Medicinal, Aromatic and Poisonous Plants Research Center[1], Department of Pharmaceutical Chemistry[2], College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, Department of Pharmacognosy[2], School of Pharmacy, University of Mississippi, USA

# Spectrofluorimetric determination of manzamine A in spiked human urine and plasma

A. KHALIL[1], K. EL-SAYED[1], F. BELAL[2], N. Y. KHALIL[1], M. HAMANN[3]

*Received December 9, 2002, accepted December 12, 2002*

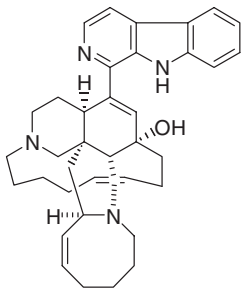
*Prof. Dr. F. Belal, Department of Pharmaceutical Chemistry, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh-11451, Saudi Arabia*
*ffbelal@ksu.edu.sa*

*Pharmazie 58: 385–388 (2003)*

The native fluorescence of manzamine A (a biologically active β-carboline marine-derived alkaloid) has been studied under different conditions. The highest fluorescence intensity was obtained in methanol. Two wavelength settings were found to be suitable for excitation, 280 nm and 340 nm; while $\lambda_{max}$ emission was constant in both cases at 387 nm. The fluorescence intensity at 340/387 nm setting was 1.6 greater than that obtained at 280/387 nm settings. The calibration curves were rectilinear over the range 0.1–2.0 and 0.5–2.5 μg/ml for the two settings, respectively. The detection limits were 0.05 μg/ml ($9.1 \times 10^{-9}$ M) and 0.1 μg/ml ($1.82 \times 10^{-8}$ M) at 340/387 nm and 280/387 nm, respectively. The proposed method was applied to the determination of manzamine A in spiked human urine and plasma samples adopting the 340/387 nm wavelength setting, the % recoveries (n = 6) were 99.61 ± 0.90 and 100.25 ± 1.63, respectively.

## 1. Introduction

Manzamines represent a group of β-carboline marine-derived alkaloids. These compounds possess a fused and bridged tetra- or pentacyclic ring system attached to a β-carboline moiety. The first member of this group, manzamine A (MZA), was isolated and reported in 1986 [1]. Manzamines are attractive candidates for bioevaluations due to their unique structures. It was previously reported that manzamines exhibit a diverse range of bioactivities including anti-inflammatory, cytotoxicity, antibacterial and antimalarial activity [2–4]. Manzamine A was a patent topic twice due to its promising anti-inflammatory and antimalarial activities [3]. *In vitro*, MZA inhibited the growth of *Plasmodium falciparium* (D6 Clone) and *P. falciparium* (W2 Clone) with $IC_{50} < 528.8$ ng/ml, [5, 6]. *In vivo* evaluation revealed that MZA prolonged the survival of *Plasmodium berghei* infected mice for more than 10 days after a single intraperitoneal administration at a dose of 50 μmol/kg and for 60 days at 100 μmol/kg with no detectable parasitemia, which was far superior to both currently used antimalarial drugs chloroquine and artemisinin [6]. The structure novelty, ideal pharmacokinetic character, bioavailability, and possible immunostimulant effect of manzamines indicated their potential as a new class of lead antimalarial drugs. A literature review revealed that nothing has been reported regarding the determination of MZA in biological fluids. This led us to study the fluorometric characteristics of the drug in an attempt to develop a simple, sensitive and reliable method for its determination in spiked human urine and plasma. The native fluorescence of the compound initiated the present study. The method developed can be readily adopted for the pharmacokinetic and pharmacodynamic studies of the drug.




Manzamine A

## 2. Investigations, results and discussion

The UV spectrum of MZA in methanol shows several $\lambda_{max}$ values; the most prominent one is that at 279 nm with a specific absorbance ($A^{1\%}_{1\,cm}$) of 242 and molar absorptivity (ε) of $1.328 \times 10^4$ L; $Mol^{-1}$; $cm^{-1}$. As a consequence, poor sensitivity will be achieved by conventional spectrophotometric measurements, and this problem is more aggravated if the compound needs to be estimated in biological fluids.

On the other hand, the MZA solution exhibited an intense native fluorescence. Different media such as water, methanol, 0.1 M HCl, 0.1 M NaOH and phosphate buffer of pH 7, were attempted. As shown in Table 1, the highest fluorescence intensity was obtained in methanol. Therefore, methanol was used throughout this study. Fig. 1

**Table 1: Effect of the medium on the fluorescence of MZA (1 μg/ml) using 280 nm for excitation**

| Medium | $\lambda_{max}$ emission | % Relative fluorescence |
|---|---|---|
| Methanol | 387 nm | 54 |
| Water | 404 nm | 48 |
| Phosphate buffer (pH 7) | 395 nm | 18 |
| HCl (0.1 M) | 465 nm | 8 |
| NaOH (0.1 M) | 387 nm | 6 |

shows the typical fluorescence spectra of MZA in methanol.

As evident in Fig. 1, there are two wavelengths for excitation, 280 nm and 340 nm, the $\lambda_{max}$ for emission in both cases was constant at 387 nm. Although the Stock shift for the 280/387 nm setting is greater (107 nm) compared with the Stock shift in the 340/387 nm setting (37 nm), the latter setting yields more fluorescence intensity (1.6 times greater than the former setting). The analytical performance data, including the working ranges, regression equations, limits of detection, correlation coefficients at the two wavelength settings are shown in Table 2.

Statistical evaluation of the two regression lines, regarding standard deviation of the residuals ($S_{y/x}$), standard deviation of the slope ($S_b$) and standard deviation of the intercept ($S_a$) are abridged in the same table. The small figures obtained point out to the high precision of the proposed method [7].

To assess the validity of the method, four replicate samples were tested at MZA concentrations of 0.5, 1.0, 1.5 and 2.0 μg/ml, mean % relative fluorescence values at 280/387 nm of $17.9 \pm 0.21$; $32.2 \pm 0.32$; $47.2 \pm 0.37$ and $60.3 \pm 0.41$, respectively, were obtained. The precision of these measurements was expressed by the relative standard deviations which were 1.173; 0.993; 0.782 and 0.78%, respectively, indicating that the results are satisfactorily reproducible.

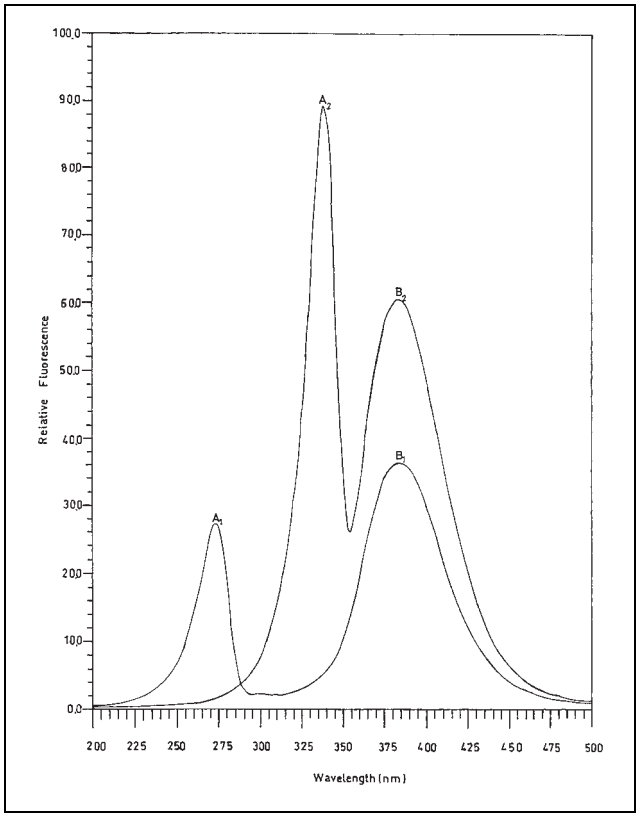



Fig. 1: Typical fluorescence spectra of MZA in methanol (2.0 μg/ml). $A_1$ and $A_2$ Excitation wavelengths. $B_1$ and $B_2$, emission wavelengths

**Table 2: Analytical performance data of the proposed method**

| Parameter | Wavelength setting | |
|---|---|---|
| | 280/387 nm | 340/387 nm |
| Working range (μg/ml) | 0.5–2.5 | 0.1–2.0 |
| Detection limit (μg/ml) | 0.1 ($1.82 \times 10^{-8}$ M) | 0.05 ($9.1 \times 10^{-9}$ M) |
| Regression equation | $F = 1.716 + 29.66\,C$ | $F = 0.252 + 38.75\,C$ |
| Correlation coefficient | 0.9994 | 0.9992 |
| Standard deviation of residuals ($S_{y/x}$) | 1.28 | 1.17 |
| Standard deivation of slope ($S_b$) | 0.57 | 0.57 |
| Standard deviation of intercept ($S_a$) | 0.48 | 0.675 |

The between-day variation was also studied by testing a sample of MZA concentration of 1.0 μg/ml on three successive days. The results are abridged in Table 3. The mean % recovery was $100.01 \pm 0.213$. The results are fairly precise.

The high sensitivity obtained by the proposed method renders it applicable to the determination of MZA in biological fluids. The proposed method could be successfully applied to the assay of MZA in human urine and plasma. The soluble proteins in case of plasma have to be precipitated by methanol followed by centrifugation. The 340/387 nm setting was adopted to get higher sensitivity and to avoid the interference encountered from plasma. The fluorescence-concentration plot is rectilinear over the

**Table 3: Between day variation**

| | Added conc. (μg/ml) | Found (μg/ml) | % Recovery |
|---|---|---|---|
| Day No. 1 | 1 μg/ml | 0.9952 | 99.52 |
| | 1 μg/ml | 1.0097 | 100.97 |
| | 1 μg/ml | 0.9879 | 98.79 |
| | 1 μg/ml | 1.0145 | 101.45 |
| | 1 μg/ml | 1.0193 | 101.93 |
| | 1 μg/ml | 1.0266 | 102.66 |
| | 1 μg/ml | 0.9879 | 98.79 |
| | 1 μg/ml | 0.9855 | 98.55 |
| $\bar{X} \pm SD$ | | | $100.33 \pm 1.5$ |
| Day No. 2 | 1 μg/ml | 1.0128 | 101.28 |
| | 1 μg/ml | 1.0029 | 100.29 |
| | 1 μg/ml | 1.0029 | 100.29 |
| | 1 μg/ml | 1.0029 | 100.29 |
| | 1 μg/ml | 0.9931 | 99.31 |
| | 1 μg/ml | 0.9833 | 98.33 |
| $\bar{X} \pm SD$ | | | $100.01 \pm 0.86$ |
| Day No. 3 | 1 μg/ml | 1.0181 | 101.81 |
| | 1 μg/ml | 0.9715 | 97.15 |
| | 1 μg/ml | 0.9870 | 98.70 |
| | 1 μg/ml | 1.0000 | 100.00 |
| | 1 μg/ml | 1.0018 | 100.78 |
| | 1 μg/ml | 0.9948 | 99.48 |
| $\bar{X} \pm SD$ | | | $99.69 \pm 1.62$ |

**Table 4: Application of the proposed method to the determination of MZA in spiked human urine and plasma**

| Material | Amount added (μg/ml) | Amount found (μg/ml) | % Recovery |
|---|---|---|---|
| Plasma | 0.20 | 0.2036 | 101.81 |
| | 0.40 | 0.4014 | 100.36 |
| | 0.80 | 0.8036 | 100.46 |
| | 1.20 | 1.1663 | 97.19 |
| | 1.60 | 1.6345 | 102.15 |
| | 2.00 | 1.9905 | 99.53 |
| $\bar{X} \pm SD$ | | | 100.25 ± 1.63 |
| Urine | 0.40 | 0.4027 | 100.69 |
| | 0.60 | 0.6000 | 100.00 |
| | 0.80 | 0.8055 | 100.68 |
| | 1.20 | 1.1850 | 98.75 |
| | 1.40 | 1.3872 | 99.09 |
| | 2.00 | 1.9689 | 98.45 |
| $\bar{X} \pm SD$ | | | 99.61 ± 0.90 |

range 0.2–2 μg/ml. Linear regression analysis of the data gave the following equation:

$$F = 15.12\,C - 1.19 \quad (R = 0.9995)$$

where F is the % relative fluorescence.
C is the concentration of MZA in plasma (μg/ml).
The results of analysis of MZA in spiked human plasma are presented in Table 4. The results obtained are satisfactorily accurate and precise. Fig. 2 shows the fluorescence spectra of spiked plasma upon addition of successive amounts of MZA.
Similarly, urine samples spiked with MZA could be successfully analyzed. The method involved just filtration of the sample solution before measurement. The 340/387 nm wavelength setting was also adopted here for the same

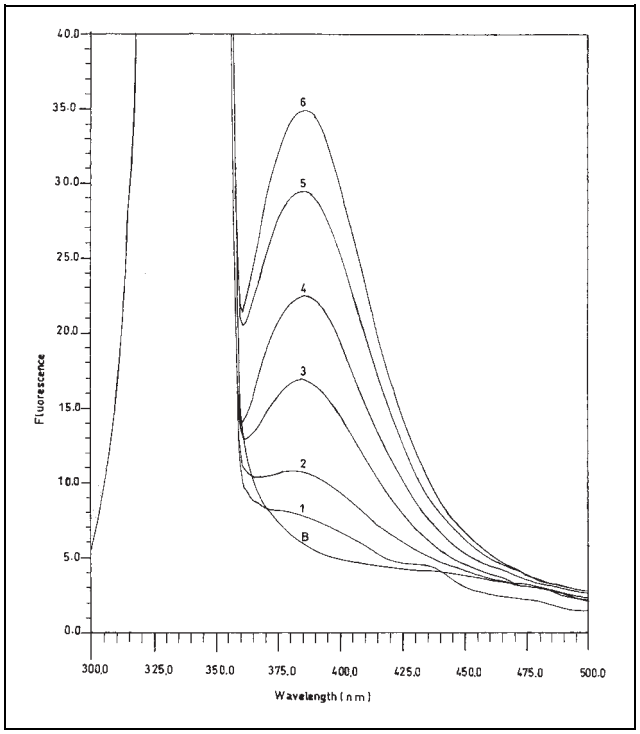



Fig. 2: Fluorescence spectra of plasma spiked with successive concentrations of MZA.
B: Blank
1. 0.2 μg/ml 2. 0.4 μg/ml
3. 0.8 μg/ml 4. 1.2 μg/ml
5. 1.6 μg/ml 6. 2.0 μg/ml

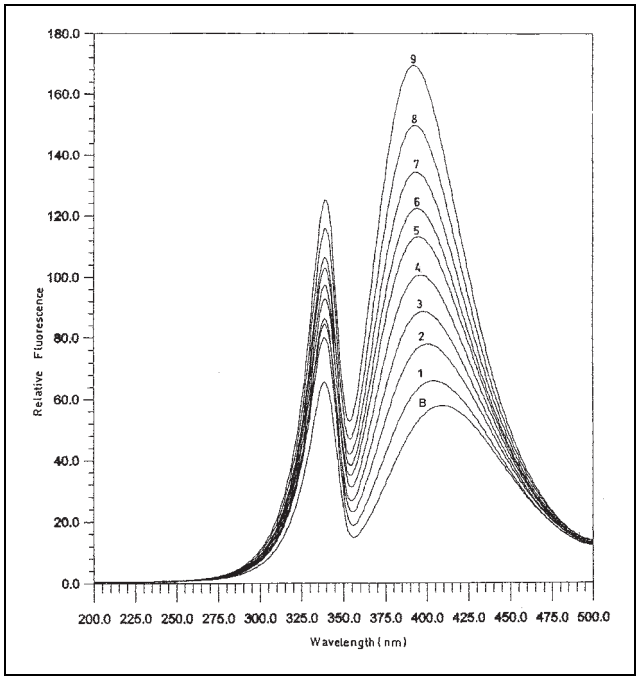



Fig. 3: Fluorescence spectra of urine spiked with successive concentration of MZA.
B: Blank
1. 0.2 μg/ml 2. 0.4 μg/ml
3. 0.6 μg/ml 4. 0.8 μg/ml
5. 1.0 μg/ml 6. 1.2 μg/ml
7. 1.4 μg/ml 8. 1.6 μg/ml
9. 2.0 μg/ml

reasons encountered in case of plasma. The fluorescence-concentration plot is rectilinear over the range 0.4–2.0 μg/ml with the following regression equation:

$$F = 60.3\,C - 0.01 \quad (R = 0.9992)$$

Fig. 3 shows the fluorescence spectra of an urine sample spiked with different amounts of MZA. The results of analysis of spiked urine samples are shown in Table 4. The results are, also, satisfactorily accurate and precise.

## 3. Experimental

### *3.1. Instrumentation*

Kontron Spectrofluorimeter, SFM 25A, equipped with a 150 Xenon – high pressure lamp and driven by a PC Pentium II Computer.

### *3.2. Materials and reagents*

All chemicals and solvents were of analytical reagent grade.
Manzamine A (MZA) was isolated from an undescribed genus of the family Petrosiidue (order Haplosclerida) collected at the Indo-Pacific. It was isolated in 0.66% dry weight yield, using silica gel 60 column (1 : 100, gradient elution) with chloroform/methanol, followed by neutral aluminium oxide column (1 : 20) using *n*-hexane-acetone or cyclohexane-ethyl acetate, gradient elution.
A stock solution (1.0 mg/ml) of MZA was prepared in methanol. The solution was stable for at least one week while kept in the refrigerator. Plasma was obtained from King Khalid University Hospital, Riyadh, Saudi Arabia and kept in the freezer until use, after gentle thawing. Urine was collected from healthy volunteers (men, ~40 years old) and kept frozen until use.

### *3.3. Calibration graph*

Transfer aliquots of the working standard solution into a series of 25 ml volumetric flasks. Complete to the mark with methanol. Measure the fluorescence at 387 nm after excitation at 280 nm or at 340 nm. Plot the relative fluorescence intensity of the solution vs the final concentration (μg/ml) to get the calibration graph. Alternatively, derive the corresponding regression equation.

### *3.4. Application to spiked human plasma*

Into a series of 10-ml measuring flasks containing 0.5 ml of plasma, aliquot volumes of MZA solution (10 μg/ml) were added, in a way that the final concentration is in the range of 0.2–2.0 μg/ml. The volume was adjusted to 10 ml with methanol. The contents were mixed well then transferred to centrifugation tubes and centrifuged at 5000 rpm for 5 min. The clear centrifugate was transferred into the measuring cuvette and the relative fluorescence intensity was measured at 340/387 nm. The concentration of MZA in plasma was determined from the corresponding regression equation.

### *3.5. Application to spiked human urine*

Into a series of 10-ml volumetric flasks containing 0.5 ml of urine, aliquots of a stock solution of MZA were added in a way that the final concentration is in the range 0.2–2.0 μg/ml. The volume was completed with methanol. The contents were mixed well and filtered. The fluorescence of the clear filtrate was measured at 340/387 nm. The concentration of MZA was determined using the corresponding regression equation.

**References**

1 Sakai, R.; Higa, T.; Jefford, C. W.; Bernardielli, G.: J. Am. Chem. Soc. **108**, 6404 (1986)
2 Kara, A. U.; High, T.; Holmes, M.: Patent, Int. Appl. WO1999-SG42 (1999)
3 Mayer, A. M. S.; Gunasekera, S. P.; Pomponi, S. H.; Sennet, S. H.: Patent, Int. Appl. WO 2000-2000US7974 (2000)
4 Zhou, B. N.; Slebodnick C.; Johnson, R. K.; Mattern, M. R.; Kingston, D. G. I.: Tetrahedron **56**, 5781 (2000)
5 El Sayed, K. A.; Kelly, M.; Kara, U. A. K.; Ang, K. K. H.; Katsuyama, I.; Dunbar, D. C.; Khan, A. A.; Hamann, M. T.: J. Am. Chem. Soc. **123**, 1804 (2001)
6 Ang, K. K. H.; Holmes, M. J.; Higa, T.; Hamann, M. T.; Kara, U. A. K.: Antimicrob. Agents Chemother. **44**, 1645 (2000)
7 Miller, J. C.; Miller, J. N.; in: Statistics for Analytical Chemistry, p. 83, Wiley, New York 1984