CASE REPORTS
REPORTES DE CASO

DETERMINATION OF VERAPAMIL THROUGH LC-ESI-MS/MS IN A CASE OF FATAL INTOXICATION

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SUMMARY

Verapamil is a synthetic derivative of papaverine, which is used therapeutically as a hypertensive, antiarrhythmic and antianginal. This study describes an analytical method for the determination of verapamil in biological matrices of blood and urine, which consists of a liquid-liquid extraction of samples for analysis using liquid chromatography-mass spectrometry (LC-ESI-MS/MS), with flurazepam as an internal standard. The method was applied to the acute fatal intoxication of a 17-year-old young woman who consumed 170 tablets of verapamil; the concentration of this medication found in the blood was 18.261mg/L and 0.369mg/L in the urine. This study also puts forth the use of LC-ESI-MS/MS in the analysis of verapamil in biological samples for applications in forensic toxicology.

Keywords: *Verapamil; Liquid chromatography with electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS); Forensic toxicology.*

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INTRODUCTION

A large number of cases of unspecified deaths which arrive at the National Institute of Legal Medicine and Forensic Science (INMLCF for it's acronym in Spanish) in Bogotá lack adequate information to establish an apparent cause of death. Toxicological analysis is used to identify the substances of habitual consumption to assist in determining the cause of death. In the experience of the Toxicology Laboratory at the INMLCF, some suicide deaths are associated with the consumption of pest-control substances and various pharmaceutical drugs.

Verapamil is a drug that acts by inhibiting slow calcium channels, which are dependent on the voltage of cardiac muscle cells, reducing the intracellular ion concentration. This medication is classified as a calcium blocker which acts on the vascular system, heart, and conduction tissue; it has clinically useful effects in relaxing blood vessels, reducing the need for the heart to pump with such force; it also increases blood flow and oxygenation of the heart while diminishing the electrical activity to control heart rate **(1,2,3,4)**.

Verapamil is administered orally and the therapeutic dosage is determined by pharmacokinetics as well as the clinical actions and characteristics of the patient. Overdosing can be fatal, which is why the analysis to determine biological matrices is very important in forensic toxicology to establish the cause of death.

A quick and simple method was developed to determine the quantity of verapamil in blood and urine using LC-ESI-MS/MS. The analytical methodology was obtained by reviewing articles that analyze these substances **(5,6,7,8)**, which were then applied to a case of fatal intoxication presumably caused by this drug.

Presentation of the case

A young woman of 17 years of age with a 53kg weight was brought to the emergency room due to intoxication symptoms after consuming a mixture of drugs including metformin, gemfibrozil and verapamil, which produced vomiting, tonic-clonic seizures, abundant secretions from the airways and finally, a cardio-respiratory arrest.

In the medical-legal examination, the patient presented signs of minor blunt trauma in the inferior extremities with different stages of evolution. There were also nonspecific signs, both internal and external, of marked hypoxia, pulmonary oedema and cerebral and pulmonary lesions suggesting pulmonary hemorrhaging. From the medical forensic opinion, the findings were nonspecific and were not enough to determine the cause of death; therefore the toxicological analysis was necessary, considering that, along with the cadaver, 17 packs of 120mg of verapamil were found, which correspond to 170 tablets (20.4g), 3 packs of 850mg of metformin, which correspond to 30 tablets (25.5g), and 4 packs of 600mg of gemfibrozil, which correspond to 40 tablets (24g). The research was then narrowed down to verapamil since literature reports a lethal dose for this drug, in contrast with the other substances that were ingested (Table 1).

Based on the number of tablets consumed, the volume of distribution of each substance, the concentration in plasma and the weight of the deceased, theoretical values of the concentrations of each substance in the blood were calculated supposing that they were totally absorbed.

It is not known if any other attempts at disintoxication, aside from vomiting, were made in the emergency room to eliminate the absorption of the active principles in the gastric content (Table 2). These calculations allow for the establishment of an approximate value of the concentration in the blood of each drug supposing a total absorption of these substances. As the ingested dose of verapamil was so high and the lethal dose low, it is likely that a total absorption did not occur since the organs failed quickly.

Substance in blood	Therapeutic concentration (µg/mL)	Toxic concentration (µg/mL)	Lethal concentration (µg/mL)
Verapamil	0.08-0.3	0.36	1
Gemfibrozilo	Not reported	Not reported	Not reported
Metformina	1-4	45-70	Not reported

Table 1. Therapeutic, toxic and lethal concentrations of the drugs in this study. Source: (9,10).

Substance in blood	Volume of distribution (L/kg)3,9	Dose taken (g)	Maximum concentration in blood (mg/L)
Verapamil	2-6	20.4	64.15
Gemfibrozil	Not reported	24.0	---
Metformin	1-4	25.5	120.28

Table 2. Volume of distribution, dose taken of each drug and maximum concentration in blood of the deceased. Source: (3).

$$\text{Where Cp (mg/L)} = \frac{A(mg/Kg)}{Vd(L/Kg)}$$

Cp (mg/L): Concentration in plasma.
A (mg/L): Dose taken over weight.
Vd (L/Kg): Volume of distribution for each active component.

METHODOLOGY

Procedure

The levels of the calibration curve were prepared in triplicate by taking 2mL of blank blood in test tubes and adding verapamil to obtain concentrations of 5, 10, 15, 20 and 25µg/mL and 0.1µg/mL of flurazepam (enriched blood). For the urine, the same preparation was done with concentrations of 0.1, 0.3, 0.5, 0.7, 1.0 and 1.5 µg/mL of verapamil and 0.1µg/mL of flurazepam (enriched). Flurazepam was added to the blood and urine samples of the cadaver as internal standard with a concentration of 0.1µg/mL.

Levels of enriched blood and urine, along with the respective samples from the cadaver, were submitted to liquid-liquid extraction to recover the verapamil by adding a pH 6 buffer of 4.0 mL of phosphates and 6.6 mL of extraction solvent (dichloromethane/isopropanol/ammonium hydroxide 80/20/2). They were then sub-

mitted to sonication for 30 minutes and centrifuged at approximately 2000 rpm. The upper organic layer was then transferred to clean and dry test tubes of 6 mL, evaporated at 60°C, and agitated to achieve an approximate volume of 0.5 mL. Maximum vacuum was applied until dry. Finally, the evaporated extracts were reconstituted with 50µL of ACN/H20 solution (50:50 v/v) with 0.1% formic acid.

Each solution of verapamil and flurazepam analytes were prepared at 1mg/mL in methanol, both reactions are standard. The reagents were at analytic grade with the exception of methanol and acetonitrile (ACN), which were at HPLC grade. Blank urine was obtained through volunteers and blank blood was obtained from a 50:50 dilution of concentrated red blood cells with deionized water.

Conditions of the liquid chromatography-mass spectrometry

PA liquid chromatography-mass spectrometry (LC-MS) of the Thermo Electron Corporation brand and Thermo Surveyor-LCQ Advantage Max model was used. The conditions were: Column HPLC Hypersil Gold PFP of (50mm X 2.1mm, 5µm); the temperature of the column was 40°C. An acetonitrile gradient was used with 0.1% formic acid and a 10 mm solution of ammonium formiate with 0.1% of formic acid at a constant flowrate of 200 µL/min. The programming was the following: 0-0.5 min 5% of ACN, 0.5-5.5 increase of 5-95%, 5.5-8.5 minutes remaining at 95% of ACN, 8.6-13 minute decrease of 95-5%. The solvents of the mobile phase had been vacuum filtered earlier using a hydrophilic polyvinylidene fluoride (PVDF) membrane with a pore size of 0.22µm. The injection volume was 10µL.

Mass spectometry was conducted in tandem with the ion trap analyzer using a product ion scan, equipped with an electrospray ionization source (ESI) in positive mode. The conditions were optimized for the verapamil through infusion to the mass spectrometer. The conditions of the main parameters were: capillary voltage 9.00V, source voltage of 5.00kV, capillary temperature of 160°C, lense voltage of 5V, flow of ionization gas of 55 units and flow of auxiliary gas of 15 units. Table 3 shows the values of ions (m/z), retention time (RT) and collision energy (CE) and the isolation width optimized for the identification of each composite.

RESULTS

The correlation coefficients for the calibration curve were 0.0047 and 0.9960 in blood and urine respectively, with a variation coefficient lower than 5% in each level. The concentration of verapamil found in the blood of the deceased was 18,26mg/L and 0.37mg/L in the urine. The

Composite	Transition (m/z)	CE (%)	isolation width	Polarity	RT (min)
Verapamil	455.1→303.2 455.1→165.1	35	1	positive	6.23
Fluazepam (SI)	388.1→315.1 388.1→317.1	35	1	positive	5.52

Table 3. Data of the LC-ESI-MS/MS

Source: Author.

chromatogram and calibration curve of the verapamil in blood are presented in Figure 1.

DISCUSSION AND CONCLUSIONS

A new method was established to determine verapamil in blood and urine through LC-ESI-MS/MS. The method presents linear results that comply with the acceptance criteria of bioanalytical guidelines **(11)**.

LC-ESI-MS/MS is a sensitive technique to detect verapamil in biological samples; the method is highly selective and allows for the unequivocal determination of the drug in question, which is a fundamental requirement of forensic toxicology.

According to information found in bibliographical references, the lethal concentra-

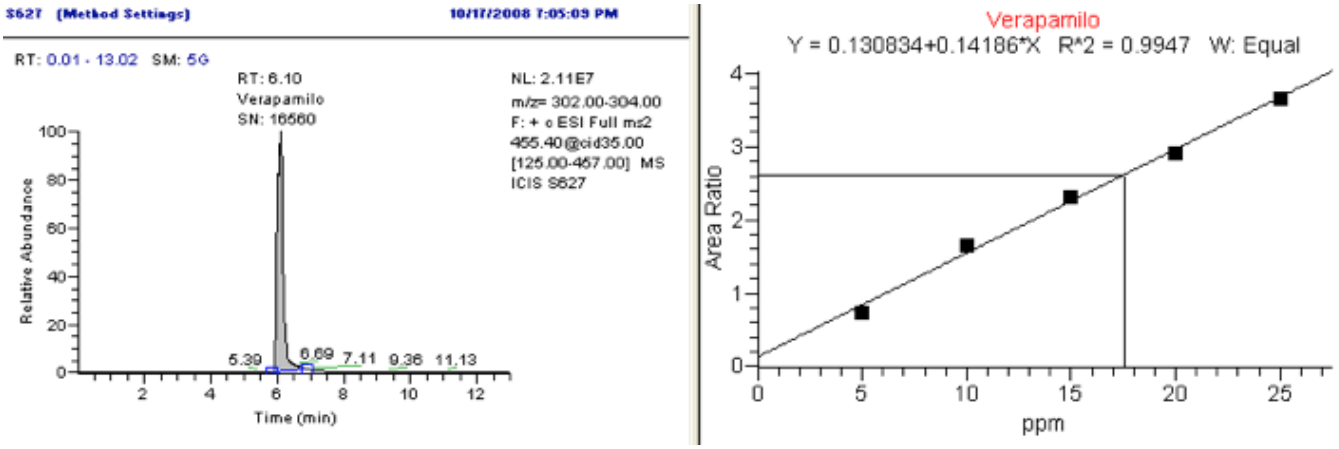

Fig 1. Chromatogram and calibration curve of the verapamil in blood (LC-MS) Source: Author.

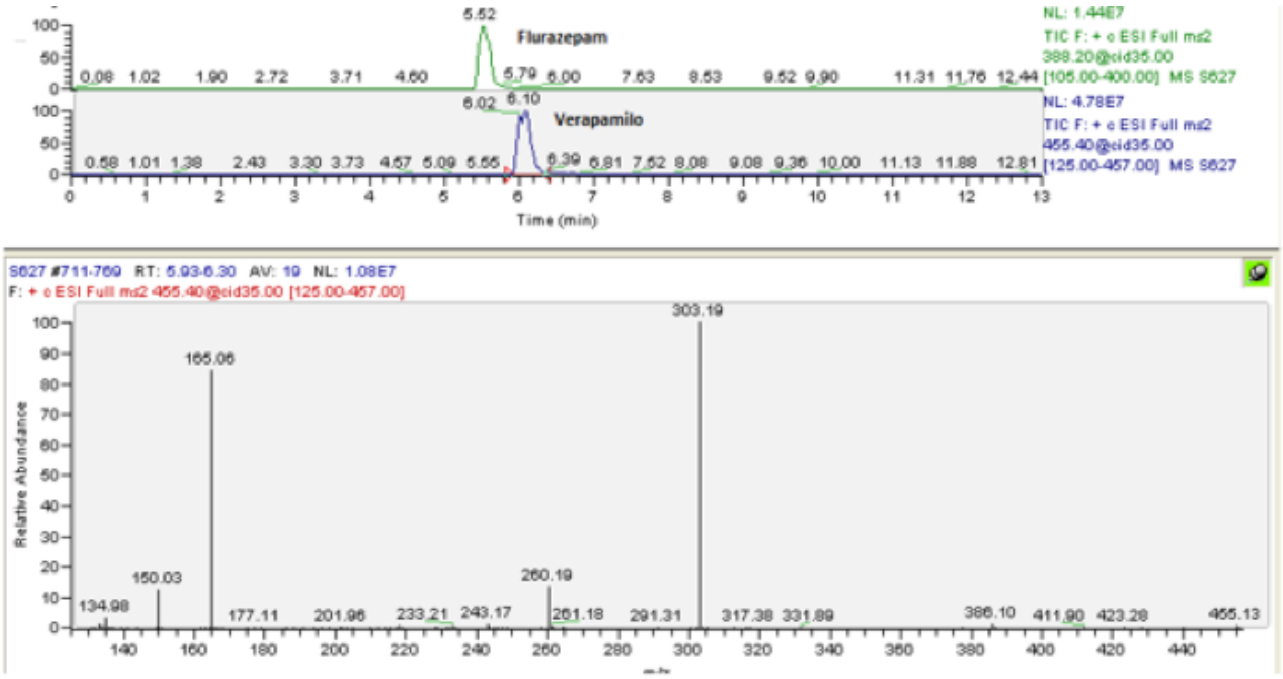

Fig 2. Chromatogram of the verapamil and flurazepam, and mass spectrum of the verapamil Source: Author.

tion of verapamil is 1μg/mL and in the results obtained, the concentration of verapamil was 18.26mg/L (18.26μg/mL) after the deceased had consumed 170 tablets of 120 mg of verapamil for a total dosage of 20.4g, which was potentially modified by vomiting. Plasma concentration levels could have theoretically reached 64.15mg/L if the drug had been completely absorbed.

The lethal dose of verapamil can be considered the cause of rapid organ failure, indicating that she suffered from an acute intoxication that led to her death.

Finally, in the forensic context, it is good practice to determine the presence of a substance with different matrices or, if there is only one sample, to undertake analyses with different techniques or assays repeated on different days **(12)**. While components are not necessarily present in all matrices (according to the variables involved in toxicokinetics), in the current case, the concentration of verapamil in blood and urine definitely proved the cause of death by intoxication with this drug.

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