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A sensitive, rapid and validated liquid chromatography – tandem mass spectrometry (LC-MS-MS) method for determination of Mimosine in *Mimosa pudica* Linn

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ABSTRACT

A rapid, sensitive and accurate liquid chromatographic tandem mass spectrometric method is described for the determination of Mimosine in *Mimosa pudica* Linn. whole plant powder. Mimosine was extracted from the plant using 1.0% HCl in water. The chromatographic separation was achieved using a Thermo Hypurity C18 (50 x 4.6 mm) 5.0 µ column interfaced with a triple quadrapole mass spectrometer. The mobile phase consisted of a mixture of Methanol: 10 mM ammonium formate buffer whose pH was adjusted to 3.00 ± 0.05 with formic acid (80:20, v/v) and was delivered at a flow rate of 1.0 mL min^{-1}. Electrospray ionization (ESI) source operated in the negative ion mode was used for the quantitation. Detection was performed using an Applied Biosystems Sciex API 3200 Mass spectrometer. The method was found to be simple, precise, accurate, fast, specific and sensitive and can be used for routine quality control analysis of Mimosine in *Mimosa pudica* Linn.

Keywords: LC-MS-MS; Mimosine; *Mimosa pudica* Linn

1. INTRODUCTION

Mimosa pudica Linn. (Fam. -Leguminosae) is commonly known as Sensitive plant in English and Lajvanti or Chuimui in Hindi language. The plant is distributed through out India especially in moist places. *Mimosa pudica* Linn. is also said to have larvicidal property [1]. It is used to treat menorrhagia and leucorrhoea [2-4]. In Ayurvedic system of medicine, *Mimosa pudica* Linn. has been described as an indispensable drug for blood pressure [5]. Phytochemical screening has revealed that the plant contains Mimosine (alkaloid), stigmasterol, leucoanthocyanidin, D-xylose and D-glucuronic acid, norepinephrine, D-pinitol, linoleic acid, oleic acid, palmitic acid, stearic acid, β-sitosterol and crocetin dimethyl ester. Of all these, the major compound present in *Mimosa pudica* Linn. is Mimosine [4]. Mimosine is used for treating the cutaneous effects of psoriasis and related skin disorders [6]. It is less soluble in methanol and ethanol, insoluble in other organic solvents, but sparingly soluble in water. It is soluble in dilute acid and base. Structure of Mimosine is shown in **Figure 1** [7].

The quality of herbal medicine that is the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and modern analytical techniques are accepted to help in circumventing this problem [8]. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization [9].

Quantitation of Mimosine from *Mimosa pudica* L. using RP-HPTLC has been reported [10]. A method using HPLC and spectrophotometric determination of Mimosine has also been reported [11,12]. Literature survey, hence, revealed that there is no method available in the

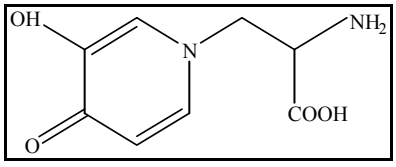

Figure 1. Structure of Mimosine.

public domain for quantitation of Mimosine from *Mimosa pudica* Linn. using an LC-MS-MS system. So, the aim of the present work was to develop a simple, fast, sensitive, precise, and accurate LC-MS-MS method for determination of Mimosine from *Mimosa pudica* L. The developed method was further validated as per ICH guidelines to indicate its suitability [13,14].

2. Experimental

2.1. Chemicals and Preparation of Standard Solutions

HPLC grade Methanol and acetonitrile were purchased from J.T. Baker, Mumbai, India. Extra pure Formic acid (99.9%) and ammonium formate was purchased from Fluka, Steinheim, Germany. High purity deionised water was prepared in-house using a Milli-Q water purification system obtained from Millipore, Bangalore, India. Mimosine standard (Purity 98%) was procured from Sigma-Aldrich (Aldrich Division; Steinheim, Federal Republic of Germany).

The stock solution A of Mimosine (1,000 µg mL^{-1}) was prepared by dissolving 25 mg of accurately weighed Mimosine in minimum quantity of 1.0% HCl in water and diluting with same solution up to the mark in a 25 mL standard volumetric flask. Further solution B of Mimosine (10 µg mL^{-1}) was prepared by transferring 0.25 mL of stock solution A and diluting with mobile phase up to the mark in a 25 mL volumetric flask. Different volumes in the range of 0.4-1.0 mL of stock solution B were transferred to 10 mL standard volumetric flasks and diluted up to the mark with the mobile phase, to provide a concentration range of 400-1000 ng mL^{-1}.

2.2. Plant Material and Preparation of Sample Solution

The plant *Mimosa pudica* L. was collected from Mumbai, Maharashtra, India and was authenticated by National Institute for Science Communication and Information Resources (NISCAIR), New Delhi, India. The collected plant material was dried at room temperature, under shade and then ground in a mixer to a fine powder. This was then passed through an ASTM BSS mesh (size 85) and stored in an airtight container at room temperature. 25 mg of the dried powder was accurately weighed, placed in a stoppered tube and 10 mL of Methanol was added. The sample was vortexed for 1-2 minutes and then mixed on a shaker for 60 minutes. The contents of the tube were then centrifuged at 4600 rpm and filtered through Whatmann No. 41 filter paper (E. Merck, Mumbai, India) and residue was collected in a 10 mL standard volumetric flask and 1.0% HCl in water was added up to the mark, the sample was vortexed for 1-2 minutes and left to stand overnight at room temperature. The content was filtered through Whatmann No. 41 filter paper and the clear supernatant was collected in a dry tube (solution C). Further solution D was prepared by transferring 1.0 mL of solution C and diluting with mobile phase up to the mark in a 10 mL volumetric flask. Solution D was used for further experiments.

2.3. Instrumentation and Chromatographic Conditions

A Hypurity C_{18}, (50 × 4.6 mm), 5 µ obtained from Thermo Electron, Mumbai, India was used for the compound retention. The mobile phase consisted of mixture of Methanol: 10mM ammonium formate buffer pH adjusted to 3.00 ± 0.05 with formic acid (80:20, v/v) and was delivered at a flow rate of 1.0 mL min^{-1} by employing a Shimadzu Prominence series (Kyoto, Japan) binary pump, at ambient temperature. Detection was achieved using an Applied Biosystems API 3200 MS-MS apparatus (Applied Biosystems, Ontario, Canada) fitted with a Turbo Ion Spray source. The instrument was interfaced with a computer running Applied Biosystems Analyst version 1.4.2 software. Electrospray ionization (ESI) was performed in the negative ion mode. The spray voltage and source temperature were −4500 V and 550°C respectively. Nitrogen was used as the collision gas. The Declustering Potential (DP), Collision Energy (CE), Entrance potential (EP), Cell Exit Potential (CXP) were optimized during tuning as −20, −24, −10, −4 eV for Mimosine. The collision activated dissociation (CAD) gas was set at 3 psi, while the curtain gas was set at 12 psi. The Applied Biosystems API 3200 LC-MS-MS apparatus was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the molecular ion m/z 197.7 to the product ion m/z 162.8 for Mimosine. The instrument response was optimized for Mimosine by infusing a constant flow of a standard solution (1000 ng mL^{-1}) via a T-piece into the stream of mobile phase eluting from the column. **Figure 2** shows the product ion mass spectra obtained from collision-induced dissociation of the deprotonated molecular ions of Mimosine.

3. METHOD VALIDATION

3.1. System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by injecting 10 µL of standard solution of Mimosine (600 ng mL^{-1}) six times. The % RSD was found to be 1.27% for Mimosine, which was acceptable as it is less than 2%.

3.2. Linearity

In order to establish linearity, standard solutions of Mi-

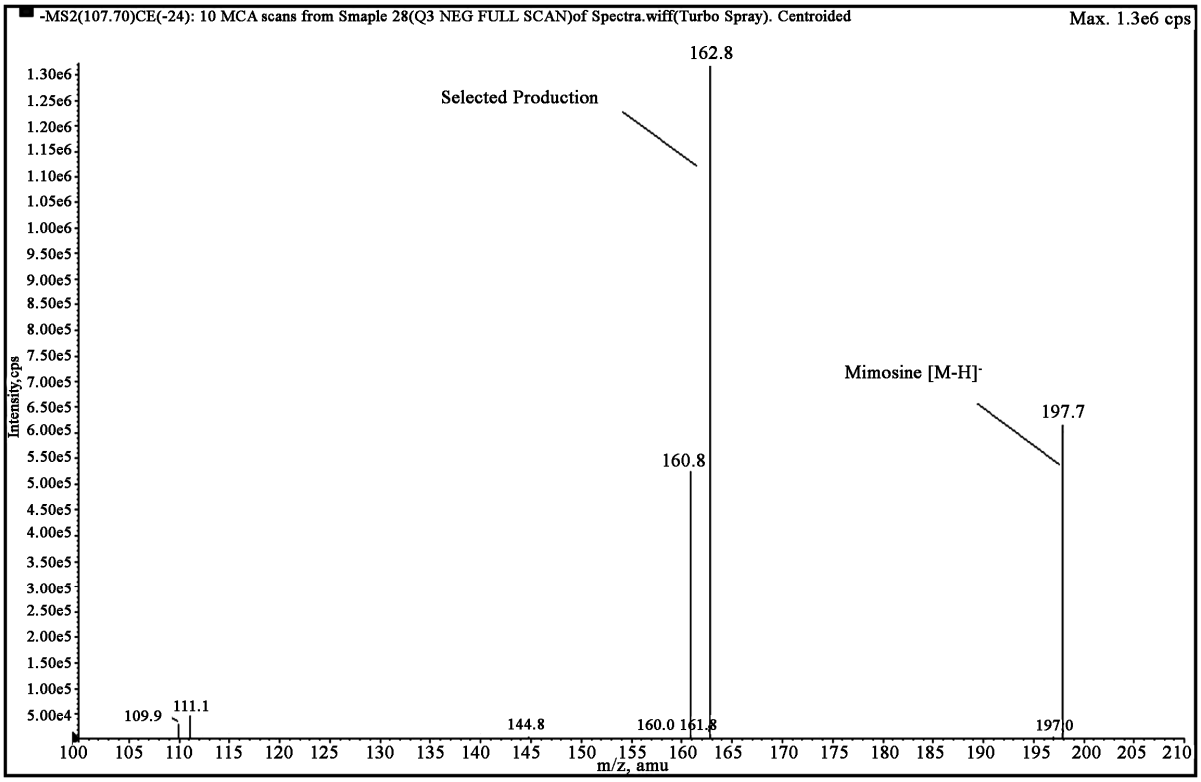

Figure 2. Representative Spectra of product ion of Mimosine.

mosine at six different concentrations (400.0, 5‰00.0, 600.0, 700.0, 800.0 and 1000.0 ng mL^{-1}) were prepared in mobile phase. Each of these solutions (10 µL) was injected and the detector response for the different concentrations was measured. A graph was plotted of drug peak area against concentration. The plot was linear in the range 400.0 ng mL^{-1} to 1000.0 ng mL^{-1} for Mimosine. The experiment was performed five times and the mean was used for the calculations. The equation of linear regression curve obtained was y = 94.4x − 1923.3, where y = (peak area), x = (concentration of Mimosine in ng mL^{-1}) with a correlation coefficient 0.9951. A typical chromatogram of standard and plant is shown in **Figure 3** and **Figure 4** respectively.

3.3. Limit of Detection and Limits of Quantitation

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. The LOD and LOQ of Mimosine were 100 ng mL^{-1} and 400.0 ng mL^{-1}, respectively.

3.4. Assay

The developed LC-MS-MS method was used for determination of Mimosine from whole plant powder of *Mimosa pudica L.* The sample working solution D (10 µL) was injected and the area of Mimosine peak was measured. From the calibration curve, the amount of Mimosine in dry powder of *Mimosa pudica* L. was calculated. The retention time of Mimosine in sample solution and in the standard solution was found to be 0.67 min. The mean assay value of Mimosine was found to be 1.938 mg/g of plant powder with % RSD as 1.55%.

3.5. Precision and Accuracy

The intra-day and inter-day precision was used to study the variability of the method. The % RSD for intra-day and inter-day precision for Mimosine were 0.66 and 1.06%, respectively. Accuracy of the method was studied using the method of standard addition. Standard Mimosine solution were added to the extract of the whole plant powder of *Mimosa pudica* L. and the percent recovery was determined at two different levels 50% and 100%. Mimosine content was determined and the percent recovery was calculated. The results of recovery analysis are shown in **Table 1**.

4. RESULTS AND DISCUSSION

The high selectivity of MS-MS detection allowed the development of a very specific and rapid method for the determination of Mimosine in *Mimosa pudica* L. whole

Table 1. Results of recovery experiment.

Standard	Level	Pre analysed sample in (ng mL^{-1})	Amount of std added to pre analysed sample in (ng mL^{-1})	Total amount of std found in (ng mL^{-1})	SD	RSD (%) (n = 7)	Recovery (%)
	0	484.59	0	479.13	6.72	1.40	98.87
Mimosine	50%	484.59	250	730.79	7.40	1.01	99.48
	100%	484.59	500	979.39	10.96	1.12	99.47
						Mean	99.28

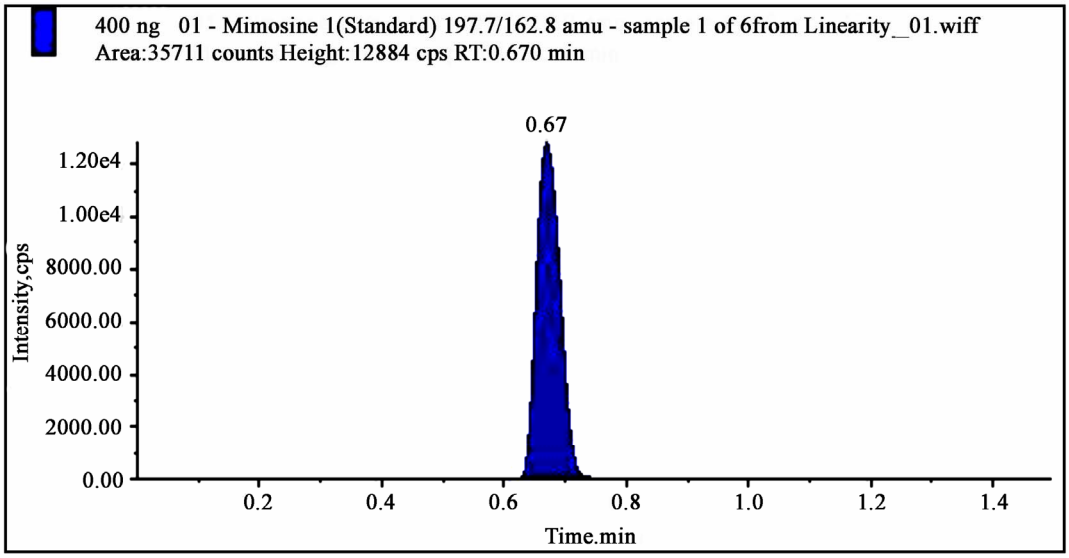

Figure 3. Representative chromatogram of standard Mimosine at LLOQ level (400 ng mL^{-1}).

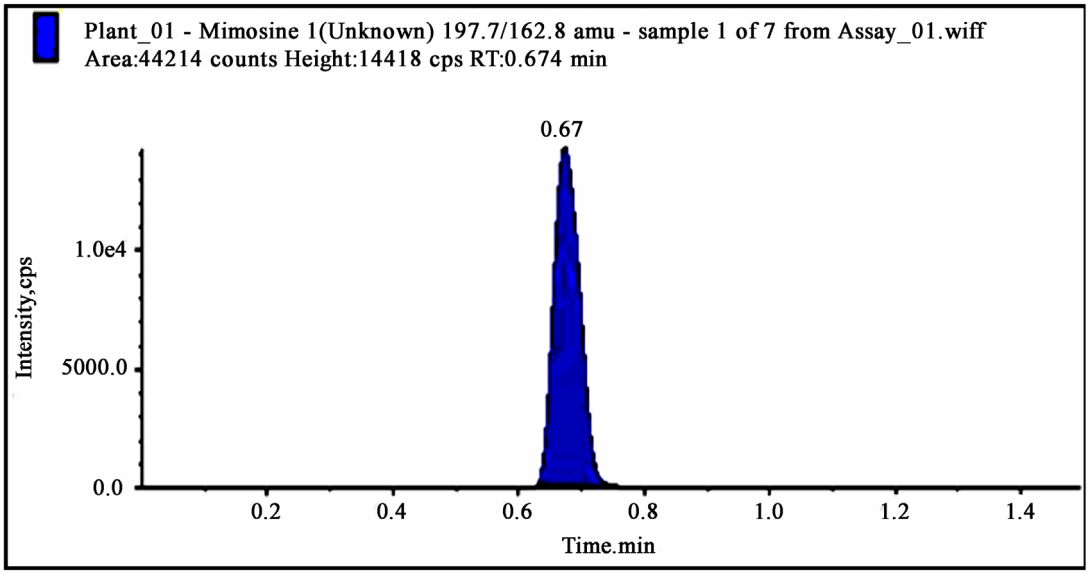

Figure 4. Representative chromatogram of plant *Mimosa pudica* L.

plant powder. During method development different options were evaluated to optimize, detection parameters and chromatography. Electrospray ionization (ESI) was evaluated to get better response of analytes as compared to atmospheric pressure chemical ionization (APCI) mode. It was found that the best signal was achieved

with the ESI negative ion mode. A mobile phase containing formic acid solution and Methanol in varying combinations was tried during the initial development stages. But the best signal for Mimosine was achieved using a mobile phase containing 10mM ammonium formate buffer pH adjusted to 3.00 ± 0.05 with formic acid in combination with Methanol (20:80 v/v). Use of a short Hypurity C_{18}, (50 mm × 4.6 mm), 5 µ column resulted in reduced run time of 1.5 min. Regression analysis of calibration data showed that the linearity of Mimosine was observer over a concentration range of 400 ng mL^{-1} to 1000 ng mL^{-1} with regression coefficient of 0.9951. The concentration of Mimosine in 1.0 g of whole plant powder of *Mimosa pudica* L. was found to be 1.938 mg.

When the method was validated in terms of instrumental precision, intra-assay precision and intermediate precision, the percent RSD values were found to be less then 2, indicating that the proposed method is precise and reproducible. The accuracy of the method was established by means of recovery experiments. The mean recovery was close to 100%, which indicates that method is accurate. The low values of %COV for replicate analyses are indicative of precision of the method. The method is specific because it resolved the standard Mimosine (Retention time = 0.67) well in presence of other phytochemicals of whole plant powder of *Mimosa pudica* L.

5. CONCLUSIONS

A new LC-MS-MS method has been developed for quantification of Mimosine from whole plant powder of *Mimosa pudica* L. The method developed with careful validation was found to be fast, simple, precise, sensitive and accurate. The linearity, precision, accuracy of the method prove that the method is easily reproducible in any quality control set-up provided all the parameters are followed accurately.

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