
**Traditional Chinese medicine —
Determination of selected *Aconitum*
alkaloids by high-performance liquid
chromatography (HPLC)**

*Médecine traditionnelle chinoise — Dosage d'alcaloïdes d'aconit
(Aconitum) sélectionnés par chromatographie liquide à haute
performance (CLHP)*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Aconitum is a genus of about 350 species of flowering plants belonging to the family of Ranunculaceae. The therapeutic use of as many as 76 species of *Aconitum* herbs in traditional Chinese medicine have been documented for a very long time. Among the *Aconitum* herbs, the most well known are processed *Aconitum carmichaelii* lateral root (附子), *Aconitum carmichaelii* root (川乌) and *Aconitum kusnezoffii* root (草乌). Of approximately 80 000 traditional Chinese medicine formulae, around 10,2 % contain *Aconitum* herbs. It is one of the most frequently used groups of herbal medicines in traditional Chinese medicine. Typical *Aconitum* herbs used in traditional Chinese medicine are shown in [Annex C](#), Table C.1.

Aconitum herbs contain *Aconitum* alkaloids which have anti-inflammatory, analgesic and cardiotonic activities. The *Aconitum* alkaloids are a double-edged sword, however. At present, international trade in *Aconitum* products is restricted to a few nations due to the high natural toxicity of crude *Aconitum* products. Unprocessed *Aconitum* is highly toxic. Expert processing is required to reduce (but not eliminate) toxicity, and where individual national regulatory schemes do not ban the herb, it is generally restricted to a high-risk category, such as Schedule 2 in the Hong Kong Chinese Medicine regulations. Also, there are sporadic cases of *Aconitum* alkaloid poisoning due to misuse reported all over the world.

Nonetheless, the toxicity of *Aconitum* herbs can be reduced dramatically with proper processing (such as repeated boiling or steaming), prolonged decoction and dose control. However, testing standards for *Aconitum* alkaloids have not yet been harmonized on an international level, and regulatory authorities in many nations do not adequately differentiate highly toxic forms from less-toxic forms (or even non-toxic forms) of *Aconitum* herbs.

Six kinds of *Aconitum* alkaloids [aconitine (AC), mesaconitine (MA), hyaconitine (HA), benzoylaconine (BAC), benzoylmesaconine (BMA) and benzoylhyaconine (BHA)] are commonly used as chemical markers for quality control of aconite, determined by the high-performance liquid chromatography (HPLC) method^[1]. The AOAC Official Method 2008.11 also requires the determination of three *Aconitum* alkaloids, AC, MA and HA, in dietary supplements and raw botanical materials by LC/UV detection with confirmation by LC/MS/MS^[2]. Nevertheless, poisoning cases are still occasionally reported. From 1989 to 2010, 140 cases of *Aconitum* poisoning, including one fatal case, were reported in Hong Kong^[3]. Additionally, 17 cases were reported in Taiwan from 1990 to 1999, 2017 cases in China from 1989 to 2008 and 121 cases in Korea from 1995 to 2007^[4]. Multiple reasons for *Aconitum* poisoning exist and include overdoses, inadequate processing, *Aconitum* contamination in other herbs, dispensing and management errors and hidden risk factors. In the 17 cases reported in Hong Kong, yunaconitine (YAC), crassicauline A (CCA) and 8-deacetyl-yunaconitine (DYA) were detected instead of AC, MA and HA in the urine samples of the *Aconitum* poisoning patients^[3,4]. As a result, these alkaloids are considered to be hidden risk factors and should be covered in laboratory screenings for toxic compounds^[5]. Therefore, a method to simultaneously determine the levels of these nine alkaloids is needed for quality control of the herb and its products in order to ensure the safe use of these medicinal materials^[6].

This document aims to build a systematic and practical international standard for the determination of *Aconitum* alkaloids with the goal of standardizing the global market, to ensure safe and effective use in clinics and to reduce cases of *Aconitum* alkaloid poisoning.

As national implementation may differ, national standards bodies are invited to modify the limit values of selected *Aconitum* alkaloids in their national standards. Examples of national and regional values are given in [Annex D](#).

Traditional Chinese medicine — Determination of selected *Aconitum* alkaloids by high-performance liquid chromatography (HPLC)

1 Scope

This document specifies methods for the determination of the selected *Aconitum* alkaloids, including aconitine, mesaconitine, hypaconitine, benzoyleaconine, benzoylmesaconine, benzoylhypaconine, yunaconitine, deacetyl-yunaconitine and crassicauline A.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

World Health Organization, *Quality control methods for herbal materials*, 2011

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

4 Abbreviated terms

For the purposes of this document, the following abbreviated terms apply.

AC	aconitine
BAC	benzoyleaconine
BHA	benzoylhypaconine
BMA	benzoylmesaconine
CAS	chemical abstracts service
CCA	crassicauline A
CRS	chemical reference substance
DYA	8-deacetyl-yunaconitine
ESI	electrospray ionization
HA	hypaconitine