Analysis of strychnine from detoxified Strychnos nux-vomica seeds using liquid chromatography–electrospray mass spectrometry

Young Hae Choi a, You-Min Sohn a, Chul Young Kim a, Kwan Yul Oh b, Jinwoong Kim a,∗

a College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Republic of Korea
b GreenTek21 Co., Ltd., Shinsu-dong 1, Mapo-ku, Seoul 121-742, Republic of Korea

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Abstract

The content of strychnine from Strychnos nux-vomica seeds was analyzed and compared to processed seeds by the HPLC–ESI/MS method. Using this technique, levels as low as 1 ng of strychnine were detected. In contrast to conventional UV detectors, this method also made it possible to discriminate brucine. This study resulted in finding the content of strychnine in detoxified seeds to be one tenth of unprocessed Strychnos nux-vomica seeds.

Keywords: Strychnos nux-vomica seeds; Detoxification; HPLC–ESI/MS

1. Introduction

Strychnos nux-vomica L. (Loganiaceae) seeds have been used in activating the channels, alleviating pain, reducing swelling, and moving the blood in oriental medicine (Benoky and Gamble, 1986). The pharmacological effects of this plant have also been known to increase spinal reflexes and stimulate respiratory and sensory centers of the cerebral cortex (Chung and Shin, 1989). In oriental medicine, Strychnos nux-vomica seeds have been used in combination with aconite roots to treat spasms, numbness, or weaknesses associated with wind damp painful obstructions, and with myrrh to treat trauma-induced pain, swelling, fractures and sprains topically, and with sophora roots to treat severe and painful swelling of the throat (Bensky and Gamble, 1986).

The main constituents of Strychnos nux-vomica seeds were known to be alkaloids, such as strychnine, brucine, and vomicine (Fig. 1) (Han, 1988). Larger doses of strychnine are known to be a deadly poison, which leads to violent muscular convulsions. In addition, strychnine is known to be severely toxic in human beings, although small doses of this compound can give subjective feelings of stimulation (Samulesson, 1992).

Therefore, the detoxification method has been used to reduce the toxicity of Strychnos nux-vomica seeds in traditional oriental medicine. The contents of the major alkaloids, such as strychnine and brucine declined significantly with increased amounts of isostrychnine, isobrucine, strychnine N-oxide, and brucine N-oxide using thermal treatment (Cai et al., 1990).

Previously, the decreased amount of strychnine was measured by a conventional TLC method (Cai et al., 1990). In this paper, we established the optimal detection method of strychnine using high performance liquid chromatography, equipped with an electrospray ionization mass spectrometer (HPLC–ESI/MS). The amount of strychnine in unprocessed and detoxified Strychnos nux-vomica seeds was compared using this method.

2. Methodology

2.1. Plant material

The seeds of Strychnos nux-vomica L. were obtained from the Korea Export and Import Federation of Drugs, Seoul, Korea. The authenticity was confirmed by one of the authors. A voucher specimen (SNUSNS-001) has been deposited at the Herbarium of Medicinal Plant Garden at the College of Pharmacy at Seoul National University. The plant materials were dried at 40 °C for 24 h.

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2.2 Chemicals and standards

HPLC grade methanol, acetonitrile, chloroform, and water were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). NH₄OH (28%) was obtained from Duksan Chemical Co. (Yongin, Kyungki-Do, Korea). Strychnine and ammonium acetate was purchased from Sigma (St. Louis, MO, USA).

2.3 Detoxification of Strychnos nux-vomica seeds

Strychnos nux-vomica seeds were detoxified using traditional methods described in the literature (Chung and Shin, 1989). The Strychnos nux-vomica seeds (10 g) were roasted with sea sands (10 g) until the seeds became dark yellow. Then, these seeds were boiled in water for 10 min and then dried. The dried materials were parched with sesame oil turning the seeds to a pale yellow color.

2.4 Extraction

Pulverized plant materials (100 mg) were extracted with methanol (50 mL) at 40°C for 30 min in an ultrasonic apparatus and then filtered. The methanolic extract was evaporated to dryness and the residue was dissolved in water (10 mL) and washed three times with chloroform (10 mL). The aqueous phase was adjusted to a pH of 9.5 using NH₄OH (28%) and extracted three times with 10 mL chloroform. The organic phase was evaporated to dryness and the residue was dissolved in 10 mL methanol. The final solution was filtered using a 0.45 μm nylon membrane filter (Alltech, Deerfield, IL, USA) and diluted 20 times. All results were based on five experiments.

2.5 HPLC–ESI/MS analysis

The HPLC–ESI/MS system used in this experiment consisted of Agilent 1100 series LC/MSD Trap equipped with a binary pump, degasser, photodiode array detector, column oven, automatic sample injector, and an ion-trap mass spectrometer (Agilent, Waldbronn, Germany). Separation was performed on a Zorbax Bonus RP-18 (150 mm × 2.1 mm, particle size 5 μm; Agilent) at 40°C. A linear gradient was used for the mobile phase (acetonitrile–20 mM ammonium acetate) changing from 30:70 to 70:30 within 30 min. The flow rate of the mobile phase was 0.2 mL/min. Five micro-liter of sample solution was injected through the automatic sample injector.

Mass spectrometer conditions were optimized in order to achieve maximum sensitivity. Capillary voltage, nebulizing gas pressure (N₂), drying gas (N₂) flow rate, and drying temperature were set at 1.1 kV, 10 psi, 4.0 L/min, and 325°C, respectively. Full scan spectra from m/z 100 to 1500 in the positive ion mode were obtained (scan time 0.1 s).

3. Results and discussion

The ESI-MS conditions were optimized using the authentic compound, strychnine. Optimum parameters for the ESI-ion trap mass spectra, such as capillary voltage, skimmer, octopole, and trap drive were determined by the direct
infusion method. The ionization efficiency of the compound that obviously affected the sensitivity in ESI-MS was tested by comparing the various mobile phases. The best sensitivity of strychnine was achieved on ESI-MS in the positive ion mode using 20 mM ammonium acetate–acetonitrile during the mobile phase.

The most abundant ion was found at \( m/z \, 335 \,[M + H]^+ \), signal due to the quasi-molecular ion in the mass spectra (Fig. 2). One nanogram of the compound on the column can be detected using this method. However, strychnine was not detectable at this concentration with an UV detector. In addition to the increased sensitivity, ESI-ion trap MS could detect strychnine and brucine, separately, from overlapped peaks using extracted ion chromatogram targeting each quasi-molecular ion at \( m/z \, 335 \,[M + H]^+ \) for strychnine and \( m/z \, 395 \,[M + H]^+ \) for brucine (Fig. 3).

Fig. 3. Chromatograms of total ion current (A), extracted ions current of \( m/z \, 335 \) (B), and \( m/z \, 395 \) (C), and mass spectrum of the peak 1 (D) in the total ion current chromatogram of *Strychnos nux-vomica* seeds extract.

Fig. 4. MS/MS spectrum of strychnine, \( m/z \, 335 \,[M + H]^+ \), obtained from unprocessed (A) and detoxified (B) *Strychnos nux-vomica* seeds and proposed fragmentation scheme of \( m/z \, 264 \).
From these results, MS detection was operated in the positive ion mode in the scan range from $m/z$ 100 to 1500 and the analysis of strychnine was performed using the extracted ion chromatogram (EIC) targeting at $m/z$ 335. The quantity of strychnine in *Strychnos nux-vomica* seeds was determined by a calibration curve using the standard strychnine solutions in the range of 0.2–14.0 μg/mL (a linear correlation coefficient of 0.9996). Based on this calibration curve, the amount of strychnine in *Strychnos nux-vomica* seed was found to be 4.31 (±0.49) mg/g.

The content of strychnine in the detoxified *Strychnos nux-vomica* seeds was also analyzed using the HPLC–ESI/MS method developed in this experiment. In the MS/MS spectrum of the $m/z$ 335 [M + H]$^+$ of unprocessed and detoxified *Strychnos nux-vomica* seeds, both spectrum showed the same base fragment ion peak at $m/z$ 264 [M + H – C$_3$H$_5$NO]$^+$ (Fig. 4). This result suggested that strychnine remained in the detoxified *Strychnos nux-vomica* seeds. As a result of this analysis of strychnine, the content in the detoxified *Strychnos nux-vomica* seeds was found to be 0.411 (±0.028) mg/g by HPLC–ESI/MS, which was dramatically reduced 10 times in comparison to the unprocessed *Strychnos nux-vomica* seeds. In addition to the reduction of strychnine, the contents of brucine and vomicine were also greatly reduced in the processed seeds (Fig. 5).

These results suggested that the HPLC ESI/MS methodology has been confirmed as an important tool to analyze strychnine and other alkaloids in *Strychnos nux-vomica* seeds. HPLC ESI/MS might detect strychnine down to the nanogram level. Also, the traditional detoxification method in oriental medicine for *Strychnos nux-vomica* seeds resulted in a significant reduction of strychnine.

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References


