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Cardiovascular drugs and suicide death: Determination of carvedilol, amlodipine, doxazosin and diltiazem in two fatal cases

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ABSTRACT

A number of medical conditions are identified as risk factors for suicide death; in particular, cardiovascular illnesses are recognized as a major suicide risk factor. In this case, self-poisoning is the common method of suicide and cardiovascular drugs are among the major medications associated with fatal overdose, with calcium channel blockers being one of the most common agents. The present study describes two different fatal suicide cases involving four cardiovascular drugs: carvedilol, doxazosin and amlodipine (case 1) and diltiazem (case 2). The concentrations of the target cardiovascular drugs in the different biological specimens (central and femoral blood, urine, liver, brain) are presented, giving information about the potentially fatal data and the distribution of the drugs in the body. The study led to the implementation of a fast, sensitive and simple method for the detection and quantification of the four commonly prescribed cardiovascular drugs in post-mortem specimens including fluids and tissues for forensic purposes. The method was fully validated. The toxicological results of the studied cases are discussed, along with the autopsy results, histopathological evidence, and circumstances of death. The toxicological findings presented in the study provide new data regarding cardiovascular drugs in different post-mortem specimens, which will contribute to the currently limited knowledge about the toxicological profile of cardiovascular drugs and their distribution.

1. Introduction

Both suicide and cardiovascular disease (such as hypertensive heart disease, ischemic heart disease, heart failure) are commons cause of death [1,2]. Petersen et al. [2] showed a detailed cohort study on the association between heart diseases and suicide showing that specific disorders were found to be associated with an elevated rate of suicide, especially in the first time after diagnosis. Suicide is a serious public health problem; indeed, 703,000 people die due to suicide worldwide every year. In Europe, the crude suicide rate in 2022 was 13 per 100,000 population [3]. It is well known that certain medical conditions have been identified as risk factors for suicide death, including cancer, cardiovascular disease and other chronic illnesses, as well as history of a psychiatric disorder [4]. So medical illness is an important contributor to suicide risk, being a significant factor in about 50 % of suicides in people older than 50 years old [1]. As highlighted by Hawkins et al. [4], several studies have reported that individuals with cardiovascular disease experience suicidal ideation at rates varying between 8 % and 17 %. In this context, self-poisoning is a common method of suicide, but there is only few studies fully characterizing suicide methods in the presence of major physical health conditions. Hawkins et al. [4] demonstrated a higher proportion of suicide deaths due to self-poisoning in patients with cardiovascular disease compared with others suicide decedents without cardiovascular disease. It is important to note that a previous report published by the American Association of Poison Control stated that cardiovascular drugs are among the major medications associated with fatal overdose, with calcium channel blockers being among the most common agents [5]. Furthermore, cardiac medications were identified as lethal at a significantly higher proportion in cardiovascular disease self-poisoning than in people without disease, who died of self-poisoning [4]. Patients with cardiovascular disease have greater access to certain medications with the possibility of being lethal in overdose. Taking into account the fundamental contributory role that cardiovascular drugs can play in causing death, determining the concentration of cardiovascular drugs in post-mortem fluids and tissues is very important for the correct determination of the cause of death. In fact, even though cardiovascular drugs are widely used in clinical practice, and it is known that they are potentially lethal in over-dosage, there is scarce data on the

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toxic/lethal concentrations in the literature [6–25], and there is very little information in the literature on the toxicological profile in relation to cause of death. However, most previously published reports have generally focused only on blood (without site of collection specified) and/or urine biological samples and mostly presented complex sample preparation [6–31]. In this study, we describe two fatal suicidal overdoses involving four cardiovascular drugs: carvedilol (CRVD), doxazosin (DOX), amlodipine (AML) (case 1) and diltiazem (DLTZ) (case 2). We will present analytical data and case circumstances for these suicide deaths, determining the target compounds in different post-mortem specimens (such as peripheral and central blood, urine, brain, liver, gastric contents) by means of a simple, fast and sensitive procedure using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2. Case histories

2.1. Case 1

A 56-year-old man (weight 73 kg, height 176 cm) was found unresponsive in his bed at approximately 8 p.m. by his sister, with a suicide note left. When the emergency medical services arrived on the scene at 9 p.m., they found no signs of life and detected a condition of rigor and livor mortis. No resuscitative efforts were made. The man was pronounced dead at the scene. Several empty bottles of prescription medications were found near the corpse: one bottle of DOX (30 tablets per bottle at 2 mg each); a second bottle of DOX (20 tablets per bottle at 4 mg each); one bottle of AML (14 tablets per bottle at 10 mg each); two bottles of CRVD (30 tablets per bottle at 25 mg each). The sister had not heard from the man since the previous evening (6 p.m.). Anamnestic data were available: epilepsy, heart disease and hypercholesterolemia under pharmacological treatment, and a history of depression were reported. A judicial autopsy was ordered by the prosecutor to establish the cause of death. The autopsy was performed 3 days after death and the corpse was maintained at 4 °C until the autopsy. The corpse was in good condition, well nourished. At the external examination, no traumatic lesions or evidence of violence were observed. At the autopsy, cerebral and pulmonary edema were revealed, and all the viscera appeared congested. Subpleural petechiae were observed. Aortocoronary sclerosis was found; the right coronary artery showed a critical stenosis. Partially dissolved tablets were found in the stomach contents (280 mL). The histological examination showed sclerosis of the myocardium, in particular in the posterior part of the right wall. Histological investigation of other organs revealed no other significant findings. Biological fluids (peripheral blood collected from femoral vein, urine, gastric content) and tissues (brain and liver) were collected for toxicological analyses during the autopsy.

2.2. Case 2

A 57-year-old woman (weight 84 kg, height 163 cm) was found unresponsive in bed at approximately 1 p.m. by her husband. When the emergency medical services arrived on the scene at 1.10 pm, they found no signs of life and detected a condition of rigor and livor mortis. No resuscitative efforts were made. The woman was pronounced dead at the scene. Eight empty blister units of DLTZ 200 mg, sustained release, were found near the corpse. The husband had not seen the woman since the previous evening. Little anamnestic data were available: previous gastroplasty surgery for obesity and a history of hypertensive heart disease, under pharmacological treatment, were reported. She had already attempted suicide in the past. A medical autopsy was ordered to establish the cause of death. The autopsy was performed 5 days after death; the corpse was maintained at 4 °C until the autopsy. The corpse was in good condition, obesity class 1, well nourished. At the external examination, no traumatic lesions or evidence of violence were observed. At the autopsy, cerebral and pulmonary edema were revealed, and all the viscera appeared congested. Subpleural petechiae were observed. Partially dissolved tablets were found in the stomach contents (50 mL). The histological examination showed sclerosis of the myocardium in the septum, fibro-fatty myocardial replacement, and focal signs of subendocardial contraction bands. Histological investigation of other organs revealed no other significant findings. Biological fluids (central and peripheral blood, collected from the heart and femoral vein, respectively; gastric contents) and tissues (brain and liver) were collected for toxicological analyses during the autopsy.

3. Materials and methods

3.1. Samples

Biological fluids and tissue samples of the two autopsy cases were routinely taken during autopsy at the University Center of Legal Medicine of Modena and Reggio Emilia, were immediately frozen and were kept at −20 °C until analysis. The collected samples were stored in polystyrene tubes with sodium fluoride (~1 % w/v). The analyses were performed within 10 days from autopsy. The case specimens (blood, brain, liver, gastric contents and urine) were subjected to a routine systematic toxicological analysis (STA) used in our laboratory. Peripheral blood was screened for volatiles by headspace gas chromatography with flame ionisation detection. Blood and urine were screened using Syva EMIT immunoassay (Siemens, Newark, DE, USA) for drugs of abuse, including amphetamines, ecstasy, barbiturates, benzodiazepines, buprenorphine, methadone, cocaine, cannabinoids, opiates, valproic acid and phencyclidines. A comprehensive screening for pharmaceuticals and drugs of abuse, including NPS and fentanyl analogues (a total of 299 analytes included metabolites), was performed on samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and full scan gas chromatography-mass spectrometry (GC-MS). Analytes, MRM transitions (LC-MS/MS) and method sensitivity are listed in Supplementary Table S1. As a preliminary result of the STA, case 1 screened positive for DOX, AML, CRVD, 7-aminoclonazepam, lacosamide; case 2 screened positive for DLTZ, phenobarbital, diazepam, nordazepam. Therefore, we focused our attention on the detection and quantitation of the four cardiovascular drugs: CRVD, DOX, AML and DLTZ. We evaluated the distribution of these analytes in the different collected specimens by the LC–MS/MS method validation for the simultaneous determination of the four analytes in all collected biological samples of the victims.

3.2. Chemicals and reagents

DOX, CRVD, AML and DLTZ were purchased from Merck KGaA (Darmstadt, Germany). As internal standard (IS), carvedilol-d5, amlodipine-d4 and prazosin obtained from LGC (Milan, Italy) were used. All the solvents used for high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) were of LC–MS purity grade (Baker-VWR, Milan, Italy) and the chemicals used for preparation were of analytical grade (Carlo Erba, Milan, Italy). Phree Phospholipid Removal Tabbed 1 mL Cartridges were purchased from Phenomenex (Bologna, Italy).

3.3. Preparation of working solutions, calibration and quality control samples

Initially, separate stock solutions of DOX, AML and CRVD were prepared in methanol at a concentration of 1 mg/mL. A mixture of all analytes at 2000 ng/mL in methanol was obtained through the dilution of these indicated solutions and the stock solution of DLTZ hydrochloride (1 mg/mL in acetonitrile). Additional working standard solutions were prepared for the calibration at 1000 ng/mL, 500 ng/mL, 100 ng/mL and 10 ng/mL. A single internal standard stock solution of Carvedilol-d5, Amlodipine-d4 and Prazosin was also prepared at a

concentration of 1 mg/mL. The internal standard working solution (IS) at 500 ng/mL was obtained by appropriate dilution of stock solutions with methanol. Carvedilol-d5 was used for quantification of CRVD and DLTZ; Amlodipine-d4 was used for AML, and Prazosin was used for DOX. All solutions were stored at −20 °C. The preparation of calibration samples was carried out using a pooled blank sample, checked before analysis. Precellys Evolution, Bertin (AlfaTech, GE, Italy) was used to homogenise the body tissue (brain and liver). The brain or liver tissue was added to a 7 mL plastic tube containing 10 steel balls and then homogenised at 10,000 rpm up to 30 s. Calibration samples were obtained through the addition of the appropriate volumes of the working solutions to 200 µL blank fluids or 1 g homogenised blank tissue to final concentrations of 1, 10, 50, 100, 500, 1000, 1500, 2000 ng/mL (or ng/g). The calibration samples were analysed in triplicate. Quality control samples (QC) were prepared using the same method as the calibrators to final concentrations of 1 ng/mL (ng/g), 100 ng/mL and 1000 ng/mL (ng/g) for body fluid and tissue specimens.

3.4. Sample preparation

For body fluids (peripheral and central blood, urine and gastric contents), 200 µL matrix aliquots, including all types of samples (blank, QC, calibrator and case samples), 20 µL of IS solution and 1 mM acetonitrile-methanol solution (70:30 v/v) with 0.1 % formic acid were added. For tissue samples (brain and liver), 40 µL of IS solution and 2 mL of ammonium formate, and 1 mM acetonitrile-methanol solution (70:30 v/v) with 0.1 % formic acid were added to 1 g of all the homogenised tissue samples. All samples were mixed and centrifuged for 10 min at 3500 x g. The total supernatant for body fluids and 1 mL of supernatant for tissues were purified through Phree phospholipid removal tubes (1 mL). The extract obtained was evaporated to dryness under nitrogen at 40 °C. All residues were reconstituted with 150 µL of mobile phase solution, consisting of 95 % of mobile phase A [ammonium formate 1 mM and 0.1 % formic acid in water] and 5 % of mobile phase B [acetonitrile/methanol (70/30) added with ammonium formate 1 mM and 0.1 % formic acid]. The samples were transferred to microvials and a 10 µL aliquot of each sample was injected for analysis.

3.5. Instrumentation

High-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) was performed using the Agilent 1200 LC System (Agilent Technologies, Palo Alto, CA, USA) coupled with a 4000 QTRAP (AB SCIEX, Foster City, CA, USA) with an electrospray ionization (ESI) Turbo V™ Ion Source. The autosampler was maintained at room temperature and the injector needle was washed with methanol:0.05 % formic acid in water (1:9 v/v) prior to any injection. Samples were analyzed on a Synergi 4 µm Polar-RP (150 × 2.0 mm; 4 µm particle size) (Phenomenex, Torrance, CA, USA). The mobile phase was composed of (A) ammonium formate 1 mM and 0.1 % formic acid in water (pH 3.0) and (B) ammonium formate 1 mM acetonitrile-methanol solution (70:30 v/v) with 0.1 % formic acid]. The following gradient program was used: 0.0–20.0 min linear gradient from 5 % to 95 % (B); 20.0–23.0 min isocratic at 95 % (B), 23.0–23.2 min linear gradient from 95 % to 5 % (B). Between each run, a pre-equilibration period of 6.8 min was applied. The column was set to 35 °C and the flow rate was 0.25 mL/min. The injector needle was washed with methanol: 0.05 % formic acid in water (1:9 v/v). The ESI source settings were: ion-spray voltage: 5500 V; source temperature: 450 °C; nebulization and heating gas (air): 40 psi and 40 psi, respectively. The acquisition was performed in multiple reaction monitoring (MRM), which was optimised using nitrogen as collision gas and a dwell time of 100 ms. Nitrogen was produced by a gas generation system (Nitrogen Generator model 75–72, Whatman Inc., MA, USA). The MRM transitions and optimized parameters are shown in Table 1. Analyst software (version 1.6.1, AB SCIEX, Foster City, CA, USA) was used for instrument control, data acquisition and qualitative data analyses. Quantitative data analyses were performed using MultiQuant software (version 3.0.2.).

Table 1
MRM transitions and LC–MS/MS parameters optimized for the target analytes.

Analyte	MRM transitions (*m/z*)	DP$ (Volt)	CEϒ (v)	R_Tþ (minutes)	ISρ
CRVD₪	407.5→**224.4**[a], 283.1[b], 100.0[b]	60	45, 30, 40	15.81	CRVD-d_5
DLTZ₡	415.0→**178.0**[a], 370.0[b]	60	30, 25	14.67	CRVD-d_5
AML₵	409.3→**294.0**[a], 238.0[b]	60	18.5, 14	14.99	AML-d_4
DOX₸	452.3→**344.4**[a], 247.0[b]	60	43, 30	14.88	PRZS
CRVD-d_5£	412.1→**104.8**[a]	60	28	15.86	-
AML-d_4£	413.1 → **238.1**[a]	60	15	15.02	-
PRZS&	384.0 → **247.0**[a]	60	38	14.62	-

CRVD₪: CARVEDILOL; DLTZ₡: DILTIAZEM; AML₵: AMLODIPINE; DOX₸: DOXAZOSIN; CRVD-d5£: CARVEDILOL-d5; AML-d4£: AMLODIPINE-d4; PRZS&: PRAZOSINE; ρIS: internal standard; $DP: declustering potential; ϒCE: collision energy; þRT retention time; a quantifier transitions are highlighted in bold characters; b qualifier transitions are used for confirmation purposes.

3.6. Method validation

The method was validated according to standard practice for method validation in forensic toxicology, ANSI/ASM Standard 036, First Edition 2019 [32]. The calibration model, bias, intra- and inter-day precision, carryover, interferences, matrix effects, limit of quantitation (LOQ)/limit of detection (LOD), recovery, dilution integrity and stability were evaluated. All studied matrices have been validated. The calibration model was explored over a range of 1–2000 ng/mL (ng/g) for all compounds in both the fluid and tissue specimens. Calibration curves were generated from the peak-area ratio of each analyte quantifier transition to the deuterated IS; the ratio was then plotted on the y-axis against the nominal analyte concentration to generate the standard curves by the method of least squares using a weighted (1/x) linear regression model. Bias and precision were assessed by analysing low quality control at 1 ng/mL (LQC), medium quality control at 100 ng/mL (MQC) and high-quality control at 1000 ng/mL (HQC) in triplicate over the course of five different days. The acceptable bias range was within ± 20 % of each QC level. The precision was evaluated by analysis of variance (ANOVA) for the coefficient of variance (%CV) of within-run and between-run values, which should not exceed 20 % for each QC level. LODs were determined by injecting decreasing analyte concentrations in the different matrices. The LOD was considered to be the lowest concentration at which the analyte response could be differentiated from that of background noise and had a signal-to-noise ratio ≥ 3 on the lowest MRM transition. The LOQ was the lowest calibrator concentration on the curve and was monitored by analysing nine replicate samples for matrices, three replicates over three separate days, for bias and precision values (within-run and between-run) to be within 20 %. The matrix effect (ME) and extraction recovery (ER) were evaluated with a set of three different samples at low (LQC), medium (MQC) and high (HQC) concentration with ten different authentic samples: the neat standard (set 1), blank matrix spiked with target analytes after extraction (set 2) and blank matrix spiked before extraction (set 3). The ME was evaluated comparing the average peak areas (X) of set 2 to those of set 1 as follows: ME (%) = [(X area of set 2 /X area of set 1) −1] x 100. The ME% should be within ± 25 %. Extraction recovery was estimated by comparing the average peak areas of set 2 to those of set 3, expressed as percentages. The carryover effect was assessed by injecting extracts of blank matrix samples after analyses of calibration samples spiked at the upper limit of quantitation. Carryover was deemed not present if the negative samples did not exhibit a signal greater than that of the LOD.

Matrix interference studies were performed by using a total of ten different blank post-mortem samples, for each matrix, without the addition of an internal standard. Interference studies were performed by analysing a low QC specimen spiked with common medicines, metabolites and other common drugs of abuse at high concentrations. Specimens were considered to be free from interference if a response did not meet the criteria required for an identification, which included the correct retention time and qualifier transition ratio. The dilution integrity was assessed as follows: blank specimens for all matrices were spiked at 5 times the highest validation sample and mixed with additional blank matrices to achieve a 5-fold (n = 6), 10-fold (n = 6), 15-fold (n = 6) dilution. All the samples obtained were analysed against the calculated calibration curves to assess if the criteria performance was still met. QC samples after 24 and 48 h on the autosampler were re-analyzed in triplicate to assess the stability parameters. The analyte was considered stable until the average concentration values, after 24 and 48 h, compared to the time zero average concentration values fell outside of the method's acceptable bias.

4. Results

A 1/x-weighted calibration model was determined for the quantifier transition and the coefficient of determination was $r^2 > 0.998$ for DOX and DLTZ and $r^2 > 0.995$ for AML and CRVD. The linear range of this model was 1–2000 ng/mL (ng/g) for all analytes in fluid and tissue. As the limit of quantification (LOQ) for all the analytes, the lowest calibration point was chosen. LODs were calculated at 0.5 ng/mL (ng/g) for all the analytes. Overall accuracy was better than 11.7 %, 12.5 %, 14.0 % and 8.3 % for DOX, DLTZ, AML and CRVD, respectively. The imprecision was between 1.3 % and 14.3 % inter-batch and 1.4–13.2 % intra-batch. No interference was detected during endogenous, exogenous and IS studies. Carryover was not observed. Matrix effects were less than 14.3 % for all compounds, and recovery was higher than 81.2 % for CRVD and AML, higher than 83.4 % for DLTZ, and higher than 80.2 % for DOX. The data validation is shown in Table 2. All dilution controls were within the acceptable range of method validation, with the intra-batch and inter-batch imprecision less than 10 % and the accuracy within the range ± 20 % for all analytes. The stability was tested and the QC sample analyses were within the acceptable bias range of method validation. The validated method was applied to the peripheral and central blood, urine, gastric contents, liver and brain tissue collected during the autopsy of the studied case. Table 3 gives a summary of the concentrations of the analytes measured in all the matrices. In case 1, the presence of CRVD, DOX and AML was found in all analyzed matrices. The concentration ranges of CRVD, DOX and AML in different specimens were tested at 0.094–1.763 mg/L (mg/kg), 0.109–4.936 mg/L (mg/kg), and 0.869 − >20.0 mg/L (mg/kg), respectively. In case 2, the presence of DLTZ was found in all analyzed matrices. The concentration range of DLTZ in all matrices was tested at 16.6–26.5 mg/L (mg/kg). Moreover, Table 3 shows other findings revealed at the routine systematic toxicological analyses. In case 1, all specimens were positive to 7-aminoclonazepam (metabolite of clonazepam) and lacosamide. Instead, in case 2, phenobarbital, diazepam and its metabolite (nordazepam) were detected in all the analysed matrices, and ethanol at concentration of 0.35 g/L was also found in the peripheral blood in case 2.

5. Discussion

The study led to the implementation of a fast, sensitive and simple method for the detection and quantification of the four commonly prescribed cardiovascular drugs in post-mortem specimens including fluids and tissues for forensic purposes. The sensitive method was fully validated. In this study, the concentrations of the target cardiovascular drugs in the different post-mortem specimens are presented, giving information about the potentially fatal data and the distribution of the drugs in the body during fatal intoxication. Data for all the studied analytes are presented in Table 3. Considerations of the levels of CRVD, DOX, AML and DLTZ calculated can be found in the published data, though only AML and DLTZ data regarding lethal intoxication are available. In fact, there is no information on the toxicological profile of CRVD and DOX in relation to the cause of death. This lack of information in the scientific literature produces an evident difficulty in the interpretation of cases involving cardiovascular drugs as cause of death.

AML is a competitive calcium channel antagonist of the dihydropyridine group that is used to treat hypertension, angina and heart failure by causing peripheral vasodilatation and reducing afterload. It acts primarily on voltage-gated (L-type) calcium channels and in overdose can have more effects on the heart by reducing contractility and slowing atrio-ventricular conduction. Peak plasma concentrations occur between 6 and 12 h after ingestion and it has an elimination half-life of 35–50 h [18]. It is used with a daily prescribed dose of 5–10 mg [33]. Therapeutic dosage range of AML usually is 0.001–0.025 mg/L [22]. Regarding fatal cases, Table 4 summarizes the published cases involving AML giving an overview on deceased characteristic and AML concentration in different specimen: AML reported overdose has typically been associated with suicidal intent and have involved doses of between 50 and 500 mg. In particular, Morini et al. [10] reported a fatal intoxication involving AML. The peripheral blood concentration (0.17 mg/L) was in the toxic range, though not particularly high. The authors affirm that even the uncontrolled intake of a less toxic calcium channel blocker can lead to death. Furthermore, the authors demonstrated a significant degradation of AML in biological matrix storage at −20 °C (99 %). This evidence leads the authors to assert that this aspect may lead to an underestimation of the measured concentration. The authors state that the use of fluoride is always advisable in order to avoid in vitro degradation of AML, and, in case of delayed autopsy, the concentrations of the analyte in biological fluids should be interpreted with caution. Furthermore, Alvarez et al. [24] studied the post-mortem concentrations of AML in order to define more precisely the fatal and non-fatal post-mortem concentrations. The AML concentrations were measured in femoral whole blood by LC-MS/MS. Based on the conclusions of the autopsy and toxicological results, the data were classified into 2 groups: G1 non-toxic death (22 cases) and G2 fatal poisoning (13 cases) involving AML alone or as part of a multidrug poisoning with a conclusion of toxic origin. The observed range concentration of AML was 7–128 ng/mL in G1 (mean 58.73 ng/mL) and 100–737 ng/mL in G2 (mean 295.63 ng/mL).

CRVD is a non-selective β-adrenoreceptor antagonist with additional α1-adrenoreceptor antagonist activity. CRVD's α1-adrenoreceptor-antagonist activity presents a theoretical risk for additional hypotension, especially in the case of overdose. As is well known, therapeutic concentrations are linearly related to the administered dose [34]. The published data have reported a therapeutic range of 20–150 (300) ng/mL [35]. Regarding post mortem blood concentration, the median therapeutic values were reported at 0.018 mg/L, while the upper percentile (90th, 95th, 97.5th) concentrations, that indicate possible overdose levels, where calculated at 0.080 mg/L, 0.13 mg/L and 0.30 mg/L respectively [36]. Concerning toxicity, despite its wide use, there are only two published reports of CRVD overdose. However, in only one [25] there is a quantitative determination of CRVD in serum, in which the authors described a survival case with a massive CRVD ingestion. An 84-year-old man with severe Alzheimer's dementia and hypertension was discovered by his wife in their car to have chewed and swallowed a full bottle of her CRVD (sixty 6.25-mg tablets) and part of a bottle of simvastatin (fifteen 20-mg tablets). The man rapidly developed symptoms and was transported promptly to the hospital by ambulance. Quantitative confirmation showed a CRVD serum concentration of 472 ng/mL. The typical threshold for CRVD toxicity in overdose is 50 mg however, even if beta-blocker toxicity is usually the result of overdose patients with cirrhosis represent a special et-risk group that can have toxicity also at standard doses [37].

DOX is a selective alpha-1 adrenergic reversible non-competitive antagonist which is used to treat resistant hypertension by inhibiting

Table 2
Bias and precision data for CRVD, DLTZ, AML and DOX in all studied matrices by LC–MS/MS.

Matrix	Analyte	Low QC[$]					Medium QC					High QC				
		Bias (%)	Imprecision CV[Ψ] (%)		RE[¥] (%)	ME[ϒ] (%)	Bias (%)	Imprecision CV[Ψ] (%)		RE[¥] (%)	ME[ϒ] (%)	Bias (%)	Imprecision CV (%)		RE (%)	ME (%)
			Intra-batch	Inter-batch				Intra-batch	Inter-batch				Intra batch	Inter -batch		
Blood	CRVD₪ (QC = 0.001, 0.1, 1 mg/L)	-5.1	4.0	6.1	87.2	1.1	-4.2	8.4	5.0	89.3	5.7	-7.9	2.5	2.4	83.4	1.2
	DLTZ₡ (QC = 0.001, 0.1, 1 mg/L)	-1.6	4.1	4.3	86.0	10.7	-3.5	4.0	6.2	87.4	6.7	3.1	4.5	4.6	85.2	7.7
	AML₵ (QC = 0.001, 0.1, 1 mg/L)	1.0	4.0	8.3	87.9	9.9	1.9	7.9	3.5	89.2	7.1	1.7	6.7	4.9	84.2	3.7
	DOX₸ (QC = 0.001, 0.1, 1 mg/L)	3.4	7.8	5.4	81.8	-8.3	3.9	8.7	4.9	81.2	-12.3	4.8	7.4	1.3	81.9	-4.7
Brain	CRVD₪ (QC = 0.001, 0.1, 1 mg/Kg)	6.2	8.7	4.3	85.6	-4.5	6.7	7.2	10.4	84.9	-2.2	7.7	9.4	3.4	88.2	-7.0
	DLTZ₡ (QC = 0.001, 0.1, 1 mg/Kg)	4.9	2.4	7.4	83.4	-10.2	7.8	10.0	3.3	84.5	-7.5	5.6	8.4	7.8	85.3	-6.4
	AML₵ (QC = 0.001, 0.1, 1 mg/Kg)	11.0	4.3	4.8	88.1	4.9	10.7	5.9	8.9	87.3	10.6	12.3	4.6	8.7	87.0	10.1
	DOX₸ (QC = 0.001, 0.1, 1 mg/Kg)	8.2	5.8	4.9	87.2	3.2	11.7	9.1	7.2	84.2	7.5	10.4	7.6	9.	83.5	4.3
Liver	CRVD₪ (QC = 0.001, 0.1, 1 mg/L)	6.8	9.2	13.5	81.2	-13.1	7.3	10.2	10.0	82.0	-11.7	6.0	13.2	10.0	83.0	-10.2
	DLTZ₡ (QC = 0.001, 0.1, 1 mg/Kg)	12.5	10.7	9.8	84.1	-10.0	9.7	11.0	10.0	88.1	-6.2	10.0	7.0	9.8	84.0	-4.9
	AML₵ (QC = 0.001, 0.1, 1 mg/Kg)	10.3	9.4	11.3	84.2	-9.3	12.0	11.7	9.4	87.2	-10.6	14.0	9.8	10.3	89.2	-9.2
	DOX₸ (QC = 0.001, 0.1, 1 mg/Kg)	9.2	8.3	10.5	85.3	-2.4	10.3	7.8	8.2	88.2	-4.3	10.2	5.3	6.7	88.3	1.2
Gastric content	CRVD₪ (QC = 0.001, 0.1, 1 mg/L)	4.5	1.9	10.0	86.4	-10.0	8.3	4.9	4.8	84.3	-10.2	6.3	8.7	9.4	88.2	-13.4
	DLTZ₡ (QC = = 0.001, 0.1, 1 mg/L)	3.5	6.4	7.9	85.2	-10.7	10.2	6.1	5.9	88.9	-8.8	5.3	7.4	9.3	85.2	-6.7
	AML₵ (QC = = 0.001, 0.1, 1 mg/L)	7.3	12.0	8.2	84.3	14.3	12.2	8.4	9.3	85.0	10.3	10.0	11.0	7.3	81.2	10.5
	DOX₸ (QC = = 0.001, 0.1, 1 mg/L)	9.4	4.0	7.4	82.1	10.4	10.7	9.2	14.3	80.9	12.4	6.0	13.0	4.3	80.2	12.5
Urine	CRVD₪ (QC = = 0.001, 0.1, 1 mg/L)	2.1	3.4	1.9	91.8	-3.3	4.2	2.0	3.4	89.9	-2.3	6.8	4.1	2.9	92.1	-1.9
	DLTZ₡ (QC = = 0.001, 0.1, 1 mg/L)	3.1	1.4	4.5	85.9	-1.1	4.5	5.0	3.9	89.9	-4.1	4.3	5.1	3.2	89.0	-3.5
	AML₵ (QC = = 0.001, 0.1, 1 mg/L)	6.2	2.3	6.1	85.0	-6.0	5.3	10.0	8.2	87.0	-4.2	6.0	8.1	9.4	85.4	-4.1
	DOX₸ (QC = = 0.001, 0.1, 1 mg/L)	7.0	4.3	10.0	86.1	-7.9	4.9	8.5	10.4	84.9	-2.2	4.5	7.9	7.5	89.0	-2.1

CRVD₪: Carvedilol; DLTZ₡: Diltiazem; AML₵: Amlodipine; DOX₸: Doxazosin.

Table 3
CRVD, DLTZ, AML and DOX concentrations in post-mortem specimens and other findings.

		PERIPHERAL BLOOD (mg/L)	CENTRAL BLOOD (mg/L)	URINE (mg/L)	GASTRIC CONTENT (mg/L)	BRAIN (mg/Kg)	LIVER (mg/Kg)
Case 1	**CRVD₪**	0.500	N/Aζ	0.094	positive	0.067	1.763
	DOX₸	0.910	N/Aζ	0.109	positive	0.435	4.936
	AML₡	1.278	N/Aζ	0.869	positive	0.984	>20.000
	OTHER FINDINGS	Clonazepam N/A 7-aminoclonazepam 0.157 Lacosamide 3.820	N/Aζ	Clonazepam N/A 7-aminoclonazepam 0.062 Lacosamide 4.373	Lacosamide	Clonazepam N/A 7-aminoclonazepam 0.118 Lacosamide 2.315	Clonazepam N/A 7-aminoclonazepam 0.682 Lacosamide 1.427
Case 2	**DLTZ₡**	16.600	20.200	N/Aζ	positive	16.700	26.500
	OTHER FINDINGS	Ethanol 0.35 g/L Phenobarbital 0.870 Diazepam < LLOQ Nordazepam 0.018	Phenobarbital 1.800 Diazepam < LLOQ Nordazepam 0.029	N/Aζ	/	Phenobarbital Diazepam Nordazepam	Phenobarbital Diazepam Nordazepam

CRVD₪: Carvedilol; DOX₸: Doxazosin; AML₡: Amlodipine; DLTZ₡: Diltiazem; N/Aζ: not Available

Table 4
AML review of potentially toxic or lethal cases.

Author and references	Cases	Deceased	Toxicological circumstantial data	AML concentration
Sklerov et al. [13]	2 cases	44-year-old; male; Caucasian; pre-existing heart condition	Ingestion of three bottles of prescription medication containing AML	Central blood 2.4 mg/L Brain 5.4 mg/kg Liver: 68.8 mg/kg Urine: 1.1 mg/L Stomach contents: 583 mg/kg
		66-year-old; female; African American; history of depression	Bottle originally contained 60 AML tablets found empty (dose unspecified by investigator)	Central blood: 0.95 mg/L Peripheral blood: 0.87 mg/L Liver: 91.1 mg/kg Bile: 69 mg/L
Johansen et al. [22]	1 case	50-year-old; male	N/Aζ	Peripheral blood: 2.3 mg/L Liver: 8.7 mg/kg Muscle tissue: 2.9 mg/kg Stomach contents 17 mg/kg ⇒ 0.1 mg
Cosbey and Carson [7]	1 case	15 years-old; female	Ingestion of 140 mg of AML and an unspecified dose of mefenamic acid	Central blood: 15.4 mg/L Peripheral blood: 2.7 mg/L Gastric contents: 24 mg/L
Morini et al. [10]	1 case	50-year-old; male; history of improper self-medication hypertensive heart disease, under pharmacological treatment	N/A	Peripheral blood: 0.17 mg/L Urine: 1.73 mg/L
Linnet et al. [9]	1 case	N/A	N/A	Peripheral blood: 0.94 mg/kg

N/Aζ: not Available

the binding of noradrenaline. It is a potent vasodilator and can cause postural hypotension in patients. Peak plasma concentrations occur 2–3 h after dosing with an elimination half-life of 22 h [18]. The published data have reported a therapeutic range of 10–150 ng/mL [35]. Concerning toxicity, there are only two survival cases regarding DOX overdosage reported in the international literature. However, in both articles, the authors could not obtain serum/blood concentrations, and the diagnosis and management of the patient was established based on the history obtained from the patient and relatives [19,20]. As previously stated, no such cases of a suicidal/accidental lethal death involving CRVD and DOX have been available. Therefore, we conducted a comparison with the only reported published data.

DLTZ is a calcium-channel blocker frequently used for treatment of angina pectoris, hypertension, and cardiac arrhythmias [11]. The therapeutic range is established between 0.030 and 0.25 mg/L [35]. DLTZ is currently the cause of acute and chronic poisoning due to overdose [11]. In fact, though many cases of acute poisoning are reported in the literature, cases of lethal outcome are rare, so a lack of data is available on post-mortem tissue concentration [11]. Regarding fatal cases involving DLTZ, reported overdose has typically been associated with suicidal intent and has involved blood concentrations >2 mg/L and Table 5 summarized the published cases involving AML giving an overview on deceased characteristic and AML concentration in different specimen. As is well known, from a forensic point of view, an accurate collection of circumstantial data, followed by a thorough autopsy, histological investigation and toxicological investigation, is always essential to lead to a correct medical-legal diagnosis and to allow the cause of death to be identified. A careful multidisciplinary evaluation of all data is necessary to assess the contributory role of the substance intake in fatal cases. For both cases under examination, the analysis of the circumstantial data (several empty bottles of prescription medications: two bottles of DOX, one bottle of AML and two bottles of CRVD for case 1, and eight empty blister units of DLTZ for case 2) quickly directed the investigations toward the hypothesis that the cause of death had been a drug intoxication with the goal to commit suicide. For case 1, further confirming the self-poisoning hypothesis, a suicide note was found near the corpse. For case 2, the anamnesis reported that the victim had a previous suicide attempt. The pathological findings of the decedents, including congested and edematous lungs and cardiac abnormalities, and partially dissolved tablets in the stomach contents, are consistent with death attributable to drug poisoning. In both cases, the histopathological findings show cardiac pathology: in case 1 coronary artery disease with cardiac sclerosis and in case 2 septum sclerosis and focal fibro-fatty replacement. No significant findings in other organs, in particular the liver, were observed. For the cases presented, the toxicological analysis was indispensable to confirm the cause of death, because it gave proof of the presence of elevated concentrations of the cardiovascular drugs in

Table 5
DLTZ review of potentially toxic or lethal cases.

Author and references	Cases	Deceased	Toxicological circumstantial data	DLTZ concentration
Kaliciak et al. [23]	1 case	elderly woman	N/Aξ	Blood: 6.7 mg/L Urine: 5.4 mg/L Vitreous fluid 5.5. mg/L Liver: 79 mg/kg
Roper et al. [16]	4 cases	58-year-old; male; ischemic heart disease	N/A	Blood: 8 mg/L
		female; history of depression	N/A	Blood: 4 mg/L
		21-year-old; male; asthmatic, alcohol abuse	25 DLTZ 60 mg tablets and 10 propanolol 80 mg tablets	Blood: 1.5 mg/L
		68-year-old; male	N/A	Blood: 2.5 mg/L Gastric contents:5.4 mg
Engelhart et al. [21]	1 case	78-year-old; female; history of hypertensive heart disease	N/A	Blood: 5.9 mg/L Urine: 11.7 mg/L Gastric contents: 2.8 mg/L Bile: 4.0 mg/L
Kalin et al. [15]	1 case	39-year-old; male	N/A	Blood: 6.9 mg/L Urine: 4.7 mg/L
Della Casa et al. [17]	1 case	16-year-old; female	14 DLTZ 120 mg retard tablets	Blood: 12 mg/L Liver: 2.6 mg/kg Brain: 6.2 mg/kg Bile: 18.3 mg/L Urine: 2.6 mg/L Gastric contents: 8.9 mg/kg
Cantrell et al. [6]	1 case	65-year-old; male; aortic stenosis	6 DLTZ 360 mg slow-release tablets	Central blood: 6 mg/L Peripheral blood: 5 mg/L
Romano et al. [11]	1 case	60-year-old; male	40 DLTZ 200 mg slow-release capsules	Blood: 31 mg/L Brain: 33.1 mg/kg Lung: 179.5 mg/kg Heart: 41.8 mg/kg Liver: 182 mg/kg Kidney: 49.2 mg/kg Bile: 294.9 mg/L
Morini et al. [10]	1 case	57-year-old; female; history of myocardial infarction, arterial hypertension and dyslipidaemia	N/A	Central blood: 23.4 mg/L Peripheral blood: 13.4 mg/L Urine: 4.5 mg/L

N/Aξ: not Available

question and enabled a comparison with published post-mortem blood concentrations for fatal cardiovascular intoxication. Case 1 correlates well with reported post-mortem blood concentrations for AML overdose. Instead, no references were available for DOX and CRVD concentrations in fatal cases, but the toxicological findings were noticeably higher than the therapeutic concentrations. However relative to CRVD, our results in peripheral blood are significantly higher than calculated concentrations reported by Ketola et al. at upper PM percentiles [36]. In our case co-administered drugs, DOX, AML and CRVD were also found at high concentrations, as the interaction between these medications is well known as the mechanism of action. Concentrations in the different organs are also presented in this study, giving information about the distribution of the drugs in the body during acute intoxication. The highest concentration for AML was found in the liver tissue (>20 mg/kg), while the lowest concentration was found in the urine at 0.869 mg/L; the highest concentration for CRVD was found in the liver tissue (1.763 mg/kg), while the lowest concentration was found in the brain at 0.067 mg/kg, which could indicate a low passage through the blood-brain barrier; the highest concentration for DOX was found in the liver tissue (4.936 mg/kg), while the lowest concentration was found in the urine at 0.435 mg/L. Evidence for post-mortem redistribution was unavailable because of the absence of central blood collection. All these concentrations result from a single case, and more data are necessary to draw conclusions related to drug distribution. The case presented is the first reported fatal poisoning attributable to the combination of the three cardiovascular drugs mentioned. We reported for the first time new data related to DOX and CRVD intoxication concentrations in different post-mortem specimens (both fluids and tissues). The medical examiner ruled the cause of death as mixed drug intoxication with atherosclerotic coronary vascular disease as a possible contributing factor. The manner of death was ruled suicide. Case 2 presented in our study correlates well with reported post-mortem blood concentrations for DLTZ overdose. The detected concentrations in peripheral and central blood were tested at 16.6 mg/L and 20.2 mg/L, respectively. The findings in case 2 demonstrate that the cause of death was determined by acute poisoning of DLTZ alone. The overview of previously published cases shows that only four fatal cases are reported involving DLTZ alone, with a blood concentration of 6.7 mg/L, 12 mg/L, 13.4 mg/L, and 31 mg/L. DLTZ was found in high concentration in our case 2. The only reported case with a higher concentration was described by Romano et al. [11], where the DLTZ was a slow-release drug and the patient died after 20 h of hospitalisation. The authors affirm that the high concentration can be ascribed to an inefficacy of gastric lavage and to low blood pressure from the DLTZ, causing lower metabolism and elimination of the drug. The findings in our case 2 demonstrated that the cause of death was determined by acute DLTZ poisoning. DLTZ concentrations in the different organs are presented in this study, giving information about the distribution of the drug in the body during acute intoxication. The highest concentration was found in the liver tissue (26.5 mg/kg), while the lowest concentration was found in the peripheral blood (16.6 mg/L). The medical examiner ruled the cause of death as DLTZ intoxication, with myocardial fibrosis as a possible contributing factor. The manner of death was ruled suicide. All the concentrations result from single cases, and more data are necessary to draw conclusions related to cardiovascular drug distribution.

6. Conclusions

The current study presents post-mortem lethal reference values in two different cases for concentrations of AML, DOX, CRVD and DLTZ in fluids and tissues, which could be useful in forensic toxicology practice. These values contribute knowledge to the scarce data reported in the international literature. The present cases further confirm that, especially when no data is available regarding toxic doses or concentration levels, a careful multidisciplinary evaluation of all data (circumstantial information, autopsy, histological/toxicological investigation) is necessary to assess the contributory role of the substance intake in fatal death. To the best of our knowledge, this is the first description of a fatal intoxication involving also CRVD and DOX with fluid and tissue concentrations. The two different fatalities were associated with a massive suicidal overdose of cardiovascular drugs. These overdoses can be assumed as directly involved in the actual cause of death. Because many suicide attempts are reported in patients treated with cardiovascular drugs, it is important in all cases of sudden death to perform a toxicological analysis, especially in heart disease patients treated with these drugs, even for widely used and prescribed drugs such as those presented herein.

Ethical approval

This study was performed with human cadavers. This article does not contain any studies with (living) human participants or animals performed by any of the authors. The data was acquired as part of a forensic judicial investigation or health authority investigation and in accordance with Italian Police Mortuary Regulation.

Informed consent

n.a.

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CRediT authorship contribution statement

Federica Palazzoli: conceptualization, methodology, validation, writing – original draft, writing – review. **Anna Laura Santunione**: conceptualization, investigation, writing– original draft, writing – review. **Patrizia Verri**: formal analysis and methodology. **Daniele Vandelli**: formal analysis and methodology. **Valentina Castagnetti**: writing, formal analysis and methodology. **Carlotta Profeta**: formal analysis and methodology. **Enrico Silingardi**: supervision.

Declaration of Competing Interest

The authors (A. L. Santunione, F. Palazzoli, P. Verri, D. Vandelli, V. Castagnetti, C. Profeta, E. Silingardi) declare that they do not have any financial or other relationships that might lead to a conflict of interest.

Data availability

n.a.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2023.115831.

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