CASE REPORT

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A Non-fatal Case of Intoxication with Foxglove, Documented by Means of Liquid Chromatography-Electrospray-Mass Spectrometry

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ABSTRACT: The non-fatal self-poisoning of a 36-year-old female patient, who ingested a concoction of foxglove (*Digitalis Purpurea*), is presented. On the admission, initial symptoms were nausea and vomiting, abdominal pain, and cardiovascular shock with sinus bradycardia. Blood and urine were assayed for 17 cardiotonic hetorosides, using a highly specific LC-MS procedure. Serum and urine specimens were collected over five days and analyzed by liquid chromatography-electrospray-mass spectrometry (LC-ES-MS). This accurate procedure allowed the determination of the digitalis glycosides and their metabolites in serum and urine. The serum concentrations of digitalis glycosides were maximum on the first day (gitoxin 13.1 ng/mL, digitoxin 112.6 ng/mL, digitoxigenin 3.3 ng/mL, and digitoxigenin mono-digitoxoside 8.9 ng/mL) and decreased over five days. We observed a peak gitaloxin level (112.6 ng/mL) on the fifth day only. After administration of atropine as well as dimeticone, alginic acid, and metoclopramide, health status improved. The peak urine concentrations were reached at hour 30 and were respectively 91.3 and 69.9 ng/mL for gitaloxin and digitoxin, while those of digitoxigenin, digitoxigenin mono-digoxoside and gitoxin were lower (respectively 0.7, 1, and 5.6 ng/mL). The patient was discharged on the fifth day when there were no residual symptoms.

KEYWORDS: forensic science, forensic toxicology, cardiotonic glycosides, liquid chromatography-electrospray-mass spectrometry, *Digitalis Purpurea*

Many digitalic plants including common oleander, foxglove, lily of the valley, yellow oleander and red squill contain potentially cardiotoxic glycosides (1), which can cause fatal poisoning in humans. We report a case of self-poisoning by ingestion of a concoction of foxglove (*Digitalis purpurea*). This plant is highly toxic since all parts contain several potent cardiotoxic glycosides and metabolites, namely lanatoside C, digitoxin, gitoxin, digitoxigenin, digitoxigenin monodigitoxoside, and digitoxigenin bisdigitoxoside (2).

Poisoning with either of yellow oleander, squill, and foxglove has been reported (3–6). However, in these published studies, the relevant cardiac glycosides and their metabolites were not followed systematically. Symptoms seen in these cases generally included gastrointestinal (nausea, vomiting, abdominal pain, and anorexia), and cardiovascular disorders (hypotension, bradycardia, heart block, and ventricular arrhythmias) (3,4,7). Other known side effects include neurosensorial symptoms (headache, dizziness, and blurred vision) and altered mental status (confusion, delirium) (1,8).

Intoxication with therapeutic digitalis glycosides requires treatment with digoxin- or digitoxin-specific Fab fragments. However, the efficacy of the Fab therapy is questionable because of the risk of cross-reactivity with a variety of plant cardiac glycosides (5) and with compounds unrelated to digoxin and digitoxin (9). Immunoassays commonly used for the routine therapeutic drug monitoring of digoxin and digitoxin present the same lack of specificity and can give false results in clinical specimens in the presence of metabolites (10,11).

Digitalis cardiac glycosides may be analyzed also by several separative techniques such as thin-layer or gas chromatography (12), and above all, high performance liquid chromatography (HPLC) that has proven to be a selective and sensitive technique (2,11). Liquid chromatography-atmospheric pressure ionization-mass spectrometry appeared to be the method of choice for forensic toxicological investigations necessitating the simultaneous determination of a few *Digitalis* heterosides and metabolites (13,14).

The aim of our work was to identify and quantify cardiotonic glycosides and their metabolites in a case of non-fatal self-poisoning with a foxglove concoction. For this purpose, we designed a specific and sensitive method using LC-MS. Moreover, to the best of our knowledge, it is the first time that a *Digitalis purpurea* poisoning is analytically documented.

Case History

A 36-year-old female ingested a concoction of foxglove leaves that she had prepared in a suicidal purpose. Prior to admission, the symptoms were nausea and vomiting, and an initial examination (few hours after intoxication) revealed a sinus bradychardia (38 beats per minute-bpm-) and a blood pressure of 140/80. After 1 mg

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of atropine I.V., the heart rate increased to 100 bpm. The apparent digitoxin level in serum was then measured at 162 nmol/L (124 μg/L) using a Microparticule Enzyme Immuno-Assay (Abbott laboratories, Canada).

After admission in the emergency department, the pulse rate had decreased again down to 38 bpm, with a blood pressure of 130/70. Blood chemistry showed normal blood potassium (4.2 mEq/L) and creatinine (90 μmol/L). On the second day (at hour 30), the patient had other episodes of bradychardia. She complained of abdominal pain, nausea, and vomiting. After administration of atropine as well as dimeticone, alginic acid, metoclopramide, health status improved and ECG abnormalities disappeared. Blood and urine were sampled each day for toxicological analyses over five days (from hours 8 to 100). The patient was discharged on the fifth day, as there were no residual symptoms and the ECG was normal.

Materials and Methods

Chemicals and Reagents

α- and β-acetyldigoxin, digitoxigenin mono-digitoxoside, and digoxigenin bis-digitoxoside were obtained from Nativelle (France). Convallatoxine, digitoxigenin, digitoxin, digoxigenin, digoxin, gitoxin, oleandrin, strophantidin, and di-amino phenylsulfone (internal standard, IS) were purchased from Sigma (France). Acetyldigitoxin and deslanosid were obtained from Sandoz (France), gitaloxin from Nycomed Christianes (Belgium), lanatoside C from Serva (Germany), methyldigoxin and proscillaridin from Boehringer-Manheim (Germany). Standard stock and working solutions were prepared in deionized water at the following concentrations: 1 g/L, 1, 0.1, and 0.01 mg/L. All stock and working solutions were stored at +4°C for a maximum of one month. All solvents used were of analytical grade. Sodium hydroxide (25%), ammonium chloride, ether and isopropanol were obtained from Prolabo (France), formic acid and ammonium formate from Sigma (France). Methanol, acetonitrile, and chloroform were purchased from Carlo Erba Reagenti (France) and ammoniac from Merck (France).

Extraction Procedure

To 2 mL of serum were sequentially added 50 μL of a 100 μg/L IS working solution, 1 mL of a saturated ammonium chloride solution (pH 9.5), and 3 mL acetonitrile. After 30 s vortex-mixing, 8 mL of an ether-chloroform-propanol-2 (30:40:30; v/v/v) mixture were added to the supernatant, then extraction was performed by shaking for 15 min and centrifuging at 3000 rpm (1600 g) for 5 min. The organic phase was filtered through a Watmann silicone treated filter paper (pore diameter of 125 mm) and evaporated under a gentle stream of nitrogen. The dry extract was dissolved in 25 μL mobile phase.

Chromatographic and Mass Spectrometric Conditions

The chromatographic material consisted of a Perkin-Elmer autosampler (SA 200), two series 200 Perkin-Elmer micro-pumps, and a Nucleosil C18 (150 by 1 mm i.d., 5 μm particule size) reversed phase column. The mobile phase was a gradient of acetonitrile in 2 mM ammonium formate (pH 3; 5 mM) with a constant flow rate of 40 μL/min: the percentage of acetonitrile was set at 25% for 1 min, then raised to 80% in 20 min, held at 80% for 1 min, and then decreased to 25% in 3 min.

The mass spectrometer was a Perkin Elmer Sciex API-100 single quadrupole instrument, equipped with an Ionspray® source. It was used in the positive ionization mode. The main MS conditions were as follows: nebulization gas flow 0.95 l/min; curtain gas flow 1.16 1/min; orifice voltage 50 V; and ionspray voltage 3500 V. MS data were collected in the selected ion monitoring (SIM) mode with a dwell time of 100 ms per ion monitored. For each analyte, the pseudo-molecular ion $(M + H)^+$ and one or two fragment ions were selected for quantitation and confirmation, respectively (Table 1). Concentrations were determined by linear curve fitting of analyte to IS peak area ratios.

The detection limit (LOD) was determined by injecting extracts of serum fortified with decreasing concentration of the analytes, as the lowest concentration giving a response of three times the average of the baseline noise defined from three unfortified samples. The limit of quantitation (LOQ) was determined as the lowest concentration giving a response that could be quantified with an inaccuracy and an interassay relative standard deviation (RSD) of less than 20%.

Results and Discussion

The major symptoms of digitalic intoxication were present in the clinical case presented herein: mainly bradychardia, nausea, vo-

TABLE 1—*Ions selected (m/z) and relative retention times of the 17 cardiac glycosides.*

Cardiac Glycosides	Ion of Quantitation	Ions of Confirmation		Relative Retention Time
Acetyldigoxin	824.2	635.2	375.5	3.0
α and β acetyldigitoxin	840.3	823.5	805.3	1.8
Convallatoxin	568.7	551.2	405.6	0.7
Deslanoside	960.7	651.2	489.6	1.0
Digitoxigenin	375.5	392.3	339.8	2.1
Digitoxigenin mono-digitoxoside	375.5	522.5	505.7	2.3
Digitoxin	375.5	782.2	635.2	2.6
Digitoxigenin	391.6	373.4	408.4	0.7
Digoxigenin bis-digitoxoside	651.3	668.8	521.8	1.1
Digoxin	798.3	781.5	521.1	1.3
Gitaloxin	826.5	284	419.5	2.4
Gitoxin	798.3	373.4	408.4	2.0
Lanatoside C	1002.5	805.3	651.3	1.4
Methyldigoxin	812.5	795.5	—	1.8
Oleandrin	594.6	577.1	373.4	2.7
Proscillardin	531.6	367.6	—	1.9
Strophantidin	422.4	405.6	359.4	0.9

miting, malaise, weakness, confusion, and disorientation. Nevertheless, the serum potassium level was normal (4.2 mEq/l). The toxicological analysis of the serum revealed the presence of digitoxin, digitoxigenin, and digitoxigenin mono-digitoxoside, while gitaloxin, digitoxin, digitoxigenin, digitoxigenin mono-digitoxoside, and gitoxin were detected in urine.

The evolution of concentration could be monitored in the serum samples collected from 8 h after the intoxication and over five days. At hours 8 and 70, two peak digitoxin levels with respective concentrations of 112.6 ng/mL and 75.3 ng/mL and two peak gitoxin levels with respective concentrations of 13.1 ng/mL and 7.1 ng/mL, were noted. We observed only a peak gitaloxin level of 112.6 ng/mL at hour 100. The long elimination half-life of digitoxin (about 100 h) due to enterohepatic circulation can explain the serum concentration measured five days after the ingestion (see Fig. 3). The maximum serum concentrations of digitoxigenin and digitoxigenin mono-digitoxoside obtained at hour 8, were respectively 3.3 and 8.9 ng/mL and decreased over five days. The peak urine concentration of gitaloxin and digitoxin were respectively 91.3 and 69.9 ng/mL at hour 30, while those of digitoxigenin, digitoxigenin mono-digitoxoside and gitoxin were lower (respectively 0.7, 1, and 5.6 ng/mL). These results illustrate that digitoxin undergoes a complex metabolic degradation generating digitoxigenin, digitoxigenin mono-digitoxoside, and gitoxin and show that digitoxin is also partly excreted unchanged in urine. The identification of these different digitalis glycosides, particularly digitoxin and its relevant metabolites using a specific procedure, confirmed foxglove ingestion. Therapeutic cardiotonic glycosides possess a narrow therapeutic index, and then suicidal ingestion in a patient taking digitoxin should be considered as serious. The most commonly reported therapeutic range for digitoxin is 15 to 30 ng/mL. Digitoxin concentrations above 40 ng/mL are associated with a high incidence of toxicity. Nevertheless, toxic effects are difficult to predict because of a variety of factors that predispose to *Digitalis* poisoning (age, sex, and heart disease, . . .) (7). In this case, the maximum peak concentrations obtained were higher than 70 ng/mL and lead to serious intoxication.

Figure 1 and 2 show SIM chromatograms obtained respectively from a serum spiked at 200 ng/mL and from the serum sample collected on day 1 (at hour 8). The chromatographic resolution was satisfactory for the 17 cardiotonic heterosides. The simple extraction procedure used only requires protein precipitation with acetonitrile and a single-step liquid/liquid extraction. Extraction recoveries were consistently high (from 68% to 98%) (see Table 2). The relatively poor sensitivity of this method for gitaloxin is due to a low ionization efficiency (confirmed by tests on solutions of the pure compound).

One of the main advantages of mass-spectrometry over other detection systems is the gain in specificity obtained by monitoring characteristic ions for each of the digitalis glycosides and by using ratios of confirmation ions to their respective quantitation ions, in addition to relative retention times (see Table 1). Moreover, the thermolability and nonvolatility of cardiac glycosides precludes the use of GC/MS. The high selectivity of mass spectrometry allows

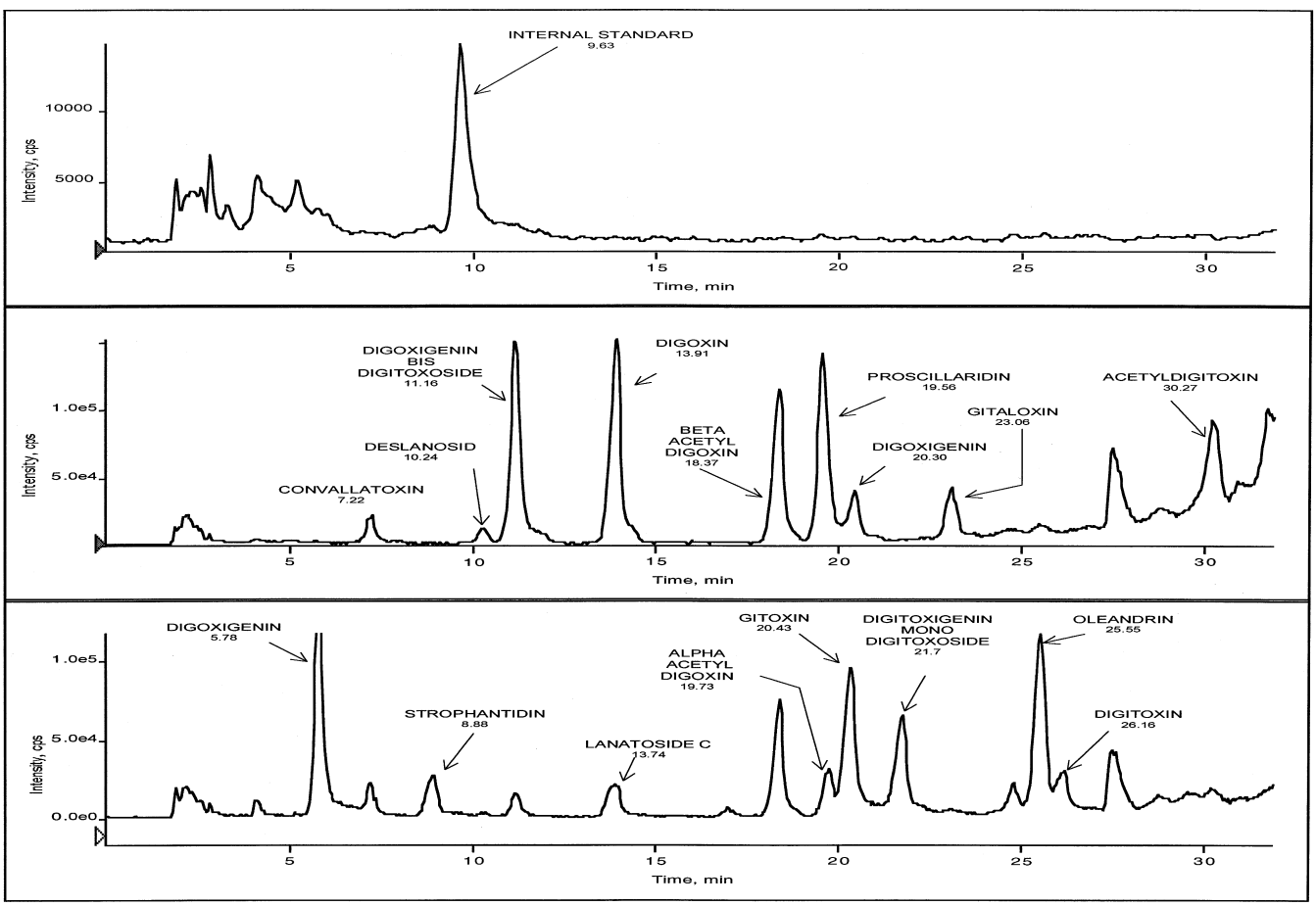
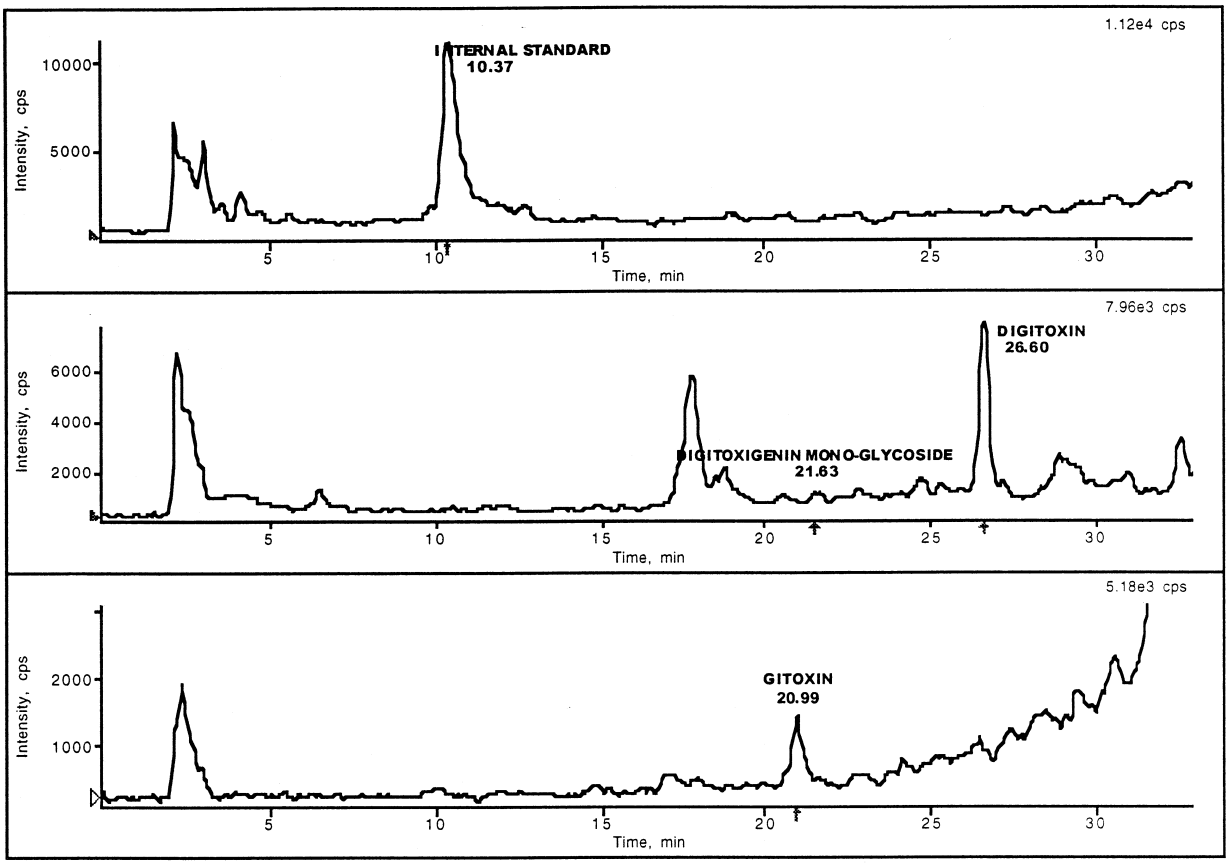


FIG. 1—*Selected-ion recontructed chromatograms of an extract from a serum sample spiked with the 17 cardiac glycosides (at 50 ng/mL).*



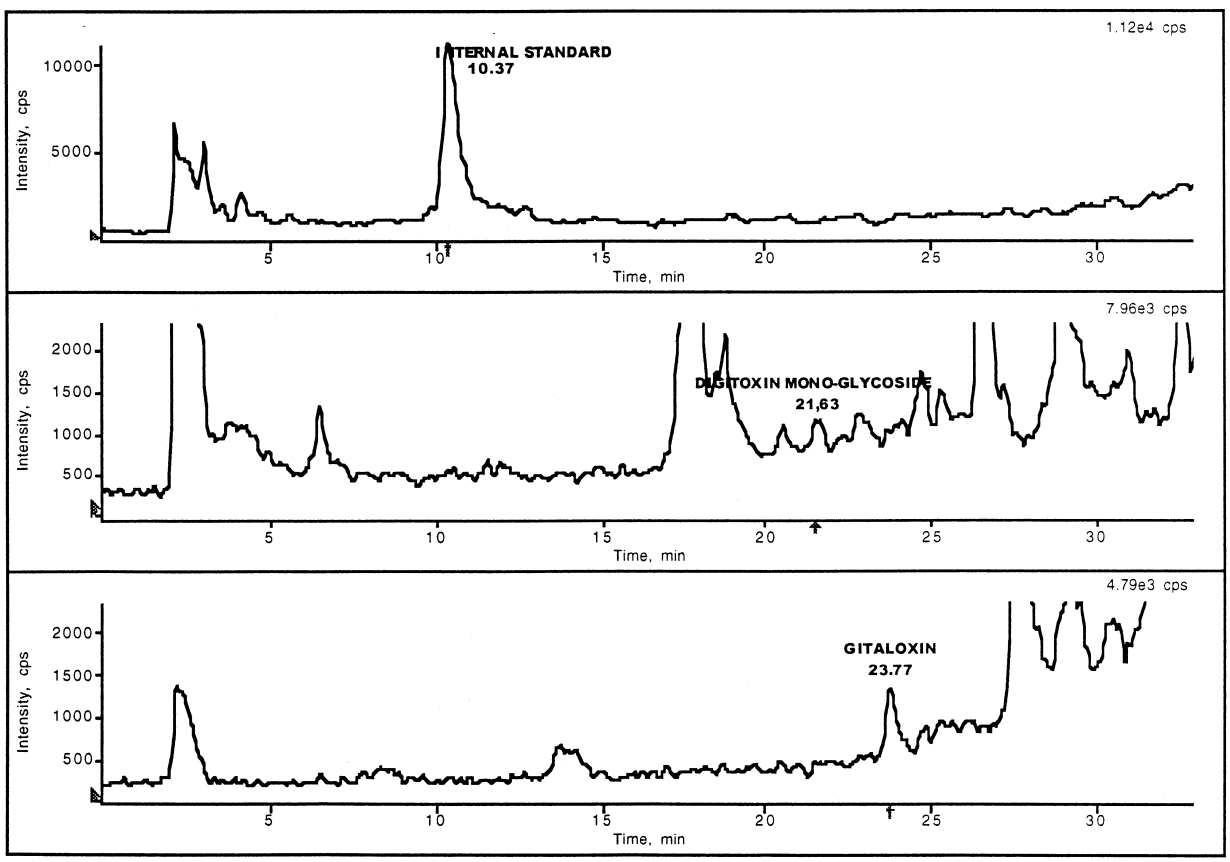


FIG. 2—*Selected-ion recontructed chromatograms of a serum extract from a case of self-poisoning with* Digitalis Purpurea *(at H 8).*

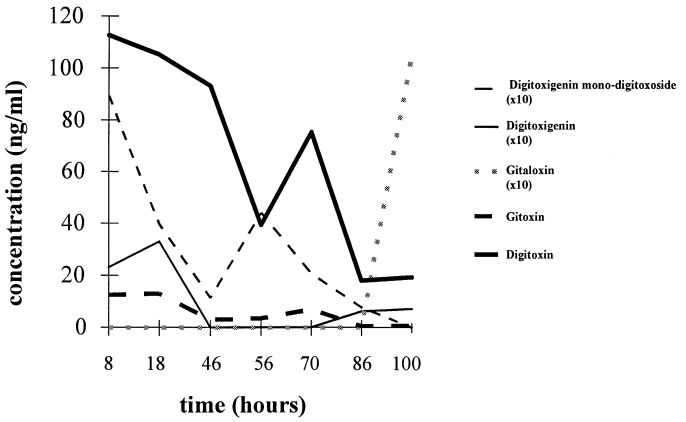


FIG. 3—*Revolution of digitoxin, gitoxin, gitaloxin, digitoxigenin, and digitoxigenin mono-digitoxoside concentrations in serum over a five day period (from H 8 to H 100) after intoxication.*

TABLE 2—*Detection and quantitation limits, linearity range, and extraction recovery for the 17 cardiac glycosides.*

Compounds	LOD ng/mL	LOQ ng/mL	Range	Recovery (%)
Acetyldigoxin	10	20	20–100	67.8
α and β acetyldigitoxin	2	5	5–100	84.9
Convallatoxin	5	10	10–100	73.3
Deslanosid	1	2	2–100	70.3
Digitoxigenin	2	5	5–100	86.1
Digitoxigenin mono-dig.	5	10	10–100	88.8
Digitoxin	5	10	10–100	76.4
Digitoxigenin	5	10	10–100	86.1
Digoxigenin bis-dig.	2	5	5–100	93.3
Digoxin	1	2	2–100	94.0
Gitaloxin	10	20	20–100	89.1
Gitoxin	2	5	5–100	97.2
Lanatoside C	2	5	5–100	83.9
Methyldigoxin	1	2	2–100	93.8
Oleandrin	5	10	10–100	80.0
Proscillardin	5	10	10–100	85.0
Strophantidin	5	10	10–100	98.6

the selective determination of digitalis glycosides and their metabolites in biological samples to determine the source of the plant material ingested when their determination is of forensic interest. However, this LC-ES-MS procedure is not suitable for therapeutic monitoring of digitalis glycosides and derivative because of too high of a LOQ for digoxin and, to a lesser extent, digitoxin. To our knowledge, this is the first intoxication case with a *Digitalis purpurea* concoction in which the compounds were identified, quantitated, and their excretion monitored. The LC/MS method presented should be used in forensic cases requiring the absolute identification of the compounds responsible for digitalic-like symptoms.

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