Tropical Journal of Pharmaceutical Research October 2014; 13 (10): 1675-1680
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i10.15

Original Research Article

Online Monitoring for *Strychnos Nux-vomica* Parching in Sands and Chemical Compositional Analysis by Ultra Performance Liquid Chromatography - Linear Trap Quadrupole -Orbitrap- Mass Spectrometry

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Received: 18 June 2014 *Revised accepted: 11 September 2014*

Abstract

Purpose: *To provide an online and non-contact method to monitor the dynamic temperature of the nux-vomica parching in sands, and also to develop a rapid screening and comparison of the compounds in crude and processed nux-vomica.*
Methods: *Seeds of nux-vomica were parched in sands by an herbal medicine roaster with an online-type and non-contact temperature measurement system (ONTMS). Processing was monitored by ONTMS and the processing temperature was accurately controlled within the range 200 - 220 °C. Ultra performance liquid chromatography - linear trap quadrupole -orbitrap-mass spectrometry method in positive ionization mode was established in this work. The samples were extracted by ultrasonication with 50 % methanol for 30 min. Chromatographic separation was achieved on an Acquity UPLC HSS T3 column using a gradient program with acetonitrile and 1 % formic acid as the mobile phase and a diode array detector set at 254 nm for quantification.*
Results: *The surface color of the processed seeds of nux-vomica were brown or dark brown and the cross-section bulged with small bubble which meets the requirements of Chinese traditional processing methods. Fourteen major compounds from crude and processed nux-vomica were identified simultaneously by HPLC-DAD. According to the MS fragmentation pathways and by comparing with reference literature, the structures of eleven of the compounds were confirmed.*
Conclusion: *Methods using ONTMS are more accurate for parching nux-vomica in sands and UPLC-LTQ-Orbitrap-MS is a rapid and straightforward method for compound profiling in crude and processed nux-vomica.*

Keywords: *Strychnos, Nux vomica, Parching in sands, Online-type and non-contact temperature measurement system , Ultra performance liquid chromatography - linear trap quadrupole - orbitrap - mass spectrometry , Compound profiling*

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INTRODUCTION

The dried ripe seeds of *Strychnos nux-vomica* L. are called nux vomica used as a traditional folk medicine in China. It is mainly used to cure diseases such as swelling pain, trauma, rheumatoid arthritis, bone fracture, myasthenia gravis, and facial nerve paralysis [1]. The main chemical composition of Strychno nux-vomica seeds were alkaloids, such as strychnine and

brucine, which have been proved to be the main bioactive components responsible for the pharmacological and toxic properties [2,3].

The processing of traditional Chinese herbal medicine is a common procedure and usually applied before the prescription. In China, seeds of Strychnos nux-vomica need to be processed in hot sands for toxicity reduction before clinical practice as described in the Chinese pharmacopoeia [1,4]. Contents of the major toxic alkaloid were significantly decreased because of the high temperature treatment [5]. Processed Strychnos nux-vomica appears to become more effective than its crude product because toxic alkaloids are transformed into nitrogen oxidation or isoforms derivatives after processing procedure [5,6].

An online-type and non-contact temperature measurement system (ONTMS) was invented by our research team and had been patented (PR China Patent No.: ZL200520200614.0). In the sand parching process of *Strychnos nux-vomica*, it could display and control the temperature of the herbal medicine roaster.

HPLC-MS method used to analyze the content of strychnine from *Strychno nux-vomica* seeds and comparison to processed seeds and quantitative determination of strychnine residues in urine have been published [7,8]. In this paper, we use ONTMS to display and control the temperature of the herbal medicine roaster in the sand parching process of nux-vomica. Then the changes in the chemical compositions changes from nux-vomica to the sand parching process was analyzed by UPLC-MS method.

EXPERIMENTAL

Chemicals and reagents

Seeds of *Strychnos nux-vomica* were purchased from Sichuan Chinese Medicine Yinpian Company Limited. The sand (50kg) were purchased from the local market in Chengdu, passed through sieve for 300 – 600μm and washed clean with water. Acetonitrile, methanol, formic acid of HPLC grade were purchased from Thermo Fisher Scientific (China).

Strychnos nux-vomica parching in sands

Sand (300 – 600 μm) was placed in a herbal medicine roaster with online-type and non-contact temperature measurement system (ONTMS, Haishan Pharmaceutical Equipment Company Limited. in Hangzhou) for heating. The ONTMS was connected to computer and temperature of processing procedure was collected. The nux-vomica seed were put into roaster when the temperature of sands increased to 210 ^{o}C, and then parched for about 8 min with the roaster temperature set between 200 – 220 oC. The roaster was controlled to pour burnt nux-vomica into a sieve to remove the sands, and burnt nux-vomica seeds were obtained.

Sample preparation

Crude nux-vomica and the burnt sample were powdered by a mill and sieved through a No. 50 mesh sieve (inside diameter 355 μm ± 13 μm). An amount of 0.5 g was accurately weighed into 50 mL conical flask respectively, and 25 mL of 50 % methanol was added to each conical flask. The mixtures were placed into an ultrasonic bath (40 kHz) for 30 min at room temperature and then filtered. The filtrate was diluted to 10mL with 50 % methanol and filtered through 0.22 μm cellulose membrane filters prior to injection.

UPLC- LTQ-Orbitrap-MS conditions

UPLC was performed using an UltiMate 3000 UPLC system (Thermo, USA). All samples separation was performed on an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 150 mm). The mobile phase consisted of A (acetonitrile) and B (0.8 % formic acid in aqueous solution). Gradient elution was as follows: 0 – 2 min, 5 % A; 2 – 25 min, 5– 30 % A. The injection volume was 1 μL, and the column temperature was maintained at 30 °C. The flow rate of the mobile phase was 0.2 mL / min and Photodiode array detector (DAD) detection wavelength was 254 nm.

LTQ Orbitrap Elite MS (Thermo, USA) was operated in positive ionization mode using the following operating parameters: spray voltage 3.5 kV, sheath gas flow rate 35 arb, aux gas flow rate 10 arb, sweep gas flow rate 1 arb, capillary temperature 340 ^{o}C, capillary voltage 3.5 kV. Accurate mass spectra of $[M+H]^{+}$ ions were recorded from 50 to 800 m/z and collision energy was 35 eV.

RESULTS

Online monitoring of *Strychnos nux-vomica* parching in sands

From temperature - time curve collected by ONTMS, the temperature decreases about 40 ^{o}C when the nux-vomica was placed in the herbal medicine roaster. It took about 4 min before the temperature was back to original and it then took

4 min for parching with the temperature 200 - 220 °C.

In this study, accurate processing methods and ONTMS were used to process the nux-vomica. The surface color of the processed products was brown or dark brown and cross-section bulged with small bubble. These meet the requirements of traditional processing methods of nux-vomica according to Chinese pharmacopoeia.

Optimization of UPLC- LTQ-Orbitrap-MS conditions

Extraction conditions, including extraction methods, extraction solvents and extraction time were assessed based on single factor experiments in order to obtain optimal extraction efficiency for all the constituents. The optimal extraction efficiency was obtained by ultrasonication extraction with 50 % methanol for 30 min. Photodiode array detector (DAD) was used in UPLC analysis and the optimum monitor wavelength at 254 nm was selected from the full range spectra by comparing the sample separation under different wavelength. Several binary solvent gradients were compared with respect to separation efficiency of each compound. To enhance peak resolution, formic acid, acetic acid and phosphoric acid were used as modifiers added to the mobile phase. As described in the experimental section, a gradient solvent system with formic acid as modifier was developed after several trials and a total of 13 compounds were detected within 25 min.

In the experiment, the target compounds showed much higher response signals in positive ionization mode than in negative ionization mode. Hence, positive ionization mode was finally selected to detect these compounds. The MS conditions, including spray voltage and collision energy were optimized to achieve better detection of the compounds. As a result, positive ionization mode, spray voltage 3.5 kV and collision energy 35 eV was selected, and results of the UPLC-LTQ-Orbitrap-MS assay was presented in Table 1.

UPLC- LTQ-Orbitrap-MS analysis of authentic compounds

The processed nux-vomica was compared with the unprocessed sample under the same chromatographic conditions. Ten peaks of unprocessed sample and eleven peaks of processed sample were detected. Based on the recorded m/z values and additional fragment information in the positive mode, with reference literatures of strychnos nux-vomica [6,9-14], the peak identification results are shown in Fig 2 and presented in Table 1.

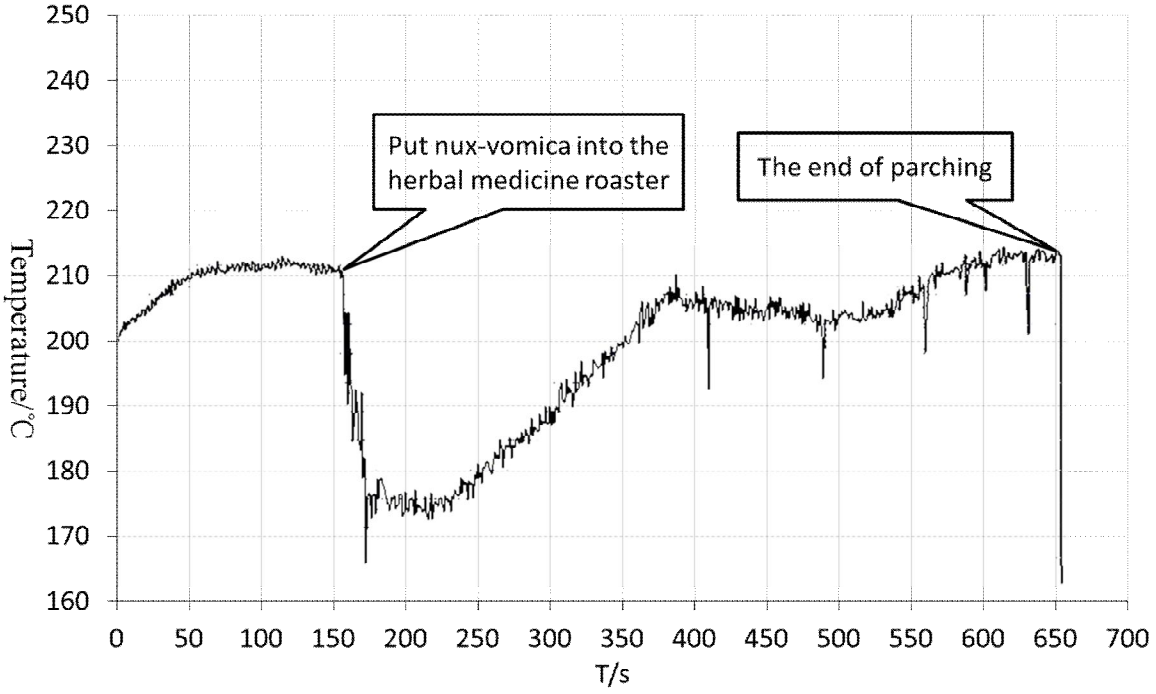

Fig 1: Temperature variation in the sand parching process of nux-vomica

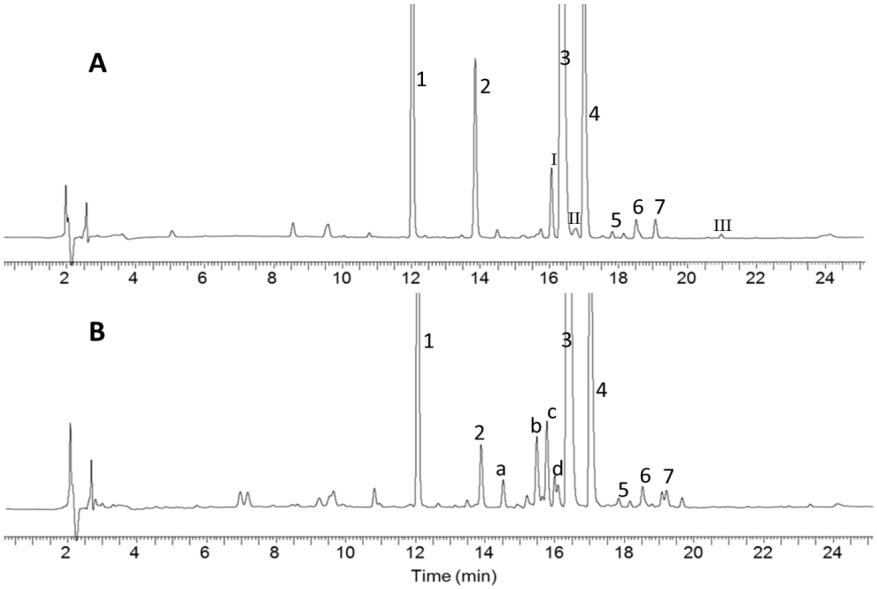

Fig 2: Representative UPLC chromatogram of (A) unprocessed nux-vomica and (B) processed nux-vomica

Table 1: Results of the UPLC-LTQ-Orbitrap-MS assay

Peak no.	T_R(min)	$[M+H]^{+}$/(m/z)	Fragment ions(+)/(m/z)	Identification
1	12.09	394.1726	341,215,197,179,151,137	Unidentified
2	13.92	355.1039	337,253,181,163,145,117	Chlorogenic acid
a	14.54	349.1926	331,306,210,184	Strychnine-N-oxide
b	15.53	349.1926	321,264,206,183	Isostrychnine-N-oxide
c	15.85	335.1768	317,272,197,144	Isostrychnine
d	16.07	395.1983	367,332,280,204,122	Isobrucine
I	16.17	408.1883		Unidentified
3	16.48	335.1769	307,264,222,184,153,122	Strychnine
II	16.83	411.1933	376,320,204,112	Pseudobrucine
4	17.04	395.1983	367,324,282,244,190,146,108	Brucine
5	17.89	365.1876	347,308,264,219,179,148	Methyl pseudostrychnine
6	18.65	365.1875	337,294,252,214,166,124	3-methoxystrychine
7	19.15	381.1835	363,324,306,280,252	Vomicine
III	21.11	443.1988		Unidentified

Fragmentation pathway was analyzed for the chemical profiling of the sample. Seven peaks (1, 2, 3, 4, 5, 6 and 7) in the unprocessed sample were the same as in the processed sample. Peak 1(m/z 394.1726) was unidentified, peak 2-7 was respective for Chlorogenic acid, Strychnine, Brucine, Methyl pseudostrychnine, 3-methoxy strychnine and Vomicine.

Four new peaks (a, b, c and d) in the processed sample were identified: peak a was Strychnine-N-oxide, peak b was Isostrychnine-N-oxide, peak c was isostrychnine and peak d is isobrucine. Three peaks (I, II and III) in the unprocessed sample disappeared, peak II was Pseudobrucine, peak I (m/z 408.1883)and peak II (443.1988) were unidentified.

DISCUSSION

The traditional processing methods of nux-vomica described in the Chinese pharmacopoeia were mainly relied on experience and the temperature of parching in sands was determined manually. The ONTMS provided a method to measure the dynamic temperature change during processing procedure. Based on this, a method of accurate temperature control to process nux-vomica was established. The processed products have stable quality due to the accurate control and are also conductive to the inheritance of traditional processing methods. A sensitive and reliable UPLC-LTQ-Orbitrap-MS method has been developed for the determination of compounds in crude and processed nux-vomica. Two peaks (II and III) were not detected in the sample of crude nux-

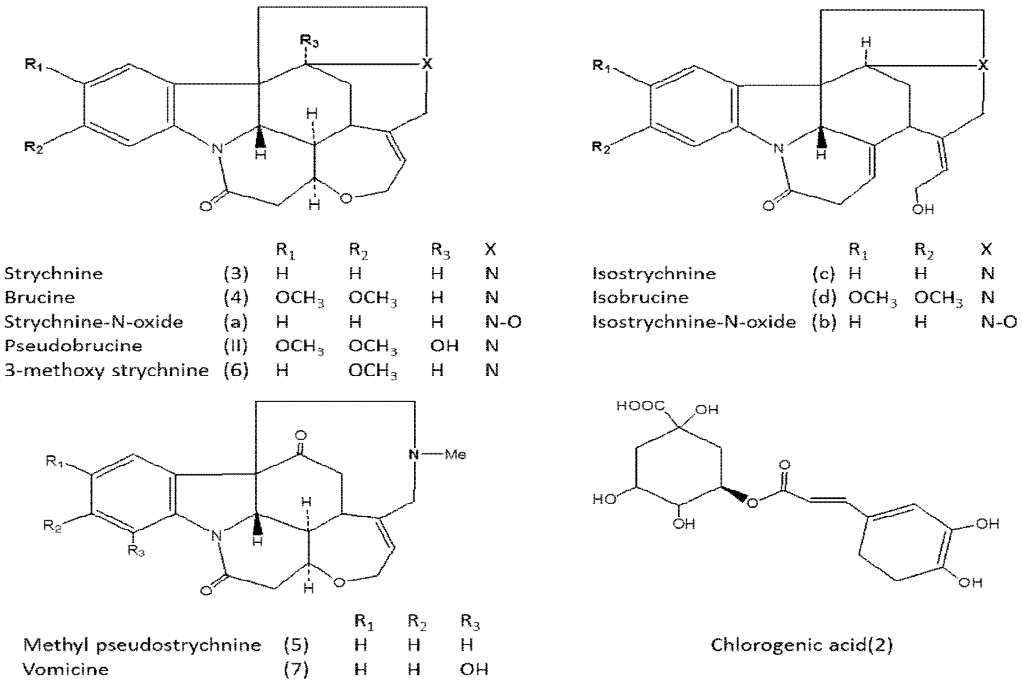

Fig. 3 Structure of compounds

vomica, further investigation on the corresponding structures (Fig 3) would be of profound significance. Meanwhile, development of new separation techniques are necessary to distinguish the isomer compounds. Nevertheless, the direct analysis by UPLC-LTQ-Orbitrap-MS provided a rapid screening and comparison on the compounds in the seeds of crude and processed nux-vomica.

CONCLUSION

ONTMS provided an online and non-contact method to monitor the dynamic temperature of the nux-vomica parching in sands; UPLC-LTQ-Orbitrap-MS is a rapid and straightforward method to generate compounds profiles in the seeds of crude and processed nux-vomica.

ACKNOWLEDGEMENT

This work was supported by TCM Industry-specific Project of 2010, 2012(No.201107008) and Three-year action development Plan of TCM in Shanghai(No. ZYSNXD-CC-BZH).

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