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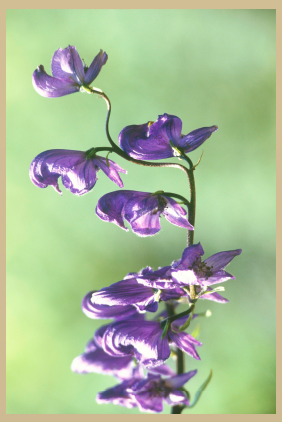
Detection of the decomposition of aconitine in Aconitum napellus mother tincture V.2a and identification of the main cleavage products using HPTLC-MS



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Aconitine, the main alkaloid of Aconitum napellus is one of the most toxic phytochemicals. The toxicity decreases from aconitine to its degradation products induced by hydrolytic cleavage of acetate and / or benzoate groups. So the decomposition of aconitine in aged tinctures is of great interest when used for pharmaceutical purposes.

Sample preparation for HPTLC: 2 ml of the tincture were mixed with 2 ml of water *R* and 1 ml of diluted ammonia solution *R* 3. The mixture has been put onto a kieselguhr cartridge and eluted twice with 8 ml of ether *R*. The extract was evaporated to dryness under reduced pressure. The residue was dissolved in 0,5 ml of methanol *R*.

HPTLC method: The sample solutions were applied on a HPTLC silica gel 60 (20 x 10 cm) glass plate (Merck, Darmstadt, Germany) by ATS4 (CAMAG, Berlin, Germany). Using a mobile phase of cyclohexane / ethylacetate / diethylamine (70:20:10 v/v/v) the plate was developed twice over a migration distance of 60 mm. For detection the plate was sprayed with a mixture of a solution of tartaric acid in water and the solution of iodine and iron-III-chloride in acetone. Evaluation in vis.

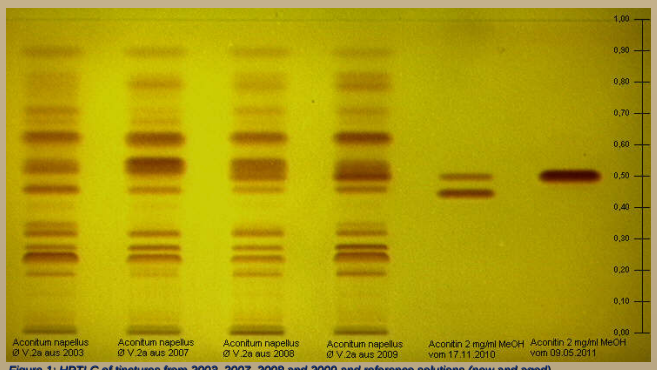

Figure 1: HPTLC of tinctures from 2003, 2007, 2008 and 2009 and reference solutions (new and aged)

Sample preparation for HPLC: The visible zones (Figure 2) were marked on an underivatised plate at the matching Rf value and extracted with methanol *R* (2 min, flow rate 0,3 mL·min^{-1}) using a TLC - MS - interface (CAMAG, Berlin, Germany). All sample solutions of the zones were separately collected in glass vials.

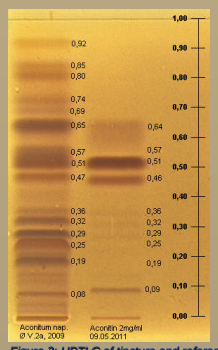

Figure 2: HPTLC of tincture and reference solution showing the extracted zones

Instrumentation for HPLC:
HPLC was performed with a Thermo Fisher Scientific Accela (Thermo Fisher Scientific, Dreieich, Germany) instrument with diode-array detector. The equipment was controlled by means of Xcalibur, Version 2.07. Compounds were separated on a 100 x 200 mm i.d., 1,9 µm particle, Hypersil Gold RP 18 column (Thermo Fisher Scientific) maintained at 25 °C.

Table 1: Gradient for HPLC measurement

Time	Percentage mobile phase B (%)	Remarks
0 → 30	2 → 50	Start of linear gradient 2-50 % B in 30 min
30 → 30,1	50 → 100	Washing step
30,1 → 35	100	
35 → 37	100 → 2	
37 → 43	2	Reequilibration

The mobile phase was a multi step, 43 min, linear gradient prepared from 0,1% HCOOH (A) and CH_3CN (B) (Table 1); the flow rate was 0,35 mL·min^{-1}. The volume of sample solution injected was 5 µL. Peaks were recorded in the range of 200 – 600 nm.

Instrumentation for HPLC-MS analysis:
The HPLC system described above was coupled with an LTQ Orbitrap XL (Thermo Fisher Scientific, Dreieich, Germany).
Ionisation was performed with an heated electrospray interface equipped with a metal needle kit. The conditions used for mass spectrometry were as follows: capillary voltage 35 V, spray voltage 3.00 kV, tube lens 120 V. Nitrogen was used as drying gas. The capillary temperature was set at 300 °C. Collision-induced dissociation spectra (CID) were obtained with a collision energy of 35 and an activation time of 30 ms. Full scan spectra from m/z 150-2000 were obtained in positive mode. Aconitine (Figure 3) was used for the optimization of ionization parameters.

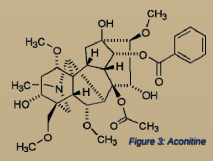

Figure 3: Aconitine

Table 2: LC/MS data of Aconitum napellus tincture

HPTLC zone (Rf) tincture	HPLC Retention time (min)	$[M+H]^+$ (m/z)	Major fragment ions ms^2
0.08	7.2	500	482 / 468 / 450 / 436 / 418
	7.9	500	482 / 468 / 450 / 436 / 418
	19.0	618	586 / 558 / 536 / 526
0.47	19.8	618	586 / 568 / 536 / 522
	21.9	632	600 / 582 / 568 / 550
0.51	16.3	604	n.d.
	19.7	618	600 / 586 / 568 / 554 / 536 / 522
	20.3	586	568 / 554 / 536 / 522
	21.7	646	n.d.
0.65	11.4	480	462 / 430 / 412 / 402

Table 3: LC/MS data of an aged reference solution of aconitine

HPTLC zone (Rf) reference solution	HPLC Retention time (min)	$[M+H]^+$ (m/z)	Major fragment ions ms^2
0.09	19.1	618	586 / 558 / 536 / 526 / 494
0.46	17.9	590	572 / 558 / 540 / 526 / 508 / 494 / 476 / 444
	19.8	618	586 / 568 / 554 / 536 / 522
0.51	16.3	604	586 / 572 / 554 / 540 / 522
	19.7	618	600 / 586 / 568 / 554 / 536 / 522
	20.2	586	568 / 554 / 536 / 522 / 504
	21.7	646	586 / 568 / 554 / 536 / 526 / 522
0.64	9.6	482	464 / 450 / 432
	19.8	618	600 / 586 / 568 / 554 / 537 / 522 / 504
	20.2	586	568 / 554 / 536 / 522 / 504 / 490

Comparison of the significant zones:
1) The zones at Rf 0,08 and 0,09 are corresponding in the detected mass 618 at a retention time of 19,0 and 19,1 minutes. A possible identity of the substance according to the detected mass and its major fragments is described in literature as N-deethyl-aconitine [5]. The detected mass at the retention times 7,2 or 7,9 could be identified as aconine.
2) The zones at Rf 0,47 and 0,46 are corresponding in the detected mass 618 at a retention time of 19,8 minutes. A possible identity of the substance according to the detected mass and its major fragments is described in literature as 8-deacetylyunaconitin [3].
3) The zones at Rf 0,51 are corresponding in all of the four detected masses at different retention times. The identity of the substance with m/z 646 could be proofed according to its fragmentation as aconitine [3]. The detected mass at the retention time 16,3 could be identified as benzoylaconine.
4) The zones at Rf 0,65 and 0,64 do not correspond in their detected masses.

Conclusion:
The main products of the reference substance aconitine solved in methanol *R* could be detected in aged tincture of Aconitum napellus as well. Most of the examined zones were a mixture of substances with different masses.

Literature References: [1] Van Wyk Wink Wink, Handbuch der Arzneipflanzen, wissenschaftliche Verlagsgesellschaft mbH Stuttgart, *[2] Hänsel Sticher, Pharmakognosie / Phytopharmazie 9. Auflage, Springer, [3] Neue Wirkstoffe aus der Flora des Himalaya? Identifizierung von Diterpenalkaloiden aus Akonitum-Arten, Inauguraldissertation, Jakob Maier, Universität Basel 2004, [4] Hikoto Ohta, Yasuo Seto, Noriko Tsunoda, Yutaka Takahashi, Kenji Matsuura, Kunio Ogasawara, Journal of Chrom. B, 714 (1998) 215-221, [5] Yuguang Wang, Shengqi Wang, Yongxue Liu, Liangping Yan, Guifang Dou, Yue Gao, Journal of Chrom. B, 844 (2006) 292-300*