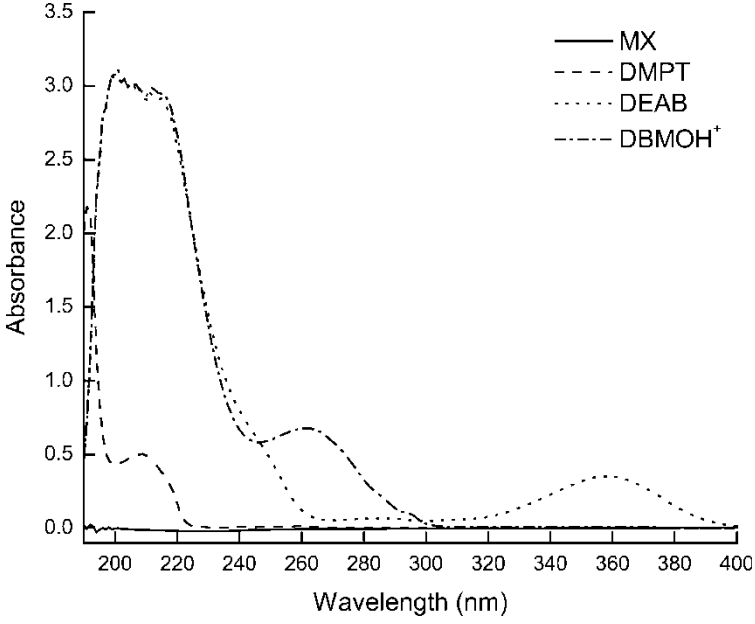

Figure 2. The UV spectra of MX, DMPT, DEAB, and $DBMOH^+$. The working solutions were prepared by dilution of each individual solution to 4.0 mL with 15 mM phosphate buffer at pH 2.5. MX: 40.0 μL of 5.00 mM MX in water; DMPT: 20.0 μL of 10.0 mM DMPT in 0.333% acetic acid; DEAB: 40.0 μL of 5.00 mM DEAB in 33.3% formic acid; and $DBMOH^+$: 40.0 μL of 50.0 mM MX in water reacted with 40.0 μL of 5.00 mM DEAB in 33.3% formic acid at room temperature.

standard (DMPT), the MX derivative ($DBMOH^+$), and the derivatizing agent (DEAB) were longer at the low pH values (Figures 3A and 3B) than those at the high pH values (Figures 3C and 3D). Since the pKa values of DMPT, $DBMOH^+$, and DEAB are 5.63, 6.28, and 3.36, these compounds are fully protonated at pH $\leq$ 3.0. Therefore, single peaks were observed for DMPT, $DBMOH^+$, and DEAB (Figures 3A and 3B). As the pH values exceeded the

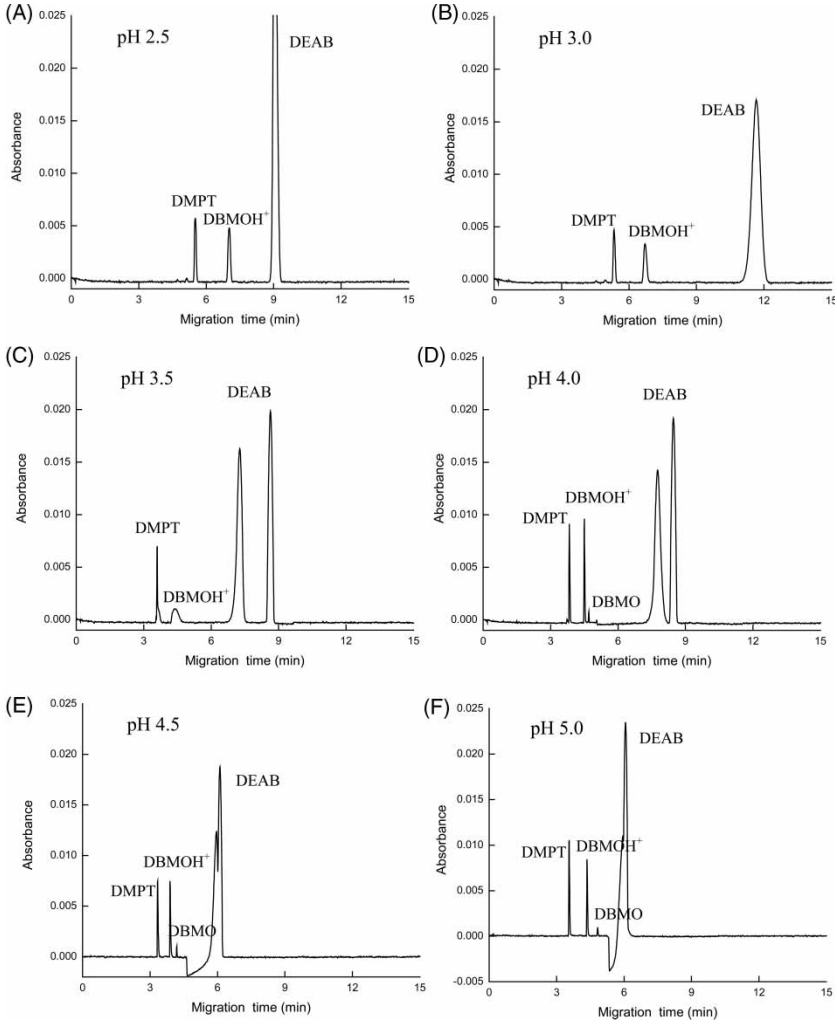

Figure 3. The effect of buffer pH on the separation. The separation buffer was 20 mM phosphoric acid and the applied voltage was 15 kV. The sample solution was prepared by the mixture of equal volumes (40.0 μL) of 5.00 mM MX, 100 mM DEAB, and 10.0 mM DMPT, and the dilution to 4.0 mL with the separation buffer. Other conditions were the same as those in the "Instrumentation" under the "Experimental" section.

pKa of DEAB, a gradual deprotonation took placed. As the results, dual peaks of DEAB were observed at pHs 3.5–4.5 (Figures 3C-3D), and these peaks were merged together at pH 5.0 after total deprotonation (Figures 3F). For the subsequent analytical procedure, a pH of 2.5 was chosen for the separation of DMPT, $DBMOH^+$, and DEAB.

Effect of Applied Voltage

Applied voltage influences the apparent mobility and the resolution of analytes, as well as the current of separation. In this work, our experimental data (not shown) indicated that the current obeyed Ohm's law when the applied potential $\leq$25 kV with 15 mM phosphate buffer (pH 2.5) as the separation buffer. As shown in Figure 4, the migration time of DMPT, $DBMOH^+$, and DEAB decreased as the applied potential increased. For instance, the migration time of $DBMOH^+$ decreased rapidly from 10.40 to 3.65 min as the applied potential increased from 10 to 25 kV. Meanwhile, the resolution of the analytes was almost unchanged as the applied potential increased from 10 to 20 kV, and the resolution decreased only as the applied potential further increased. Therefore, an applied potential of 20 kV

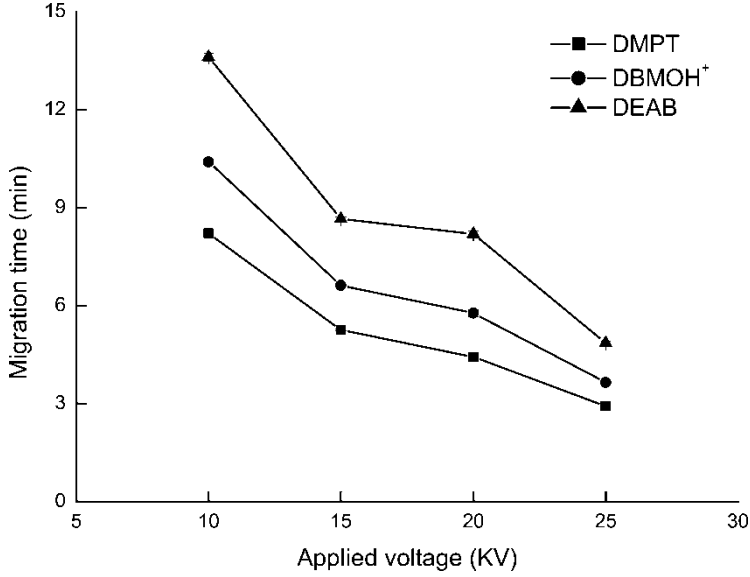

Figure 4. The effect of applied voltage on the migration time and the resolution of DMPT, $DBMOH^+$, and DEAB. The experimental conditions were the same as those in Figure 3, except the separation buffer was 15 mM phosphoric acid at pH 2.5.

was chosen for the analytical procedure with the consideration of analysis time and separation efficiency.

Internal Standard

CE is a well-known technique for small sample analyses, and typical injection volumes of CE are in the range of picoliters to nanoliters.[11] To eliminate injection error and construct a calibration plot, an internal standard was used for calibration in this work.

Due to its structural similarity to the derivatizing agent DEAB, *N,N*-diethyl-*p*-toluidine (DEPT) was first tested as an internal standard for the study. However, the experimental results (not shown) indicated that DEPT did not resolve well with the MX derivative ($DBMOH^+$). Therefore, a smaller compound, *N,N*-dimethyl-*p*-toluidine (DMPT) was chosen as the internal standard for calibration.

Under the optimum conditions (pH 2.5, applied voltage 20 kV), the peaks of DMPT, $DBMOH^+$, and DEAB, were well resolved and the peak areas could be easily integrated (Figure 5). The migration order was DMPT, $DBMOH^+$, and DEAB at 4.2, 5.4, and 7.7 min, respectively.

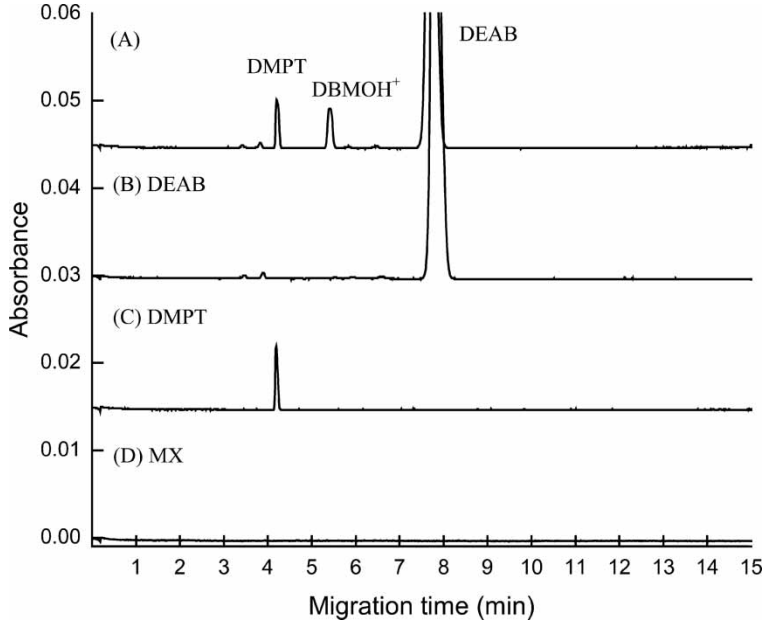

Figure 5. The representative electropherograms of (A) the reaction mixture, (B) DEAB, (C) internal standard DMPT, and (D) MX. The experimental conditions were the same as those in Figure 4.

Analytical Performance

The intra-assay and inter-assay precision, as well as the accuracy of the method, were determined within runs (n = 6) and between runs (n = 3) at three MX concentrations (10.0, 50.0, and 250 μM) with a fixed concentration of internal standard (100 μM). As shown in Table 1, the intra-assay and inter-assay precision expressed in terms of percent relative standard deviations of the relative migration time (RMT) and the relative peak area ratio (RPA)[12] were ≤0.2 and 1%, and ≤4 and 2%, respectively. The intra-assay and inter-assay accuracy defined as the measured value divided by the accepted value of MX multiplied by 100 were 98.4–104% and 99.6–102%, respectively.

The linearity of the method was established over the concentration range of 5.00–500 μM MX (Table 2). The mean calibration curve based on tripli-

Table 1. The intra- and inter-assay precision and the accuracy of the method

		MX added (μM)		
		10.0	50.0	250
Within-run (n = 6)	RMT[a] (mean ± SD) (RSD)	1.296 ± 0.003 0.2%	1.287 ± 0.003 0.2%	1.270 ± 0.003 0.2%
	RPA[b] (mean ± SD) (RSD)	0.216 ± 0.008 4%	0.970 ± 0.010 1%	4.639 ± 0.032 0.7%
	MX Measured (μM)	10.4	50.5	246
	Accuracy (%)	104	101	98.4
Between-run (n = 3)	RMT (mean ± SD) (RSD)	1.296 ± 0.009 0.7%	1.286 ± 0.013 1%	1.273 ± 0.010 0.8%
	RPA (mean ± SD) (RSD)	0.210 ± 0.005 2%	0.981 ± 0.021 2%	4.670 ± 0.043 0.9%
	MX Measured (μM)	10.1	51.1	249
	Accuracy (%)	101	102	99.6

[a]RMT = (Migration time of methoxyamine derivative ($DBMOH^+$)/Migration time of internal standard (DMPT))

[b]RPA = ((Peak area of $DBMOH^+$/Migration time of $DBMOH)^+$/(Peak area of DMPT/Migration time of DMPT)); the concentration of DMPT was fixed at 100 μM.

Table 2. The mean calibration values of three separate calibration curves

	RPA	
MX (μM)	Mean ± SD	RSD (%)
5.00	0.122 ± 0.007	6
10.0	0.203 ± 0.003	1
25.0	0.494 ± 0.009	2
50.0	0.990 ± 0.01	1
100	1.87 ± 0.03	2
250	4.69 ± 0.05	1
500	9.43 ± 0.09	1

cate measurements gave a mean calibration equation by the method of least squares: $Y = 0.0188X + 0.0206$ with a correlation coefficient of 1.00, where X is the concentration of MX and Y is the relative peak area of the MX derivative ($DBMOH^+$) to the internal standard (DMPT).

The limit of detection (LOD) and the limit of quantitation (LOQ) of the method for MX, defined as $3.3 \times (SD/S)$ and $10 \times (SD/S)$, where SD (=0.0140) is the standard deviation of the blank and S (=0.0188) is the slope of the mean calibration curve, were 2.50 and 7.50 μM, respectively.

CONCLUSIONS

A simple and quantitative CZE-UV method was developed for the analysis of MX in pharmaceutical products. The method has a wide linear calibration range, low limits of detection, and high precision and accuracy. It is a rapid and cost effective method that may be used for the quality control of MX in bulk and formulation.

REFERENCES

1. Gerson, S.L.; Liu, L. Methoxyamine or PARP Inhibitor Potentiation of Temozolomide Anti-Cancer Activity. PCT Int. Appl., 2001, WO 2001022199 A2.
2. Gerson, S.L.; Liu, L. Methoxyamine Combinations in the Treatment of Cancer. US Pat. Appl. Publ., 2002, US2002198264 A1.
3. Liu, L.; Yan, L.; Donze, J.R.; Gerson, S.L. Blockage of abasic site repair enhances antitumor efficacy of 1,3-bis-(2-chloroethyl)-1-nitrosourea in colon tumor xenografts. Mol. Cancer Ther. **2003**, *2*, 1061–1066.
4. Liu, L.; Nakatsuru, Y.; Gerson, S.L. Base excision repair as a therapeutic target in colon cancer. Clin. Cancer Res. **2002**, *8*, 2985–2991.

5. Taverna, P.; Liu, L.; Hwang, H.-S.; Hanson, A.J.; Kinsella, T.J.; Gerson, S.L. Methoxyamine potentiates DNA single strand breaks and double strand breaks induced by temozolomide in colon cancer cells. Mutat. Res. **2001**, *485*, 269–281.
6. Iversen, P.E.; Lund, H. Polarographic determination of hydroxylamines and some hydrazine derivatives. Anal. Chem. **1969**, *41*, 1322–1324.
7. Korpela, T.K.; Makela, M.J. Spectrophotometric measurement of hydroxylamine and its O-alkyl derivatives. Anal. Biochem. **1981**, *110*, 251–257.
8. Wang, E.; Struble, E.; Liu, P.; Cheung, A.P. A new sensitive HPLC assay for methoxyamine and its analogs. J. Pharm. Biomed. Anal. **2002**, *30*, 415–427.
9. Yang, S.; Liu, L.; Gerson, S.L.; Xu, Y. Measurement of anti-cancer agent methoxyamine in plasma by tandem mass spectrometry with on-line sample extraction. J. Chromatogr. B **2003**, *795*, 295–307.
10. Liao, Y.-C.; Yang, S.; Syu, M.-J.; Xu, Y. Development and validation of an HPLC-UV method for the analysis of methoxyamine using 4-(diethylamino)benzaldehyde as a derivatizing agent. J. Pharm. Biomed. Anal. **2005** (in press).
11. Xu, Y. Capillary electrophoresis in clinical chemistry. In *Encyclopedia of Analytical Chemistry: Instrumentation and Applications*; Meyers, R.A., Ed.; John Wiley & Sons Ltd., 2000; 1181–1199.
12. Altria, K.D.; Frake, P.; Gill, I.; Hadgett, T.; Kelly, M.A.; Rudd, D.R. Validated capillary electrophoresis method for the assay of a range of basic drugs. J. Pharm. Biomed. Anal. **1995**, *13*, 951–957.

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