INTERNATIONAL STANDARD

ISO 23191

First edition
2020-07

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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Reference number
ISO 23191:2020(E)

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Published in Switzerland

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Foreword

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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.

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Six kinds of *Aconitum* alkaloids [aconitine (AC), mesaconitine (MA), hypaconitine (HA), benzoylaconine (BAC), benzoylmesaconine (BMA) and benzoylhypaconine (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.

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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, benzoylaconine, 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

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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

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

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HPLC high-performance liquid chromatography

JAC jesaconitine

MA mesaconitine

MRM multiple reaction monitoring

MS mass spectrometer

TS test solution

UV DAD ultraviolet diode array detector

YAC yunaconitine

5 Principle

The test solutions (TSs) are analysed by HPLC on a reverse-phase column packed with octadecylsilane bonded silica gel, with 0,1 % formic acid as the mobile phase A, acetonitrile as the mobile phase B and by mass spectrometer (MS) or ultraviolet diode array detector (UV DAD).

Aconitum alkaloids determinable by this method are shown in Table 1. Relevant structural formulae of *Aconitum* alkaloids are given in Figure 1.

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Table 1 — *Aconitum* alkaloids determinable by HPLC method

Name	Molecular formula	CAS no.	Molar mass g/mol
AC	$C_{34}H_{47}NO_{11}$	302-27-2	645,74
MA	$C_{33}H_{45}NO_{11}$	2752-64-9	631,71
HA	$C_{33}H_{45}NO_{10}$	6900-87-4	615,71
BAC	$C_{32}H_{45}NO_{10}$	466-24-0	603,78
BMA	$C_{31}H_{43}NO_{10}$	63238-67-5	589,68
BHA	$C_{31}H_{43}NO_9$	63238-66-4	573,67
YAC	$C_{35}H_{49}NO_{11}$	70578-24-4	659,76
DYA	$C_{33}H_{51}NO_{10}$	110011-77-3	645,79
CCA	$C_{35}H_{49}NO_{10}$	79592-91-9	643,76

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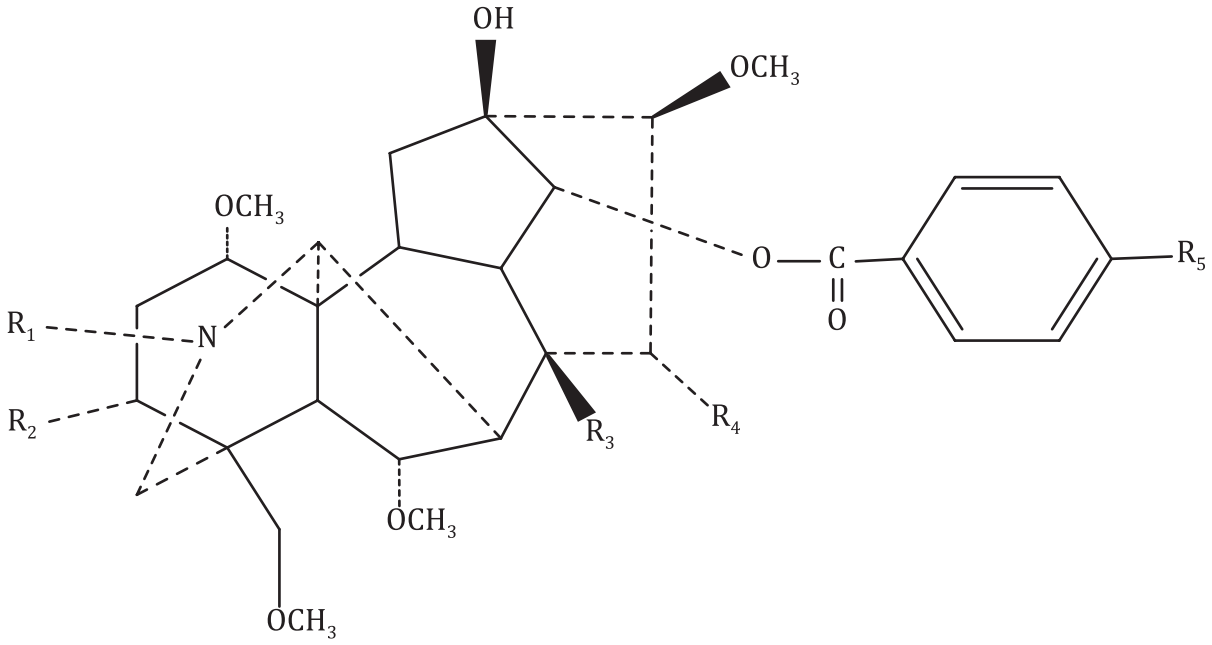

Key

	AC	HA	MA	BAC	BHA	BMA	YAC	DYA	CCA
R_1	CH_2CH_3	CH_3	CH_3	CH_2CH_3	CH_3	CH_3	CH_2CH_3	CH_2CH_3	CH_2CH_3
R_2	OH	H	OH	OH	H	OH	OH	OH	H
R_3	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	OH	OH	OH	$OCOCH_3$	OH	$OCOCH_3$
R_4	OH	OH	OH	OH	OH	OH	H	H	OH
R_5	H	H	H	H	H	H	OCH_3	OCH_3	OCH_3

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Figure 1 — Structural formulae of selected *Aconite* alkaloids

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6 Reagents

Use only reagents of recognized analytical grade and water conforming with grade 3 as specified in ISO 3696, unless otherwise specified.

— **isopropanol**, C_3H_8O, AR grade;

— **dichloromethane**, CH_2Cl_2, AR grade;

— **acetonitrile**, CH_3CN. HPLC grade;

— **ethyl acetate**, $C_4H_8O_2$. AR grade;

— **methanol**, CH_3OH. HPLC grade;

— **purified water**, deionized;

— **ammonia TS**.

7 Apparatus

Use the usual laboratory apparatus and the following:

— **liquid chromatograph**, fitted with electrospray ionization (ESI) MS or DAD;

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— **chromatographic column**, of stainless steel, 2,1 mm × 100 mm, with 1,7 µm particle size packing of octadecylsilane bonded silica gel;

— **ultrasonic bath**, power 300 W, frequency 40 kHz;

— **one-mark pipettes**, of capacity 5 ml, 25 ml and 50 ml;

— **conical flasks**, of capacity 100 ml, with glass stopper;

— **vacuum system** (e.g. Büchner flask, Vac-Elut system 1 or peristaltic pump);

— **analytical balance**, capable of weighing to the nearest 0,001 g;

— **microporous membrane**, 0,22 µm, organic phase.

8 Sampling

Sampling shall be carried out in accordance with the method described in the World Health Organization's *Quality control methods for herbal materials*, *General advice on sampling*. Samples not less than 250 g shall be taken from each batch randomly. Sampling of *Aconitum* genus shall be conducted as follows:

a) from a batch of five containers or packaging units, take a sample from each;

b) from a batch of six to 50 units, take a sample from five;

c) from a batch of over 50 units, sample 10 %, rounding up the number of units to the nearest multiple of 10. For example, a batch of 51 units would be sampled as for 60, i.e. take samples from six packages;

d) from each container or package selected, take three original samples from the top, middle and bottom of the container or package;

e) the three original samples shall then be combined into a pooled sample which shall be mixed carefully;

f) the average sample is obtained by quartering:

   — from the pooled sample, adequately mix into an even and square-shaped heap;

   — divide this diagonally into four equal parts;

   — take two diagonally opposite parts and mix carefully;

   — repeat the process as necessary until the required quantity, to within ± 10 %, is obtained;

g) using the same quartering procedure, divide the average sample into four final samples, taking care that each portion is representative of the bulk material.

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9 Test procedures

9.1 General

The HPLC-MS method applies and the HPLC-DAD method is optional. If the analytical results according to the HPLC-MS method and the HPLC-DAD method are equal, both methods can be applied. If the analytical results are different, the HPLC-MS method shall be used and is preferred for DYA, YAC and CCA because of its high sensitivity. The procedures can be modified if the accuracy and precision are validated. Other similar methods can be applied if they are validated.

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