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Determination of aconitine-type alkaloids as markers in *fuzi* (*Aconitum carmichaeli*) by LC/(+)ESI/MS^3

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ABSTRACT

LC/(+)ESI/MS^3 was used to determine aconitine, mesaconitine, and hypaconitine as target markers in crude methanol extracts of (i) the raw lateral roots of *Aconitum carmichaeli*, (ii) roots treated by three different refining processes, and (iii) eight generally available traditional Chinese medicine (TCM) preparations containing *fuzi* (treated lateral roots of *A. carmichaeli*). The optimal ionization behavior resulted when using electrospray ionization (ESI) in positive-ion mode with 0.005% TFA as an additive in the mobile phase. The consecutive reaction monitoring (CRM) mode provided additional improvements in selectivity, which was exploited to minimize the noise and interference problems.

Employing this approach, aconitine and mesaconitine were found to decompose readily during the refining processes, but hypaconitine remains present at the same content, presumably because of its characteristic chemical structure. Thus, treated and untreated *fuzi* samples can be distinguished by monitoring the ratio of aconitine and mesaconitine to hypaconitine. The limits of detection (LODs) for these three markers were 0.05, 0.08, and 0.03 ng/ml. The linearity range for the three marker compounds was 0.1–1000 ng/ml. The analysis time was 12 min per sample.

1. Introduction

Aconite ("Carmichael's Monkshood," *Aconitum carmichaeli*) is widely distributed over the southwest provinces of China. Its lateral roots and those of other near-relative species of the same genus share the common name *fuzi*, which is one of the most useful herbal medicines. The raw lateral roots of aconite cannot be used directly because of their high content of aconitine-type alkaloids; hence, a pretreatment process that reduces their toxicity is necessary. Each of the three major kinds of pretreated *fuzi* on the market – *bai-fu-pian*, *hei-shun-pian*, and *yen-fu-zi* – has its own characteristic refining processes [1].

The pharmacological effects of *fuzi* are a regeneration of vigor with dispelled damp; it is used in many Chinese medicinal preparations for the treatment of colds, polyarthralgia, diarrhea, heart failure, beriberi, and edema. The main ingredients in the lateral roots of *A. carmichaeli* are a series of alkaloids sharing a common C_{19}-norditerpenoid skeleton; the major toxic ingredients – aconitine, mesaconitine, and hypaconitine (Fig. 1) – are also active agents of this herbal medicine [2], even though they can result in fatal ventricular fibrillation. The mechanism of its toxicity has been reported to be the activation of the sodium channel function in cells [3]. The reported LD_{50} value of aconitine for mice per oral injection is 1.8 mg/kg body weight [4]. Based on this high toxicity and pharmacological activity, refining processes are necessary to reduce the toxicity in most Chinese medicinal preparations.

Several methods have been developed for the analysis of aconitine-type alkaloids in the lateral roots of aconite, using gas chromatography (GC) [5], high-performance liquid chromatography (HPLC) [6], or capillary electrophoresis (CE) [7]. Nevertheless, these methods have limited applicability because of their low sensitivities and selectivities. In recent years, mass spectrometry (MS) has been employed for the analysis of the alkaloids in the lateral roots of aconite because of its high selectivity. Four toxic aconitine-type alkaloids – aconitine, mesaconitine, hypaconitine, and jesaconitine – were determined in blood and urine by LC/MS operated in the selected ion monitoring (SIM) mode after solid phase extraction (SPE) [8]. Subsequently, a series of these alkaloids was analyzed using matrix-assisted laser desorption ionization (MALDI)/time-of-flight (TOF) mass spectrometry on the basis of their typical fragmentations [9]. The complete fragmentation pathways of such aconitine-type alkaloids were later determined using electrospray ionization tandem mass spectrometry (ESI/MS^n) which was also employed for their qualitative analysis

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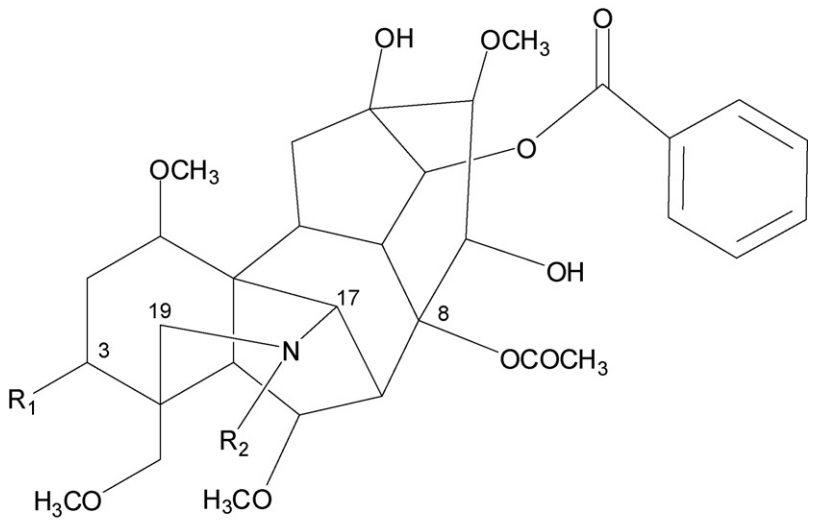
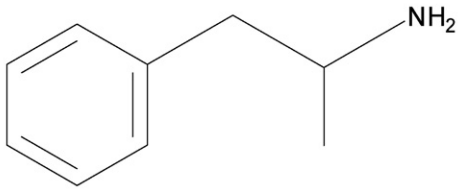


Aconitine: R_1=OH, R_2=C_2H_5 (MW=645) ;

Mesaconitine: R_1=OH, R_2=CH_3 (MW=631) ;

Hypaconitine: R_1=H, R_2=CH_3 (MW=615)



Amphetamine (used as IS)

Fig. 1. Chemical structures of aconitine, mesaconitine, hypaconitine, and amphetamine (IS).

[10]. These toxic alkaloids in aconite pills were determined using LC/MS/MS operated in selected reaction monitoring (SRM) mode [2], although the LOD was fairly high (0.3 μg/ml).

Even though various reports outlined above have appeared in recent years for the analysis of aconitine-type alkaloids, there remains a need to devise a simple quality control method for analyzing *fuzi*-containing Chinese herbal preparations that allows the levels of the three major toxic alkaloids to be estimated simultaneously without sample pretreatment. Thus, the aim of this study was the development of a simple and specific LC/MS^3 method, exhibiting high precision and selectivity, for the identification and quantification of low levels of aconitine-type alkaloids. Using this technique in conjunction with the improved selectivity of the consecutive reaction monitoring (CRM) mode of MS, we analyzed four *fuzi* samples with and without treatment and eight Chinese medicinal preparations containing *fuzi*.

2. Experimental

2.1. Materials

Aconitine was purchased from Sigma (St. Louis, MO, USA). Mesaconitine and hypaconitine were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), China. The purities of the three standards were above 99.8%. Amphetamine was used as the internal standard (IS). Analytical-grade trifluoroacetic acid (TFA), acetic acid (AA), formic acid (FA), methanol, and acetonitrile were purchased from Merck (Gibbstown, NJ, USA). The treated *fuzi* samples *hei-shun-pian* and *bai-fu-pain* were purchased from a local market in Taiwan Taichung city. Raw *fuzi*, the treated *fuzi* sample *yen-fu-zi*, and eight Chinese medicinal preparations containing *fuzi* (*yow-guei-wan*, *shy-nin-tang*, *fu-tzyy-li-chong-tang*, *guey-fuh-dih-huang-wan*, *jen-wu-tang*, *jih-sheng-shenn-chin-wan*, *sheau-shium-ming-tang*, and *guey-jy-shaur-yuh-jy-muu-tang*) were provided by the Chuang-Song-Zong Pharmaceutical Company.

2.2. Preparation of samples

Each ground crude *fuzi* sample powder (0.2 g) was sonicated in methanol (30 ml) at 25 °C for 30 min. The mixture was then centrifuged at 3000 rpm for 5 min. The supernatant was collected, filtered through a 0.2 μm PVDF syringe filter, and concentrated to a final volume of 10 ml. An aliquot (1 ml) of the sample solution was mixed with an equal volume of the IS solution (2 μg/ml) prior to analysis.

Each of the dry powder Chinese medicinal preparations (1.0 g) was treated as described above, except that they were concen-