RAPID TARGET AND UNTARGET ANALYTICAL METHOD FOR ALKALOIDS ANALYSIS IN HERBAL EXTRACTS

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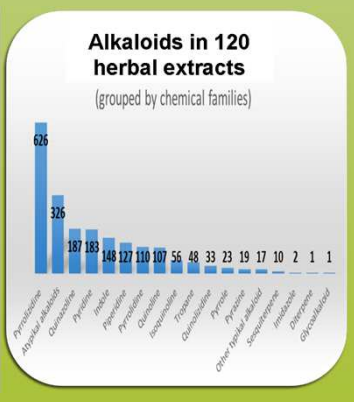
Overwiew
A method for analyzing herb extracts was developed and it enabled the identification of about 300 alkaloids, belonging to 14 chemical groups. An aliquot (2.5 g) of minced herb was extracted with 20mL water /methanol /formic acid (94.5:94.5:1) solution, sonicated and centrifuged. Subsequently an automatic clean up solid-phase extraction of the extracts and the analyses for the presence of alkaloids using ultra-high-pressure liquid chromatography coupled to heated electrospray ionization Quadrupole/High-Resolution Mass (Orbitrap) was performed. The procedure was validated using 33 commercial alkaloids and alkaloids N-oxides. The method was applied to the analysis of 120 herbal plants . The most frequently occurring groups were pyrrolizidine, atypical, quinazoline and pyridine alkaloids.

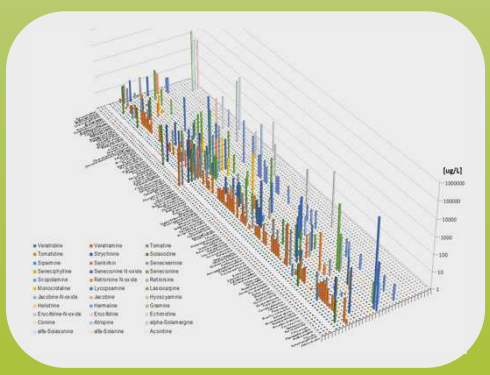
Introduction
Alkaloids are nitrogenous organic basic compounds produced by plants, not only as waste products of metabolic processes, but also for many biological functions (*e.g.*, pest defence, protection from photodegradation, aid in the growth of the seeds [1]). Over ten thousand alkaloids have been isolated from nature [2] and divided in two groups: atypical alkaloids (non heterocyclic) and typical alkaloids (heterocyclic). Typical alkaloids, in according to their ring structure, can be classified into the following main groups: pyrrole, pyrrolidine, tropane, pyrrolizidine, piperidine, quinoline, isoquinoline, aporphine, quinolizidine, indole, indolizidine, pyridine, imidazole, and purine. Some of them are responsible for the beneficial effects of traditional medicines, but some may instead have the harmful effects of poisons [3]. In particular pyrrolizidine alkaloids (PAs) have hepatotoxic, mutagenic, and carcinogenic effects [4] and in accordance with the German Federal Institute for Risk Assessment (BfR) a daily intake limit of 0.007 µg/kg body weight (0.42 µg/60 kg adult) was identified for 1,2- unsaturated Pas. Different studies revealed clearly that some herbal preparations may contain high amounts of PAs exceeding current recommendations [4,5].

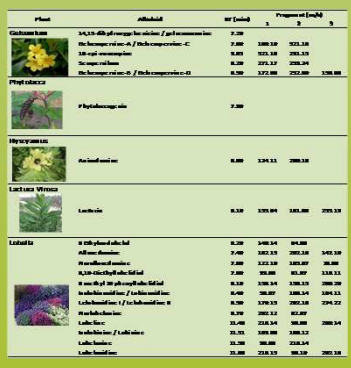
Methods
On-line concentration/purification was performed with a SolEx HRP spe cartridge, while the chromatographic separation with an Kinetex PFP analytical column, managing the water/0,1% formic acid/5mM ammonium acetate - methanol/0,1% formic acid/5mM ammonium acetate gradient elution in 15 minutes from 30% to 100% of organic solvent. The mass spectrometer was operated in positive ion mode using the following parameters: sheath gas flow rate set at 30 arbitrary units; aux gas flow rate at 10 arbitrary units; spray voltage at 3.5 kV; capillary temperature at 330 °C; aux gas heater temperature at 300 °C; Mass spectra were acquired in full MS-data dependent MS/MS analysis (full MS–dd MS/MS) at mass resolving power of 140.000. Thirty three alkaloids were quantified *vs* analytical standards; 45 were successfully identified using the exact masses and the isotopic patterns, and the chromatographic retention times were confirmed analysing the natural extracts of plants well documented in literature. Other roughly 200 alkaloids were tentatively identified by searching through a database built using literature information.

Results
The method was linear up to alkaloid concentration of 400/1000 µg L^{-1} with R^2 always > 0,99, and limits of detection ranging 0.1-5 µg L^{-1}. Accuracy (as recoveries) ranged from 80 to 120% for the 72% of compounds, and the precision (RSD%) was generally <4% in all the quantitation range. The most frequently present were pyrrolizidine, atypical, quinazoline and pyridine alkaloids. Regarding target alkaloids, the largest amounts were those of alpha-Solamargine, Lasiocarpine and Lycopsamine, while the most frequently detectable alkaloid was Tomatidine.

Compounds	Ret.Time (min)	Linearity range	R^2	DL (ug/L)	Precursor (M+H)+ (m/z)	NCE	Products (m/z)
Monocrotaline	4,25	0.01-1000	0,996	0,100	326,15980	60	94,0; 120,0
Eruciflorine	4,80	0.01-1000	0,997	0,100	350,15980	60	94,0; 120,0
Lycopsamine	5,01	0.01-1000	0,998	0,100	300,18050	35	138,0; 94,0
Eruciflorine-N-oxide	5,41	0.01-1000	0,997	0,100	366,15470	60	94,0; 118,0
Scopolamine	5,76	0.01-1000	0,994	0,010	304,15430	35	138,0; 156,1
Jacobine	5,95	0.01-400	0,990	0,100	352,17540	50	120,0; 155,0
Jacobine-N-oxide	5,95	0.01-400	0,994	0,100	368,17030	50	120,0; 296,0
Retrorsine	6,08	0.01-400	0,995	0,100	352,17540	50	94,0; 120,0
Retrorsine N-oxide	6,10	0.01-400	0,990	0,010	368,17030	60	94,0; 120,0
Gramine	6,19	0.01-1000	0,999	1,000	175,12290	35	130,0; 119,0
Seneciphylline	6,67	0.01-400	0,999	5,000	334,16490	50	94,0; 120,0
Coniine	6,69	0.01-500	0,991	5,000	128,14330	45	69,0
Heliotrine	6,75	0.01-250	0,991	5,000	314,19610	35	138,0
Atropine	6,78	0.01-250	0,993	0,100	290,17500	50	124,1; 93,0
Hyoscyamine	6,78	0.01-250	0,993	5,000	290,17507	45	124,1; 93,0
Senecionine	7,02	0.01-800	0,995	0,010	336,18055	50	94,0; 120,0
Seneciverine	7,02	0.01-400	0,993	0,010	336,18055	50	94,0; 120,0
Senecionine N-oxide	7,08	0.01-450	0,993	0,100	352,17546	50	94,0; 120,0
Strychnine	7,09	0.01-400	0,996	0,100	335,17540	60	184,0; 264,1
Senkirkine	7,30	0.01-800	0,997	0,010	366,19110	50	122,0; 168,0
Echimidine	7,37	0.01-500	0,999	1,000	398,21730	35	120,0; 83,0
Sipeimine	7,60	0.01-800	0,998	0,010	430,33150	50	138,1
alfa-Solasonine	7,73	0.01-250	1,000	5,000	884,50020	45	253,1; 359,5
alfa-Solanine	7,78	0.01-250	1,000	5,000	868,50500	50	157,1; 396,3
alpha-Solamargine	7,78	0.01-500	1,000	5,000	868,50520	50	157,1; 396,3
Lasiocarpine	7,90	0.01-400	0,996	0,100	412,23290	35	128,0; 220,1
Harmaline	7,92	0.01-1000	0,990	0,100	215,11788	50	120,0; 220,1
Veratramine	8,12	0.01-800	0,995	0,100	410,30530	35	256,2; 84,0
Veratridine	8,55	0.01-1000	0,996	5,000	674,35340	50	279,1; 149,0
Tomatidine	9,87	0.01-800	0,995	0,100	416,35230	60	161,1; 255,2
Tomatine	9,87	0.01-1000	0,995	0,010	416,35230	50	161,1; 255,2
Solasodine	9,94	0.01-450	0,996	0,010	414,33666	60	157,1; 70,0
Aconitine	10,39	0.01-2000	1,000	0,100	646,32210	50	105,0; 218,1







Conclusion
The use of a liquid chromatography-high resolution mass (Orbitrap) made it possible to rapidly describe a very broad profile of the alkaloids characterizing herbal plants, allowing to quantify 33 target alkaloids (commercially available as standards), and to identify more than other 250 untargeted ones (thanks to the high selectivity of the spectrometer in determining exact mass and isotopic profile).

Reference
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