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# Gas chromatography-mass spectrometry determination of matrine in human plasma

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**Abstract**

A method was developed for the quantification of matrine in human plasma using a liquid–liquid extraction procedure followed by gas-chromatography-mass spectrometry (GC/MS) analysis. Deuterated matrine, an internal standard of the analysis, was spiked into the plasma samples before extraction. Linear detection responses were obtained for matrine concentrations ranging from 10 to 500 ng/ml. The intra-day and inter-day precision ranged from 0.4 to 4.0% and 1.0–3.5%, respectively. The intra-day accuracy was between −7.3 and 4.5%. The limit of quantification for matrine was 23 ng/ml. The extraction efficiency averaged about 38%. The validated GC/MS method will be used to quantify matrine in human plasma samples collected in a clinical trial study.


*Keywords:* Matrine

## 1. Introduction

The roots of *Sophora tonkinensis*, *S. alopecuroides*, and *S. flavescens* have a wide range of pharmacological and toxicological activities [1]. Therefore, these roots have been used as traditional herbal medicine in China, Japan, and Korea to treat diseases such as cancer, viral hepatitis, cardiac arrhythmia, asthma, and skin problems [2].

About 2% of the *S. flavescens* roots (dry weight) are composed of alkaloids with matrine (Fig. 1a) and oxymatrine as the major components [3]. Several minor alkaloids including sophoranol, sophocarpine, and sophordine also have been found in the *S. flavescens* roots [4].

Although the *S. flavescens* roots have been used for disease treatment, very little is known of the absorption, distribution, metabolism and excretion of matrine and oxymatrine in humans. After oral administration to human or animals, oxymatrine was converted by the gastrointestinal tract and liver to matrine [5]. In the only pharmacokinetic study reported for humans [6], a large dose of pure matrine (6 mg/kg) was infused intravenously to the experimental subjects and blood samples were withdrawn at specific time points post-dosing. A HPLC/UV method was used to determine matrine concentrations in the blood, which ranged from 1 to 6 μg/ml [6]. However, the HPLC/UV method probably is not sensitive enough for detecting matrine in the plasma of humans receiving an oral dose of the *S. flavescens* roots due to the small amount of matrine in *S. flavescens* roots and the low bioavailability of matrine in humans. The concentrations of matrine in the blood of humans after ingestion of *S. flavescens* roots is presently unknown but they are expected to be much lower than 1 μg/ml.

The purposes of the present work were to develop and validate a sensitive GC/MS method, which could be used to detect matrine in the plasma of human at low ng/ml concentrations. The novel GC/MS method used deuterated matrine as the internal standard and liquid–liquid extraction for sample preparation.

## 2. Experimental

### 2.1. Reagents and chemicals

Chemicals were purchased from the following sources: matrine from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

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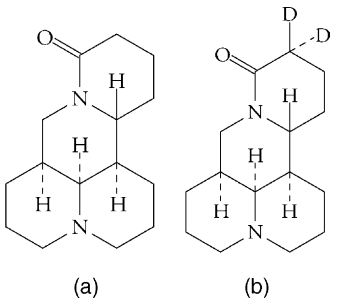


Fig. 1. Chemical structures of: (a) matrine and (b) its corresponding deuterated analogue.

Toluene and hydrochloric acid from Anachemia Co. (Montreal, Que., Canada). Petroleum ether and sodium sulfate from BDH (Toronto, Ont., Canada). Sodium hydroxide solution (1 M) was from VWR (West Chester, PA, USA). Butanol from Caledon (Georgetown, Ont., Canada). Sodium deuteroxide (40%, in water), deuterium oxide and deuterated chloroform from CDN Isotope (Pointe-Claire, PQ, Canada). Deuterated ethyl alcohol and absolute tetrahydrofuran from Aldrich (Milkwankee, WI, USA). Human plasma from the blood bank of the Royal Columbian Hospital (New Westminster, BC, Canada).

### 2.2. Preparation of the deuterated internal standard

Deuterated derivative of matrine (14, 14-$D_2$-matrine) (Fig. 1b) was prepared as follows: matrine (100 mg), 40% sodium deuteroxide (0.5 ml), deuterium oxide (5 ml), deuterated ethyl alcohol (5 ml) and absolute tetrahydrofuran (15 ml) were mixed in a 50-ml round bottom flask equipped with a reflux condenser. The mixture was refluxed for 4 days. After cooling to room temperature, the mixture was dried in a rotatory evaporator. The residues were re-dissolved in 5 ml of deuterated chloroform and the isotopic composition of matrine was assessed by GC/MS. The procedure was repeated until both hydrogens at position 14 of the matrine molecule were replaced by deuterium. The reaction mixture was extracted with deuterated chloroform, passed through a column of sodium sulfate, and dried in a rotary evaporator. The residues were re-dissolved in petroleum ether for recrystallization. The crystals were collected and stored in a desiccator.

### 2.3. Equipment and chromatographic conditions

A Hewlett-Packard 5890 series II gas chromatograph coupled to a 5971 mass spectrometric detector was used in the study. Chromatographic separation was performed using a 5% diphenyl-95% dimethylpolysiloxane capillary column (30 m × 0.25 mm × 0.25 μm, HP-5 MS). Helium was used as the carrier gas under a head pressure of 50 psi. The injector and transfer line temperatures were set at 250 and 280 °C, respectively. The initial oven temperature was set at 110 °C, maintained for 1 min and then increased to 220 °C at a rate of 30 °C/min and maintained for 1 min. The temperature was further increased to 300 °C at a rate of 15 °C/min and maintained for 3 min. Ionization was performed by electron impact with 70 eV.

Matrine and deuterated matrine were analyzed in the selected ion monitoring (SIM) mode; the ions *m/z* 248 and *m/z* 250 were selected to monitor matrine and deuterated matrine, respectively, due to their abundance and specificity. The ion *m/z* 249 also was recorded but was not used in matrine quantification.

### 2.4. Analytical procedure

#### 2.4.1. Calibration curve and quality control samples

Standard solutions of matrine were prepared in doubly distilled water. A new set of standard solutions was prepared for each batch of samples. Calibration and quality control (QC) standards [7] were prepared by mixing various amounts of unlabeled matrine with a fixed amount of deuterated matrine (250 ng) in 1 ml of human plasma. Standard concentrations of 10, 15, 25, 50, 100, 250, and 500 ng/ml were used to prepare the calibration curve. QC sample concentrations were 10, 50, 100, and 250 ng/ml.

#### 2.4.2. Extraction procedure

An aliquot (50 μl) was removed from a stock solution of deuterated matrine (5000 ng/ml) and put into a 10-ml screw-capped glass centrifuged tube containing either a calibration or a QC standard (Section 2.4.1). NaOH (0.5 ml, 1 M) was added to make the solution alkaline. After adding 3 ml of toluene:butanol (v/v 7:3), the centrifuge tube was capped and shaken on a mechanical shaker for 15 min. The centrifuge tube was centrifuged at 3000 rpm to separate the layers. The organic layer was removed and put into a new screw-capped tube containing 0.5 ml HCl (0.25 M). The centrifuge tube was shaken on a mechanical shaker and then centrifuged to separate the layers. The organic layer was discarded. The remaining aqueous layer was made alkaline with the addition of 0.5 ml NaOH (1 M). After the addition of 200 μl toluene:butanol (v/v 9:1), the mixture was shaken again on the mechanical shaker. The organic layer was separated by centrifugation and transferred to a GC vial for GC/MS analysis.

#### 2.4.3. Validation of the method

*2.4.3.1. Linearity.* The area ratios of matrine (*m/z* 248) and deuterated matrine (*m/z* 250) peaks in the GC/MS chromatograms of the calibration standards were calculated. These ratios were plotted against their nominal concentrations to generate the calibration curve in Excel® using the un-weighted least-squares linear regression analysis.

*2.4.3.2. Precision and accuracy.* Precision and accuracy of the method were determined by the reproducibility of the

QC samples within and between different batches of samples. Precision was determined by the coefficients of variation (%CV). Accuracy of the method was determined by the relative error (%RE), which was calculated by the equation: (mean of determined concentration − actual concentration)/mean of determined concentration) × 100.

*2.4.3.3. Specificity.* Specificity of the method was performed by comparing the chromatograms from a blank plasma sample with a plasma sample spiked with either matrine or deuterated matrine.

*2.4.3.4. Limits of quantitation (LOQ).* The LOQ was determined as the concentrations to produce a signal that is 10-fold of the error on the regression line (see Section 2.4.3.1).

*2.4.3.5. Recovery.* To determine the recovery of matrine by the liquid–liquid extraction method, 1 ml of plasma was spiked with deuterated internal standard (250 ng) and extracted using the above procedure (see Section 2.4.2). Matrine (50 or 250 ng) was added either before or after extraction. Recovery was calculated by comparing the area ratios of the matrine and deuterated matrine peaks obtained from adding matrine before and after extraction.

*2.4.3.6. Stability.*

*Autosampler stability.* QC samples from the first day were kept on the autosampler of the GC/MS at room temperature for 24 h and injected again the next day. Matrine was quantified using the calibration curve constructed on the next day.

*Stock solution stability.* Aqueous stock solutions of matrine (1.1, 2.2, and 5.5 μg/ml) were stored in the refrigerator for 7–14 days at 4 °C. On days 7 and 14, these stock solutions were spiked into human plasma samples and extracted as described above (see Section 2.4.2). Matrine was quantified using the calibration curves constructed with stock solutions freshly prepared on days 7 and 14.

*Freeze–thaw stability.* Matrine was spiked into human plasma at 50, 100, and 250 ng/ml and stored at −80 °C temperature. The samples were subjected to three freeze–thaw cycles and then extracted. The amount of matrine in the plasma samples after three freeze–thaw cycles was determined using a newly prepared calibration curve.

*Short-term stability.* Matrine was spiked into human plasma at 50, 100, and 250 ng/ml and stored at −80 °C for 14 days and then extracted. The amount of matrine in the plasma samples after 14 days was determined using a newly prepared calibration curve.

## 3. Results and discussion

The present work describes the development and validation of a GC/MS method, which uses deuterated matrine as the internal standard to determine matrine concentration in human plasma. Although both hydrogens at position 14 of the matrine molecule (see Fig. 1a) could be activated with respect to deuterium exchange under basic conditions [8], less than 30% isotopic enrichment was achieved using sodium deuteroxide synthesized in situ from deuterium oxide and sodium at our laboratory. However, when the reaction was conducted with sodium deuteroxide and deuterated chloroform purchased from commercial sources, about 97% of the protons at position 14 of the matrine molecule were replaced by deuterium atoms. It should be noted that complete exchange of both hydrogens at position 14 was not possible and thus the deuterated matrine formed was a mixture of $D_1$ and $D_2$ isotopomers (see Table 1).

Fig. 2a and b show the mass spectra of matrine and its deuterated analogue, respectively. It is clear that the *m/z* value of the parent molecular ion has increased from 248 in the matrine spectrum (Fig. 2a) to 250 in the deuterated matrine spectrum (Fig. 2b), i.e., an increase of two units. In both mass spectra, the M-29 and M-43 peaks probably represent the M–$C_2H_5$ and M–$C_3H_7$ fragments, respectively. The 221 (M-29) and 207 (M-43) ions in Fig. 2b also provide the evidence that the C-D bonds of deuterated matrine are stable since the C-D bonds appear remaining intact even after the fragmentation process.

GC/MS analysis was conducted on human plasma samples spiked either with matrine or deuterated matrine. Typical chromatograms of matrine as monitored at *m/z* 248 and 250 are shown in Fig. 3a. Similarly, typical chromatograms of deuterated matrine are shown in Fig. 3b. As expected, matrine co-eluted with deuterated matrine under the present chromatographic conditions; both compounds had a retention time of 8.45 min. Chromatographic analysis of blank plasma (Fig 3c) confirmed that there were no endogenous peaks that co-eluted with the matrine peak. These results show that the GC/MS method is specific for the detection of matrine in human plasma.

Fig. 4 shows a typical calibration curve of the GC/MS analysis. Quantitation was based on the internal standard method. The response for matrine is linear over the concentration range of 10–500 ng/ml, with a correlation coefficient greater than 0.999. The LOQ of the method was determined to be 23 ng/ml of matrine. In addition, the LOD

Table 1
Isotopic data for matrine and the deuterated analogue

| *m/z* | Matrine (%)* | Deuterated matrine (%)* |
|---|---|---|
| 248 | 83.3 | 3.3 |
| 249 | 15.2 | 44.3 |
| 250 | 1.5 | 52.4 |

* Data expressed as percentage of total corrected area obtained using selected ion monitoring mode of GC/MS.

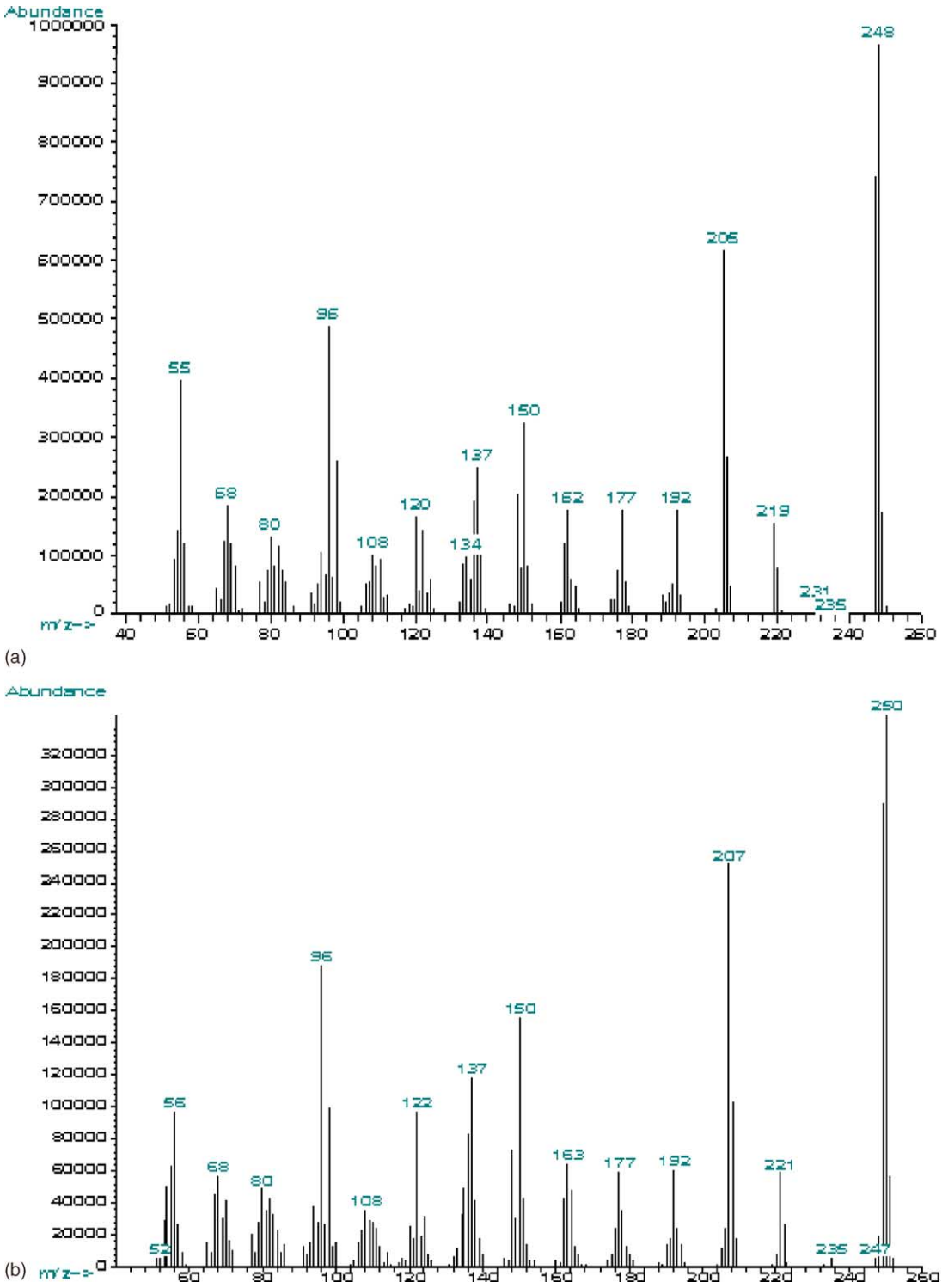


Fig. 2. Mass spectra of: (a) matrine and (b) deuturated matrine.

was found to be 7 ng/ml. As compared to the LOQ of the HPLC/UV method (about 1.25 μg/ml) [6], the GC/MS method markedly improves the LOQ of matrine by about 54-fold.

Inter- and intra-day precisions were estimated by comparing the QC samples extracted on the same day and on a different day. The intra-day precision (% coefficient of variation) was found to range from 0.4 to 4.0% CV (see Table 2). The inter-day precision was found to range from 1.0 to 3.5% CV. In addition, the accuracy (% residual error) was found to range from −7.3 to 4.5% RE (see Table 2). Recovery of matrine by the extraction procedure was found to be about 38%.

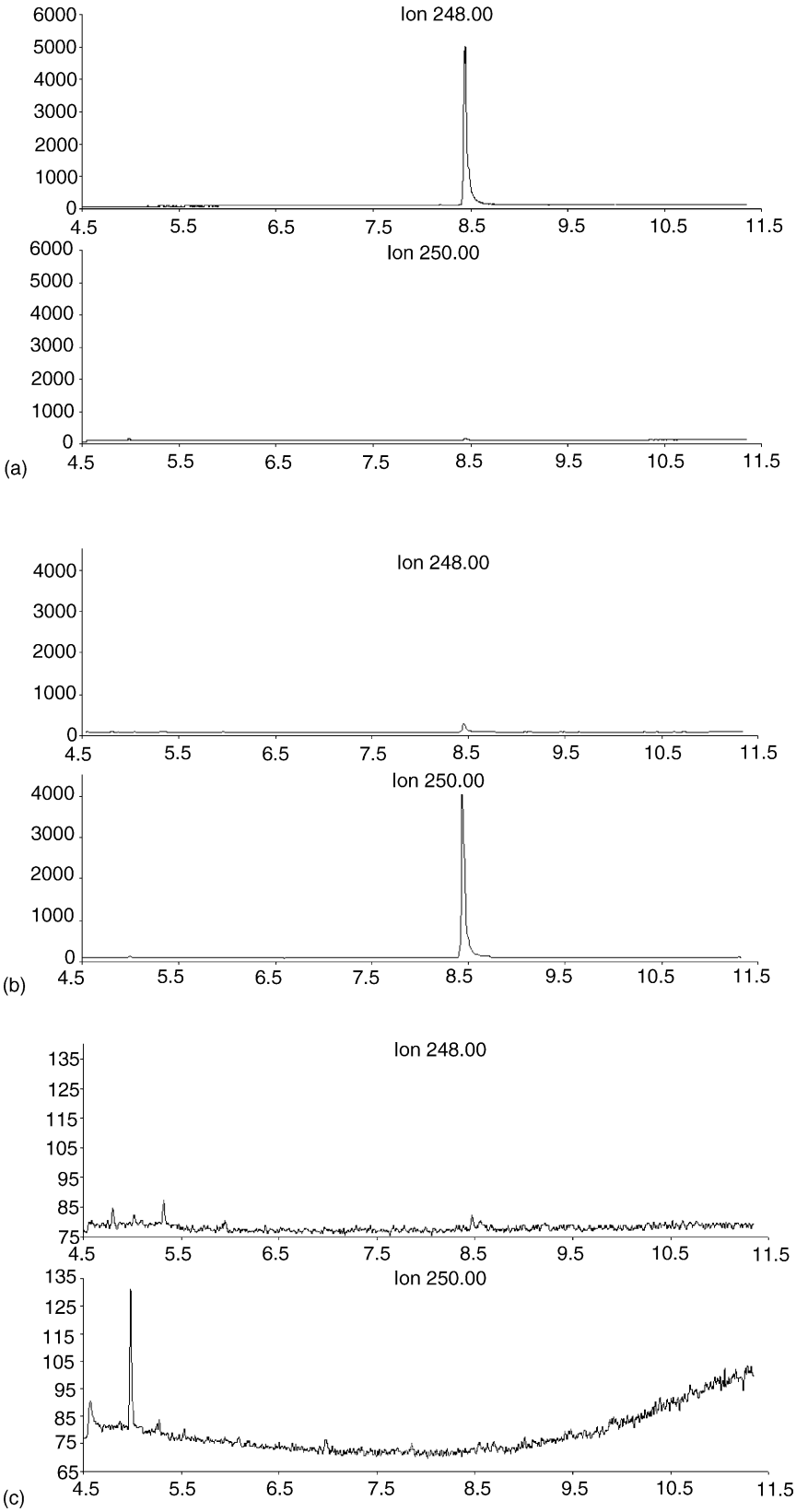


Fig. 3. (a) Gas chromatograms of human plasma spiked with matrine. (b) Gas chromatograms of human plasma spiked with deuterated matrine. (c) Gas chromatograms of blank human plasma. In each case, the top chromatogram is monitored at *m/z* 248 in the selected ion monitoring mode; the bottom chromatogram is monitored at *m/z* 250. The *x*-axis represents time in minute, and the *y*-axis represents ion abundance.

Table 2
Accuracy and precision of QC sample pools over a 4-day period*

| Concentration | Day 1 | | Day 2 | | Day 3 | | Day 4 | |
|---|---|---|---|---|---|---|---|---|
| | %RE | %CV | %RE | %CV | %RE | %CV | %RE | %CV |
| QC-10 (ng/ml) | 0.0** | 4.0 | −7.3 | 3.9 | 0.3 | 4.2 | −6.0 | 2.1 |
| QC-50 (ng/ml) | 2.2 | 2.9 | 1.6 | 0.7 | −4.4 | 2.9 | 0.0 | 2.6 |
| QC-100 (ng/ml) | 1.9 | 2.2 | 4.5 | 3.2 | 1.4 | 4.5 | 3.2 | 0.9 |
| QC-250 (ng/ml) | 0.4 | 0.4 | 3.2 | 2.2 | −0.2 | 0.7 | 2.4 | 0.7 |

* Accuracy (% residual error); precision (% coefficient of variation).
** Mean of triplicate samples.

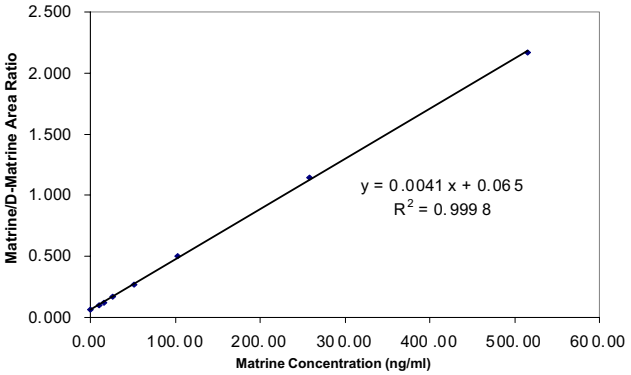


Fig. 4. Calibration curve of matrine.

Table 3
Stability of matrine and deuterated matrine stored in autosampler*

| Matrine concentration | Peak area ratio of 248/ 250 | |
|---|---|---|
| | 0 h | 24 h |
| 10 (ng/ml) | 0.105 ± 0.004 | 0.101 ± 0.001 |
| 50 (ng/ml) | 0.278 ± 0.008 | 0.268 ± 0.003 |
| 100 (ng/ml) | 0.493 ± 0.011 | 0.481 ± 0.003 |
| 250 (ng/ml) | 1.124 ± 0.005 | 1.098 ± 0.009 |

* Results represent mean + S.D. ($n = 3$).

The stability of matrine was established for the GC/MS autosampler, freeze–thaw cycle, storage time of the stock and sample solutions. The amount of matrine remaining after all these tests was found to be very close to 100%. In other words, matrine was not degraded under these conditions. For instance, the stability data of matrine and deuterated matrine in plasma samples stored in the autosampler for 24 h is given in Table 3. In each QC sample pool, the ratios of the peak area obtained at *m/z* 248 to that at *m/z* 250 are compared at 0 h and 24 h. Since there is no significant change in this ratio (at 99% confidence level), we conclude that both matrine and deuterated matrine are stable.

In conclusion, a validated GC/MS method has been developed for the detection of matrine in human plasma. Results of the study show that the GC/MS method is sensitive, specific, accurate, and precise. This method will be used to determine the pharmacokinetic profile of matrine in the plasma of human volunteers after receiving an oral dose of the *S. flavescens* roots.

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