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Rapid and sensitive quantitation of the antiproliferative agent mitoguazone in small volumes of plasma by high-performance liquid chromatography with ultraviolet detection

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Abstract

Mitoguazone is an antiproliferative agent used in chemotherapy. This study describes a simple and sensitive high-performance liquid chromatographic method for the determination of mitoguazone in 100 μl of plasma. Samples were deproteinized with 100 μl of a solution of internal standard (amiloride, 10 μg/ml) in acetonitrile. An aliquot of the supernatant was injected onto the column. HPLC separation was achieved on a silica column with the mobile phase of methanol–50 m*M* potassium phosphate buffer (pH 3)–triethylamine (80:20:0.3, v/v), at a flow-rate of 1 ml/min. The eluent was detected at 320 nm. The retention time was about 5.5 min for amiloride and 12 min for mitoguazone. No endogenous substances were found to interfere. Calibration curves were linear from 0.25 to 50 μg/ml. The absolute recoveries of mitoguazone and amiloride were both greater than 84%. The limit of quantitation was 0.25 μg/ml. The intra- and inter-day precision (expressed as RSD) was 5.8%, or less, and the accuracy was 94.7% of the nominal concentration. The method is suitable in pharmacokinetic investigation and monitoring mitoguazone concentration.

Keywords: Mitoguazone

1. Introduction

Mitoguazone [methylglyoxal bis(guanylhydrazone), Fig. 1] is an antiproliferative agent used in combination chemotherapy. Mitoguazone was recognized as an antineoplastic and cytotoxic agent and it gained considerable attention during the 1960s. Early clinical trials with daily dosing schedules of mitoguazone resulting in severe dose-related toxicity, therefore it was discarded from clinical use. However, recent clinical trials of mitoguazone on weekly dosing regimens have shown favorable antitumor activity with minimal toxicity in patients with solid tumors and lymphomas. Thus, there has been a renewal of clinical interest in this drug [1–5].

Mitoguazone is an organic polycation, and it is a potent inhibitor of polyamine biosynthesis through specific inhibition of *S*-adenosylmethionine decarboxylase. In spite of the long-standing availability of mitoguazone, the exact mechanism underlying its antiproliferative effect is not fully understood, and

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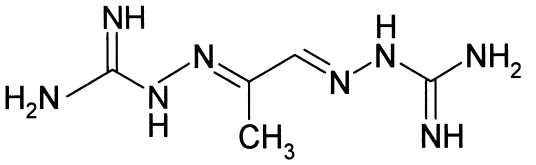
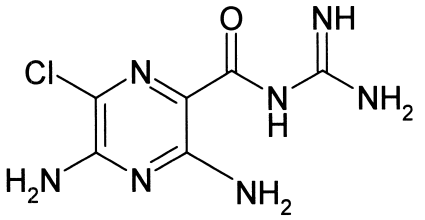


Mitoguazone



Amiloride

Fig. 1. Structures of mitoguazone (pK_a: 7.63, 9.05) and internal standard amiloride.

information on its pharmacokinetic characteristics in the body is still limited [2,4]. The serious side effects associated with mitoguazone including myelosuppression, mucositis and other toxicities, are related to high blood concentrations of the drug [2,5]. As the toxicity of mitoguazone is dose-related, therapeutic monitoring on its blood level would be very useful in controlling and minimizing its side effects. To address all these issues, a suitable assay for quantitation of mitoguazone is deemed necessary.

Several high-performance liquid chromatography (HPLC) methods were used for the determination of mitoguazone in biological fluids [6–11]. Previously described methods suffer from several disadvantages, such as lack of sensitivity [6], use of complex pretreatment procedures which are tedious and time consuming [6,8–11], need of the use of ultrafiltration [6,7], and requirement of relatively large sample volume [6,8–10].

For pharmacokinetic studies, a suitable sensitive method that allows an accurate measurement of low concentration of mitoguazone in plasma is needed. For routine drug monitoring in cancer patients and AIDS patients with lymphoma, assays that require small sample volumes are very useful. In this report, we present a simple, sensitive and specific HPLC method to determine mitoguazone concentration in human plasma. The limit of quantitation (LOQ) of this validated method was 0.25 μg/ml using 100 μl of plasma. The applicability of this assay was demonstrated in animal pharmacokinetic studies.

2. Experimental

2.1. Chemicals and reagents

Mitogauzone dihydrochloride (lot 100K3656) and amiloride (lot 98H1169) (Fig. 1) were purchased from Sigma (St. Louis, MO, USA). All chemicals were analytical-grade reagents and used as received without further purification. HPLC-grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Triethylamine (lot 72670) was from Riedel-de Haen (Germany). Milli-Q reagent water (Millipore, Bedford, MA, USA) was used in the preparation of mobile phase. Human plasma was obtained from the Blood Bank of National Cheng Kung University Hospital (Tainan, Taiwan).

2.2. Instrumentation and chromatography

The HPLC system consisted of a Hitachi L-7100 pump, a Hitachi L-7200 autosampler, a Hitachi L-7400 UV detector (Tokyo, Japan), and an SISC data station (Scientific Information Service, Taipei, Taiwan). The analytical column, Hypersil HS silica 5 μm, 25 cm×4.6 mm I.D., was protected by a precolumn (Hichrom silica H5, 10×3.2 mm I.D.). The mobile phase comprised 0.05 *M* KH_2PO_4 (pH 3)–methanol (20:80, v/v), and 0.3% triethylamine. The prepared mobile phase was filtered through a 0.45-μm Millipore filter and degassed ultrasonically before use. Analyses were run at a flow-rate of 1 ml/min at ambient temperature. The detector wavelength was set at 320 nm, and peak areas were measured.

2.3. UV spectrometry

The UV spectra of mitoguazone (5 μg/ml) in aqueous solutions with various pH values (1–12) were recorded using a Hitachi U2010 spectrophotometer. The effects of mobile phase additives triethylamine (0.1, 0.2, 0.3%) and sodium heptane sulfonate (2 m*M*) on the UV spectrum of mitoguazone in the mobile phase [0.05 *M* KH_2PO_4 (pH 3)–methanol, 20:80] were also investigated.

2.4. Standards and controls

Master stock solutions of mitoguazone (1 mg/ml in water, as base) and the internal standard amiloride (10 mg/ml in methanol) were prepared monthly and kept tightly sealed at −80 °C. The stock solution of mitoguazone was diluted with drug-free plasma to give the calibration standards at concentrations of 0.25, 0.5, 1.25, 2.5, 5, 12.5, and 50 μg/ml mitoguazone. The quality controls were prepared independently at concentrations of 0.25, 0.5, 5 and 50 μg/ml prior to the start of sample collection and stored at −80 °C until used. The working solution of amiloride was obtained by diluting the stock solution in acetonitrile to 10 μg/ml. A complete calibration curve was generated with each analytical run.

2.5. Sample preparation

The samples to be analysed were removed from the freezer and thawed. Calibration standards, controls, and unknown samples were pipetted into 1.5-ml microcentrifuge tubes and processed as a batch. To 100-μl aliquots of human plasma samples were added a 100-μl aliquot of the internal standard working solution. After vortex-mixed for 30 s and upon centrifugation at 15 850 *g* for 10 min, an aliquot of the supernatant was injected onto the column for HPLC analysis.

2.6. Quantitation

The model for the calibration curve of mitoguazone used the peak area ratio (PAR) of mitoguazone to amiloride and the mitoguazone concentration (C), as given in the following equation: PAR=slope·C+(y-intercept). The slope and y-intercept were determined by a nonlinear least-squares program (WinNonlin, Professional Version 2.1, Pharsight, Mountain View, CA, USA), using nominal concentrations and measured PARs from calibration standards. A weighting scheme of $1/y^2$ was used. Mitoguazone concentrations were estimated from PARs using the formula: C=[PAR−(y-intercept)]/slope.

2.7. Precision, accuracy and limit of quantitation

Intra-day precision was evaluated by analysing the spiked controls six times over 1 day in random order, while inter-day precision was evaluated from the analysis of each control once on each of 6 different days. Assay precision (RSD) was assessed by expressing the standard deviation of the measurements as a percentage of the mean value. The accuracy was estimated for each spiked control by comparing the nominal concentration with the assayed concentration. The lower LOQ was the lowest non-zero concentration level, which could be accurately (relative error <20%) and reproducibly (RSD<20%) determined [12]. Assay selectivity was examined in relation to interference from endogenous substances in drug-free plasma.

2.8. Recovery

Absolute recoveries of 0.5, 5 and 50 μg/ml concentrations of mitoguazone in plasma were determined by assaying the samples as described above and comparing the peak areas of both mitoguazone and amiloride with those obtained from direct injection of the compounds dissolved in aqueous supernatant of processed blank plasma.

2.9. Stability

Freeze–thaw stability of mitoguazone (0.5, 5 and 50 μg/ml) in plasma samples was determined for three freeze–thaw cycles. The post-preparative stability of mitoguazone in processed samples left at ambient temperature (ca. 20 °C) and 4 °C in autosampler vials was followed for 24 h. The short-term stability of mitoguazone in plasma at 4 °C was studied for 24 h, and the stability of mitoguazone in

plasma samples stored at −20 °C in screw cap vials was followed for 720 h.

2.10. Pharmacokinetic application

The assay was applied to a single dose (75 mg/kg) pharmacokinetic study in rats. Male Sprague–Dawley rats were obtained from the Animal Breeding Center of National Cheng Kung University. The study protocol complied with the Institutional Guidelines on Animal Experimentation of National Cheng Kung University. After intravenous bolus administration, blood samples for analytical determinations were collected at specific time intervals for 8 h. Plasma samples were stored at −20 °C until analysis. The pharmacokinetic parameters of mitoguazone were determined by compartmental analysis. Nonlinear regression modeling (WinNonlin, Professional Version 2.1, Pharsight) was used to fit the measured mitoguazone plasma concentrations to a two-compartment model with first-order elimination.

3. Results

3.1. UV absorption

As mitoguazone is a hydrophilic base with two pK_a values of 7.63 and 9.05, the potential effect of pH value on its UV spectrum deserves a systematic investigation (Fig. 2). In the aqueous solution with pH less than 4, the UV spectra of mitoguazone were similar and showed a distinct absorption maximum (λ_{max}) at about 283 nm. The absorptivity decreased and λ_{max} shifted to 285 nm as the pH value increased to 7.5. At pH 8, the spectrum changed remarkably with the λ_{max} occurred at 295 nm and a ''shoulder'' around 320 nm. When the pH values of solutions were greater than 9.1, the absorption intensity increased as the pH increased. At pH values greater than 11, the spectra of mitoguazone were similar and showed a distinct λ_{max} at about 328 nm (Fig. 2A). These phenomena could be explained by the degree of ionization and species distribution of mitoguazone under various pH values. The adjustment of solution to pH less than 5.6 resulted in mitoguazone existing predominantly as di-cation form with a λ_{max} of 283 nm, whereas adjustment of solution to pH greater than 11 resulted in mitoguazone existing mainly as non-ionic form with a λ_{max} of 328 nm. For pH values between 5.6 and 11, mitoguazone existed as a mixture of non-ionic, mono- and di-cation forms, accordingly the shape and intensity of its UV spectrum changed as a function of the extent of species existed. In the mobile phase system of 0.05 *M* KH_2PO_4 (pH 3)–methanol (20:80), the UV spectra of mitoguazone remained unchanged in the presence of ion-pair reagent sodium heptane sul-

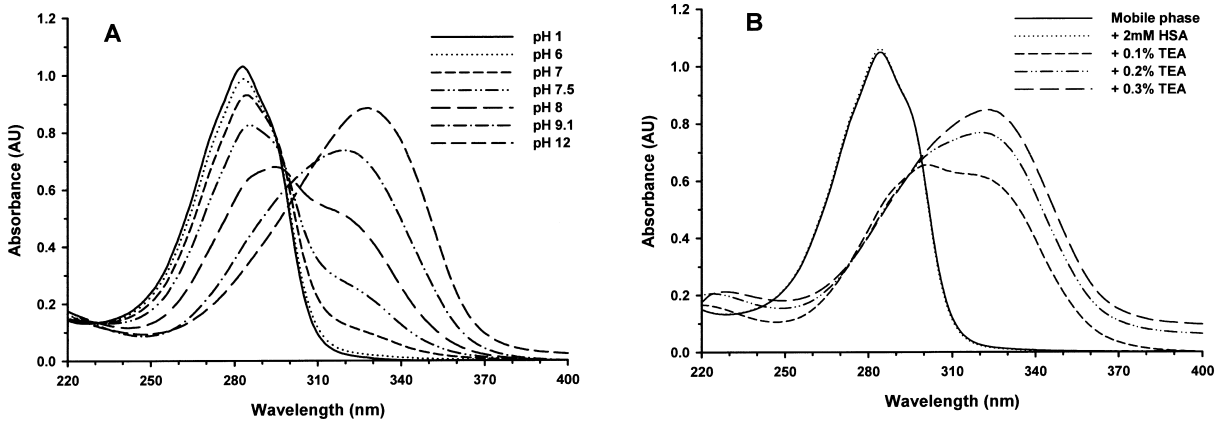


Fig. 2. The UV spectra of mitoguazone (5 μg/ml) in aqueous solutions with various pH values (A). Also shown are the spectra of mitoguazone in the mobile phase [0.05 *M* KH_2PO_4 (pH 3)–methanol, 20:80] in the absence and the presence of triethylamine (0.1, 0.2, 0.3%) and sodium heptane sulfonate (2 m*M*).

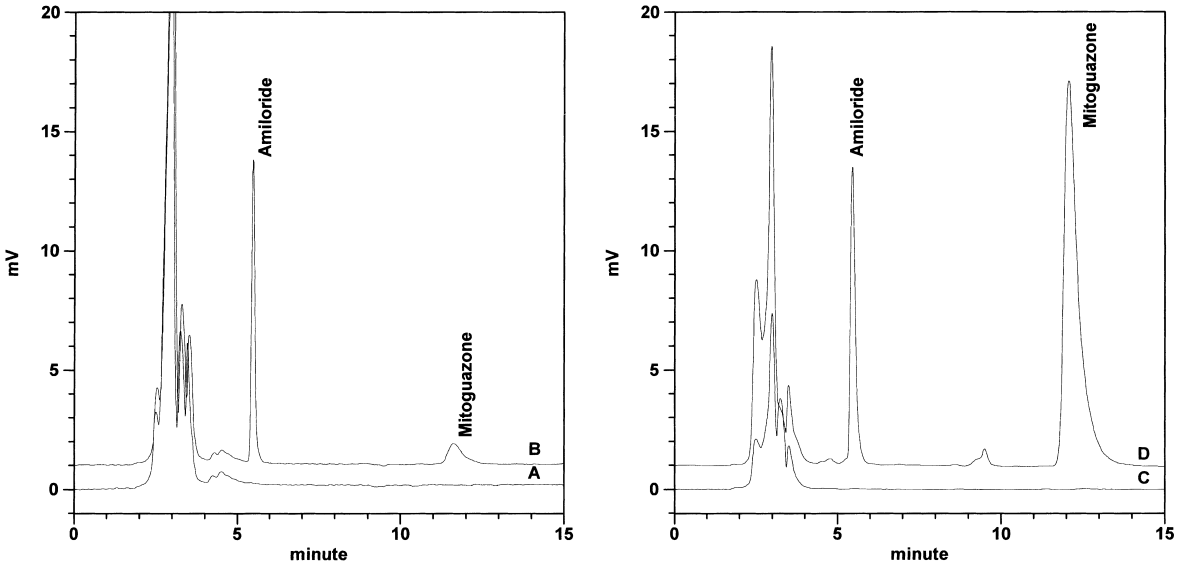


Fig. 3. HPLC chromatograms of a pooled blank plasma sample (A) and blank plasma fortified with 0.25 μg/ml mitoguazone and the internal standard (B); the chromatograms of extracts from pre-dosing (C) and 8 h (D) plasma samples from a rat after intravenous bolus of 75 mg/kg mitoguazone are also shown.

fonate. However the addition of triethylamine shifted the spectrum toward that for higher pH (Fig. 2B).

3.2. Chromatography

Fig. 3 shows chromatograms of a pooled blank plasma (A) and blank plasma fortified with 0.25 μg/ml mitoguazone and the internal standard (B). Fig. 3C and D represent chromatograms of extracts from pre-dose and 8 h, respectively, after administration of 75 mg/kg mitoguazone to a rat. The concentration of mitoguazone was 3.05 μg/ml. Amiloride and mitoguazone were eluted after 5.4 and 12 min, respectively. The total run time was less than 15 min. As can be seen in Fig. 3, a good separation of mitoguazone and the internal standard was achieved under the chromatographic conditions specified in the Experimental section.

The simple protein precipitation by acetonitrile was sufficient to isolate mitoguazone and amiloride from plasma without any interfering endogenous peaks. The method is specific for mitoguazone. No interfering peaks were observed at the retention of mitoguazone when blank pre-dose rat plasma samples were analyzed (Fig. 3C).

3.3. Calibration and validation

The calibration curves were linear from 0.25 to 50 μg/ml. The mean (±SD) regression equation for 14 replicated calibration curves constructed using 100 μl of plasma samples on different days was: PAR = $(1.29\pm0.15)\cdot C+(-0.01\pm0.03)$, $r^2=0.996\pm0.001$.

Precision and accuracy (0.25–50 μg/ml) were investigated by replicated analyses of spiked controls (Table 1), and in all cases the within-day and between-day precision was acceptable at an RSD of 5.8% or less. In addition, accuracy was within 5.3% when compared with nominal concentrations across this range. From this experiment the LOQ of the method was determined to be 0.25 μg/ml, with the within-day imprecision and error of 4.7% and 5.3%, and the between-day imprecision and error of 5.8% and −0.2%. Nevertheless, mitoguazone concentration at least down to 0.025 μg/ml could be still detected while preserving the signal-to-noise ratio well above 3.

Satisfactory assay sensitivity was assisted by relatively high and reproducible recoveries for mitoguazone and amiloride as shown in Table 2. The suitability of amiloride as the internal standard is demonstrated as the relative recovery of amiloride,

Table 1
Within-day and between-day accuracy and precision for the determination of mitoguazone in 100-μl plasma aliquots ($n=6$)

$C_{nominal}$ (μg/ml)	Within-day			Between-day		
	C_{est} (μg/ml)	RSD (%)	Error (%)	C_{est} (μg/ml)	RSD (%)	Error (%)
0.25	0.26	4.7	5.3	0.25	5.8	−0.2
0.5	0.49	3.3	−1.2	0.49	2.6	−1.2
5	5.04	3.7	0.8	4.94	1.4	−1.2
50	49.04	2.5	−1.9	50.36	3.8	0.7

$C_{nominal}$: Nominal concentration; C_{est}: estimated concentration.

Table 2
Recovery of mitoguazone in spiked plasma in the presence of internal standard (mean±SD, $n=6$)

Concentration (μg/ml)	Absolute recovery (%)		Relative recovery (%)
	Mitoguazone	Amiloride	
0.5	96.0±6.7	83.8±5.9	114.6±4.4
5	91.6±3.2	84.2±3.0	108.8±3.1
50	85.2±2.6	86.7±2.4	98.2±1.2

determined by comparing the peak area ratios (mitoguazone/amiloride) of the processed samples with that of unprocessed control samples, was close to 100%.

Long-term stability of mitoguazone in frozen plasma has been investigated previously for up to 1 year and 5 months [4,9]. The present study showed that mitoguazone was stable in plasma at 4 °C for up to 24 h and at −20 °C for at least 720 h (Table 3). The mean concentration for the quality control samples (0.5, 5 and 50 μg/ml) was within ±10% of nominals for mitoguazone following the third freeze–thaw cycle. It has been reported that prolong exposure to perchloric acid and potassium hydroxide during sample preparation may cause significant loss of mitoguazone [8,10]. In this study, mitoguazone was stable in processed samples left at ambient temperature and 4 °C for up to 24 h, indicating that treatment with acetonitrile did not affect the stability of mitoguazone (Table 3).

Table 3
Stability of mitoguazone in spiked plasma and post-preparative samples (mean±SD, $n=3$)

Conditions		Remained (%)		
		0.5 μg/ml	5 μg/ml	50 μg/ml
Stability in plasma				
4 °C	24 h	94.8±1.2	93.0±3.6	87.7±0.5
−20 °C	168 h	103.6±4.6	101.4±8.4	92.8±0.9
	720 h	98.7±2.3	97.2±1.3	97.0±4.6
Freeze–thaw stability				
−20 °C/20 °C	Cycle 1	91.7±3.9	95.2±6.3	94.7±2.3
	Cycle 2	97.0±8.6	92.6±2.7	94.7±1.0
	Cycle 3	105.0±2.9	106.1±1.7	100.3± 1.3
Post-preparative stability				
4 °C	24 h	101.5±7.4	95.1±1.8	91.3±1.5
20 °C	24 h	104.1±3.0	104.9±7.0	103.6±2.5

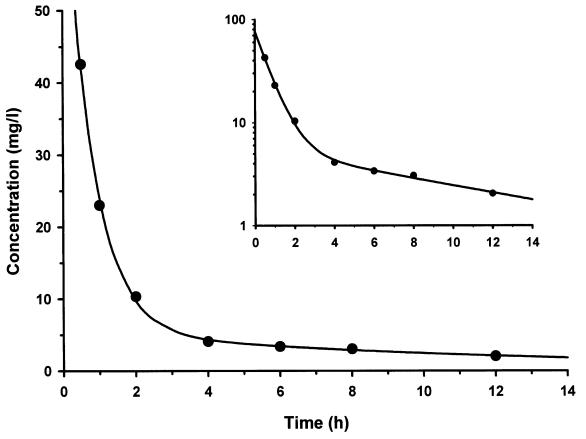


Fig. 4. The plasma concentration–time profile of mitoguazone after intravenous bolus of 75 mg/kg mitoguazone to a male rat. The solid line represents fitted plasma concentrations according to a two-compartment model using WinNonlin Pro 2.1, Pharsight, USA.

3.4. Application

The assay was applied to a preliminary pharmacokinetic experiment in rats. A single bolus dose of 75 mg/kg of mitoguazone was administered intravenously to a male rat. Blood samples were collected at scheduled intervals. The plasma concentration–time profile is illustrated in Fig. 4. The pharmacokinetic parameter estimates of clearance, volume of distribution and terminal elimination half-life were 10.2 ml/min/kg, 4.5 l/kg and 8.6 h, respectively. These indicated that mitoguazone distributed widely into tissues and was cleared slowly from the body. The results showed that this simple and rapid method is sufficiently sensitive to follow blood level of mitoguazone. The present assay has also been applied successfully to study the hepatic disposition of mitoguazone in isolated perfused rat liver [13].

4. Discussion

4.1. Method development

Mitoguazone level in biological fluids has been analyzed by HPLC exclusively on a reversed-phase column with C_{18} bond phase [6–11]. A recent study has used a C_8 column for the analysis of mitoguazone [14]. In most of these assays, ion pair reagents are employed in the mobile phase, and the pH value of the buffer solution/mobile phase varies from 3 to 4.3. Mitoguazone has high molar absorbance at 283 nm in acidic medium (Fig. 2A), and the presence of alkyl sulfonic acids does not affect its absorption spectrum (Fig. 2B), therefore all the reported HPLC assays used UV detection at 280–285 nm. It has been suggested that at a pH lower than 4.3, ion pair reagents may not be necessary for the analysis of mitoguazone [6]. In the present study, we used a normal-phase silica column with a simple phosphate buffer (pH 3)–methanol system. The basic additive triethylamine was used to suppress the peak tailing; consequently UV at 320 nm was employed for the detection of mitoguazone.

Marsh et al. [9] and Rizzo et al. [4] used serotonin (5-hydroxytryptamine) as the internal standard for HPLC assay of mitoguazone. However, it is an endogenous substance and it bears little structure similarity to mitoguazone. The mitoguazone homolog ethylglyoxal-bis(guanylhydrazone) was used as the internal standard, however, it was not commercially available [11]. In the present study, amiloride was chosen as the internal standard because it is commercially available and its peak was sufficiently separated from that of mitoguazone. Amiloride has a guanidine group and its structure is similar to mitoguazone (Fig. 1). Under physiological pH 7.4, amiloride also existed in cationic form. Like mitoguazone, amiloride is hydrophilic, and does not bound substantially to plasma proteins.

Because of its high polarity and basicity, mitoguazone is particularly difficult to extract into organic solvents. Even when the sample was adjusted to pH 11, the recovery of extraction was still less than 10% [9]. Therefore, previous studies used deproteinization agents or ultrafiltration in sample pretreatment. It has been reported that deproteinization with perchloric acid resulted in time-dependent, irreversible loss of mitoguazone, and acetonitrile completely precipitated mitoguazone as well as protein [8]. Notably, as illustrated in this study the use of acetonitrile resulted in quantitative and high recoveries for both mitoguazone and amiloride.

Following intravenous infusion of 600 mg/m^2 of

Table 4
Analytical characteristics of reported HPLC methods for the determination of mitoguazone in plasma/serum*

Ref.	Column	Mobile phase	Internal standard	Flow-rate (ml/min)	Run time (min)	UV (nm)	Volume (ml)	Pretreatment	LOD# (ng/ml)
This study	Silica	[50 m*M* phosphate buffer (pH 3)–CH_3OH, 2:8]+0.3% TEA	Amiloride	1	15	320	0.1	CH_3CN	25 (5)
[6]	C_{18}	30 m*M* acetate buffer (pH 4.3)–CH_3OH: gradient	No	1	~5	285	1	Ultrafiltration	400 (3)
[7]	C_{18}	[50 m*M* phosphate buffer (pH 3)–CH_3OH, 6:4]+1 m*M* SHS	No	1	15	280	0.4	CH_3CN+ethanol, ultrafiltration	100 (3)
[8]	C_{18}	CH_3OH–[200 m*M* acetate buffer (pH 4.5)+20 m*M* SOS +40 mg/l NaN_3] (66.7:100)	No	3	3	283	1.5	PCA	3.7 (2)
[9]	C_{18}	[CH_3OH–water, 17.5:82]+2.5 m*M* SPS+0.5% acetic acid	5-HT	2	7.5	280	1	CX	50 (?)
[10]	C_{18}	30 m*M* acetate buffer (pH 4.3)–CH_3OH (95:5)	No	2	10	283	1	PCA	250 (?)
[11]	C_{18}	[5 m*M* SPS–5 m*M* SHS (9:1)]–CH_3OH (68:32)	EGBG	2	20	280	0.25	CH_3OH, CCl_4	30 (2)

* CX: Cation exchange, EGBG: ethylglyoxal-bis(guanylhydrazone), 5-HT: 5-hydroxytryptamine, PCA: perchloric acid, SHS: sodium heptane sulfonate, SOS: sodium octane sulfonate, SPS: sodium pentane sulfonate.

LOD: Limit of detection, the signal-to-noise ratio is indicated in parentheses.

mitoguazone over 30 min, the peak plasma concentration of mitoguazone in AIDS related non-Hodgkin's lymphoma patients ranged from 6.5 to 43 μg/ml and the average concentration 192 h post-infusion was about 0.25 μg/ml [4]. Mitoguazone concentrations as low as 0.5 μg/ml have been reported to competitively inhibit *S*-adenosylmethionine decarboxylase [4]. Based on these considerations, we have validated the assay over clinical relevant concentration range of 0.25–50 μg/ml.

Due to the clinical status of patients with malignancies, especially in AIDS-related lymphoma patients and young children, it is often difficult to obtain suitable amount of blood from these patients. Thus, in this study efforts were made to reduce considerably the amount of plasma sample needed, from 0.25 to 1.5 to 0.1 ml (Table 4), for the quantitation of mitoguazone. This is very useful in reducing the blood collection, offering the possibility to make sufficient numbers of blood samples for pharmacokinetic study, and minimizing the amount of blood-derived biological waste that need to be disposed with care.

In conclusion, a new HPLC method for the determination of mitoguazone has been developed. The method offers the sensitivity and selectivity for monitoring therapeutic concentration of mitoguazone. The method is flexible and required only 100 μl plasma, making it suitable for studying the pharmacokinetics of mitoguazone in cancer patients, AIDS patients with lymphoma, children, and small animals. The application of this method was demonstrated in a pharmacokinetic study in rats.

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References

[1] E. Aurlien, H. Holte, S. Kvaloy, E. Jakobsen, L.S. Rusten, G. Kvalheim, Eur. J. Haematol. Suppl. 64 (2001) 14.

[2] S. Ekelund, P. Nygren, R. Larsson, Biochem. Pharmacol. 61 (2001) 1183.

[3] A.M. Levine, A. Tulpule, D. Tessman, L. Kaplan, F. Giles, B.D. Luskey, D.T. Scadden, D.W. Northfelt, I. Silverberg, J. Wernz, B. Espina, D. Von Hoff, J. Clin. Oncol. 15 (1997) 1094.

[4] J. Rizzo, A.M. Levine, G.R. Weiss, T. Pearce, M. Kraynak, R. Mueck, S. Smith, D.D. Von Hoff, J.G. Kuhn, Invest. New Drugs 14 (1996) 227.

[5] D.D. Von Hoff, Ann. Oncol. 5 (1994) 487.

[6] M. Koskinen, H. Elo, P. Lukkari, M.-L. Riekkola, J. Chromatogr. B. 685 (1996) 141.

[7] A. Negro, R. Méndez, J. Martin-Villacorta, A.I. Ortiz, D. Ordóñez, J. Liq. Chromatogr. 14 (1991) 2409.

[8] D.L. Gildersleeve, M.C. Tobes, R.B. Natale, Clin. Chem. 31 (1985) 1979.

[9] K.C. Marsh, A.J. Repta, L.A. Sternson, J. Chromatogr. 187 (1980) 101.

[10] M.G. Rosenblum, T.L. Loo, J. Chromatogr. 183 (1980) 363.

[11] J. Roboz, K.T. Wu, R.D. Hart, J. Anal. Toxicol. 4 (1980) 127.

[12] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.

[13] E-G. Lin, C.-L. Cheng, C.-H. Chou, in: Proceedings of the Annual Conference of the Pharmaceutical Society of ROC, December 2001, Taipei, 2001, p. 112.

[14] C.E. Thomson, M.R. Gray, M.P. Baxter, J. Pharm. Biomed. Anal. 15 (1997) 1103.