

Journal of Chromatography B, 733 (1999) 181-229

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Poisoning by plant material: review of human cases and analytical determination of main toxins by high-performance liquid chromatography–(tandem) mass spectrometry

Yvan Gaillard*, Gilbert Pepin

Laboratoire d'Expertises TOXLAB, 18 Rue André Del Sarte, 75018 Paris, France

Abstract

The authors have reviewed the main toxic plants responsible for human deaths throughout the world. Forty plants (genera or species) were listed in order to establish an inventory of the active molecules that could be identified, the already published analytical methods and the reported human fatal cases. In a second step, the authors have developed a general method for the detection of various toxins in whole blood by high-performance liquid chromatography coupled to mass spectrometry or tandem mass spectrometry. Sample preparation was realized by liquid–liquid extraction at pH 9.5 for oleandrine, taxol and the alkaloids. These latter compounds were divided into two groups following their chemical properties and could be subsequently purified by acid/base clean up. Cyanogenic compounds and atractyloside were isolated by precipitation of the protein content with acetone and purified for atractyloside by washing with chloroform. Separation of the drugs occurred under reversed-phase conditions on a C₁₈ analytical column $150 \times 2 \text{ mm}$ I.D. (5 µm particle size) using two different mobile phases. The first one, formiate buffer 2 mM acidified at pH 3.0, was used for the separation of atractyloside, oleandrine, taxol, the cyanogenic molecules and some alkaloids. A gradient elution mode was chosen using acetonitrile or acetonitrile–methanol (50:50, v/v) as the eluting solvent. Detection under positive ionization mode was the mode of choice for all compounds except for atractyloside (negative ions) and for taxol (mixed mode available). Application to real forensic cases has been demonstrated. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Toxic plants; Oleandrine; Taxol; Alkaloids; Atractyloside; Cyanogenic compounds

Contents

Introduction	184	
General strategy for chromatographic analysis		
2.1. Reagents	185	
2.2. Instrumentation	186	
2.2.1. HPLC-MS	186	
2.2.1.1. Cyanogenic compounds	186	
2.2.1.2. Alkaloids (group A)	186	
2.2.1.3. Alkaloids (group B), atractyloside, oleandrine and taxol	187	
(General strategy for chromatographic analysis	

0378-4347/99/\$ – see front matter © 1999 Published by Elsevier Science B.V. All rights reserved. PII: S0378-4347(99)00181-4

^{*}Corresponding author.

2	2.2. HPLC-MS-MS	187
	libration	196
	mple preparation and extraction procedures	196
	.4.1. Cyanogenic glycosides	196
2	.4.2. Alkaloids (group A)	196
	.4.3. Alkaloids (group B) – Oleandrine and taxol	198
	.4.4. Atractyloside	199
	tions	199
	prus precatorius–Jequirity bean–Fabaceae	199
	.1.1. Active molecules	199
	.1.2. Analytical toxicology and human poisonings	199
	enus Aconitum–Monkshood–Ranonculaceae	199
	2.1. Active molecules	199
	2.2. Analytical toxicology	199
	2.3. Human poisonings	200
	ractylis glummifera–Glue thistle–Asteraceae	200
	3.1. Active molecules	201
		201
	3.2. Analytical toxicology and human poisonings	
	ropa belladona–Deadly nightshade–Solanaceae	201
	4.1. Active molecules	201
	.4.2. Analytical toxicology	201
	4.3. Human poisonings	202
	uccharis cordifolia–Asteraceae	202
	.5.1. Active molecules	202
	.5.2. Analytical toxicology and human intoxications	203
	cuta virosa and related species (douglasii, maculata, occidentalis)-Water hemlock-Oenanthe crocata and related species	
(4	aquatica, javanica)-Hemlock water dropwort-Apiaceae	203
	.6.1. Active molecules	203
3	.6.2. Analytical toxicology	203
3	.6.3. Human poisonings	203
3.7. Ca	olchicum automnale–Meadow saffron–Liliaceae	204
3	.7.1. Active molecules	204
3	7.2. Analytical toxicology	204
3	.7.3. Human poisoning	204
	mium maculatum–Poison hemlock–Apiaceae	205
	.8.1. Active molecules	205
	.8.2. Analytical toxicology	206
	8.3. Human poisonings	206
	mvallaria majalis–Lily-of-the-valley–Liliaceae	206
	.9.1. Active molecules	206
	9.2. Analytical toxicology and human poisonings	200
	<i>Coriaria myrtifolia</i> –Coriariaceae	200
	.10.1. Active molecules	200
		200
	.10.2. Analytical toxicology and human poisonings	200
	Datura stramonium and related species (arborea, ferox, inermis, innoxia, metel, sanguinea, sauveolens)-Jimsonweed or	207
	norn apple–Solanaceae	207
	.11.1. Active molecules	207
	.11.2. Human poisonings	207
	Genus Dieffenbachia–Araceae	207
	Digitalis purpurea and related species (grandiflora, lanata)-Foxglove-Scrophulariaceae	207
3	.13.1. Active molecules	207
3	.13.2. Analytical toxicology	208
3	13.3. Human poisonings	208
3.14. 0	Genus Dioscorea-Dioscoreaceae	209
3.15. C	Genus Duboisia–Duboisia–Solanaceae	209
3.16. E	<i>Supatorium rugosum</i> and related species (<i>cannabinum</i>)–White snakeroot–Asteraceae	209
	16.1. Active molecules	209
	.16.2. Analytical toxicology and human poisonings	209

3.17.	Ferrula communis and related species (chiliantha, tingitana)-Apiaceae	209
	3.17.1. Active molecules	
	3.17.2. Analytical toxicology and human poisonings	209
3.18.	Gelsemium sempervirens and related species (elegans, rankinii)-Carolina jessamine-Loganiaceae	210
	3.18.1. Active molecules	
	3.18.2. Analytical toxicology and human poisonings	
3.19.	Hyoscyamus niger and related species (albus, falezlez, muticus, reticulatus)-Henbane-Solanaceae	210
	3.19.1. Active molecules	210
	3.19.2. Human poisonings	210
3.20.	Jatropha curcas	210
	3.20.1. Active molecules	210
	3.20.2. Analytical toxicology and human poisonings	210
3.21.	Lobelia inflata and related species (polyphylla, portoricensis)-Lobeliaceae	211
	3.21.1. Active molecules	211
	3.21.2. Analytical toxicology and human poisonings	211
3.22.	Nerium oleander and N. indicum, Thevetia peruviana and T. nerifolia-Common or yellow oleander-Apocynaceae	211
	3.22.1. Active molecules	211
	3.22.2. Analytical toxicology	211
	3.22.3. Human poisonings	211
3.23.	Nicotiana glauca-Tobacco tree-Solanaceae	211
	3.23.1. Active molecules	211
	3.23.2. Analytical toxicology and human poisonings	211
	Pachyrhizus erosus	212
3.25.	Pausinystalia yohimbe–Yohimbe–Rubiaceae	212
	3.25.1. Active molecules	212
	3.25.2. Analytical toxicology and human poisonings	213
3.26.	Pilocarpus microphyllus-Rutaceae	213
	3.26.1. Active molecules	213
	3.26.2. Analytical toxicology and human poisonings	213
	Genus Philodendron-Araceae	213
3.28.	Genus Polygonatum–Solomon's seal–Liliaceae	213
	3.28.1. Active molecules	213
	3.28.2. Analytical toxicology and human poisonings	213
3.29.	Prunus laurocerasus-Black cherry-Rosaceae	213
	3.29.1. Active molecules	213
	3.29.2. Analytical toxicology	214
	3.29.3. Human poisonings	214
3.30.	Rauwolfia serpentina and related species (tetraphylla, vomitoria)-Apocynaceae	214
	3.30.1. Active molecules	214
	3.30.2. Analytical toxicology and human poisonings	
3.31.	Ricinus communis-Castor oil plant-Euphorbiaceae	214
	3.31.1. Active molecules	214
	3.31.2. Analytical toxicology	215
	3.31.3. Human poisonings	215
3.32.	Solanum dulcamara and related species (dimitatum, malacoxylon)-Woody nightshade or bittersweet-Solanum pseudo-	
	capsicum-Jerusalem berry-Solanaceae	215
	3.32.1. Active molecules	215
	3.32.2. Analytical toxicology	215
	3.32.3. Human poisonings	215
3.33.	Spartium junceum and Cytisus scoparius-Fabaceae	215
	3.33.1. Active molecules	215
	3.33.2. Analytical toxicology	215
	3.33.3. Human poisonings	216
3.34.	Strychnos nux vomica and S. ignacii–Loganiaceae	216
	3.34.1. Active molecules	216
	3.34.2. Analytical toxicology	216
	3.34.3. Human poisonings	216
3.35.	Genus <i>Taxus</i> –Yew–Taxaceae	217

183

		3.35.1. Active molecules	217
		3.35.2. Analytical toxicology	217
		3.35.3. Human poisonings	217
	3.36.	Trichosanthes kirilowii-Cucurbitaceae	219
		3.36.1. Active molecules	219
		3.36.2. Analytical toxicology and human poisonings	219
	3.37.	Tripterygium wilfordii-Celastraceae	219
	3.38.	Veratrum viride and related species (album, californium, japonicum)-False or white hellebore-Liliaceae	219
		3.38.1. Active molecules	219
		3.38.2. Analytical toxicology	219
		3.38.3. Human poisonings	220
	3.39.	Xanthium sibiricum-Asteraceae	220
		3.39.1. Active molecules	220
		3.39.2. Analytical toxicology and human poisonings	220
	3.40.	Genus Zigadenus-Death camus-Liliaceae	220
4.	Precis	ion and recovery	220
	4.1.	Cyanogenic molecules	220
		Alkaloids, oleandrine and taxol	220
	4.3.	Atractyloside	220
5.	Linear	ity	221
6.	Accur	acy	221
7.	Limits	of detection: LOD	221
8.	Chron	natographic separations	221
9.	Foren	sic applications of authors' own experience	222
		Case 1	222
	9.2.	Case 2	223
	9.3.	Case 3	223
10	. Conc	lusion	226
Re	ferenc	28	226

1. Introduction

The incidence of plant exposure was about 1.5% in France, 5% in Belgium, 6.5% in Italy, 7.2% in Switzerland and 6% in Turkey following different retrospective studies of the last 20 years [1]. Between 1985 and 1994 in the United States, these exposures rank fourth only, behind intoxications involving pharmaceuticals, household cleaning agents, and cosmetics [2,3]. Human fatal cases are the fact of about a maximum of 40-50 genera throughout the world. The total number of deaths however, is very difficult to establish and must certainly be underestimated since all cases were not always well documented on the analytical and forensic point of views and thus rarely published. Nevertheless, it seems that the mortality might be important in contrast to other causes of poisonings. In South Africa, 2% of the people admitted for acute poisoning died, compared to 15% of the patients poisoned by traditional plant medicine [4].

A multiresidue screen for the quantitative and

qualitative determination of alkaloids in plant material, animal ingesta, tissue and biological fluids is described by a veterinary diagnostic laboratory [7]. The samples are extracted using 5% ethanol in ethyl acetate and purified by back extraction in 0.5 mol/l HCl. A final clean-up procedure using polymeric C_{18} column is applied. Alkaloids are analyzed by gas chromatography (GC)-nitrogen-phosphorus detection for quantitative analysis and by GC-mass spectrometry (GC-MS) and thin layer chromatography (TLC) for qualitative analysis. The method recovered the six tested alkaloids (atropine, coniine, nicotine, retrorsine, solanidine and strychnine) in the range of 72-113% at the 1 μ g/g level. Limits of detection however, are theoretically contained between 25 and 200 ng/g that made the method not very sensitive. Besides, non-volatile compounds cannot be assayed by the presented technique. Foukaridis et al. [4] have developed an efficient multi-isocratic high-performance liquid chromatography (HPLC) system coupled with photodiode array UV detection for the identification of poisoning by

plant folk medicine of Black South Africa. These two works however, are an exception. In forensic toxicology, general screenings for toxic plant molecules are remarkably absent. Few methods exist punctually as for the tropane alkaloids, for nicotine and its metabolites, for colchicine or for the cardiac glycosides. For a large number of low concentrated toxins however, the methods are generally not sensitive enough to enable the detection of numerous cases. That is why, considering these problems, the authors have developed an efficient HPLC–MS (and MS-MS) method for the detection of several plant toxins of particular interest in forensic science.

In the present paper, the authors have listed the most toxic plant genera responsible for human fatalities. Since it was the author's willing to detail the plants solely responsible for accidental and homicidal cases, the narcotics, stimulants and hal-

Table 1

List of the plants that are outside the scope of the present study

lucinogens were not treated in this review (see Table 1) [5,6]. The poisons used for hunting were also not envisaged. In the case of conflict between tribes, the warriors might resort to different poisons that are not so short acting toxins. Death was supposed to be the result of several hours or days of a painful agony. The different plants used both for hunting or war were listed in Table 1 [5].

2. General strategy for chromatographic analysis

2.1. Reagents

Methanol, acetone, acetonitrile, chloroform, isopropanol, formic acid, ammonium formiate (NH_4COOH), 30% ammonia solution (NH_4OH),

Classes	Botanical names
Narcotics	Myristica fragrans, Nymphaea ampla, N. caerulea, Papaver somniferum.
Stimulants	Ephedra species, Erythroxylon coca, Sida acuta, S. rhombifolia.
Hallucinogens	 Acorus calamus, Andenanthera colubrina, A. peregrina, Ariocarpus fissuratus, Banisteriopsis caapi, B. inebrians, B. rusbyana, Brugmansia arborea, B. aurea, B. insignis, B. sanguinea, B. suaveolens, B. versicolor, Cannabis sativa, Coriaria thymifolia, Coryphanta compacta, Cymbopogon densiflorum, Desfontania spinosa, Echinocerus salmdyckianus, E. triglochidiadus, Epithelantha micromeris, Erythrina americana, Galbulimima belgraveana, Gormotega keule, Heimia salicifolia, Helicostylis pedunculata, H. Tomentosa, Homalomena species, Ipomoea violata, Justicia pectoralis, Kaempferia galanga, Latua publiflora, Lagochilus inebrians, Lochroma fuschioides, Lophophora diffusa, L. Wiliamsii, Mammilliaria craigii, M. grahamii, M. senilis, Mandragora officinarum, Mesembryanthemum expansum, Methysticodendron amesianum, Mimosa hostilis, Mucuna pruriens, Nephelium topengii, Oncidium cebolleta, Pernettya furens, P. parvifolia, Pachycereus pecten-arboriginum, Pancratium trianthum, Peganum harmala, Phytolacca acinosa, Salvia divinorum, Scirpus species, Senecio cardiophyllus, Sofora secundiflora, Tabernanthe iboga, Tetraptis methustica, Trichocerus pachanol, Turbinea corymbosa, Virola calophylla, V. Calophylloida, V. elongata, V. theiodora, Zornia latifolia.
Hunting poisons	Abuta species, Acokanthera schimperi, Capsicum annum, Chondrodendron tomentosum, Cissampelos species, Cocculus laurifolius, Curarea toxifera, C. candicans, Erytrina species, Lophopetalum toxicum, Physostigma venenosum Sciadotenia species, Strychnos toxifera, S. diaboli, S. lethalis, S. Castelnaena, S. rondetelioides, Telitoxicum species.
War poisons	Colliguaja species, Euphorbia species, Hippomane mancinella, Hura species, Sapium species, Schoenobiblus peruvianus.

ammonium chloride (NH₄Cl) chlorhydric acid 0.5 mol/l and sodium hydroxide 1 mol/l of analytical grade were from Carlo Erba (Milan, Italy).

Formiate buffer 10 m*M*, pH 8.2 of the mobile phase A was prepared by dissolving 630 mg of NH_4COOH in 1 l of distilled water and adjusted to pH 8.2 with 30% NH_4OH solution.

Formiate buffer 2 m*M*, pH 3.0 of the mobile phase B was prepared by dissolving 126 mg of NH_4COOH in 1 l of distilled water and adjusted to pH 3.0 with pure formic acid.

Mobile phase C was a mixture of acetonitrile-methanol (2:1, v/v).

A basic buffer was prepared for the liquid–liquid extraction from a saturated solution of ammonium chloride adjusted to pH 9.5 with 30% NH_4OH solution.

Internal Standard $(I.S._1)$ solution was realized by dissolving 1 g of lidocaine free base in 1000 ml of methanol (1 mg/ml). A subsequent dilution was made at 1 μ g/ml (I.S.₂). Lidocaine was a gift from Laboratoires Sepval (Laval, France).

Cevadine, lobeline, senecionine and veratridine were purchased from Extrasynthèse (Genay, France). Aconitine, amygdalin, anabasine, arecoline, atractyloside, atropine, colchicine, emetine, eserine, crotaline, pilocarpine, prunasin, reserpine, retrorscine, scopolamine, sparteine, strychnine, taxol and yohimbine were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Stock solutions were prepared in methanol (or in acetonitrile for aconitine due to its unstability in protic solvent) at 1 mg/ml. Subsequent dilutions at 10, 1 and 0.1 μ g/ml were necessary for blood standard calibration.

2.2. Instrumentation

2.2.1. HPLC-MS

The HPLC–MS system included a series LC 200 pump from Perkin Elmer (Les Ulis, France), a series 200 autosampler PE and an API-165 Perkin Elmer-Sciex (Toronto, Canada) mass spectrometer equipped with an electrospray-type IonsprayTM ionization device. Chromatographic separation was performed on an Uptisphere TM ODSB C₁₈ column, 150×2.0 mm I.D. (5 μ m particle size) interchrom from Interchim (Montluçon, France) with a 20 mm length guard column. Split ratio to MS was 1:3.

2.2.1.1. Cyanogenic compounds. Cyanogenic compounds were separated under isocratic conditions with acetonitrile-formiate buffer 2 mM, pH 3.0 (10:90, v/v) as the mobile phase delivered at a flow-rate of 200 µl/min. Retention times (RTs) were 2.9 min for lidocaine $(I.S._1)$, 4.4 min for amygdalin and 5.6 min for prunasin. Relative RTs (RRTs) were 1.51 and 2.02 for amygdalin and prunasin, respectively. Chromatographic run time was 8 min. The instrument was operated in the positive ionization mode with an electrospray voltage of +4.85 kV while the orifice voltage was set at +130 V. Determination of the two compounds was realized using the following ions m/z 480.3 and 325.2 for amygdalin (sodium adduct of the molecular weight 457.4, and fragment), m/z 318.1 and 313.1 for prunasin (sodium and ammonium adducts of the molecular mass 295.2), and m/z 235.1 and 86.0 for I.S., (protonated ion of the molecular mass 234.3 and fragment). Dwell time was 600 ms per ion.

2.2.1.2. Alkaloids (group A). A gradient elution mode was estimated necessary for the separation of all other compounds which were divided into two major groups with different chromatographic conditions corresponding to different chemical properties. Group A included alkaloids with a pKa value ranging from 6 to 9. Initial conditions were 80% solvent B (formiate buffer 10 mM, pH 8.2) and 20% solvent C (acetonitrile-methanol; 2:1, v/v) maintained for 1 min then linearly increased to 90% C at time 10 min and held for 12 min. Chromatographic duration was 22 min. Flow rate of the mobile phase was 250 μ l/min. Re-equilibration of the analytical column was done at 250 µl/min during 15 min. RTs were 5.8 min for anabasine, 6.4 min for crotaline, 9.2 min for arecoline, 9.8 min for pilocarpine, 13.9 min for scopolamine, 14.2 min for retrorscine, 14.6 min for atropine, 15.4 min for eserine, 15.9 min for senecionine, 16.0 min for sparteine, 17.4 min for yohimbine, 18.3 min for lobeline, 18.7 min for lidocaine (I.S.,) and 19.2 min for emetine. RRTs were 0.31 for anabasine, 0.34 for crotaline, 0.49 for arecoline, 0.52 for pilocarpine, 0.75 for scopolamine, 0.76 for retrorscine, 0.78 for atropine, 0.83 for eserine, 0.85 for senecionine, 0.86 for sparteine, 0.93 for yohimbine, 0.98 for lobeline and 1.03 for emetine. The instrument was operated in the positive

ionization mode with an electrospray voltage of +5.22 kV while the orifice voltage was set at +60 V. The mass-to-charge ratios corresponding to the protonated molecules of anabasine (m/z 163.1), arecoline (m/z 156.2), atropine (m/z 290.3 and fragment at m/z 124.0), crotaline (m/z 326.1), emetine (m/z 481.4), eserine (m/z 338.3), pilocarpine (m/z 304.3 and fragment at m/z 156.2), scopolamine (m/z 336.3), sparteine (m/z 235.2), yohimbine (m/z 355.3) and I.S.₁ (m/z 235.2) were monitored with a dwell time of 300 ms each (see Figs. 1 and 2).

2.2.1.3. Alkaloids (group B), atractyloside, oleandrine and taxol. Group B included alkaloids with a pKa value less than 6.5, the cardiotonic glycoside oleandrine, the pseudo-alkaloid taxol and the diterpenic heteroside atractyloside. Initial conditions were 80% A (formiate buffer 2 mM, pH 3.0) and 20% acetonitrile maintained for 1 min then linearly increased to 90% acetonitrile at time 20 min and held for 5 min. Chromatographic duration was 25 min. The equilibration time between two consecutive samples analyzed in series was set at 15 min. The instrument was operated with a voltage of +5.2 kV applied to the sprayer during experiment with the positive ion detection mode and with a voltage of -4.2 kV during experiment with the negative ionization detection. This latter mode was the detection mode of choice of atractyloside which is a dipotassium salt of a 19-norkaur-16-en-18-oic acid. A double negative ion charge must thus be expected for some ions leading to a z value of 1 or 2. Taxol, a highly oxygenated compound could be successfully analyzed either in negative or in positive ionization modes. Orifice voltage was set at +60 or -60 V depending upon the polarity of the sprayer. The run time was divided into three different periods corresponding to the following molecules: period A from time 0 to 9 min corresponding to the acquisition of lidocaine (I.S.,) and strychnine, period B from time 9 to 18.5 min corresponding to the acquisition of colchicine, veratridine, cevadine, aconitine and reserpine, and period C from time 18.5 to 25 min corresponding to the acquisition of oleandrine and taxol. RTs were 2.6 min for I.S., 2.9 min for strychnine, 14.2 min for colchicine, 14.9 min for

veratridine, 15.3 min for cevadine, 16.0 min for aconitine, 17.0 min for reserpine, 20.5 min for oleandrine and 22.4 min for taxol. RRTs were, respectively, equal to 1.12 for strychnine, 5.46 for colchicine, 5.73 for veratridine, 5.88 for cevadine, 6.15 for aconitine, 6.53 for reserpine, 7.88 for oleandrine and 8.69 for taxol. The mass-to-charge ratios corresponding to the protonated molecules (and fragment when available) of lidocaine (m/z)235.1 and fragment at m/z 86.0), strychnine (m/z335.2), colchicine (m/z 400.3), veratridine (m/z674.4), cevadine (m/z 592.3), aconitine (m/z 646.4 and fragment at m/z 586.3=loss of acetate), reserpine (m/z 609.3), oleandrine (m/z 577.5 andfragments at m/z 433.3 and 373.2), and taxol (m/z854.6 and fragments at m/z 569.5 and 509.4) were monitored with a dwell time of 400 ms each. Figs. 3 and 4 displays the full scan mass spectrum of oleandrine and taxol under the previously described chromatographic conditions. Fig. 5 shows a chromatogram of an extract of a blood standard spiked with 1 ng/ml of the alkaloids. Fig. 6 displays a chromatogram of an extract of a blood standard spiked with 10 ng/ml of oleandrine and taxol.

Negative experiments could be conducted for an ultimate confirmation of the presence of taxol. Fig. 7 gives a full scan, negative ions mass spectrum of the drug. This latter mode was also the mode of choice for the detection of atractyloside, an acidic compound with two sulfate groups. Fig. 8 displays the full scan mass spectrum of the molecule. Lidocaine was used as the external standard and acquired under positive ionization mode during the first 5 min of the run, using the ions m/z=235.1 and 86.0 with a dwell time of 600 ms each. In a second period from 5 to 25 min, the polarity was inverted and the ions m/z =725.4, 645.1, 343.2 and 322.4 were monitored with a dwell time of 500 ms each. RTs were 2.6 min for lidocaine and 18.2 min for atractyloside. RRT was 6.97.

2.2.2. HPLC-MS-MS

The HPLC-MS-MS system included a series P4000 pump from Thermo Separation Products (Les Ulis, France), a series AS100XR autosampler from TSP that were interfaced using a SN4000 device to a triple quadrupole TSQ 7000 tandem mass spectrometer from Finnigan (Manchester, UK). The mass



Fig. 1. Chromatogram of an extract of a whole blood standard spiked with 100 ng/ml of each molecule. Peaks: 1=eserine (m/z 162.0), 2=senecionine (m/z 336.2), 3=emetine (m/z 481.4), 4=retrorscine (m/z 352.2), 5=I.S., lidocaine (m/z 235.1).



Fig. 2. Chromatogram of an extract of a whole blood standard spiked with 100 ng/ml of each molecule. Peaks: 1=sparteine (m/z 235.1), 2=1.S., lidocaine (m/z 235.1), 3a=atropine fragments 1 (m/z 290.3), 3b=atropine fragment 2 (m/z 124.2), 4=yohimbine (m/z 355.3), 5=lobeline (m/z 338.3).



Fig. 3. Chromatogram (above) and positive ions full scan mass spectrum (below) from 300 to 900 amu of oleandrine (molecular mass = 576.7). Ammonium adduct at m/z 594.4. Ion m/z 559.2=577.3-H₂O. Ion m/z 517.4=577.3-Acetate. Ion m/z 433.3=594. -l-arabinose. Ion m/z 373.2=594.4-l-arabinose acetate.



Fig. 4. Chromatogram (above) and positive ions full scan mass spectrum (below) from 300 to 900 amu of taxol (molecular mass = 853.8). Ion m/z 836.4=854.3-H₂O. Ion m/z 776.3=836.3-Acetate. Ion m/z 569.4 (T series)=854.3-286.2 (S series, which corresponds to the lateral chain on the carbon 13 of the dimethylamino- α -hydroxy benzene-propanoic acid). Ion m/z 551.3=569.4-H₂O. Ion m/z 509.3=569.4-Acetate. Ion m/z 387.2=509.3-Benzoate.



Fig. 5. Chromatogram of an extract of a whole blood standard spiked with 1 ng/ml of each molecule. Peaks: $1 = \text{colchicine} (m/z \ 400.3)$, $2 = \text{veratridine} (m/z \ 674.4)$, $3 = \text{cevadine} (m/z \ 592.3)$, $4 = \text{aconitine} (m/z \ 646.4)$, $5 = \text{reserpine} (m/z \ 609.3)$.



Fig. 6. Chromatogram of an extract of a whole blood standard spiked with 10 ng/ml of each molecule. Peaks: 1 = oleandrine (a) m/z 577.5, (b) m/z 433.3, (c) m/z 373.2, 2 = taxol (a) m/z 854.6, (b) m/z 569.5, (c) m/z 509.4.



Fig. 7. Chromatogram (above) and negative ions full scan mass spectrum (below) from 300 to 900 amu of taxol (molecular mass = 853.8).



Fig. 8. Chromatogram (above) and negative ions full scan mass spectrum (below) from 300 to 900 amu of atractyloside (molecular mass = 803.0).

spectrometer was equipped with an electrospray atmospheric pressure ionization device. Chromatographic separation was performed on an inertsil[™] 5 ODS-3 C_{18} column, 150 mm $\times 3.0$ mm I.D. (5 μm particle size) chromsep from chrompack (Les Ulis, France) with a 10 mm length guard column using a mixture of 2 mM ammonium formiate pH 3 (solvent A) and methanol delivered at a flow-rate of 500 μ l/min as the mobile phase. Initial condition was 25% methanol for 1 min linearly increased to 90% methanol at time 20 min and held for 5 min. Chromatographic run time was 25 min. Re-equilibration was done at 600 µl/min during 15 min. No split ratio to MS was applied. The instrument was operated in the positive ionization mode with a voltage of +4.5 kV applied to the sprayer during all experiments. The heated capillary was set at 280°C under a voltage of +50 V. The collision gas was argon at 1.7 mTorr. Collision energies were -35 eV for colchicine, -40 V for lidocaine (I.S.₂) and sparteine, -45 V for aconitine, -50 V for strychnine and -60V for cevadine and veratridine. Dwell time per parent ion was set at 700 ms. RTs were 2.0 min for sparteine, 5.2 min for I.S.₂, 5.6 min for strychnine, 15.4 min for aconitine, 16.0 min for veratridine, 16.8 for colchicine and 17.0 min for cevadine. RRTs were, respectively, 0.39, 1.05, 2.99, 3.07, 3.23 and 3.27. Parent ions corresponding to the protonated molecules of the seven compounds were selected with a mass resolution of 2.5 amu. These were m/z235 for lidocaine and sparteine, m/z 335 for strychnine, m/z 400 for colchicine, m/z 592 for cevadine, m/z 646 for aconitine and m/z 674 for veratridine. Two daughter ions were chosen (when available) based upon criterion of specificity and abundance and collected by the third quadrupole with a mass resolution of 2.5 amu. These daughter ions were m/z 86 for lidocaine, m/z 98 for sparteine, m/z 156 and 184 for strychnine, m/z 438 and 456 both for cevadine and veratridine and m/z 368 and 586 for aconitine. Dwell time per daughter ion was set at 350 ms. Fig. 9 shows a chromatogram of an extract of a whole blood standard spiked with 0.1 ng/ml of each drug.

2.3. Calibration

Drug-free blood samples were collected from screened volunteers. Biological matrices were

pooled, extracted and analyzed by the present methods. No peak was obtained which corresponded to the compounds of interest.

Standard solutions (in methanol or in acetonitrile) were added producing concentrations of 0.1, 1, 10 and 100 ng/ml for alkaloids, oleandrine and taxol. The range of whole blood calibration was set up in the same way but at the concentrations of 0.5, 5 and 50 μ g/ml for the two cyanogenic compounds and at concentrations of 0.1, 1 and 10 μ g/ml for atractyloside.

2.4. Sample preparation and extraction procedures

Table 2 summarizes the different extraction procedures used for the different toxins.

2.4.1. Cyanogenic glycosides

Amygdalin and prunasin were isolated from the biological fluid by extraction with two volumes of acetone. To 1 ml of standard or unknown whole blood, was added 10 μ l of I.S.₁ methanolic solution (1 mg/ml) together with 2 ml of acetone, and vortex mixed for 2 min. After centrifugation, the acetone extract was evaporated to dryness at 40°C under a stream of nitrogen. Reconstitution was performed by adding 50 μ l of acetonitrile–water (50:50, v/v) to the dry residue, then vortex mixed for 10 s. The extract was transferred to a microtube Eppendorf type from ATGC (Paris, France), and centrifuged at 7500 g for 3 min. This important step gives a clear extract separated from oily residues and impurities. At last, 20 μ l were injected in the chromatograph.

2.4.2. Alkaloids (group A)

A 2 ml volume of whole blood was alkalinized with 2 ml of a saturated NH₄Cl solution at pH 9.5. A 100 µl volume of I.S.₂ solution (1 µg/ml) was added, followed by 10 ml of chloroform–isopropanol (95:5, v/v). After gentle inversion of the glass tube for 10 min, the tube was centrifuged at 2000 g for 5 min. The ionizability of alkaloids in acids is the one general feature of these compounds; thus acid/base cleanup was subsequently applied. The organic layer was transferred to another screw cap glass tube, then back-extracted with 5 ml HCl 0.5 mol/l for 10 min. After centrifugation at 2000 g for 5 min, the acidic layer was neutralized with NaOH 1 mol/l and made alkaline with 4 ml of pH 9.5 saturated NH₄Cl



Fig. 9. Chromatogram of an extract of a whole blood standard spiked with 0.1 ng/ml of each molecule. Peaks: 1 = sparteine (daughter ion m/z 98), 2 = lidocaine (daughter ion m/z 86), 3 = strychnine (sum of daughter ions m/z 156+184), 4 = aconitine (sum of daughter ions m/z 368+586), 5 = veratridine (sum of daughter ions m/z 438+456), 6 = colchicine (sum of daughter ions m/z 282+310), 7 = cevadine (sum of daughter ions m/z 438+456).

Table 2		
Extraction	procedures	used

Compounds	Sample volume	Extraction
Cyanogenic glycosides:	1 ml	Add 2 ml acetone and vortex mix for 2 min
amygdalin and prunasin		Centrifuge and evaporate the acetonic extract
		Reconstitute with 50 μ l of acetonitrile–water (50:50, v/v)
		Centrifuge at 7500 g for 3 min
		Inject 20 µl
Alkaloids group A:	2 ml	Add 2 ml of saturated NH ₄ Cl solution at pH 9.5
anabasine, arecoline,		followed by 10 ml of chloroform-isopropanol (95:5, v/v)
atropine, crotaline,		Agitate for 10 min and centrifuge
emetine, eserine,		Back extract the organic phase using 5 ml of
lobeline, pilocarpine,		0.5 mol/1 HCl
retrorscine, scopolamine,		Agitate for 10 min and centrifuge
senecionine, sparteine,		Neutralize acidic layer with 1 mol/l NaOH and make it alkaline
yohimbine		with 4 ml of the pH 9.5 saturated NH ₄ Cl solution
		Re-extract with 10 ml chloroform
		Agitate for 10 min and centrifuge
		Evaporate to dryness and reconstitute with 30 µl acetonitrile
		Inject 20 µl
Alkaloids group B:	2 ml	Add 2 ml of saturated NH_4Cl solution at $pH=9.5$
oleandrine and taxol:		followed by 10 ml of chloroform-isopropanol (95:5, v/v)
aconitine ^a , cevadine ^a ,		Agitate for 10 min and centrifuge
colchicine ^a , oleandrine,		Evaporate to dryness and reconstitute with 30 µl of
reserpine ^a , strychnine ^a ,		acetonitrile-water (50:50, v/v)
taxol, veratridine ^a		Centrifuge at 7500 g for 3 min
		Inject 20 µl
Atractyloside	1 ml	Add 1 ml acetone and vortex mix for 2 min
		Centrifuge and decant the supernatant
		Add to the supernatant 3 ml chloroform and agitate for 5 min
		Remove the chloroform–acetonic phase
		Evaporate the aqueous phase
		Reconstitute the dry residue with 40 μ l of acetonitrile–water (50:50, v/v)
		Centrifuge at 7500 g for 3 min
		Inject 20 µl

^a For an ultimate confirmation, these alkaloids could be purified using the alkaloids group A procedure.

solution. Re-extraction in 10 ml of chloroform was finally conducted for 10 min. The chloroformic phase was evaporated to dryness under a stream of nitrogen at 40°C. The dried residue was dissolved in 30 μ l of acetonitrile from which 20 μ l was injected into the chromatographic system.

2.4.3. Alkaloids (group B) – Oleandrine and taxol

A 2 ml volume of whole blood was alkalinized with 2 ml of a saturated NH₄Cl solution at pH 9.5. A 100 μ l volume of I.S.₂ solution (1 μ g/ml) was added, followed by 10 ml of chloroform–isopropanol (95:5, v/v). After gentle inversion of the glass tube

for 10 min, the tube was centrifuged at 2000 g for 5 min. Alkaloids could be successfully purified following the above-described procedure. However, the acid/base cleanup is not valid for cardenolides and for taxoids. On the other hand, considering the high molecular masses of the compounds of interest, further purification does not allow us to obtain a better signal-to-noise ratio than a single liquid–liquid extraction. The organic phase was thus evaporated to dryness and reconstituted in 30 μ l of acetonitrile–water (50:50, v/v) then vortex mixed for 20 s. The extract was transferred to a microtube Eppendorf type, and centrifuged at 7500 g for 3 min. Finally, 20 μ l was injected in the chromatographic instrument.

2.4.4. Atractyloside

To 1 ml of whole blood was added 1 ml of acetone in order to precipitate the protein content. After agitation during 1 min, the tube was centrifuged and the supernatant decanted in another glass vial followed by the addition of 3 ml of chloroform. The acetone-chloroform phase that has been consequently formed was able to remove the remaining fat by gentle inversion of the tube during 5 min. After centrifugation the clean aqueous upper phase can be isolated and evaporated to dryness under a stream of nitrogen at 90°C in the presence of 50 µl of I.S., methanolic solution. The dry residue was reconstituted with 40 µl of acetonitrile-water (50:50, v/v) then vortex mixed for 20 s. The extract was transferred to a microtube Eppendorf type, and centrifuged at 7500 g for 3 min. Finally, 20 µl was injected in the analytical column.

3. Applications

3.1. Abrus precatorius-Jequirity bean-Fabaceae

3.1.1. Active molecules

This Indian native tropical plant produces ornamental seeds called: crab's eyes, coral peas, paternoster beans, lucky bean or minnie-minnies. The seeds contain one of the most toxic substances of plant origin, abrin whose toxicity is similar to that of ricin.

3.1.2. Analytical toxicology and human poisonings

A radioimmunological method for the measurement of abrin has been proposed [8]. Though the plant is widely distributed throughout the world, human poisoning is very rare and no fatal case has been reported yet [5].

3.2. Genus Aconitum–Monkshood–Ranonculaceae

3.2.1. Active molecules

The amounts of alkaloids (0.5-3%) greatly vary depending on the species, place of origin or time of harvesting. The main drug, aconitine is a methylated and benzoylated ester of a hexacyclic norditerpenic amino alcohol, aconine. Other drugs are hypaconitine, mesaconitine, lycaconitine, jesaconitine,

neopelline, napelline, neoline. bikhaconitine. methyllycaconitine, talatisamine, atisine and lappaconitine [5]. The major alkaloid may vary following the species: aconitine in A. napellus, hypaconitine in A. septemtrionale, hypaconitine and mesaconitine in A. carmichaelii, mesaconitine in A. kuznezofii, bikhaconitine in A. ferox, talatisamine in A. kongboense, atisine in A. anthora and A. heterophyllum, lycaconitine in A. vulparia. Some isoquinoleic alkaloids or phenethylamine derivatives could also be present: higenamine in A. japonicum, magnoflorine in A. vulparia and A. napellus, coryneine in A. Carmichaelii and N-methyl adrenaline in A. nasutum. The most poisonous aconites are A. balfourii and A. deinorrhizum [9,10].

3.2.2. Analytical toxicology

The determination of aconitine in A. kongboense has been realized by thin TLC-fluorometry [11]. A method of TLC-densitometry was established in order to detect mesaconitine, aconitine and hypaconitine in aconite roots. Powdered samples were alkalinized by ammonia and macerated with ether. The spots were stable for more than 24 h [12]. An HPLC method was also described for the analysis of the same three alkaloids in plant material [13]. A GC-MS in the selected ion-monitoring mode was used to detect jesaconitine in blood in a fatal accidental overdose following the ingestion of the Japanese aconite Torikabuto [14]. However, due to poor chromatographic behaviors, some authors have suggested the conversion of the alkaloids into their trimethylsilyl (TMS) derivatives. This resulted in well-defined peaks on selected ion recording. Good linear response over the range of 100 pg to 7.5 ng of the pure compounds was demonstrated [15]. Determination of aconitine, mesaconitine, hypaconitine and jesaconitine, in blood and urine samples has been realized using HPLC combined with UV detection and MS [16]. These alkaloids were recovered very efficiently by solid-phase extraction using a styrene polymer resin. Elution was achieved using acetonitrile-HCl. HPLC-UV separation was done on a reversed-phase column under isocratic conditions using a mixture of tetrahydrofuran and 0.2% trifluoroacetic acid. Limits of detection in blood were 50 ng/ml for the four compounds, whereas due to severe interfering peaks it was quite impossible to obtain the detection in urine even at high concentrations. HPLC–MS separation was done on C₁₈ 150×4.6 mm column using tetrahydrofuran–0.3% trifluoroacetic acid–glycerin as the mobile phase. The protonated molecular ions were m/z=676 for jesaconitine, 646 for aconitine, 632 for mesaconitine and 616 for hypaconitine, and also the degradation ions of either methoxylphenylcarbonyl cation (m/z=135) or phenylcarbonyl cation (m/z=105). The detection limits were 2.5 ng injected in scan mode and 100 pg injected in the selected monitoring mode [16]. Fig. 10 displays the selected ion monitoring chromatogram of a blood extract spiked with *Aconitum* alkaloids.

3.2.3. Human poisonings

In western countries, intoxication is very unlikely. One fatal case was noticed in South France in 1975 where aconite was confused with an edible grass frequently consumed in Catalonia, *Molopospermum peloponesiacum* [17]. In Germany, a fatal report was registered in a 20-month-old girl [18]. On the contrary, fatalities are frequent and numerous in eastern Asia. In the sole province of Sichuan in China, for the period 1980–1984, 72 deaths were reported distributed between 35 murders, 16 suicides and 21 accidental ingestions [19]. Six hundred cases were reported in China during the last 35 years [20]. The harmful effects of these Chinese herbal



Fig. 10. Selected ion monitoring chromatograms of solid-phase extracts of blood and urine samples spiked with *Aconitum* alkaloids. Solid-phase extracts of blood (2 ml) and urine (5 ml) spiked with *Aconitum* alkaloids (100 ng each) were applied to a HPLC–FAB-MS system, selectively monitoring the corresponding protonated molecular ions of m/z 676 (jesaconitine), 646 (aconitine), 616 (hypaconitine) and 632 (mesaconitine). Reprinted from Ref. [16] with permission.

medicines have also become a cause of serious concern among the medical profession in other countries bordering China, such as Taiwan and Hong Kong [21]. In this town, three fatalities were reported in 1994 by But et al. [22] and two by Dickens et al. [23] for the same year. Several authors underline the absence of strict legislation upon the dispensing of these medicines together with an incorrect analytical monitoring in the way of preparing the roots. This worrying phenomenon might additionally occur in the Asiatic populations of large metropolis throughout the world. In Japan, in a 61-year-old poisoned man, jesaconitine was detected in blood by GC-MS, as well as aconitine, hypaconitine and mesaconitine in urine [14]. Other Japanese authors reported a case of homicidal poisoning by aconite. Jesaconitine was detected in the stomach contents, plasma and urine at concentrations of 5.48, 0.43 and 1.07 ng/ml, respectively [24]. Quantitation of Aconitum alkaloids in a blood sample taken from a patient intoxicated by aconite roots gave the following levels of 80, 250 and 50 ng/ml for aconitine, jesaconitine and mesaconitine, respectively [16]. In a person who made a suicide attempt with an aconite extract, the plasmatic concentrations measured 2 h after ingestion were the following: 1.72 ng/ml for aconitine, 0.22 ng/ml for benzoylaconine, 0.31 ng/ml for aconine, 4.04 ng/ml for mesaconitine, 0.68 ng/ml for benzoylmesaconine, 0.19 ng/ml for mesaconine, 0.60 ng/ml for hypaconitine, 0.19 ng/ml for benzoylhypaconine and 0.20 ng/ml for hypaconine [25].

3.3. Atractylis glummifera–Glue thistle–Asteraceae

3.3.1. Active molecules

The main toxins are represented by glycosides with a diterpenic genin derived from kaurene derivatives: atractyloside, carboxyatractyloside, parquine and carboxyparquine [9]. The mechanism of toxicity is quite comparable to the toxins of *Amanita phaloides*. Wedeloside is an aminoglycoside from a carboxyatractyligenin hydroxylated in carbon 13. It has been isolated from another asteraceae *Wedelia asperrima* responsible for livestock poisoning in Canada, and from *Wedelia glauca* in Argentina. The structure of all these molecules is very similar to that of the carboxylated kaurene of a hepatotoxic solanaceae found in South America, *Cestrum parqui* [9].

3.3.2. Analytical toxicology and human poisonings

No chromatographic procedure has been elaborated so far. An enzyme immunoassay has been developed for the detection of atractyloside of *Callilepsis laureola* [26]. Travelers in the ancient Arabian Empire have reported the use of powdered root of glue thistle in couscous dishes for homicidal purposes. Intoxications however, were rather due to the accidental ingestion of the root, especially by the children, who confused the plant with species of wild artichoke, *Scolymus hispanocus*. In Algeria, ten children were involved in a case of collective poisoning, only two survived [27]. Other cases were reported concerning adults after they confused the plant with a medicinal thistle, *Centaurea ornata* [9].

3.4. Atropa belladona–Deadly nightshade– Solanaceae

3.4.1. Active molecules

The deadly nightshade produce tropane alkaloids, esters of the troponic acid, tropanol or scopanol, which are *l*-hyoscyamine mainly present in the fresh plant, atropine (*d*,*l*-hyoscyamine) mainly present in the dried plant and, scopolamine. The whole plant is toxic (between 0.3 and 1% of alkaloids) but the drugs are rather located in the leaves and the berries. L-hyoscyamine and atropine represent 90–95% of the total alkaloid content whereas scopolamine represents only 5–10%. Minor alkaloids are tigloytropeine, aposcopolamine, apoatropine, hydroxy-hyoscyamine and tigloyloxytropane so as pyrrolidine alkaloids, hygrine and hygroline. The plant leaves also contain, in a very low quantity, a coumarine drug: scopoletol [5].

3.4.2. Analytical toxicology

Several chromatographic methods have been developed for the analysis of tropane alkaloids by GC–MS after trimethylsilylation [28] or after hydrolysis and formation of heptafluorobutyryl derivatives [7]. Ion-pair HPLC techniques may be useful to achieve excellent peak shape and separation of the compounds. A thermospray HPLC–MS method for the analysis of hyoscyamine and scopolamine in plant cell culture samples was described by Auriola et al. [29]. Fig. 11 displays a chromatogram of a root cell culture sample of *Hyoscyamus muticus* and the detection of hyoscyamine and scopolamine. The alkaloids were separated on a polymeric reversed-phase column with an acetonitrile–ammonium acetate buffer 0.1 mol/l, pH 10.4 eluent. Selected ion recording of the protonated molecules were m/z= 304 for scopolamine and m/z=290 for hyoscyamine. Xu et al. [30] have developed a sensitive HPLC–MS-MS method for the determination of hyoscyamine in human plasma after liquid–liquid extraction over the linearity range 20–500 pg/ml. The drugs eluted on a C₁₈ 50×3 mm I.D., 3 µm particle size using a mixture of acetonitrile–10 mM ammonium



Fig. 11. SIR chromatogram of a root cell culture sample of *Hyoscyamus muticus*. Compounds monitored: homatropine (A) at m/z 276 (internal standard), hyoscyamine (B) at m/z 290, and scopolamine (C) at m/z 304. Reprinted from Ref. [29].

acetate buffer. Daughter ions of scopolamine and hyoscyamine were m/z=138 and 124, respectively. The limit of quantification of the method was 20 pg/ml.

3.4.3. Human poisonings

A case of serious atropine poisoning caused by consumption of 20-25 berries of deadly nightshade which commenced with psychosis in a boy of 9 years was described [31]. Another case occurred in an elderly but healthy man [32] and another in a selfpoisoned student [33]. A woman was poisoned after drinking stinging nettle tea. Analyses of the tea specimen proved bad defilement among other things with elements of the deadly nightshade [34]. Collective intoxication of eight persons (including four adults) occurred after accidental ingestion of ripened nightshade berries [35]. Measured concentrations of atropine in plasma about 10 h after ingestion were 24, 31 and 217 ng/ml in three out of the four hospitalized patients. An oral dose of 50 mg of atropine sulfate in humans has been reported fatal [29], but there is a lack, in the literature, of fatal overdoses due to plant material documented with toxicological findings. Some papers however, described lethal cases from atropine poisoning [28]. In a case of overdose due to drug abuse of atropine, post mortem concentrations were 0.4 µg/ml in blood, 11 μ g/ml in urine and 0.7 μ g/g in liver.

3.5. Baccharis cordifolia-Asteraceae

3.5.1. Active molecules

The plant material contains highly toxic macrocyclic trichothecene glucosides. The principal metabolites, roridin A, D, E, verrucarrin A and miotoxin A, E, F and G are identical to the major metabolites produced by the fungi *Myrothecium verrucaria* and *M. roridum* [36]. The acute toxicity observed in animals eating the plant is clearly due to these toxins both in free form and as glucose conjugates. The origins of these potent fungal toxins in the plant may be due to trichothecene production by associative fungi or by the plants themselves, which have acquired the biosynthetic genes, via horizontal transfer from a fungus [36].

3.5.2. Analytical toxicology and human intoxications

This Brazilian toxic plant, which is responsible for numerous cases of livestock poisoning in southern Brazil and Argentina, has not yet been responsible for human intoxication. The polar toxic roridins and the isomeric baccharinoids were analyzed on a reversed-phase HPLC column from crude samples and ionized under thermospray conditions. Mass spectra indicated the major formation of the molecular ion-ammonium adducts. Minimum detectable amounts were determined to be 2-5 ng injected [37]. The same authors have investigated the use of GC-MS using negative chemical ionization for the measurement of several naturally occurring and synthetically modified simple trichothecenes after conversion under their trifluoroacetyl, pentafluoropropionyl or heptafluorobutyryl esters. Determination of low trace, typically 0.5-2.0 pg injected, proved feasible after a short clean-up procedure on silica-gel cartridges [38,39]. Analysis of human biofluids has not been described.

3.6. Cicuta virosa and related species (douglasii, maculata, occidentalis)–Water hemlock–Oenanthe crocata and related species (aquatica, javanica)– Hemlock water dropwort–Apiaceae

3.6.1. Active molecules

About 20 species of *Cicuta* have been identified and all are believed to be extremely poisonous. Its habitat is slightly more restricted than that of poison hemlock. Water hemlock (also called beaver poison, cowbane and five-finger root) requires more moisture conditions. It is usually found growing in the water of streams, ditches, lakes, rivers or marshy subatlantic areas of France and the United Kingdom for the species *virosa*, *maculata* in the eastern North America, *douglasii* in the north and *occidentalis* in the western United States. The toxic principle of water hemlock, cicutoxine (heptadeca-4,6-diene-8,10,12-triene-1,14-diol) is a highly unsaturated long chain alcohol. Other drugs are cicutol, falcarindiol and their isomers.

Oenanthe (from the Greek *anthos*, flower and *enos*, wine) grows in cool and moist climates of Europe, America and Asia. The hemlock water dropwort possesses a big tuber (dead men's fingers)

resembling that of a celery or a dahlia root. When it is cut in transverse section, the yellow oily liquid that appears turns brown under the action of air (this yellow liquid gave the name of *crocata*, in reference with saffron). The main toxin is closely related to cicutoxine and has received the name of oenanthotoxine (heptadeca-4,6-diene-2,8,10-triene-1,14diol). Other drugs are oenanthetol, oenanthetone and their isomers. Concentration of the toxins is maximal in winter and at the start of spring [9,40].

3.6.2. Analytical toxicology

Oenanthotoxine was isolated from tubers of *Oenanthe crocata* by refluxing with ether for several hours. Separation of the different toxic compounds could be achieved by thin layer chromatography or reversed-phase HPLC on C18 column using a mixture of methanol-water. UV analysis of the HPLC effluent exhibit highly structured UV spectra containing several λ_{max} at 253, 268, 297, 315 and 338 nm [41]. Mass spectrometry was realized on the bands of thin layer chromatography separation. Oenanthotoxine shows a mass spectrum in the electronic impact mode with main ions at m/z=115, 43, 128, 141, 41, 153, 258 (molecular ion), 55, 91 and 71 [41]. No analytical procedure for the determination of the drugs in human biofluids has been reported, nor for the toxins of Cicuta species.

3.6.3. Human poisonings

Since the concept 'returning to nature' and the assumption by some writers that all things natural are good, many people are experimenting with leaving off the land and eating edible plants, roots and berries. Unfortunately, water hemlock has roots that look like parsnip roots and have a celery odor that make the plant adequate for confusing it with wild parsnip (Pastinaca sativa), wild carrot (Daucus carota) wild celery (Apium graveolens), artichokes, sweet potatoes or sweet anise. Five fatal cases were reported in the United Sates between 1979 and 1988. Landers et al. [42] have described a fatal ingestion of two large rhizomes of the water hemlock plant in a 22-year-old man. Another member of the same group who ate only one rhizome recovered after a critical period of five grand mal seizures.

Hemlock water dropwort is probably the most poisonous plant in the British Isles. The roots are the

most toxic part of the plant and have been eaten in mistake for the roots of several other plant species with nine fatal cases out of 13 intoxication reported in the United Kingdom between the start of the century and 1978 [43]. The plant was also used as a poisonous decoction for criminal purposes [5]. Additionally, two fatal cases were noticed in France and Italy [44,45]. More recently, King et al. [41] presented three separate accidents of poisoning by absorption of the tubers, with two of these cases involving a fatality. One fatality was the fact of three young people who were subsisting on a diet of natural vegetation. The second case involved a young female student whose intention was to test the plant in the hope of producing hallucinogenic effects. No post-mortem concentrations are available in the literature.

3.7. Colchicum automnale–Meadow saffron– Liliaceae

3.7.1. Active molecules

Though more than 30 molecules are naturally occurring compounds found in the meadow saffron, only three of them have been studied and used in therapy: colchicine, colchicoside and demethyl-3colchicine. Concentrations of colchicine in flowers is contained between 0.08 to 0.6%, 0.03 to 0.06% in the bulb and 0.2 to 1.06% in seeds. Minor alkaloids are androcymbine, autumnaline, colchicilline, colchifoline, cornigerine and their demethylated derivatives. Other Liliaceae such as the glory lily, Gloriosa superba (known in Sri Lanka for its suicidal use) and Gloriosa rothschildiana would present the same toxicity [9]. The meadow saffron could be easily mistaken with the common crocus. This phenomenon is somewhat dramatic in some areas of Turkey where the population celebrates each year during the spring the traditional 'crocus-day'. Death of young children confusing the bulb of the two plants is unfortunately not uncommon [1].

3.7.2. Analytical toxicology

The most recently published methods used HPLC with either single-wavelength UV or photodiode array detection. Separation occurred on a C_{18} column [46,47]. Mobile phases consisted of a mixture of acetonitrile-methanol-0.1 mol/l KH₂PO₄-5 µmol/l

pentane sulfonic acid [47], pH 6.0 or acetonitrilemethanol-0.1 mol/l KH₂PO₄, pH 7.6 [48]. Liquidliquid extraction was conducted at pH 8.0 using dichloromethane as the organic solvent in both cases. The limits of detection were 1 ng/ml using a 3-ml blood sample in case of classic UV detection at 350 nm or 5 ng/ml using a 1-ml blood sample in case of photodiode array detection. Colchicine however, possesses a very short half-life of 20 min. On the other hand colchicine is rapidly taken into cells and bound to intracellular microtubular proteins. Considering that death can occur at any stage after ingestion, and more often after a long agony (more than 40 h), many reports were unable to detect postmortem blood concentration of the drug. That is why an HPLC-MS method was reported by Tracqui et al. [49] to insure a better sensitivity in the detection of forensic cases for which the identification of the toxin would be of major importance. HPLC separation was performed on a C₁₈ column, 250×1 mm I.D. while elution was achieved isocratically with a mobile phase of acetonitrile-2 mM ammonium formiate buffer pH 3.0. Fig. 12 displays an HPLC-MS chromatogram from a 1-ml blood extract in a suspected fatality. Colchicine was detected at m/z =400 (with small m/z 422 and 438 corresponding to sodium and potassium clusters) and the limit of detection was 0.6 ng/ml.

3.7.3. Human poisoning

The most serious poisonings result from the suicidal ingestion of colchicine tablets. Although several papers report colchicine overdoses, drug measurement in fatalities has rarely been realized. In most cases, the drug was detected at the admission in intensive care unit in ante-mortem blood, but often none was found in post-mortem samples [50]. Ingestion as little as 7 mg has been reported to be fatal whereas subjects have survived 50 mg of the drug. In a fatal case due to the ingestion of 7.5 mg of colchicine, serum ante-mortem concentration was 21 ng/ml 6 h after absorption [50]. In a 20-mg overdose, ante-mortem concentration in plasma was 250 ng/ml 2 h after administration [47]. In two cases, after intravenous injection of colchicine, serum concentrations of 170 and 240 ng/ml were measured before death [51]. For all these cases, none was detected in post-mortem samples. In a 45-year-old



Fig. 12. (a) HPLC–ISP-MS chromatogram from a blood extract (1-ml sample) in a suspected fatality; TIC recording (m/z 380–405); (b) HPLC–ISP-MS reprocessed chromatogram, extracted from the previous data by selecting m/z 383 and 400. Measured colchicine concentration: 96 ng/ml blood. Reprinted from Ref. [49] with permission.

male, ante-mortem plasma concentration was 60 ng/ ml 3.3 h after hospitalization (but 2-3 days after absorption). Reported post-mortem concentrations were 30 ng/ml in blood and 4200 ng/ml in bile (while nothing was detectable in liver, in vitreous humor and stomach content) [52]. In another case of suicide, post-mortem examination revealed the following colchicine levels: 62 ng/ml in femoral blood, 2921 ng/ml in bile, 1024 ng/ml in urine, 20 ng/ml in vitreous humor, 12 ng/g in liver and 29 ng/g in heart [48]. Poisonings by plant materials were reported in Switzerland or in Turkey [1,3]. Literature reported the case of two adults who died following the ingestion of leaves of meadow saffron administered by a healer who confused the plant with bear's garlic, Allium ursinum [5]. Very recently, in a sub apenenic area of Italy, a married couple had gathered wild vegetables (confused with Tragopogon pratensis, a common edible plant) that were subsequently cooked and ingested at dinner. The man died during the episode, colchicine concentrations being, respectively, 16 and 1600 ng/ml in blood and urine 20 h after the ingestion in ante-mortem samples and 12 and 90 ng/ml, respectively, on the third day of hospitalization [53].

3.8. Conium maculatum–Poison hemlock–Apiaceae

3.8.1. Active molecules

Four species of *Conium* are recognized throughout the world, however, one species *maculatum* was famous in Europe during antiquity and the herb grows also widely in America. The piperidinic alkaloids of the poison hemlock are formed by transamination of alanin and 5-ceto-octanal leading to the synthesis of gamma-coniceine and then to others alkaloids which are: coniine (2-propylpiperidine), *N*-methyl coniine, conhydrine, pseudoconhydrine, conhydrinone, *N*-methyl pseudoconhydrine and 2-methyl piperidine. Coniine and gamma-coniceine are the predominant alkaloids [9,40]. Gamma-coniceine predominates in the vegetative stage of plant development, while coniine predominates in the mature plant and seeds. Both drugs are toxic and teratogenic. Coniine is a volatile compound; thus its conservation in desiccated plant is not optimum. This drug has also been identified in volatile compounds produced by a carnivorous plant *Sarracenia flava*, a few nanograms of the substance could thus paralyze an ant [5]. An organized movement named 'The Hemlock Society' promotes euthanasia/assisted suicide [54,55].

3.8.2. Analytical toxicology

A GC–MS method was used in three fatal cases of hemlock ingestion in Australia. Gamma-coniceine was identified in full scan mode, main ions were m/z=97, 41, 110, 70 and 125 [52]. A GC–MS technique for the detection of coniine in plant material, rumen, liver and urine was described after a triple liquid–liquid extraction [7]. Main m/z ions are 84, 56, 85, 70, 126 and 127.

3.8.3. Human poisonings

Concoction of poison hemlock was introduced for executions in the latter half of the fifth century before Christ and was employed by the Thirty Tyrants, the 1-year government of Athens installed after the Peloponnesian War. The trial of Socrates was well documented by narratives of Plato and Xenophon. The poison potion was probably realized by mixing an extract of the plant into wine. However, accepting that the hemlock dose was responsible for death, it seems the placid death scene depicted in the Phedo not acceptable. Plato must have been selective in his description certainly because it must have been a very ignominious end for such a venerable philosopher. Socrates' last words were addressed to his slave and friend Crito: 'Crito, we owe a cock to Asclepius; please pay the debt, and be smart with it'. As a matter of fact, the Greek behavior wants the patient to give a cock in devotion to his doctor when he estimated he was cured, and Socrates could be healed in the allegorical point of view of 'the pervasive illness that is life itself'. A complete and detailed discussion of Socrates' end is reported in the excellent paper of Scutchfield and Genovese [55].

Fatal intoxications were very rarely reported, one case occurred in the United Kingdom, one in the

United States and three recently in Australia [5,56,57]. Post mortem measurements were not available. Drummer et al. [56] identified gamma-coniceine in the blood (three times) and gastric contents (twice) by using a GC–MS technique.

3.9. Convallaria majalis–Lily-of-the-valley– Liliaceae

3.9.1. Active molecules

The whole plant contains cardiac glycosides that have digitalis-like activity. These cardenolid glycosides are: desglucocheirotoxol, cannogenol-3-*O*-alpha-L-rhamnoside, cannogenol-3-*O*-beta-D-allomethyloside, convallatoxin, convallatoxol, convalloside, locundioside and periguloside [9]. Two flavonoids are also present in the inflorescence of the plant: isorhamnetin and quercetin.

3.9.2. Analytical toxicology and human poisonings

Assays of convallatoxin, locundioside, desglucocheirotoxol and periguloside have been reported in plant material [58,59]. Though several methods for *Digitalis*-like cardiac glycosides have been described in human biofluids that could theoretically be transposed to the analysis of *Convallaria*glycosides, no specific method was reported for toxicological purposes. Lily-of-the-valley has been responsible for sudden collapse in animals but no fatality has been reported for humans [5].

3.10. Coriaria myrtifolia-Coriariaceae

3.10.1. Active molecules

The main toxin, coriamyrtin whose structure is related to picrotoxine is a highly potent toxic molecule. Corianin has been also isolated from *Coriaria japonica* [5].

3.10.2. Analytical toxicology and human poisonings

Several serious intoxication caused by ingestion of the fruits of the plant known as *Coriaria myrtifolia* have been reported especially in Spain [60]. Evolution was good in all cases. No analytical procedure has been reported yet in biological materials. 3.11. Datura stramonium and related species (arborea, ferox, inermis, innoxia, metel, sanguinea, sauveolens)–Jimsonweed or thorn apple– Solanaceae

3.11.1. Active molecules

Alkaloids are the same as in *Atropa belladona* at a concentration of 0.2 to 0.6% in the whole plant (0.3-0.6% in the leaves, 0.5-0.6% in the stern, 0.3% in the seeds and in the flowers) distributed as scopolamine for one third, atropine and *l*-hyoscy-amine for two thirds. Vitale et al. [61] have also identified two other alkaloids in the *ferox* species, which were: 7-beta-hydroxy-6-beta-propenyloxy-3-alpha-tropoloxytropane and 3-phenylacetoxy-6-beta-7-beta-epoxytropane (3-phenylacetoxyscopine).

3.11.2. Human poisonings

The toxicity of jimsonweed is known for a long time since it is made partly responsible for the losses undergone by the army of Marcus-Anthony in 36 BC [62]. In North Africa, the species metel was powdered and accommodated in cooked dishes for criminal purposes [5]. In the ancient Ottoman Empire, travelers have reported that the women of the harem were useful themselves of the species inermis and innoxia to escape a few hours the monitoring of the Sultan's eunuchs. In Europe with the Middle Ages, the crushed leaves mixed with snuff 'the soporific', were used by the brigands in order to rob their victims. Seeds thrown on the hearths of the drying ovens of the public baths were used for the same intentions. In South America, Daturas were sacred plants allowing the divination and religious practices. Actually, due to its easy availability, teens are using jimsonweed as a drug. Plant parts such as the flowers called angel's trumpet or angel tulip can be chewed, and seed pods, commonly known as pods or thorn apples or devil's apples can be eaten [63,64]. The visual hallucinations were similar to those reported by LSD users. Associated with cannabis, it is named green dragon. In South America nowadays, decoctions of the seeds are used as incapacitating agent for rapes and robberies [65]. Thin layer chromatography and ultra violet scan detection of blood and urine was employed for the detection of scopolamine in a case of self-induced Datura sauveolens poisoning [66]. Urich et al. [62] reported a case of collective intoxication by a tea made by a botanist amateur who was besides the only one to die at the time of the episode. Scopolamine and atropine were identified in the urine of the decedent by GC–MS. In India, Frohne et al. [67] have reported 2728 deaths imputed to overdose of *Datura* between 1950 and 1965. In South America and in the Caribbean, the species *arborea* and *sanguinea* are traditionally used as a hallucinogen [68].

3.12. Genus Dieffenbachia-Araceae

The common houseplant, dieffenbachia, causes painful edematous swelling of the oral mucus membranes when chewed. The numerous needles, raphides, of calcium oxalate, which are contained in the specialized cells, iodioblasts, in the plant, and proteases have both been implicated. When stimulated, the iodioblasts fire the raphides with some force for a distance of two to three cell lengths. One possibility is that the local toxicity of dieffenbachia may be caused by a combination of this mechanical release of the raphides, which results in traumatic injury, and the effects of a chemical carried on their surfaces [69]. Some investigators believe the active inflammatory portion of the soluble fraction to be trypsine-like; it seems more likely that the agent is either histamine or kinine-like in nature. Although numerous literature citations promote their toxicity, there are few case reports which substantiate a cause effect relationship between ingestion and resultant symptomatology. A retrospective review of such cases reported to the regional poison information center of Pittsburgh described 188 cases for which philodendrons accounted for 67.5% and dieffenbachias for 32.5%. A 78.2% involved children aged 4-12 months [70]. During 1976, 485 cases of dieffenbachia poisoning were reported in the United Sates. In 1990, however, the number increased to 4124 [70].

3.13. Digitalis purpurea and related species (grandiflora, lanata)–Foxglove–Scrophulariaceae

3.13.1. Active molecules

The toxicity of *Digitalis purpurea* is due to some extremely potent cardiotonic glycosides. The wild plant may content 0.1 to 0.3% of these cardenolides

while concentration of the drugs in D. lanata could reach 1% of the total mass. Glycosides are divided into five series; A, B and E for D. purpurea and A, B, C, D, E for D. lanata. Digitoxin, the main heteroside of the A series of D. purpurea contains a 3,14-dihydroxylated genins bonded in position 3 to an oligoside. Primary heterosides, purpureaglucoside A, B and E are highly unstable compounds that can easily be hydrolyzed during the drying process of the fresh plant leading, respectively, to the formation of digitoxin, gitoxin and gitaloxin [9]. Primary heterosides of *D. lanata* are lanatosides for which the molecule of digitoxose linked to that of glucose occurs under an acetyl derivative. The loss of the terminal glucose molecule from the lanatoside A leads to the formation of acetyldigitoxin which could itself be transformed under alkaline conditions into digitoxin. Lanatoside C leads to the formation of acetyldigoxin and then to the production of digoxin whereas lanatoside D leads to the formation of acetyldiginatin and then to the production of diginatin.

3.13.2. Analytical toxicology

A solid-phase extraction on a C₁₈ modified poly-(styrene-divinylbenzene) polymer was described for the HPLC analysis of more than 50 cardenolides in about 2 mg of dried leaf powder of Digitalis lanata [71]. A high-performance TLC was developed for the determination of digoxin and related compounds in digoxin drug substance and tablets [72]. Radioimmunoassay and fluorescence polarization immunoassay were developed for rapid and accurate measurement in serum or plasma. There is now considerable data in the literature on serum digoxin levels in therapy. For post-mortem examinations, these methods are limited to varying degrees by non-specific interference from endogenous digoxin-like factors which are normally present in the blood of patients with certain diseases and interference from digoxin metabolites with varying degrees of cardioactivities and varying degrees of cross-reactions with the antibody [73]. A more specific method was recommended that combined the selectivity of HPLC with the sensitivity of immunoassay [74]. Tzou et al. [73] proved that a precolumn fluorescence derivatization HPLC method was suitable for pharmacokinetic quantitation of digoxin and its metabolites in human

fluids. The drugs were extracted with a cyclodextrin solid-phase extraction column. Fluorescent derivatives were formed by reaction with 1-naphtoyl chloride and secondly isolated using cyclodextrin and C_1 columns sequentially. Determination by HPLC was realized on a normal-phase silica column with hexane-methylene chloride-acetonitrilemethanol as the mobile phase. Concentrations as low as 0.25 ng/ml of both digoxin and its metabolites were determined with acceptable precision and accuracy. Just recently, Lacassie et al. [75] reported the simultaneous detection in post-mortem samples of 17 glycosides of main forensic interest by HPLC-MS. Separation occurred on a C18 column using a mixture of acetonitrile-2 mM ammonium formiate buffer pH 3 as the mobile phase. Limits of detection however were not suitable for therapeutic drug monitoring.

3.13.3. Human poisonings

During the last few years of Vincent Van Gogh's life, his paintings were deeply characterized by halos and the dominant color yellow. Some authors alleged that he might have experienced a digitalis poisoning [76,77]. Xanthopia due to overdosage of digitalis is well documented. Nevertheless, though it was established that foxglove was used to treat epilepsy during the latter part of the 19th century, no evidence of usage by the painter can be substantiated. The artist admitted to episodes of heavy drinking of absinthe (Artemisia absinthium) and other substances with a chemical connection to thujone like camphor and therebentine, containing pinenes and terpenes inducting an acute intermittent phorphyria. It is possible that he developed abnormal craving for terpenes, a sort of pica, that would for part explain his strange behavior and manners [78].

Intoxications by therapeutic overdosage or suicide attempts by means of pharmaceutical formulations are not rare while ingestion of plant material is very uncommon. Unwitting use of the foxglove plant for brewing tea resulted in cardiac glycoside toxicity in few occurrences [5]. One other case was due to intentional overdose of a homemade foxglove extract (maceration with vodka) [79]. One case was a herbalist mistake and two resulted from the confusion of the plant with *Ajuga decumbens* or with *Symphytum officinale* [80]. Many authors reported on the post mortem concentrations of digoxin after overdose and therapeutic use. Blood levels were higher than 10.0 ng/ml in cases of acute poisoning while concentrations in autopsy specimens of persons who had been under digoxin maintenance therapy ranged from less than 0.2 to 8.2 ng/ml. A third group could be isolated from the previous two, for concentrations varying between 2.0 and 10.5 ng/ml. This group comprises poisoning that could not definitely be supported by historical information or police investigation and death attributed to other causes for which the deceased showed evidence of dehydration or renal failure [81–83].

3.14. Genus Dioscorea-Dioscorea-Dioscoreaceae

Dioscorea are tropical plants used throughout the world for the extraction of diogenin, used as a natural precursor for the synthesis of steroids. Other exploited natural molecules for this purpose are: hecogenin, smilagenin and sarsapogenin. Most of the species (*alata, batatas, esculenta* and *opposita*) are sources of edible roots for human consumption, others are used in the industry of steroids (*composita, deltoidea, florinbunda, mexicana, pantaica, spiculi-flora* and *zingiberensis*). One species, however, remains toxic, even after cooking: *Dioscorea simulans* for which a young male student died after eating a cooked root tuber of this plant [8,19].

3.15. Genus Duboisia–Duboisia–Solanaceae

Australian native trees *Duboisia leichhardtii* and *D. myrporoïdes* contain high concentrations of atropine-like alkaloids especially *l*-hyoscyamine and scopolamine. They are used in plant biotechnology for the production of plant-derived pharmaceuticals [84]. Though these trees are highly potent toxic materials, no fatality was reported in literature. To date, occupational exposure to the dried plant material resulted in two clinical observations [85]. Plant abuse, as an intoxicant and hallucinogen was also reported at a higher frequency with central effects and peripheral anticholinergic effects comparable to those of jimsonweed abuse.

3.16. Eupatorium rugosum and related species (cannabinum)–White snakeroot–Asteraceae

3.16.1. Active molecules

Among plant toxicants excreted in the milk of lactating animals is tremetol or tremetone, the toxins of white snakeroot and rayless goldenrod (*Happlopapus heterophyllus*). The active components of *Eupatorium cannabinum* (crofton weed) are the pyrrolizidinic alkaloids: echinatine, lycopsamine, intermedine, rinderine and supinidine [9].

3.16.2. Analytical toxicology and human poisonings

No analytical procedure has been reported in biological specimens. These plants have been responsible for intoxication of cows and their suckling calves and for many human fatalities in early colonial times in the USA [5]. The plant is also famous for having provoked the death in 1818 of Nancy Lincoln, the mother of the future president of the United States, aged 8 years at that time [5,86]. It still continues to poison animals particularly horses and goats.

3.17. Ferrula communis and related species (chiliantha, tingitana)–Apiaceae

3.17.1. Active molecules

The whole plant contains ferulenol, hydroxy-ferulenol, ferprenin and isoferprenin that have an antithrombinic activity [87].

3.17.2. Analytical toxicology and human poisonings

To date, no human fatality has been reported though several cases were reported concerning sheep and cows, particularly in North Africa. Indeed, human poisoning could always be a possibility. In Iran and Iraq, some atoxic species of *Ferrula* are used as perfumes or condiments. Ferulenol has been quantified in sheep plasma was achieved using HPLC on a C_{18} 250×4.6 mm analytical column and fluorescence detection [81]. The mobile phase was methanol–ammonium acetate/triethylamine buffer, pH 5.6 (82:18, v/v). Plasma was purified (after protein precipitation with acetonitrile) by solid-phase extraction on a mixture of fluorisil and aluminum oxide. Limit of detection was 0.1 μ g/ml.

3.18. Gelsemium sempervirens and related species (elegans, rankinii)–Carolina jessamine– Loganiaceae

3.18.1. Active molecules

Carolina jessamine is native of eastern United Sates and is cultivated in the South. Seventeen indole alkaloids were isolated from the various species of Gelsemium including: gelsemine, gelsevirine, 21-oxogelsemine, gelsedine, 14-beta-hydroxygelsedine, gelsenicine, humantenidine, humantenirine, 11-hydroxyhuman-humantenine, koumine, koumidine. kumantenidine, kumantenine, kumantenmine, rankinidine, N-desmethoxyrankinidine and 11-hydroxyrankinidine [88,89]. Two pregnane derivatives: 12beta-hydroxy-5-alpha-pregn-16-ene-3,20-dione and 12-beta-hydroxy-5-alpha-pregn-16-diene-3,20-dione have been also extracted from Gelsemium sempervirens and found to be the principal cytotoxic entities of the plant [90].

3.18.2. Analytical toxicology and human poisonings

A reversed-phase HPLC method for the detection of various alkaloids has been reported for the quality control of *Gelsemium* preparations but no procedure suitable for analytical toxicology has been published [91]. Poisoning is unlikely to occur though some cases were described for young children who were attracted by the brightly yellow flowers [5]. No human fatality has been noticed for this herb.

3.19. Hyoscyamus niger and related species (albus, falezlez, muticus, reticulatus)–Henbane– Solanaceae

3.19.1. Active molecules

Alkaloids are the same as in *Atropa belladona* but at a lower concentration in the whole plant, i.e. between 0.05 and 0.15%. Scopolamine represents the main drug (around 50% of the total alkaloid content) which is usually sedative and euphoric, in contrary to *l*-hyoscyamine, which is stimulatory and hallucinatory. *Hyoscyamus albus* and *falezlez* contain 0.7– 1.5% alkaloid.

3.19.2. Human poisonings

Documented cases of human henbane poisoning are difficult to find. Perhaps the most startling occurred in 1881 when Hyoscyamus falezlez was used by the Touareg to poison a large part of the French Flatters expedition in the Hoggar in Algeria [92]. This case was a precursor of modern incapacitating agents that take place in the chemical warfare arsenal. The toxin was administered in dates. In 1910, 25 men and women suffered from the effects of eating the root of henbane given by mistake for horseradish [93]. More plentiful cases can be found in the Turkish literature where the problem is endemic [94,95]. Deaths are not uncommon in children, who, when fresh vegetables are scarce, eat the leaves, which taste like lettuce. An identical observation was made in the Negev in Israel, concerning Bedouin children with the species reticulatus [94]. More recent cases are deliberate chewing of the flowers of henbane in the hope of producing euphoria. These cases indicate a more widespread use of this plant among the drug-taking community [96]. Detection in biological specimens was reported in a case of a self-induced scopolamine overdose [28]. Post-mortem concentrations were 0.13 μ g/ml in blood, 43 μ g/ml in urine and 0.08 μ g/g in liver.

3.20. Jatropha curcas

3.20.1. Active molecules

a possibility.

Jatropha curcas which is also known as purging nut tree, contains a toxic lectin named curcin. A dried extract of the seeds administered intraperitoneally to mice produced death for doses as low as 1 mg/kg. The authors noticed the similarity of the lesions found in the mice to those generally attributed to ricin [97].

3.20.2. Analytical toxicology and human poisonings

So far we are concerned, no analytical procedures have been described yet. Several acute poisonings due to the accidental ingestion of the seeds by children were described but all made a rapid and uneventful recovery [98]. However, due to its toxicity, death related to *Jatropha curcas* could always be

3.21. Lobelia inflata and related species (polyphylla, portoricensis)–Lobeliaceae

3.21.1. Active molecules

Lobeline is the main alkaloid of the species *inflata* while *polyphylla* contains essentially nor-lobelanidine. Minor components are meso-lobelanine and meso-lobeladidine [99].

3.21.2. Analytical toxicology and human poisonings

An assay of lobeline was proposed in plant material [100]. A reversed-phase HPLC method was published for the detection of lobeline in rat plasma and brain tissue [101]. Human poisoning was rare with no fatal case.

3.22. Nerium oleander and N. indicum, Thevetia peruviana and T. nerifolia–Common or yellow oleander–Apocynaceae

3.22.1. Active molecules

The common oleanders contain a mixture of poisons including glycosides that are extremely toxic and are found in all parts of the plant. The main one being oleandrin, other compounds include neriine (or neroside), oleandroside, and digitoxigenin. *Thevetia peruviana* contains thevetin and thevetoxin. Among other minor glycosides of poor activity, we could enumerate adynerigenin, uzarigenin, adigoside and glyco-stropeside. Leaves from *Nerium oleander* were shown to contain 0.018 to 0.425% oleandrin [9].

3.22.2. Analytical toxicology

Several authors showed that the cardiac glycosides from *Nerium oleander* partially cross-react with digoxin or digitoxin immunoassays. However, this cross-reactivity has not been correctly established and it was demonstrated that it changes from one kit to another [102–104]. The response of the serum digitoxin fluorescence polarization immunoassay with serum specimens containing various concentrations of oleandrin is linear but it is impossible to predict the degree of toxicity from the measured digitoxin level. On the other hand, a two dimensional thin layer chromatography analysis of ingesta of oleandrin has been described [105]. An HPLC method for the analysis of oleandrin in oleander poisoning was developed after an ExtrelutTM column extraction over the range 50–10 000 ng/g in whole blood [106]. The state of the art in this area however, is the method developed by Tracqui et al. [107] who described a HPLC–MS assay of oleandrin on a C₁₈ column (150×2.0 mm) using a gradient of acetonitrile–2 m*M* ammonium formiate buffer, pH 3.0. Fig. 13 displays a chromatogram from an extract of blood in a non-fatal *Nerium oleander* poisoning. Based upon its specific fragmentation/cluster formation at m/z=618, 594 and 557, the quantification limit of oleandrin in whole blood was 0.4 ng/ml.

3.22.3. Human poisonings

Several non-fatal poisonings were described in the literature including young children in accidental ingestion or adults in accidental or voluntary intoxications [5]. Indian authors reported two fatalities with the ingestion of more than four seeds and when arrival in an intensive care unit was done after 5 h after intake [102]. A woman died after drinking herbal tea prepared from oleander leaves that she believed to be eucalyptus [104]. On a post-mortem specimen, the measured serum digoxin level (by a radioimmunoassay technique) was 6.4 ng/ml. In a non-fatal self-poisoning involving ingestion of five leaves of oleander, the measured blood concentration of oleandrin at admission in the hospital was 1.1 ng/ml by an HPLC–MS technique [107].

3.23. Nicotiana glauca-Tobacco tree-Solanaceae

3.23.1. Active molecules

The toxicity of *Nicotiana tabacum* and *Nicotiana rustica* will not be considered in the present paper. The primary alkaloid of the tobacco tree is anabasine that possesses structural similarity to nicotine and the same molecular weight. Other tobacco alkaloids include anatabine, nicotine, nornicotine, metanicotine, dihydrometanicotine, 2,3'-bipyridyl and myosmine.

3.23.2. Analytical toxicology and human poisonings

Although very toxic due to the occurrence of anabasine throughout the plant, very few poisonings have been noticed. The sole fatality was reported by Castorena et al. [108] who described a triple liquid–



Fig. 13. Chromatogram from the patient's first blood sample. Upper graph: TIC recording (m/z 520–630); arrow: location of oleandrin. Lower graph: reprocessed chromatogram from the same sample, in selected ion monitoring at m/z 577 (gray line) and 594 (black line). Peak at 8.24 min: oleandrin, concentration 1.1 ng/ml. Reprinted from Ref. [107] with permission.

liquid extraction (1-chlorobutane at pH 10, re-extraction with HCl 0.1 mol/l) of body fluids followed by a GC-MS analysis in a young self-overdosed adult male. The base ion of anabasine is m/z=84with an apparent m/z=162 molecular ion. Additional small fragments are present for anabasine as compared to the mass spectrum of nicotine. The measured anabasine postmortem concentrations were 1.15 μ g/ml in blood, 73.8 μ g/ml in urine, 11.0 $\mu g/g$ in the brain and 2.0 mg/g in the leaves of some specimens of Nicotiana glauca that have been taken in the field where the deceased was found. When compared to nicotine poisoning cases, the blood concentration of 1.15 µg/ml is much lower than the measured postmortem concentrations of nicotine. As a matter of fact, these reported levels ranged from 5.0 μ g/ml to more than 5000. Indeed, this might suggest a higher toxicity of anabasine in humans than nicotine. Another GC-MS method has

been described for the simultaneous measurement of several tobacco alkaloids in urine of smokers and smokeless tobacco users after conversion of the secondary amine alkaloids to tertiary amine alkaloids by reductive alkylation using an aldehyde and sodium borohydride [109].

3.24. Pachyrhizus erosus

A man cooked and ate about 200 g of the seeds of this poisonous plant after mistaking them for kidney beans [19]. He died 11 h after ingestion.

3.25. Pausinystalia yohimbe-Yohimbe-Rubiaceae

3.25.1. Active molecules

This African native tree contains 1-6% alkaloids in the bark. Together with the main presence of yohimbine, other identified drugs occurred under the form of isomers of yohimbine: corynanthine, pseudoyohimbine, allo-yohimbine and epiallo-yohimbine; derivatives with a heteroyohimbane compounds skeleton like ajmalicine or tetracyclic derivatives like corynantheine.

3.25.2. Analytical toxicology and human poisonings

Overdose of this drug is very uncommon, and concerns either suicide attempts or aphrodisiac experiences. In this aim, yohimbine is known as a street drug under the denomination 'yo-yo'. Psychiatric manifestations were observed in a 31-year-old male who alleged to have taken 75 tablets of 2 mg of yohimbine. Measured by HPLC-PDA on a REMEDITM apparatus, serum concentration of yohimbine was 0.12 g/ml, 1.5 h after ingestion [110]. One fatal poisoning of a child by yohimbine was noticed in the literature [111].

3.26. Pilocarpus microphyllus-Rutaceae

3.26.1. Active molecules

Several plants of the genus *Pilocarpus* contain the toxic alkaloids pilocarpine, isopilocarpine, pilocarpidine, jaborine and jaboridine that possess an imidazole skeleton [9]. The main species being *microphyllus*, some other minor representatives of the genus are: *jaborandi*, *pennatifolius*, *trachylophus* and *racemosus*.

3.26.2. Analytical toxicology and human poisonings

A sensitive assay of pilocarpine in biological fluids has been developed involving a separation of the compounds on a cyanopropyl silica column and a fluorescence derivatization using 4-bromomethyl-7methoxycoumarin [112]. The limit of detection was 1 ng/ml. Death related to the absorption of plant material has not been reported in the literature. A paper, however, described two fatal cases of hospital inpatients who were poisoned by pilocarpine administered through their food that had been adulterated [113]. A case of a suicide attempt was realized by drinking and self-injecting pilocarpine eyedrops in a young male adult [114].

3.27. Genus Philodendron-Araceae

Philodendrons and dieffenbachias are members of the araceae family including about 1800 species. Philodendron is probably the most common houseplant in the United States and in Europe. This plant, like dieffenbachia, contains calcium oxalate crystals located in ampoule-shaped raphide ejector cells. A paper reported that the raphides are coated with a proteolytic enzyme as determined by thin layer chromatography, ion-exchange resin and nuclear magnetic resonance. A death is reported in the literature concerning an 11-month-old child who had chewed the leaves of a philodendron plant and developed oropharyngeal erosions and dysphagia. Unexpected sudden death occurred 17 days after the ingestion and was attributed to vagotonia secondary to the esophageal lesions caused by the leaves [115].

3.28. Genus Polygonatum–Solomon's seal– Liliaceae

3.28.1. Active molecules

The cardiotonic effect and hypoglycemic effect of various species of Solomon's seal (*multiflorum*, *odoratum*, *officinale*, *prattii*, *sibiricum* and *vertic-cilatum*) have been studied over the past years [116]. Hypoglycemic effect is due to glucoquinine. Steroid glycosides of the roots are represented by paratiosides A–C, prosapogenins, protiosides D1, E1 and F1 and saponin POD II [117].

3.28.2. Analytical toxicology and human poisonings

Analytical procedures are missing in the literature. No fatality was reported to date.

3.29. Prunus laurocerasus-Black cherry-Rosaceae

3.29.1. Active molecules

Amygdalin (D-mandelonitrile-beta-D-glucoside-6beta-D-glucoside) is a cyanogenic glycosides widespread in the vegetable kingdom. After intravenous administration, the greater part of the dose is excreted unchanged in the urine; after oral administration glycoside excreted in the urine is almost exclusively prunasin (that is also present in the plant). Endogenous beta-glucuronidase subsequently transforms prunasin (D-mandelonitrile-beta-D-glucoside) into R-mandelonitrile [9]. This latter unstable compound gives benzaldehyde and the highly potent toxin: cyanhydric acid. The whole plant is toxic, except the flesh of the fruit. Young leaves are more concentrated. The genera Pyracantha and Passiflora also contain low quantities of cyanogenic glycosides as well as the almonds of peaches, apricots and other Rosaceae. The presence of these toxins in natural populations clearly indicates a defensive function. Detailed ecological studies of some maritime populations of Lotus corniculatus have shown that the distribution of the cyanogenic form of this plant is directly related to the distribution and density of mollusks which graze selectively the acyanogenic form [118].

3.29.2. Analytical toxicology

Some methods published were based on the enzymatic hydrolysis of amygdalin and the determination of the product of the hydrolysis: benzaldehyde or glucose [119]. These indirect methods must be rejected because of insufficient selectivity and sensitivity. A method was described for the determination of amygdalin and prunasin in plasma ultrafiltrate and urine. Both compounds are separated by high-performance liquid chromatography on a C₁₈ column, 250×4.6 mm I.D. The eluent was 10% acetonitrile in water. Detection was done at 215 nm. Separation of amygdalin and prunasin from plasma proteins was achieved by ultrafiltration at 800 g for 20 min. The limits of detection were 0.25 μ g/ml for both compounds [120]. Linearity was investigated in the range $2-500 \ \mu g/ml$. In another paper, amygdalin, as an intact substance, was isolated by extraction with acetone while a subsequent purification step was realized by HPLC on an RP-8, 5-µm column. The collected fraction (monitored at 262 nm) was subjected to both thin layer chromatography and GC-MS after derivatization with trifluoracetic anhydride [119]. Evaluation of the amygdalin content, prunasin content, cyanide content and thiocyanate content are all necessary components associated with an appropriate determination of black cherry exposure.

3.29.3. Human poisonings

Two reports have appeared that described acute lethal encounters with the pharmaceutical formulation Laetrile[®] (amygdalin) [121,122]. In each of the reported cases, documentation of the derived cyanide was provided, however, demonstration of the ingested parent compound amygdalin was noticeably missing.

3.30. Rauwolfia serpentina and related species (tetraphylla, vomitoria)–Apocynaceae

3.30.1. Active molecules

Total alkaloids content represent 0.5 to 2.5% of the whole plant. They are divided into three groups (a) containing the yohimbane alkaloids: reserpine, rescinnamine, deserpidine, yohimbine and corynanthine, (b) containing a heteroyohimbane alkaloids skeleton: ajmalicine (also called raubasine), reserpiline, dimethoxy-ajmalicine and their isomers reserpiline and isoreserpiline so as their corresponding quaternary basis serpentine and alstonine, and (c) dihydroindole derivatives mainly represented by ajmaline.

3.30.2. Analytical toxicology and human poisonings

A death after ajmaline administration was reported [123] as well as a fatal reserpine poisoning [124], unfortunately, postmortem concentrations were not reported. An HPLC–MS with a moving belt interfaced to a magnetic sector was used for determination of reserpine, bromazepam and clopenthixol in a non-fatal case of intoxication with these drugs [125]. Separation was done on a C_{18} column 300×4 mm I.D. using methanol–0.2% ammonia (25%) as mobile phase. Ammonia was used as the reagent gas in the positive chemical ionization detection mode. The limit of detection of reserpine was 0.5 ng/ml while the measured concentration of the drug in plasma was 1 ng/ml about 17 h after ingestion.

3.31. Ricinus communis–Castor oil plant– Euphorbiaceae

3.31.1. Active molecules

The seeds contain castor oil, the mildly toxic alkaloid ricinine, and the highly potent toxalbumin

ricin. Ricin is released only if the seed husks are disrupted [126]. Since ricin is not soluble in lipids, the medicinal castor oil contains no ricin. This toxin is different from the two phyto-hemagglutinins or lectins which are also present in the seed. Studies on the physical and chemical properties of pure ricin were given by Lugnier et al. [127]: molecular mass 65 750 amu, glycoproteic nature, oses composition: 15 moles of mannose and 8 moles of N-acetylglucosamine per mole of ricin, amino acids composition: 545, bicatenery structure: the toxin is formed by two peptides A and B chains linked together by a disulfur bond. Ricin is thought to be one of the top two or three deadliest poisons available. Others as bad or worse include abrin, a similar phytotoxin found in the rosary pea and the jequirity bean Abrus precatorius, and plutonium [128].

3.31.2. Analytical toxicology

The only available method for the determination of ricin in human plasma is that of Godal et al. [8], who described a radioimmunological method for the measurement of abrin and ricin. Antiricin antibodies were labeled with isotopic iodine 125. The method was applied to a young student who ingested thirty ricin seeds in a suicidal attempt. On the first day the plasma level reached 1.5 ng/ml and then decreased slowly, the calculated half-life of ricin being approximately 8 days [129]. Ricin only appeared in urine on the third day. The levels were low: around 0.3 ng/ml.

3.31.3. Human poisonings

The first death recorded in the medical literature was published in 1900. Reported mortality rates have decreased from 13 out of 161 before World War II to 2 of 247 since 1950 [130]. Ricin came to public attention in 1978. On September, while waiting for a bus, Georgi Markov, an exiled Bulgarian broadcaster, was slightly bumped into the thigh with an umbrella. He died 3 days later. At the autopsy, a 1.5-mm sphere with two tiny holes was found in the subcutaneous tissue beneath the wound. Analysis of the particle, and similarities in the mode of death of a pig model conducted the coroner to declare that ricin was responsible [131,132].

3.32. Solanum dulcamara and related species (dimitatum, malacoxylon)–Woody nightshade or bittersweet–Solanum pseudocapsicum–Jerusalem berry–Solanaceae

3.32.1. Active molecules

Solanine is a toxic glycoalkaloid, consisting of two classes of glycosides: solanines and chaconines. These glycosides contain a similar steroid skeleton: solanidine derived from spirostane and cholestane structures. Two other molecules were identified in ripe and unripe berries as diosgenine and solasodine. The mature fruit contains the least amount of solanine, and then by increasing concentration are the stern, the leaves and the unripe berry [9].

3.32.2. Analytical toxicology

A GC–MS procedure after triple liquid–liquid extraction was reported for the detection of solanidine in plant material and biological samples of poisoned animals. The main ions are m/z=150, 204, 397, 396, 382 and 151 [7].

3.32.3. Human poisonings

Bittersweet nightshade plant ingestion is a common poisoning. In the 1993 annual report of the American association of Poison Control centers, more than 1800 solanine-containing plant exposures were reported [133]. An atypical anticholinergic toxicity from woody nightshade berry intoxication responsive to physostigmine has been described though no atropine or scopolamine has been identified in the seized berries. On the contrary, the same authors have detected diosgenine and solasodine in the fruit by using a GC–MS technique [133].

3.33. Spartium junceum and Cytisus scoparius– Fabaceae

3.33.1. Active molecules

The main drug of the two plants is constituted by sparteine. Other active molecules are 17-oxo-sparteine, lupanine, scoparoside, isolupanine, anagyrine and cytisine [9,134].

3.33.2. Analytical toxicology

A packed column GC procedure with nitrogenphosphorus detection was described for the post mortem quantitation of sparteine in fluids and tissues [135]. Basic organic liquid–liquid extraction was carrying out from 2 ml of biological fluid. The limit of detection was about 1 ng/ml. However, because of adsorption on the column support, the method is not suitable for the detection of sparteine metabolites. Sparteine isomers have been isolated in an extract of *Lupinus argentus* by thin layer chromatography and GC–MS. An HPLC method with electrochemical detection was able to determine sparteine and its 2-dehydro and 5-dehydro metabolites in urine on a CN analytical column, 250×4.6 mm, 5 m particle size with acetonitrile–methanol–phosphate buffer pH 2.5 as the mobile phase [136]. The limit of detection was 2 ng/ml for sparteine.

3.33.3. Human poisonings

Sparteine plasma levels usually range from 0.1 to 1.0 μ g/ml. It has been demonstrated that about 5% of the population is unable to metabolize sparteine correctly, leading to plasma levels twice or three times higher than usual. Very few fatal reports involving sparteine have been noted in the literature. Kintz et al. [135] reported a fatal case resulting from the suicidal ingestion of tablets of PalpipaxTM containing 200 mg of meprobamate and 75 mg of sparteine. Post mortem findings were 40.4 μ g/ml of sparteine in blood, 53.1 μ g/ml in urine, 31.6 μ g/g in liver and 54.1 μ g/g in kidneys.

3.34. Strychnos nux vomica and S. ignacii– Loganiaceae

3.34.1. Active molecules

The seed of the *Strychnos nux vomica* tree contains 1–3% of alkaloids, strychnine being the main drug together with its demethoxylated derivative, brucine. Other minor components are colubrines, icajine, novacine, vomicine, pseudo-strychnine and isostrychnine [9]. Strychnine was used in traditional medicine as a tonic and for criminal purposes. It is still used as a rodenticide explaining the occurrence of poisonings in rural areas. In China, where the powder of the seed is still used as a popular remedy, deaths related to incorrect dosage of the drug are reported [19].

3.34.2. Analytical toxicology

A rapid high-performance thin-layer chromatographic method with UV densitometry detection at 260 nm was described. Detection of 2.5 ng trace levels of strychnine and crimidine proved feasible [137]. A GC procedure equipped with thermoionic detection was reported after a two step liquid-liquid extraction (back extraction of an ether phase with acetic acid) from 5 ml of body fluid [138]. A GC-MS method for the analysis of strychnine in plant material gave the following m/z ions: 334, 335, 333, 120, 144 and 162 [7]. HPLC methods are available both in reversed-phase and ion-pair chromatography. However, competition with co-extracted basic compounds is likely responsible for the decreased retention of strychnine by ion-pair HPLC. Reversed-phase methods should thus be preferred [139].

3.34.3. Human poisonings

The reported lethal dose of strychnine is 50-100 mg. In a fatal overdose (2.25 g ingested), toxicokinetic strychnine disappearance was described by a first-order process with a half-life of 10-16 h [140]. Aikman reported 189 deaths in the United States from strychnine in 1928 when the drug became widely available in tonic or cathartic pills (also containing different amounts of belladona, cascara, alone, aspirin, podophylline, ipecac, caffein, camphor, gelsemium, quinine, iron or arsenic) [5,141,142]. Drost reported 36 suicides in Canada between 1970 and 1974 with strychnine-containing products [141]. Some religious groups have used this drug in their worship services in attempts to test their faith. In China, two fatal cases recently published were due to incorrect prescription of a non-professional herbalist. A 15-year-old boy died 15 days after receiving a daily oral dose of 0.5 g of Strychnos nux vomica powder. At autopsy, his gastrical liquid contained 1 mg/ml strychnine and 0.76 mg/ml brucine, and his liver contained 1.3 mg/g strychnine but no brucine [19]. In a 56-year-old male who ingested approximately 260 mg of strychnine sulfate, post mortem findings were: 3.3 µg/ml in whole blood, 1.4 μ g/ml in urine, 6.2 μ g/g in liver and 3.2 μ g/g in kidney [138]. Published data of the literature give the following post-mortem concentrations: 0.5-61 μ g/ml in blood, 1–3 μ g/ml in urine, 0.5–26
μ g/g in brain, 5–257 μ g/g in liver and 0.07–106 μ g/g in kidney [143]. Homicides by strychnine have become very rare. As a matter of fact, the violent mode of death aroused quickly suspicion of poisoning. In Libya however, a case was recently published of a woman poisoned by her brother-in-law who administered the toxin in one of her medication. The investigation realized after exhumation of the body revealed a strychnine concentration of 6.16 μ g/g in the liver [144]. A homicide attempt in a hospital was also reported [145].

3.35. Genus Taxus-Yew-Taxaceae

3.35.1. Active molecules

The Yew family includes in fact three genera and more than 15 species distributed in both hemispheres, eight of them being present in the northern part. The most common of the Taxus species include the Taxus baccata (English yew), the Taxus cuspidata (Japanese yew), Taxus canadensis (ground hemlock), and Taxus brevifolia (Western yew). All parts of the plant are toxic at all the time of the year except the flesh of the aril. The composition of the yew is of an extreme complexity. A hundred of taxoids (taxus diterpenoids) have been isolated which are diterpenoids esterified by various classical organic acids or beta amino acids like Winterstein's acid (3-dimethylamino-3-phenylpropionic acid). The main constituents are taxol, taxine A, B and C, taxusin, cephalomanine and 10-deacetylbaccatin III [9]. Another drug called docetaxel (N-debenzoyl-Nter-butoxycarbonyl-10-desacetyltaxol) that has already undergone phase II trials will be available in the near future [146]. In the near future, accidental poisonings, suicidal or homicidal intoxications with commercially paclitaxel and docetaxel are likely to occur.

3.35.2. Analytical toxicology

HPLC–MS has been applied to study the anticancer drug taxol (or paclitaxel) and its metabolites. Collision-induced dissociation experiments enabled the presence of hydroxylated and deacetylated metabolites of taxol to be established [147–152]. HPLC–MS-MS was used to characterize the structural metabolites of paclitaxel in rat bile. Fig. 14

displays a chromatogram of paclitaxel metabolites in rat bile. These metabolites were recognized as three dihydroxytaxols, four monohydroxytaxols, one deacetyltaxol and one containing the taxane ring [153]. These methods, however, which are well suited for the commercially available drug paclitaxel are unsuitable in case of yew poisoning. In one fatal case, the crime laboratory reported that extracts of the contents of the stomach and small intestine produced a gas chromatographic profile resembling that of an extract from a yew plant [148]. In another case, the same assertion was done by thin layer chromatography between a yew extract and extract of the duodenum of the deceased [150]. More recently, phloroglucindimethylether (3,5-dimethoxyphenol) was identified as being a good marker for poisoning from Taxus baccata. The structure of this compound was elucidated by means of GC-MS, infra red spectroscopy and ¹H nuclear magnetic resonance at 300 MHz [149]. The main ions of 3,5-dimethoxyphenol from the GC-MS analysis were m/z = 154, 125, 69, 94 and 111. The content in yew leaves was measured at 0.9 mg/g in January and 1.2 mg/g in September. Thuja occidentalis, a member of another toxic plant genus was also examined, but research of 3,5-dimethoxyphenol was unsuccessful. Finally, taxine has been directly extracted from a grinding sample of yew leaves or stomach content using a multi step liquid-liquid extraction (via methylene chloride followed by a re-extraction with 0.1 mol/l sulfuric acid). By GC-MS, taxine formed a breakdown fragment which was caused by thermal degradation of the drug in the injection port. This fragment was a fast eluting peak with a large 134 main ion [150].

3.35.3. Human poisonings

Holy wood of the Celtic people used in the manufacture of bows, staves of spears and musical instruments, this tree is also a Judaic symbol of death classically found in cemeteries of western countries. The toxicity of yew has been known since antiquity. Caesar reported how Ambiorix, king of Eburones committed suicide with ingestion of yew's leaves in *The war of the Gauls* [151]. The first appearance of yew suicide can be noticed in the medical literature in the *Lancet* in 1836 [153]. In Poland, four prisoners drank a decoction of yew needles in order to



Fig. 14. Analysis of paclitaxel metabolites in rat bile samples using HPLC–ISP-MS, with full scan analysis from m/z 800–1200. (a) TIC profile of untreated rat samples, (b) TIC profile of treated rat bile samples. Reproduced from Ref. [153] with permission.

commit suicide. Two died in prison and one on the fourth day of hospitalization [154]. Another case occurred in a young agriculture student [148]. Van Ingen et al. reported five cases [147] with the review of the literature concerning four other fatal cases previously described. The authors gave evidence of suicidal use of yew under different forms: leaves extract, chopped or whole leaves and bark. Diagnosis was always anamnestic or botanical. 3,5-Dimethoxyphenol was quantified in a case of suicide in a depressive young man. The stomach contained 20 μ g/g and cardiac blood 0.32 μ g/g [149].

3.36. Trichosanthes kirilowii–Cucurbitaceae

3.36.1. Active molecules

The root of the plant contains a protein of 234 amino acids named trichosanthine, that, like ricin, inhibits elongation in protein synthesis by acting on ribosomes.

3.36.2. Analytical toxicology and human poisonings

To our knowledge, no analytical procedure has been described for trichosanthine. Two women died from using this toxic Chinese plant as an abortifacient. The first victim died in a hospital, by filling her vagina with a mixture of Chinese herbal medicine that included 250 mg of *Trichosanthes kirilowii*. The second victim induced abortion with bits of a broken root tuber of this cucurbitaceae that she had wrapped in gauze and inserted into her vagina. She delivered a 5-month-old fetus on day 6 and died the day after [19]. A fatal case in a man contaminated by the AIDS virus was also described. He performed selfinfusion of trichosanthine from a mysterious origin [5].

3.37. Tripterygium wilfordii–Celastraceae

A young woman worker who was suffering from rheumatoid arthritis was overdosed when treated with a mixture of *Lei Gong Teng*, which is the Chinese name of this toxic plant [19]. 3.38. Veratrum viride and related species (album, californium, japonicum)–False or white hellebore–Liliaceae

3.38.1. Active molecules

The plants of the genus *Veratrum* contain alkaloids with an antihypertensive effect. Also the genera *Schoenocaulon* and *Zygadenus* are a source of *Veratrum* alkaloids. The alkaloids occur as glycosides, aglycones or in the form of esters with various acids divided into seven groups [155]. The first two most important groups are (a) alkaloids of the jervanine and veratranine type with a steroidian skeleton (veratridine, veratramine, veratrosine, jervine, pseudojervine and isorubrijervine) and (b) alkaloids with the cevanine skeleton (cevadine, cevine, zygadenine, veracerine, germine, protoverine and their esters). The whole plant is toxic though the roots and rhizomes (2% alkaloids) are more toxic than the tops (0.5% alkaloids in the leaves).

3.38.2. Analytical toxicology

Veratrum viride was employed as an American Indian potion to test the endurance of young Indian braves [156]. Veratrum alkaloids were also employed as constituents of sneezing powders. However, since several poisonings with Veratrum-containing sneezing powders have occurred, a change was made to a safer formulation [157]. The toxicological data were rarely documented. Veratrine was sometimes identified in the decoction or in the gentian wine [158]. Identification was obtained by TLC-MS from the suspect preparation, from the gastric flushing liquid, urine, or viscera [158]. Thin layer chromatography used gel layers on a glass substrate. The migration solvent was ammoniacal methanol while the alkaloids were revealed when exposed to iodine vapors. A mass spectrometer was fitted with a chemical ionization source where ammonia was used as the reacting gas. An HPLC-MS method was most recently published by Gaillard et al. [159] for the detection and quantification of veratridine and cevadine in two fatal cases of Veratrum album poisoning. The drugs eluted on a Symmetry C₈ 150×2.1 mm I.D., 5 µm particle size using a methanol-formiate buffer 2 mM, pH 3.0 linear gradient. Selected ions were m/z=674.4 for veratridine and m/z=592.4 for cevadine. Limits of detection were 0.07 and 0.10 ng/ml in whole blood for veratridine and cevadine, respectively.

3.38.3. Human poisonings

Veratrum poisoning is no longer a result of therapeutic accidents, but those resulting from the erroneous identification of the plants Veratrum and Gentiana lutea in the majority of the cases [160]. The latter plant is popularly used in West and Central Europe for the preparation of homemade gentian wine [158]. Some other confusions were described in the literature: skunk cabbage in one case (Symplocarpus foetidus) [161], pokeweed in five cases (Phytolacca americana) [161], ramps in three cases (Allium tricoccum) [5]. Three intoxications resulted from ingestion of a Veratrum album tincture [161,162], 12 cases from the inclusion of Veratrum japonicum in soup [163] and 12 others from the ingestion of Veratrum album root as tea [164]. Deaths related to Veratrum poisonings are very seldom in the literature. One death was due to a pharmacist's mistake (confusion with rhubarb powder), one was a crime, and two were subacute accidental poisonings [160]. A recent paper described two fatalities due to a massive ingestion of seeds of false hellebore. Veratridine and cevadine were identified and quantified by HPLC-MS. Measured blood concentrations were 0.17 and 0.40 ng/ ml for veratridine and 0.32 and 0.48 ng/ml for cevadine [159].

3.39. Xanthium sibiricum-Asteraceae

3.39.1. Active molecules

Toxicity is due to the presence of carboxyactractyloside. Other toxic species include *X. spinosum* and *X. strumarium*.

3.39.2. Analytical toxicology and human poisonings

As for atractyloside from *Atractylis glummifera*, no analytical method is available. Three deaths were reported. In one case, the plant was mistaken with a bud of wild soybean. The two other victims had eaten cakes made from this Chinese poisonous plant after mistaking it for sesame or soybean cake [19].

3.40. Genus Zigadenus–Death camus–Liliaceae

Significant toxicity can result from the ingestion of certain species of the zigadenus plant (*chloranthus*, *paniculatus*), herb occasionally confused with non-toxic onions. The literature contains several well-documented nonfatal human cases of zigadenus poisoning [165]. Fatal cases were recorded in interviews of 19th century western American Indians. A paper contains an account from the early 20th century of some railroad workers who died shortly after eating the bulbs [166]. Toxic alkaloids are of the same nature and structure than those found in the false hellebore.

4. Precision and recovery

4.1. Cyanogenic compounds molecules

The relative standard deviations (RSDs) of the within-day precision (n=8) are usually less than 8.2% for amygdalin and less than 5.4% for prunasin. RSDs for amygdalin in the between-day precision study (n=8) were 15.1, 5.9 and 3.0 at 0.5, 5 and 50 µg/ml, respectively. For the same concentrations RSDs of prunasin were 13.2, 5.2 and 2.9%.

The overall amygdalin recovery in whole blood was 44.5% at 5 μ g/ml while prunasin recovery was 42.2% at the same concentration (*n*=8).

4.2. Alkaloids, oleandrine and taxol

RSDs of the between-day precision study are given in Table 3 at 0.1, 1, 10 and 100 ng/ml for each molecule (when the limit of detection allowed a measure) together with the overall recovery of the drug measured at 100 ng/ml (n=10).

4.3. Atractyloside

Due to the absence of internal standard (lidocaine was used as an external standard), and to the difficulties of extraction of the drug from the matrix, the RSDs were indeed higher than for other molecules. However, they were judged sufficiently reasonable for a toxicological analysis. RSDs of the within-day precision study were 20.8, 18.7 and

11.3% at concentrations of 0.1, 1 and 10 μ g/ml, respectively (*n*=6) and 30.9, 24.5 and 18.4% at the same concentrations for the between-day precision study (*n*=8). The overall atractyloside recovery was 26.2% at 1 μ g/ml (*n*=6).

5. Linearity

Analysis of variance (ANOVA) is a powerful and very general method which separates the contributions to the overall variation in a set of experimental data and tests their significance. The sources of variations are each characterized by a sum of square (SS), i.e. the sum of a number of squared terms representing the variation in question, a number of degrees of freedom (DF), and a mean square, which is the former divided by the latter and which can be used to test the significance of the variation contribution by means of the F-test. In analytical calibration experiments, only variation in the Y-direction is considered. This variation is expressed as the sum of the squares of the distances of each calibration point from the mean Y value [167]. This is the total SS of Y_{mean} : S_{T} . There are two contributions to this overall variation. One is the SS due to the regression: S_1 , and the second source of variation is the SS about regression, i.e. residual: S_0 . The residual variation can be divided itself into two contributions: lack of fit and pure analytical error [168,169]. For example, F_{cal} (colchicine) = 1752.6 >> F_{theor} ; F_{theor} is for (1, n-2) DF = 5.32, so the source of variation is well described by the regression, $F'_{cal}(colchicine) =$ 2.02 < F'_{theor} ; F'_{theor} is for [(n-2) - n/2, n/2]DF = 5.41, so the model (linear regression) can be considered as correct. The same assertion was verified for all compounds: $F_{\rm cal}$ were always superior to 750.2 whereas F'_{cal} was always found to be inferior to 3.09.

6. Accuracy

In a first step, a blood standard of known concentration is prepared and measured n times. From the values of the peak-areas, we can calculate the mean m, and the standard deviation SD that we consider as being the true value [168]. In a second step, from the equation of the linear regression: Y = aX + b, we can calculate the measured peak-area corresponding to this point: Y_t . Then we test the accuracy by the mean of a *t*-statistic; by comparing a mean to a point as follows: $t_{observed} = (m - Y_t)/(SD/n^{1/2})$. If $t_{observed} < t_{table}$, the null hypothesis is accepted. In other words, the measured value and the true value are not different. t_{table} is given with n-1 DF [170].

Using this procedure, only internal errors were tested. Since the way for standard preparation was always the same, we cannot estimate the lack of accuracy due to the external errors (incorrect weight or volumes of standards or samples, or inaccurate dilution of primary standards).

Accuracy was tested at 1 and 100 ng/ml for alkaloids, oleandrine and taxol and at 5 μ g/ml for amygdalin and prunasin. Under these conditions, t_{observed} were always inferior to $t_{\text{table}} = 2.26$ ($\alpha = 5\%$, n = 10). Atractyloside was not evaluated.

7. Limits of detection: LOD

The limit of detection is given by the mathematical formula: $\text{LOD} = m_{\text{blank}} + 6 s_{\text{blank}}$, where m_{blank} is the mean of the blanks and s_{blank} corresponds to the standard deviation of the blanks (n = 30) [170,171].

Under these conditions, LODs were 0.08 μ g/ml for amygdalin, 0.07 μ g/ml for prunasin and 0.015 μ g/ml for atractyloside. Table 3 lists the LODs values for the other drugs.

8. Chromatographic separations

Alkaloids are weak bases with pKa values generally contained between 7 and 10. Thus, the ion-pair HPLC technique may be used to achieve better peak-shape and separation of the drugs. On the other hand, basic mobile phase can be applied on polymeric reversed-phase column. To this aim, Auriola et al. [29] have already demonstrated the interest of a basic eluent for the analysis of tropane alkaloids on a poly(styrene–divinylbenzene) copolymer as the stationary phase. Under alkaline conditions, weak bases appear neutral and ion-pairing agents can be rejected. The mobile phase B was judged sufficiently

Table 3
RSDs of the between-day precision study, recoveries and LODs for alkaloids, taxol and oleandrine $(n = 10)$

Compound	RSD (%) at 0.1 ng/ml	RSD (%) at 1 ng/ml	RSD (%) at 10 ng/ml	RSD (%) at 100 ng/ml	Overall recovery (%)	LOD (ng/ml)
Aconitine	8.8	6.1	4.8	4.2	86.4	$0.01 (0.001)^{a}$
Anabasine	b	_	11.6	8.7	49.9	1.20
Arecoline	_	19.0	8.0	5.6	63.1	0.91
Atropine	11.8	6.1	4.2	3.7	70.9	0.09
Cevadine	9.1	7.9	7.0	5.3	87.1	$0.01 (0.001)^{a}$
Colchicine	24.6	13.4	9.9	8.2	51.1	$0.09 (0.005)^{a}$
Crotaline	_	_	15.3	10.4	49.6	1.60
Emetine	_	21.0	16.0	11.2	21.8	0.19
Eserine	_	17.2	8.7	8.0	103.7	0.17
Lobeline	_	_	21.8	16.2	18.3	1.52
Oleandrine	_	14.0	8.7	7.3	53.5	0.40
Pilocarpine	_	_	7.6	6.6	68.1	1.07
Reserpine	11.7	9.5	8.8	8.1	50.3	0.02
Retrorscine	_	13.0	8.4	5.2	61.6	0.18
Scopolamine	_	12.4	9.1	6.9	64.4	0.31
Senecionine	9.3	7.4	6.7	5.9	101.0	0.04
Sparteine	-	8.4	7.2	6.0	63.3	$0.16 (0.005)^{a}$
Strychnine	10.9	9.4	6.1	5.4	100.2	$0.03 (0.002)^{a}$
Taxol	_	12.7	7.8	6.6	43.2	0.48
Veratridine	11.7	9.2	6.3	4.1	86.6	$0.02 (0.001)^{a}$
Yohimbine	-	10.1	6.9	5.2	100.2	0.32

^a Analysis realized by HPLC-MS-MS.

^b -: Not determined.

basic to achieve excellent peak-shape and separation of the 13 compounds of interest. Concentration of the formiate buffer was increased to 10 mM, authorizing a slight addition of ammonium hydroxide and a pH of 8.2 to be obtained. This alkaline eluent was compatible with the conventional C_{18} packing material. The selectivity of the mobile phase must also be adjusted. Elution with acetonitrile generally appears insufficient to provide good peak-shape. In the solvent-selectivity triangle classification, acetonitrile possesses a π^*/Σ normalized selectivity factor of 0.60. This π^* factor encodes the solvent's ability to interact with a solute by dipolar and polarization factors [172]. Acetonitrile is thus an aprotic solvent with main dipolar interaction mechanisms. Considering the chemical properties of the analytes, a slight modification of the eluent selectivity appeared necessary [173]. Methanol, which is protic and a good proton acceptor ($\alpha/\Sigma = 0.43$), was thus added to produce excellent peak-shape of the drugs under our chromatographic conditions.

For the other alkaloids with pKa values less than 6.5 (strychnine: pKa=2.3 and 8.0, colchicine: pKa=

1.7, for example) classical acidic mobile phase appeared as a good choice in terms of chromatographic behavior. This group included also more polar drugs with a number of hydroxylated and carboxylated chains (aconitine: pKa=8.1 but 11 atoms of oxygen, reserpine: pKa=6.6 and nine atoms of oxygen, oleandrine: glycoside, taxol: polar taxoids with ten atoms of oxygen, atractyloside which is an acid).

9. Forensic applications of authors' own experience

9.1. Case 1

The method was successfully applied in a delicate forensic case. A 55-year-old man was found dead at his residence. At the autopsy, the medical examiner noted an intense congestion of the internal organs, the absence of traumatic lesions as well as needle marks. Blood and urine were sampled for a detailed toxicological analysis. These analyses revealed the presence of several drugs including meprobamate 9.0 μ g/ml, paracetamol 8.8 μ g/ml, phenobarbital 17.5 μ g/ml and methyl phenobarbital 8.5 μ g/ml. These concentrations however, were insufficient to document the death. Consequently, a second series of analysis was entrusted to our laboratory approximately 6 months after the first one which showed in addition to the above mentioned drugs, acebutolol at a concentration of 0.09 μ g/ml, morphine at a concentration of 9 ng/ml, and colchicine at 0.79 ng/ml (see Fig. 15).

The daughter-in-law of the victim will confess thereafter that she had administered a medication (COLCHIMAX[™] containing 1 mg colchicine, 15 mg phenobarbital, 50 mg tiemonium iodide and 15 mg of opium powder, per tablet) to the victim. This medication, originally prescribed to the victim's wife, was mixed without the knowledge of the decedent with his food. The powerful HPLC-MS-MS presented method enabled us to establish a low, but significant, concentration of colchicine, in a complex matrix, several months after the sampling. It is thus reasonable to estimate that the many cases in the literature, for which no post-mortem findings could be reported, might be accounted for by the inadequacies of the analytical methods that were used. This case, rare as it may be, of homicide by poisoning, is a good demonstration of the interest of such a method.

9.2. Case 2

The described method can be successfully applied to the analysis of plant material. Leaves, fruits, flowers or other parts of the plant are crushed by means of a ball mill EI 4000 from the KLECO manufacturer (Visalia, CA, USA) during 30 s. A 50 mg amount is introduced into a screw cap glass tube with 2 ml of distilled water. The procedure described for blood is then applied in the same way to this powdered material. The plain leaves of Atropa belladonna gathered in the East of the United Kingdom gave a content of atropine of 4536 $\mu g/g$ and 44 μ g/g of scopolamine. The ripe berries of the same plant contained 2696 $\mu g/g$ of atropine and 24 µg/g of scopolamine. The flowers of Datura arborea collected in the Lycian coast of Turkey contained 1592 $\mu g/g$ of atropine and 1604 $\mu g/g$ of scopolamine. Needles of *Taxus baccata* picked in the South of France gave a content of taxol of 12.1 μ g/g. In the same neighborhood, the leaves of *Nerium oleander* contained 536.4 μ g/g of oleandrin.

9.3. Case 3

In the French Caribbean, two 7-year-old twin sisters suffering from psychomotor handicaps were found dead, burned in a fire that destroyed their house. The criminal origin of the fire will be quickly demonstrated. Nevertheless, the investigators found no traces of escape of the children or panic. At the autopsy, the medical examiner noted marks and wounds consecutive to physical ill treatments. Residues of black fume were found in the bronchi of the two children. The bodies were burned and the faces were particularly hit, which was the official cause of death. In the morning of the facts, the parents had been refused social help for the placement of the young girls in a specialized center. Sampling of blood, hair and gastric content were realized for a complete toxicological analysis. Carboxyhaemoglobin concentrations were, respectively, 11.3 and 11.9%. These concentrations were indeed insufficient to account for the death, but significant enough, to declare that the girls were alive at the moment of the fire. Additionally identified in the blood of the first victim were valproic acid at 14.4 μ g/ml, clonazepam at 0.9 ng/ml and cyanide 1.2 mg/l. In the blood of the second deceased valproic acid at 8.3 μ g/ml, carbamazepine at 1.9 μ g/ml and cyanide at 0.7 mg/l were identified. These findings however, do not explain the absence of reaction of the two sisters in front of the danger of the fire. This is why additional investigations were undertaken. They revealed the presence of atropine and scopolamine in the blood of the first girl at concentrations of 32.5 and 4.4 ng/ml, respectively (see Fig. 16). In the gastric content, atropine and scopolamine were measured at 22.0 and 2.1 ng/ml, respectively. Analyses carried out on the second child showed atropine concentrations of 7.5 and 18.3 ng/ml in the whole blood and in gastric content while scopolamine levels were 0.8 and 1.1 ng/ml, respectively. No other poison of plant origin was identified. The simultaneous presence of these two alkaloids undoubtedly indicated absorption of a toxic plant material such as one could easily find



Fig. 15. Chromatogram of an extract of the whole blood of the deceased man of the forensic case 1. Peaks: 1=I.S., lidocaine (daughter ion m/z 86), 2=colchicine measured at 0.79 ng/ml, (a) daughter ion m/z 282, (b) daughter ion m/z 310.



Fig. 16. Chromatogram of an extract of the whole blood of the deceased child No. 1 of the forensic case 3. Peaks: 1 = atropine measured at 32.5 ng/ml, (a) ion m/z 290.3, (b) ion m/z 124.2, 2 = scopolamine measured at 4.4 ng/ml, (a) ion m/z 304.3, (b) m/z 156.2.

under a tropical climate like *Datura arborea*, *Datura sanguinea* or *Datura sauveolens*. These plants are traditionally known in the voodoo's rites of the Caribbean. It is likely that the parents have managed a potion based on one of these plants in order to put their children to sleep. A few months later, they confessed the administration of a *Datura* species so as the infanticide by fire.

10. Conclusion

After having drawn up a non-exhaustive inventory of the principal toxic plants responsible for death in humans, the authors have developed a general and powerful method of detection of several natural poisons by HPLC-MS or HPLC-MS-MS. It is the first published method to date, allowing the determination of non-volatile alkaloids at concentrations as low as 0.1 or 0.01 ng/ml. It is also the first proposed procedure for the detection of senecionine, atractyloside, or taxol in toxicology, the first HPLC-MS technique for the detection of amygdalin, anabasine, arecoline, crotaline, emetine, eserine, lobeline, pilocarpine, prunasin, retrorscine, sparteine, strychnine and yohimbine, and the first HPLC-MS-MS technique for the detection of aconitine, cevadine, colchicine, sparteine, strychnine and veratridine. The presented method appeared essential for the resolution of some very delicate forensic cases.

References

- [1] A. Oztekin-Mat, Ann. Pharm. Françaises 52 (1994) 260.
- [2] E.P. Krenzelok, T.D. Jacobsen, Vet. Hum. Toxicol. 39 (1997) 248.
- [3] R. Jaspersen-Schib, L. Theus, M. Guirguis-Oeschger, B. Gossweiler, P.J. Meir-Abt, Schweiz Med. Wochenschr. 126 (1996) 1085.
- [4] G.N. Foukaridis, G.L. Muntigh, E. Osuch, J. Ethnopharmacol. 41 (1994) 135.
- [5] J. Bruneton, Plantes Toxiques. Végétaux Dangereux pour L'homme et les Animaux, Lavoisier, Paris, 1996.
- [6] R.E. Schultes, A. Hoffman, Les Plantes des Dieux, Les éditions du lézard, Paris, 1993.
- [7] D.M. Hostege, J.N. Seiber, F.D. Galey, J. Agric. Food Chem. 43 (1995) 691.
- [8] A. Godal, J. Olnes, A. Pihl, J. Toxicol. Environ. Health 8 (1981) 409.

- [9] J. Bruneton, Pharmacognosie. Phytochimie, Plantes Médicinales, Lavoisier, Paris, 1993.
- [10] W. Tang, G. Eisenbrand, Chinese Drugs of Plant Origin Chemistry, Pharmacology, and Use in Traditional and Modern Medicine, Springer-Verlag, Berlin, 1992.
- [11] Z. Pang, B. Wang, W. Shen, Chung Kuo Chung Tsa Chih 16 (1991) 485.
- [12] K. Wang, Y.Y. Tong, Yao Hsueh Hsueh Pao 25 (1990) 387.
- [13] Y. Tong, Chung Kuo Chung Yao Tsa Chih 15 (1990) 106.
- [14] N. Yoshioka, K. Gonmori, A. Tagashira, O. Boonhooi, M. Hayashi, Y. Saito, M. Mizugaki, Forensic Sci. Int. 81 (1996) 117.
- [15] K. Ito, Y. Ohyoma, Y. Konishi, S. Tanaka, M. Mizugaki, Planta Med. 63 (1997) 75.
- [16] H. Ohta, Y. Seto, N. Tsunoda, J. Chromatogr. B 691 (1997) 351.
- [17] A.M. Debelmas, P. Delaveau, Guide des Plantes Dangereuses, Maloine, Paris, 1983.
- [18] A. Feldkamp, B. Köster, H.P. Weber, Monatsschr. Kinderheidlkd. 139 (1991) 366.
- [19] Y.G. Zhang, G.Z. Huang, Am. J. Forensic Med. Pathol. 9 (1988) 313.
- [20] Y.T. Tai, P.P.H. But, K. Young, C.P. Lau, Lancet 341 (1993) 892.
- [21] S.T. Kolev, P. Leman, G.C. Kite, P.C. Stevenson, D. Shaw, V.S.G. Murray, Hum. Exp. Toxicol. 15 (1996) 839.
- [22] P.P. But, Y.T. Tai, K. Young, Vet. Hum. Toxicol. 36 (1994) 212.
- [23] P. Dickens, Y.T. Tai, P.P. But, B. Tomlinson, H.K. Ng, K.W. Yan, Forensic Sci. Int. 67 (1994) 55.
- [24] A. Mori, M. Mukaida, I. Ishiyama, J. Hori, Y. Okada, M. Sasaki, K. Mii, M. Mizagaki, Nippon Hoigaku Zasshi 44 (1990) 352.
- [25] M. Mizugaki, K. Ito, Y. Ohyama, Y. Konishi, S. Tanaka, K. Kurosawa, J. Anal. Toxicol. 22 (1998) 336.
- [26] S.N. Bye, T.H. Coetzer, M.F. Dutton, Toxicon 28 (1990) 997.
- [27] G. Lemaigre, Z. Tebbi, R. Galinski, S. Michowitcz, R. Abelanet, Nouv. Presse Med. 4 (1975) 2565.
- [28] G. Sticht, H. Kaferstein, M. Staak, Acta Med. Leg. Soc. 39 (1989) 441.
- [29] S. Auriola, A. Martinsen, K. Oksman-Caldentey, T. Naaranhti, J. Chromatogr. 562 (1991) 737.
- [30] A. Xu, J. Havel, K. Linderholm, J. Husle, J. Pharm. Biomed. Anal. 14 (1995) 33.
- [31] A. Lange, P. Toft, Ugeskr. Laeger 152 (1990) 1096.
- [32] G. Trabattoni, D. Visintini, G.M. Terzano, A. Lechi, Hum. Toxicol. 3 (1984) 513.
- [33] I. Perlik-Gattner, Przegl Lek 54 (1997) 464.
- [34] H. Scholz, S. Kascha, H. Zingerle, Forschr. Med. 98 (1980) 1525.
- [35] F. Schneider, P. Latun, P. Kintz, D. Astruc, F. Flesch, J.P. Tempé, Clin. Toxicol. 34 (1996) 113.
- [36] B.B. Jarvis, S. Wang, C. Cox, M. Madhusudana-Rao, V. Philip, M.S. Varaschin, C.S. Barros, Natural Toxins 4 (1996) 58.
- [37] T. Krishnamurthy, D.J. Beck, R.K. Isensee, B.B. Jarvis, J. Chromatogr. 469 (1989) 209.

- [38] T. Krishnamurthy, M.B. Wasserman, E.W. Sarver, Biomed. Environ. Mass Spectrom. 13 (1986) 503.
- [39] T. Krishnamurthy, E.W. Sarver, J. Chromatogr. 355 (1986) 253.
- [40] K.E. Panter, R.F. Keeler, D.C. Baker, J. Anim. Sci. 66 (1988) 2407.
- [41] L.A. King, M.J. Lewis, D. Parry, P.J. Twitchett, E.A. Kilner, Hum. Toxicol. 4 (1985) 355.
- [42] D. Landers, K. Seppi, W. Blauer, West J. Med. 142 (1985) 637.
- [43] M.I. Mitchell, P.A. Routledge, Clin. Toxicol. 12 (1978) 417.
- [44] J.P. Anger, F. Anger, Y. Chauvel, R.L. Girre, N. Curtes, J.P. Curtes, Eur. J. Toxicol. Environ. Hyg. 9 (1976) 119.
- [45] G. Marras, Minerva Medicoleg. 85 (1965) 83.
- [46] M.J. Ellenhorn, D.G. Barceloux, Medical Toxicology. Diagnosis and Treatment of Human Poisoning, Elsevier, Amsterdam, 1988.
- [47] Y.H. Caplan, K.G. Orloff, B.C. Tompson, J. Anal. Toxicol. 4 (1980) 153.
- [48] P. Kintz, C. Jamey, A. Tracqui, P. Mangin, J. Anal. Toxicol. 21 (1997) 70.
- [49] A. Tracqui, P. Kintz, B. Ludes, C. Rougé, H. Douibi, P. Mangin, J. Chromatogr. B 675 (1996) 235.
- [50] D. Jarvie, J. Park, M.J. Stewart, Clin. Toxicol. 14 (1979) 375.
- [51] K. Harze, Z. Rechtsmed. 93 (1984) 181.
- [52] I.M. McIntyre, A.R. Ruckiewicz, K. Crump, O.H. Drummer, J. Forensic Sci. 39 (1994) 280.
- [53] C. Lacatelli, V. Petrolini, C. Varango, R. Butera, C. Gondini, L. Manzo, VIIIth International Congress of Toxicology, Paris, France, July 5–9, 1998.
- [54] I.R. Byock, Hosp. J. 7 (1991) 51.
- [55] F.D. Scutchfield, E.N. Genovese, Pharos 5 (1997) 30.
- [56] O.H. Drummer, A.N. Roberts, P.J. Bedford, Med. J. Aust. 162 (1995) 592.
- [57] N.C. Fraser, Br. Med. J. 1 (1980) 1595.
- [58] M. Hipsz, J. Kowalski, H. Strelecka, Acta Pol. Pharm. 32 (1975) 695.
- [59] W. Kubelka, Planta Med. 4 (1971) 153.
- [60] A. Garcia-Martin, R.M. Masvidal-Aliberch, A.M. Bofill-Bernardo, S. Rodriguez-Alsina, Ann. Esp. Pediatr. 19 (1983) 366.
- [61] A.A. Vitale, A. Archer, A.B. Pomilio, J. Ethnopharmacol. 49 (1995) 81.
- [62] R.W. Urich, D.L. Bowerman, J.A. Lewisky, J.L. Pflug, J. Forensic Sci. 27 (1982) 948.
- [63] M.S. Dewitt, R. Swain, L.B. Gibson, W.V. Med. J. 93 (1997) 182.
- [64] R.C. Hall, M.K. Popkin, L.E. Mchenry, Am. J. Psychiatry 134 (1977) 312.
- [65] A. Ardilla, C. Moreno, Brain Cognation 15 (1991) 236.
- [66] E.A. Smith, C.E. Meloan, J.A. Pickell, F.W. Oehme, J. Anal. Toxicol. 15 (1991) 216.
- [67] D. Frohne, J. Pfänder, A Colour Atlas of Poisonous Plants, Wolfe Publishing Ltd, London, 1984.
- [68] R.E. Schultes, R.F. Raffauf, Vine of the Soul. Medicine Men, Their Plants and Rituals in the Colombian Amazonia, Synergetic Press, Oracle, USA, 1992.

- [69] D.G. Gardner, Oral Surg. Oral Med. Oral Pathol. 78 (1994) 631.
- [70] R. Mrvos, B.S. Dean, E.P. Krenzelok, J. Toxicol. Clin. Toxicol. 29 (1991) 485.
- [71] H. Wiegrebe, M. Wichtl, J. Chromatogr. 630 (1993) 402.
- [72] G.W. Ponder, J.T. Stewart, J. Chromatogr. A 659 (1994) 177.
- [73] M.C. Tzou, R.A. Sams, R.H. Reuning, J. Pharm. Biomed. Anal. 13 (1995) 1531.
- [74] J. Plum, T. Daldrup, J. Chromatogr. 377 (1986) 221.
- [75] E. Lacassie, P. Marquet, D. Dumont, S. Martin-dupont, J.L. Dupuy, G. Lachâtre, VIth Congress SFTA, Saint François, France, 1–6 June, 1998.
- [76] P. Burnat, Lyon Pharm. 35 (1984) 199.
- [77] T.C. Lee, J. Am. Med. Assoc. 245 (1981) 727.
- [78] W.N. Arnold, J. Am. Med. Assoc. 260 (1988) 3042.
- [79] S.A. Rich, J.M. Libera, R.J. Locke, Ann. Emerg. Med. 22 (1993) 1904.
- [80] T.Z. Chen, J.F. Den, W.J. Tsai, Vet. Hum. Toxicol. 32 (1990) 368.
- [81] V.J.M. DiMaio, J.C. Garriot, R. Putnam, J. Forensic Sci. 20 (1975) 340.
- [82] M. Selesky, V. Spieler, R.H. Cravey, H.W. Elliot, J. Forensic Sci. 21 (1976) 409.
- [83] A.P. Phillips, J. Forensic Sci. Soc. 14 (1974) 137.
- [84] G.D. Lin, W.J. Griffin, Australas Biotechnol. 2 (1992) 23.
- [85] J. Pearn, Med. J. Aust. 2 (1981) 422.
- [86] R.E. Niederhofer, J. Am. Med. Assoc. 254 (1985) 2123.
- [87] N. Tligui, G.R. Ruth, L.J. Felice, Am. J. Vet. Res. 55 (1994) 1546.
- [88] L.Z. Lin, G.A. Cordell, C.Z. Ni, J. Clardy, J. Nat. Prod. 52 (1989) 588.
- [89] J.S. Yang, Y.W. Chen, Yao Hsueh Hsueh Pao 18 (1983) 104.
- [90] Y. Schun, G.A. Cordell, J. Nat. Prod. 50 (1987) 195.
- [91] S.R. Luo, T. Li, J.S. Yang, Yao Hsueh Hsueh Pao 28 (1993) 695.
- [92] E. ElBahri, J. Belguith, S. BenYoussef, H. Bellil, Vet. Hum. Toxicol. 38 (1996) 378.
- [93] H.C. Long, Plants Poisonous To Livestock, Cambridge University Press, London, 1924.
- [94] J. Urkin, H. Shalev, S. Sofer, A. Witztum, Harefuah 120 (1991) 714.
- [95] M. Kurkcuoglu, Turk. J. Pediatr. 12 (1970) 48.
- [96] J.M. Sands, R. Sands, Med. J. Aust. 2 (1976) 55.
- [97] I. Abdu-Aguye, A. Sannusi, R.A. Alafiya-Tayo, S.R. Bhusnurmath, Hum. Toxicol. 5 (1986) 269.
- [98] P.H. Joubert, J.M. Brown, I.T. Hay, P.D. Sebada, S. Afr. Med. J. 65 (1984) 729.
- [99] A. Subarnas, Y. Oshima, S. Sidik, Y. Ohizumi, J. Pharm. Sci. 81 (1992) 620.
- [100] A. Krochmal, L. Wilken, M. Chien, Lloydia 35 (1972) 303.
- [101] C. Reavill, B. Walther, I.P. Stolerman, B. Testa, Neuropharmacology 29 (1990) 619.
- [102] D.K. Saraswat, P.K. Garg, M. Saraswat, JAPI 40 (1992) 628.
- [103] A. Gupta, P. Joshi, S.A. Jortani, R. Valdes, T. Thorkelsson, Z. Verjee, S. Shemie, Ther. Drug Monit. 19 (1997) 711.
- [104] B.E. Haynes, H.A. Bessen, W.D. Wightman, Ann. Emerg. Med. 14 (1995) 350.

- [105] F.D. Galey, D.M. Holstege, K.H. Plumlee, E. Tor, B. Johnson, M.L. Anderson, P.C. Blanchard, F. Brown, J. Vet. Diagn. Invest. 8 (1996) 358.
- [106] A. Namera, M. Yashiki, K. Okada, Y. Iwasaki, T. Kojima, Nippon Hoigaku Zasshi 51 (1997) 315.
- [107] A. Tracqui, P. Kintz, F. Branche, B. Ludes, Int. J. Leg. Med. 111 (1998) 32.
- [108] J.L. Castorena, J.C. Garriott, F.E. Barnhardt, R.F. Shaw, Clin. Toxicol. 25 (1987) 429.
- [109] P. Jacob, L. Yu, G. Liang, A.T. Shulgin, N.L. Benowitz, J. Chromatogr. 619 (1993) 49.
- [110] C. Mignot, J. Lenormand, J. Kabs, J.Y. Breurec, J.P.C. Curtes, A. Caubet, VIIIth International Congress of Toxicology, Paris, France, July 5–9, 1998.
- [111] H. Patscheider, R. Dirmhofer, Beitr. Gerichtl. Med. 30 (1973) 336.
- [112] C. Aromdee, J.P. Fawcett, R. Ledger, J. Chromatogr. B 677 (1996) 313.
- [113] S.M. Gordner, R.R. Fysh, H. Gordon, S.J. Whitaker, Br. Med. J. 293 (1986) 1285.
- [114] G.P. Pflieger, K. Patalka, Am. J. Ophthalmol. 120 (1995) 399.
- [115] M.S. McIntyre, J.R. Guest, J.F. Porterfield, Clin. Toxicol. 28 (1990) 177.
- [116] N. Hirai, T. Miura, M. Moriyasu, M. Ichimaru, Y. Nishiyama, K. Ogura, A. Kato, Biol. Pharm. Bull. 20 (1997) 1271.
- [117] T. Miura, A. Kato, Biol. Pharm. Bull. 18 (1995) 1605.
- [118] W.M. Ellis, R.J. Keymer, D.A. Jones, Experimentia 33 (1977) 309.
- [119] J. Balkon, J. Anal. Toxicol. 6 (1982) 244.
- [120] A.G. Raws, L.G. Gramberg, M. Olling, Pharm. Weekblad 4 (1982) 172.
- [121] K.T. Braico, J.R. Humbert, K.L. Terplan, J.M. Lehotay, New Engl. J. Med. 300 (1979) 238.
- [122] L. Sadoff, K. Fuchs, J. Hollander, J. Am. Med. Assoc. 239 (1978) 1532.
- [123] H.J. Wellens, F.W. Bar, E.J. Vanagt, Am. J. Cardiol. 45 (1980) 905.
- [124] P.P. Rogal, V.A. Rakitin, M.P. Boichak, A.I. Gurasov, V.G. Ivachev, Sud. Med. Ekspert 32 (1989) 51.
- [125] A.C. Tas, J. VanDerGreef, M.C. TenNoverDeBrauw, T.A. Plomp, R.A. Maes, M. Hohn, U. Rapp, J. Anal. Toxicol. 10 (1986) 46.
- [126] P.J. Aplin, T. Eliseo, Med. J. Aust. 167 (1997) 260.
- [127] A.A. Lugnier, E.E. Crepy, G. Dirheimer, Pathol. Biol. 28 (1980) 127.
- [128] R.B. Mack, North Carolina Med. J. 43 (1982) 588.
- [129] J. Kopferschmitt, F. Flesh, A. Lugnier, P. Sauder, A. Jaeger, J.M. Mantz, Hum. Toxicol. 2 (1983) 239.
- [130] A. Raubert, J. Heard, Vet. Hum. Toxicol. 27 (1985) 498.
- [131] G.W. Henry, G.R. Schwenk, P.A. Bohnert, J. Indiana State Med. Assoc. 74 (1981) 572.
- [132] B. Knight, Br. Med. J. 1 (1979) 350.
- [133] L.J. Ceha, C. Presperin, E. Young, M. Allswede, T. Erickson, J. Emerg. Med. 15 (1997) 65.
- [134] C.J. Pauerstein, Clin. Obstet. Gynecol. 16 (1973) 262.

- [135] P. Mangin, P. Kintz, A. Tracqui, B. Ludes, A.A. Lugnier, A.J. Chaumont, Acta Med. Leg. Soc. 39 (1989) 385.
- [136] J. Moncrieff, J. Chromatogr. 529 (1990) 194.
- [137] P. De Saqui-Sannes, P. Nups, P. Le Bars, V. Burgat, J. Anal. Toxicol. 20 (1996) 185.
- [138] C.L. Winek, W.W. Wahba, F.M. Esposito, W.D. Collom, J. Anal. Toxicol. 10 (1986) 120.
- [139] J.J. Johnston, E.E. Petty, I. Okuno, D.L. Nolte, J. Chromatogr. B 670 (1995) 73.
- [140] J.M. Heiser, M.R. Daya, A.R. Magnussen, R.L. Norton, D.A. Spyker, D.W. Allen, W. Krasselt, Clin. Toxicol. 30 (1992) 269.
- [141] M.L. Drost, Can. Soc. Forensic Sci. 12 (1979) 125.
- [142] D.T. Teitelbaum, J.E. Ott, Clin. Toxicol. 3 (1970) 267.
- [143] R.B. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, Davis, USA, 1982.
- [144] F.A. Benomran, J.D. Henry, Med. Sci. Law 36 (1996) 271.
- [145] M. Reardon, A. Duane, P. Cotter, Ir. J. Med. Sci. 162 (1993) 315.
- [146] S.W. Legha, S. Ring, N. Papadopoulos, M. Raber, R.S. Benjamin, Cancer 65 (1990) 2478.
- [147] G. VanIngen, R. Visser, H. Peltenburg, A.M. Van Der Ark, M. Voortman, Forensic Sci. Int. 56 (1992) 81.
- [148] L.E. Sinn, J.F. Porterfield, J. Forensic Sci. 36 (1991) 599.
- [149] F. Musshoff, B. Jacob, C. Fowinkel, T. Daldrup, Int. J. Leg. Med. 106 (1993) 45.
- [150] D.G. Lang, R.A. Smith, R.E. Miller, Vet. Hum. Toxicol. 39 (1997) 314.
- [151] Caesar, De Bello Gallico VI, Ch. 31.
- [152] J. Liu, K.J. Volk, M.J. Mata, E.H. Kerns, M.S. Lee, J. Pharm. Biomed. Anal. 15 (1997) 1729.
- [153] C. Sottani, C. Minoia, A. Colombo, M. Zucchetti, M. D'Incalci, R. Fanelli, Rapid Commun. Mass Spectrom. 11 (1997) 1025.
- [154] R. Feldman, J. Chrobak, Z. Liberek, J. Szajewski, Pol. Arch. Med. Wewn. 79 (1988) 26.
- [155] D. Grancai, Z. Grancaiova, Ceska Slov. Farm. 43 (1994) 147.
- [156] M.B. Kreig, Green Medicine, Rand McNaly & Company, Chicago, 1975.
- [157] P. Carlier, M.L. Efthymiou, R. Garnier, J. Hoffelt, E. Fournier, Hum. Toxicol. 2 (1983) 321.
- [158] R. Garnier, P. Carlier, J. Hoffelt, A. Savidan, Ann. Med. Int. 136 (1985) 125.
- [159] Y. Gaillard, G. Pépin, Forensic Sci. Int., in press.
- [160] A.M. Jaffe, D. Gephardt, L. Courtemanche, J. Emerg. Med. 8 (1990) 161.
- [161] I.N. Komchatov, G.S. Nasirov, Sud. Med. Ekspert 31 (1988) 51.
- [162] M.N. Berezhnitskii, A.P. Parashchak, Sov. Med. 32 (1969) 141.
- [163] D.A. Nelson, J. Am. Med. Assoc. 156 (1954) 33.
- [164] A. Marinov, P. Koev, N. Mircher, Vulr. Boles 26 (1987) 36.
- [165] D.G. Spoerke, S.E. Spoerke, Vet. Hum. Toxicol. 21 (1979) 346.
- [166] D.J. Wagstaff, A.A. Case, J. Toxicol. Clin. Toxicol. 25 (1987) 361.

229

- [167] J.C. Miller, J.N. Miller, Statistics For Analytical Chemistry, Ellis Horwood, Chichester, 1988.
- [168] N.R. Draper, H. Smith, Applied Regression Analysis, Wiley, New York, 1988.
- [169] J.N. Miller, Analyst 116 (1991) 3.
- [170] Y. Gaillard, C. Peyrefitte, V. Cheminel, J.M. Prévosto, J.F. Chaulet, Lyon Pharm. 46 (1995) 67.
- [171] A.B.J. Nix, D.W. Wilson, Eur. J. Clin. Pharmacol. 39 (1990) 203.
- [172] L.R. Snyder, P.W. Carr, S.C. Rutan, J. Chromatogr. A 656 (1993) 537.
- [173] K. Valko, L.R. Snyder, J.L. Glash, J. Chromatogr. A 656 (1993) 501.