Stability of Aconitine in Alcoholic Extracts
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**Purpose**
C19-Norditerpenoid alkaloids with di-ester groups at C-8 and C-14 are the most toxic alkaloids. Some Aconitum species are widely used in Chinese herbal medicine and in homeopathy especially as a tincture or an ointment, but often with a pre-treatment step such as boiling in water to reduce their toxicity by inducing hydrolysis of the ester groups e.g. acetate and/or benzoate. In the course of a comparison of small scale extraction methods for routine application to the analysis of plant materials and other products containing norditerpenoid alkaloids, the reactivity of these ester alkaloids towards various common organic solvents was examined.

**Methods**
Aconitine was purchased from Sigma-Aldrich, UK. Samples 1 mg/mL in HPLC grade methanol. HPLC used Phenomenex Luna 5µ PFP 15 x 4.5 mm column; mobile phase: 0.1% formic acid:acetonitrile (65:35), 1 mL/min; sample volume 20 µL, detection UV $\lambda = 232$ nm.

**Results**
Aconitine, freshly dissolved in methanol, showed a single peak (HPLC Rt 8 min). However, when HPLC analysis was carried out on the same sample after a week stored at 20 ºC, it showed 4 peaks (detection of the benzoate group). These 4 peaks were collected separately and the norditerpenoids were pyraconitine, methoxylated-14-benzoyl aconine, and aconitine. The experiment was then repeated using d4-MeOH in order to investigate any differences (in mass) and to determine if the compound eluting as peak 2, is formed due to loss of C2H4 or replacement of the acetate group at C8 with O-methyl group from the solvent, both a resultant loss of 28 Da. We isolated the tri-deuteriated methoxylated-14-benzoyl aconine confirming the replacement of acetate by a methoxy group from the solvent.

**Conclusion**
We showed that aconitine dissolved in methanol was degraded into 3 major products after 1 week of storage at 20 ºC. Therefore, alcoholic solvents should be avoided in any extraction procedure for aconitine and diester diterpenoid alkaloids. Medicinal formulations such as tinctures that involve extracts in ethanol are likely to suffer extensive degradation of diester alkaloids during preparation and/or storage.
We thank the Egyptian Government for a fully-funded Scholarship (to MA).