



HAL
open science

Intentional overdose of glargine insulin: Determination of the parent compound in postmortem blood by LC-HRMS

Nadia Arbouche, Alexis Walch, Jean-Sébastien Raul, Pascal Kintz

► **To cite this version:**

Nadia Arbouche, Alexis Walch, Jean-Sébastien Raul, Pascal Kintz. Intentional overdose of glargine insulin: Determination of the parent compound in postmortem blood by LC-HRMS. *Journal of Forensic Sciences*, 2023, 68 (3), pp.1077-1083. 10.1111/1556-4029.15247 . hal-04100625

HAL Id: hal-04100625

<https://hal.science/hal-04100625>

Submitted on 17 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



More Cases Solved

More meaningful results from your challenging samples with 8-color PowerPlex STR kits, such as PowerPlex® 35GY, and Spectrum CE. More capacity with convenient 4-plate drawer. More scheduling flexibility with continuous access to the plate drawer. The new Spectrum CE System gives you more of everything you need to power through your casework analyses.

Learn More »

www.promega.com/SpectrumCE

CASE REPORT

Toxicology

Intentional overdose of glargine insulin: Determination of the parent compound in postmortem blood by LC-HRMS

Nadia Arbouche PhD¹ | Alexis Walch MD¹ | Jean-Sébastien Raul PhD¹ |
Pascal Kintz PhD^{1,2}

¹Institut de Médecine Légale, 11 Rue Humann, Strasbourg, 67000, France

²X-Pertise Consulting, 42 Rue Principale, Mittelhausbergen, 67206, France

Correspondence

Nadia Arbouche, Institut de Médecine Légale, 11 Rue Humann, Strasbourg 67000, France.

Email: nadia.arbouche@hotmail.it

Abstract

Insulin glargine is a long-acting insulin analog that is converted after enzymatic cleavage of the arginine pair of the β -chain into its main metabolite M1 (21^A-Gly-insulin), which is responsible for the hypoglycemic activity. In all the overdose cases described in the literature, only M1 concentrations have been reported, whereas insulin glargine was always absent or below the limit of quantitation. In this study, we present a case of suicide of a young nurse by injection of insulin glargine in which the parent molecule was found at a toxic concentration in blood. The determination and the discrimination of insulin glargine from human insulin and other synthetic analogs in the blood specimen were performed by liquid chromatography coupled to high-resolution mass spectrometry (Waters XEVO G2-XS QToF) and extraction after precipitation in the presence of bovine insulin (internal standard), with a mixture of acetonitrile/methanol +1% formic acid followed by purification on solid phase extraction cartridges C18. Glargine insulin tested highly positive in the blood with a concentration of 1.06 mg/L. Due to the difficulty in obtaining a M1 pure standard, the metabolite could not be dosed. This unique presence of the parent molecule, reported for the first time, can be explained by inter-individual variability in the rate of conversion to metabolite. Intravenous injection versus subcutaneous injection can also explain the presence of insulin glargine. Finally, the dose injected may have been so high that saturation of the proteolytic enzymes responsible for conversion to M1 should have occurred.

KEYWORDS

diabetes, forensic toxicology, insulin glargine, LC-HRMS, postmortem blood, suicide

Highlights

- The responsible of insulin glargine hypoglycemic activity is its main metabolite M1.
- In all overdose cases reported in the literature, insulin glargine values are always below the LOQ.
- In this case, insulin glargine was found in the decedent at a toxic concentration.
- Route of administration and saturated metabolism may explain glargine in blood in an overdose case.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Journal of Forensic Sciences* published by Wiley Periodicals LLC on behalf of American Academy of Forensic Sciences.

1 | INTRODUCTION

Until the beginning of the 20th century, the only possible treatment to fight diabetes consisted of drastic food control while type I diabetic subjects died from this disease. It was not until 1921 that a group of researchers at the University of Toronto succeeded in extracting insulin from the animal pancreas for the first time and studied its action in humans. Since that time, initially insulins of animal origin and later synthetic insulins obtained from genetically modified strains of *Escherichia coli* have been the most widely used treatment for type I and type II diabetes.

Several formulations of synthetic analogs of human insulin have been proposed according to the expected pharmacokinetic profile. They can be divided into two categories: fast-acting insulins (lispro, aspart, and glulisine), developed to mimic the rapid and intense pancreatic secretion of insulin after a meal, and long-acting insulins (glargine, detemir, and degludec), characterized by a constant active concentration in the blood over long term with no concentration peak [1].

Insulin glargine (Lantus®) is a long-acting synthetic human insulin used for basal insulin therapy and available for subcutaneous injection as a pre-filled pen in dosages of 100 and 300 units/mL, once a day. It is only intended to be administered subcutaneously, as this is precisely the route of injection that allows its prolonged effect.

The addition of two arginines at the C-terminal position of the β -chain and the replacement of asparagine by glycine at position 21 of the α -chain shifts the isoelectric point of insulin glargine, making it poorly soluble at neutral pH but fully soluble at the acidic pH of the injection solution (pH4). After injection into the subcutaneous tissue (pH7.4), the acid solution is neutralized, resulting in the formation of micro-precipitates that represent a “depot” from which small quantities of insulin glargine are continuously released. The addition of zinc to the preparation further stabilizes the product by delaying the release of insulin glargine monomers and dimers, extending its duration of action. As a result, the concentration/time curve is regular, with no peak, and the duration of its action is prolonged [2].

After subcutaneous injection of Lantus®, insulin glargine is rapidly degraded at the carboxyl end of the β -chain into two active metabolites, M1 (21^A-Gly-insulin) and M2 (21^A-Gly-des-30^B-Thr-insulin). In plasma, the major circulating metabolite is the M1 metabolite and its concentration increases with the administered dose of Lantus®. The effects of subcutaneous injection of Lantus® are mainly due to exposure to the M1 metabolite, as insulin glargine and the M2 metabolite are almost never detectable after subcutaneous injection of insulin glargine [3].

Insulin overdoses are under-reported in the literature despite the large number of diabetics using this therapy. Indeed, diabetes is one of the most common chronic diseases in the world and in 2020, about 3.5 million people in France were treated with antidiabetic drugs, which represents 5.3% of the population [4].

As the prevalence of diabetes increases, cases of insulin misuse and overdose are also increasing. Compared with the cases described in the 20th century where less than 5% of cases were suicide

cases [5], in the last 20 years, 90% of insulin glargine overdoses were suicide attempts (Table 1).

It is not surprising that insulin is used for suicidal purposes since diabetic patients have a much higher risk of developing depression than the rest of the population [6].

Although at therapeutic doses insulin glargine pharmacokinetics is fairly well described, there is a lack of information in overdose situations due to the limited data on the literature.

It is because of its long-acting properties that hypoglycemia caused by insulin glargine overdose is more problematic than with other synthetic analogs of human insulin, as even if treated early, it may require a long period of follow-up in which moments of normoglycemia and moments of severe hypoglycemia will alternate [7, 8].

The causes of prolonged duration of hypoglycemia following glargine overdose could be due to delayed dose-dependent absorption due to the “depot” effect at the injection site and down-regulation of insulin receptors [9]. Studies have shown that after a therapeutic injection of 0.3 units/kg of insulin glargine, hypoglycemia persists for 24 h, whereas following an injection of 0.4 units/kg, hypoglycemia persists for approximately 30 h [9].

These results therefore suggest that the duration of hypoglycemia is probably dependent on the dose of injected insulin glargine. It can be anticipated that at higher doses, hypoglycemia can last for a longer period.

In the cases described in the literature over the last 20 years, insulin glargine was seldom identified and quantified (Table 1). When identification and dosage were reported, immunoassay methods were generally used. The identification of the human insulin analog involved in the overdose is of critical importance. First, from a clinical point of view, as previously mentioned, insulin glargine overdose can induce recurrent hypoglycemic episodes over a long period of time, and knowing the type of insulin is of great benefit to the clinician in predicting the type and duration of treatment. Second, from a medico-legal point of view in the absence of anamnestic-circumstantial data (medical prescriptions, diabetes, or diabetic family members), the accurate identification can be the only evidence of exogenous insulin administration at the scene. In a 2016 study performed by Kim et al. [10] involving insulin glargine overdose, the metabolite M1 was identified and quantified throughout the hypoglycemic state using a LC-MS/MS technology. However, the authors did not detect the parent molecule. In order to increase the specificity and sensitivity of the method and to avoid any interference with other analogs, it is suitable to use separative methods coupled to mass spectrometry or high-resolution mass spectrometry (HRMS) and to avoid immunoassay tests. To date, HRMS has only been used in a study performed by Yong-Xi Li et al. [11] on the toxicokinetic of insulin glargine in dogs and was never applied to human biological specimens.

In this report, the authors present a case of suicide by insulin glargine overdose with the first identification of the parent molecule using a high-resolution mass spectrometry system (UHPLC/Q-TOF-MS).

TABLE 1 Case reports of insulin glargine overdose.

Author	Patient	Dose (units)	Blood concentration	Injection sites	Diabetes type	Notes
Kim et al. [10]	43 male	1500+600 aspart	18.07 ng/mL 0 (7h after injection) 19.54 ng/mL (30h) 15.35 ng/mL (54h) 9.6 ng/mL (80h) LC-MS/MS	7	1	Attempted suicide
Warriner et al. [26]	26 male	2700+2100 3h after	/	1	1	Attempted suicide
Fromont et al. [15]	22 female	300+300 aspart	/	2	1	Attempted suicide
Shibutani et al. [27]	31 female	2400	7390 μ U/mL (311 ng/mL)—RIA	Multiple	1	Attempted suicide
Brvar et al. [28]	21 female	26	/	/	1	Attempted suicide
Groth et al. [9]	56 male	3300	/	Multiple	2	Attempted suicide
Fuller et al. [19]	37 male	150	/	Multiple	1	Attempted suicide
Eldred et al. [29]	40 male	600	/	2	1	/
Mork et al. [6]	39 male	388+800 lispro	3712.6 μ U/mL (156 ng/mL) (immunoassay)	>10	2	Attempted suicide
Tofade et al. [30]	33 female	300+200 aspart	/	/	Not diabetic	Attempted suicide
Barmanray et al. [31]	49 male	Unknown	356.8 μ U/mL (14.9 ng/mL) (immunoassay)	/	Not diabetic	/
Lu et al. [8]	51 female	2700	/	Multiple	1	Attempted suicide
Kumar et al. [18]	12 female	>2000	/	/	Not diabetic	Attempted suicide
Kuhn et al. [32]	55 female	100	/	1	1	Accident
Ashawesh et al. [7]	31 female	1000	/	Several	Not diabetic	Attempted suicide
Sandooja et al. [20]	27 female	900	/	Several	2	Attempted suicide
Karatas et al. [17]	45 male	3600	216 mU/L (9 ng/mL)	/	2	Attempted suicide
Thornton et al. [24]	46 female	100	/	Intravenous	2	Accident
Dogan et al. [33]	76 male	500	/	/	1	Attempted suicide

2 | CASE REPORT

A 26-year-old woman (nurse by profession) was found dead at home lying on a sofa. The woman was found in her home by a friend after he received a letter mentioning her suicidal intention.

Several syringes corresponding to insulin injection devices were found next to the body, as well as loxapine capsules. According to what has been reported to the investigators, it seems that the young woman had attempted suicide twice before this fatal outcome.

The body examination and the postmortem body scanner revealed a marked asphyxiation syndrome and nail cyanosis. A tablet corresponding to a blood glucose sensor located on the right arm suggested that the young woman was diabetic. A venous catheter fixed on the left wrist of the corpse was also found. As this was an external examination of the body and no autopsy was performed, no histological data are available. In addition, no biochemical tests were performed, in particular blood glucose, lactates, and ketone bodies, as this type of analysis was not available given the long delay between autopsy and the request of the Prosecutor. However, by

directly investigating the toxic molecule involved, indirect measurements of antemortem blood glucose are not necessary.

As the forensic pathologist was focused on a toxic death, samples of femoral blood, urine, and hair were collected for toxicological tests.

Blood and urine were stored at +4°C until analysis, while head hair was stored in an envelope at room temperature.

3 | METHODS

For the identification and quantification of insulins in blood, a previously validated and published method was used [12]. All the chemical reagents, the extraction procedure and LC-HRMS parameters are reported in the previous published paper.

Since the focus, in this case, was on insulin glargine, we point out that for glargine insulin, the sevenfold charged ion was observed at m/z 867.46288 (which deconvolutes to 6072.24016) and that glargine insulin was eluted at 2.69 min.

The methods used to perform the toxicological screening have also been described in the same article [12].

4 | RESULTS AND DISCUSSION

The method used for the analysis of the blood sample has been previously validated and published [12].

Briefly, the calibration curves showed a good linearity in the range 0–100 ng/mL with a correlation coefficient ranging from 0.9994 to 0.9999. The LOD and the LOQ are 0.5 and 1 ng/mL, respectively.

Insulin glargine was found in the femoral blood at the concentration of 1.06 mg/L. Figure 1 shows the chromatogram after the extraction of the blood specimen.

Further analyses, achieved during a complete toxicological screening, revealed the presence of tiapride (24.95 mg/L), loxapine (0.18 mg/L), and nordiazepam (0.16 mg/L). While tiapride was at a toxic level, loxapine, and diazepam were considered at therapeutic levels.

Until 20 years ago, the most widely identified antidiabetics after poisoning were biguanides and sulfonylureas. However, in the 2019 report of the American Association of Poison Control Centers, insulin cases have been multiplied by 3, surpassing now sulfonylureas intoxications [13, 14].

Insulin overdoses, whether accidental or intentional, are potentially fatal due to the alteration of central nervous system (CNS) metabolism induced by hypoglycemia, which can result in cerebral dysfunction, pulmonary edema and if not treated in time and prolonged can lead to coma and neurological death [15]. Our results indicate that the hypoglycemic agent most present in the circulation was insulin glargine.

The presence of other drugs (tiapride, loxapine, and nordiazepam) may have contributed or aggravated the hypoglycemia caused by insulin glargine. In fact, the use of antipsychotics in diabetic

patients has been shown to be associated with a higher risk of hypoglycemia [16].

Insulin overdoses remain a rare occurrence compared with the large number of diabetics under insulin therapy. In fact, the problem may be underestimated and cases under-reported, with hypoglycemia often being mistaken for an accident. This is due both to the inability/difficulty of laboratories to perform the analysis of such derivatives and to the fact that in the absence of anamnestic-circumstantial evidence (being diabetic, having a diabetic relative) the analysis is not even requested by police, judges, prosecutors, and forensic pathologists (Figure 2).

In the literature, the cases of insulin glargine overdose are limited to case reports in which the drug is often not quantified (Table 1). However, most of the cases described show that the final outcome of an insulin glargine overdose does not depend primarily on the dose injected. According to a literature review by Arem et al. [5], death can occur even at doses as low as 400 units, while patients at doses up to seven times higher can fully recover [17, 18]. Karatas et al. [17] described a case of a diabetic patient who, after attempting suicide by injecting himself 3600 units of insulin glargine, required a glucose infusion for about 120 h due to frequent hypoglycemic episodes but recovered without permanent complications. There are several factors that appear to influence the reaction and final outcome of an insulin glargine overdose; the number of the injection sites, diabetes, kidney, and liver dysfunction could reduce gluconeogenesis and the ability to convert insulin and the delay between administration and treatment [19, 20].

In the case described, neither the amount of insulin injected, nor the number of injection sites were known. After acute poisoning, there is a change in the pharmacokinetics of insulin glargine due to the slowed absorption from the injection site and the possibility of prolonged clearance.

From a clinical point of view, identification of the insulin analog responsible for the overdose is essential. In most of the cases

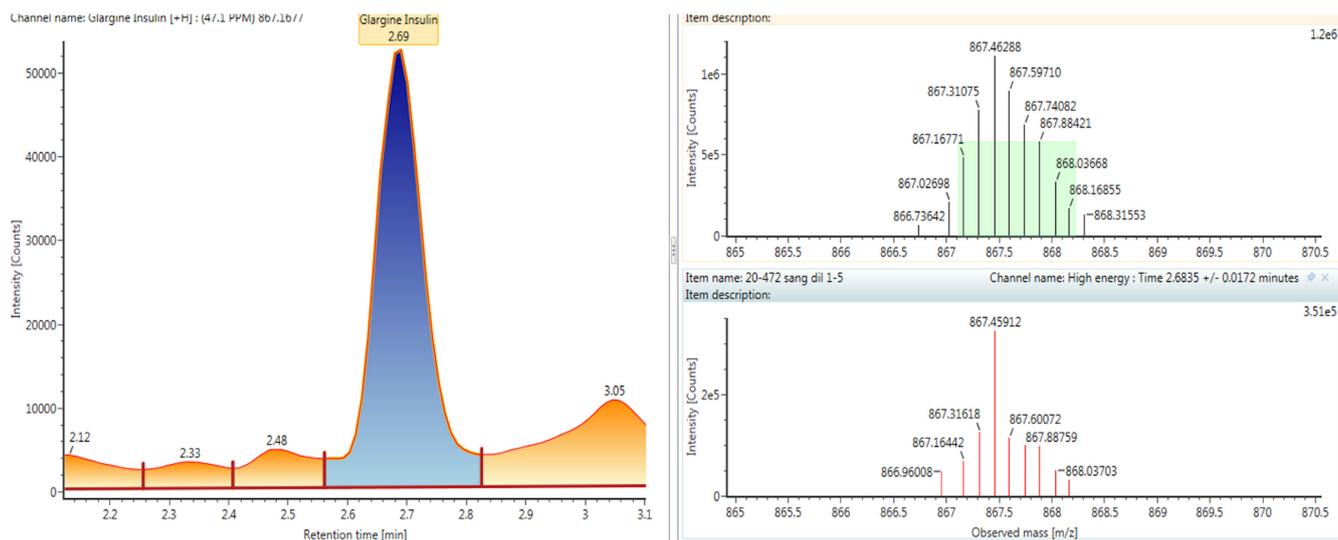


FIGURE 1 Extracted chromatogram and spectra for insulin glargine from blood specimen by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Insulin glargine: $m/z [M + 7H]^{7+}$ 867.46288. Concentration was 1.06 mg/L.

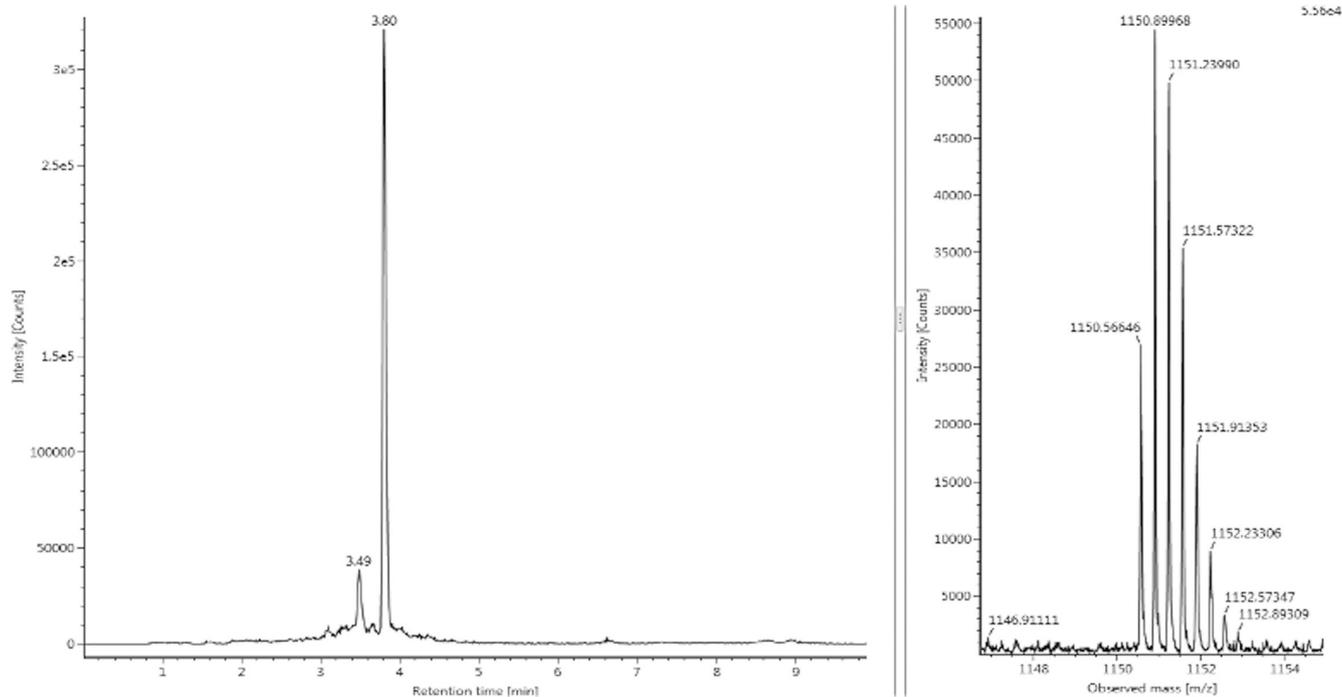


FIGURE 2 Extracted chromatogram and spectra information for insulin glargine metabolite M1 (21A-Gly-insulin). M1: $m/z [M+5H]^+$ 1150.89969.

described in the literature, insulin intake was self-reported and was not documented by subsequent analyses.

Knowledge of the type of insulin used is the key to determining the duration of treatment and the observation period required for the patient's follow-up. In most of the reported cases, insulin characterization was not performed. The latter would be essential to show the rate of decline in insulinemia and to predict when the effects of the overdose will end [6].

A patient may present to the emergency room normoglycemic and develop a hypoglycemic syndrome a few hours later due to a release of insulin from a depot. Identifying the insulin involved at an early stage and monitoring insulin levels is therefore very useful for the treatment but generally, patient management in a clinical context is mainly based on the clinical assessment of signs and symptoms.

While in clinical practice the problem is to identify the insulin analog to start the most suitable treatment, in forensic medicine, it is essential to identify the agent that caused the poisoning without any doubt, given all the legal consequences.

In forensic medicine, the cause of death can be based on circumstantial evidence such as the presence of empty syringes, testimonies, suicide letters, or even insulin/peptide C ratio by means of immunoassays (when requested).

However, immunoassays are not appropriate for *postmortem* specimens due to the lack of specificity of the antibodies which often fail to distinguish human insulin from its analogs, metabolites, and insulins of animal origin. As a consequence, in the legal context, immunoassays for insulin do not meet the criteria of quality, especially in the absence of complementary anamnestic information [12].

In our case, insulin glargine was identified and quantified using a high-resolution mass spectrometry method. This type of technology, unlike immunological methods, allows human insulin to be differentiated with certainty from all other analogs and to provide precise quantification on a wide range of biological matrices. Mass spectrometry is not affected by the problem of cross-reactivity and offers better specificity and sensitivity allowing the identification of multiple targets in a single test.

As the synthetic insulin analogs were developed with slight structural differences, their molecular characteristics remain very similar, especially in terms of molecular weight and structure. Consequently, they also have similar analytical characteristics which make their differentiation difficult even with the most modern analytical approaches. High-resolution mass spectrometry offers the possibility of separating interferences from the analyte and overcoming these limitations.

In the scientific literature, only Kim et al. [10] investigated insulin glargine in an overdose case by mass spectrometry. The authors conducted an analysis of the pharmacokinetics of this type of insulin by establishing insulin levels several times. Only the M1 metabolite of insulin glargine was identified and quantified 7 h (18 ng/mL), 30 h (19.5 ng/mL), 54 h (15.3 ng/mL), and 80 h (9.6 ng/mL) after injection. The authors observed a multiphasic peak, probably due to the delayed release from the depot or the presence of several injection sites where the absorption rate was different.

Our results for insulin glargine cannot be compared with previous data as the parent compound was never identified after overdose. The therapeutic range for this type of insulin has not yet been defined, but according to a study by Bolli et al. [3] when insulin

glargine was subcutaneously administered at a therapeutic dose of 0.3 units/kg, the M1 metabolite, mostly responsible for the glucodynamic effects, was found at a maximum concentration of 470 pg/mL. In this study, insulin glargine was never present in the blood circulation, irrespective of the injected dose. Kim et al. [10] also reported that insulin glargine is never found in circulation above the LOQ, unlike its major metabolite M1, suggesting that the parent molecule is not the main responsible for the hypoglycemic activity.

Danne et al. [21] again performed a study in 2012 involving a group of children under 6 years of age treated with insulin glargine. They analyzed blood samples taken 24 h after subcutaneous injection of insulin glargine and only identified the M1 metabolite, which was listed as the main component mediating the hypoglycemic effect. The limitation of this study was that analysis was performed on specimens collected at +24 h, and therefore, the results do not necessarily reflect the values of the parent molecule at other times.

Since buying the reference standard of the M1 metabolite is very difficult (not proposed by any vendor), it was not possible to quantify it in our case. However, we investigated the M1 metabolite in our sample and the observed mass spectrum was compared with data in the literature. We have identified a peak whose spectrum corresponds to the fivefold ion charged m/z 1150.3317 described in the paper of Yong-Xi et al. [10]. In our case, the fivefold charged ion was observed at m/z 1150.89968. The M2 metabolite was also investigated by comparing the mass spectrum with the literature data, but it was not found. This is in agreement with the study published by Bolli et al. [3].

In order to be able to explain our findings, various hypotheses can be elaborated. First of all, according to the study by Bolli et al. [3] insulin glargine, when detectable, appears very early in blood. Therefore, one possibility could be that the time between injection and death was very short. As a hypoglycemic syndrome can last for many hours before causing death, this hypothesis is not plausible. In another study conducted by Agin et al. in 2007 [22], it was shown that insulin kinetic shows some inter-individual variability which affects the rate of transformation of insulin glargine into M1 metabolite. This variability could be explained by genetic differences in the activity of the carboxypeptidases responsible for its metabolism. Indeed, in a study carried out on 69 individuals, the rate of conversion of glargine to M1 in 30 min varied between 46% and 98%.

Another hypothesis is that the injection route in our case was not the standard subcutaneous administration route, as this is the unique fatal case of intravenous insulin glargine overdose described in the literature. It is possible that the metabolism of insulin glargine is different when injected intravenously. In a study by Werner et al. [23], the kinetics of insulin glargine was compared after intravenous and subcutaneous injection. When injected intravenously, insulin glargine accounts for 15%–37% of total circulating insulin while when injected subcutaneously, the parent molecule was almost never found. Thornton et al. in 2012 [24] presented a case of a patient accidentally receiving 100 units of insulin glargine intravenously instead of subcutaneously. As the long-lasting properties of insulin glargine are due to its absorption kinetic, intravenously it acts like regular human insulin. In fact, the patient had an unremarkable course and

recovered without any parenteral glucose. Insulin glargine dosage was not performed in this case.

Finally, as proposed by Werner et al. [23], since insulin glargine was injected at very high dosage, its presence can be explained by a possible saturation of the proteolytic enzymes responsible for the conversion of insulin glargine into its metabolite. This phenomenon was also observed in a study published by Lucidi et al. [25]. The authors evaluated the metabolism of insulin glargine in diabetic patients, observing that while the parent molecule was not detectable after a therapeutic dose injection, insulin glargine could be found after a slight overdose. These results suggest that at high dosages there is a greater opportunity to find the parent molecule.

As no injection site was found in our case but only a venous catheter at the left wrist (not submitted to analysis), the possibility of an intravenous administration cannot be eliminated.

5 | CONCLUSION

The use of insulin in a forensic context is not new. Investigation of an insulin intoxication should include the assessment of various factors such as comprehensive site inspections and a full review of the medical history and subsequent biological analyses. However, toxicological tests are rarely performed. Insulin glargine is a long-lasting insulin and overdose tends to cause delayed hypoglycemic episodes. Therefore, it seems essential to be able to identify and monitor concentrations in the clinical context in order to anticipate the type and duration of treatment. In a medico-legal situation, it is instead fundamental to identify the type of insulin used, which represents evidence of the administration of exogenous insulin. In this case, it has been demonstrated how modern analytical approaches such as high-resolution mass spectrometry allows to overcome the analytical issues related to this substance and to provide adequate evidence.

In this article, the authors have presented a case of insulin glargine overdose. Insulin glargine has never been found in *postmortem* blood samples due to its rapid transformation into its major metabolite. Its presence at a toxic concentration could probably be due to the use of a route of administration different from the classic subcutaneous way, such as the intravenous route. The metabolism of this substance could indeed be different when injected intravenously. As this represents the first case of a death reported in the literature with such high insulin glargine concentration in blood, it will be necessary to document further cases to confirm the hypotheses.

REFERENCES

1. Fédération Française des Diabétiques [French Federation of Diabetics]. La découverte de l'insuline à 100 ans! [The discovery of insulin at 100 years old!]. 2021 <https://www.federationdesdiabetiques.org/federation/actualites/100-ans-de-la-decouverte-de-linsuline-decouvrez-lhistoire-et-les-combats-de-la-federation>. Accessed 27 Dec 2021

2. European Medicine Agency. Lantus, INN-insulin glargine. Annex I 2016. https://www.ema.europa.eu/en/documents/product-information/lantus-epar-product-information_en.pdf. Accessed 20 Dec 2021
3. Bolli GB, Hahn AD, Schmidt R, Eisenblatter T, Dahmen R, Heise T, et al. Plasma exposure to insulin glargine and its metabolites M1 and M2 after subcutaneous injection of therapeutic and supra-therapeutic doses of glargine in subjects with type 1 diabetes. *Diabetes Care*. 2012;35(12):2626–30. <https://doi.org/10.2337/dc12-0270>
4. Santé Publique France [Public Health France]. Le diabète en France: les chiffres 2020 [Diabetes in France: 2020 figures]. 2021 <https://www.santepubliquefrance.fr/les-actualites/2021/le-diabete-en-france-les-chiffres-2020>. Accessed 20 Dec 2021
5. Arem R, Zoghbi W. Insulin overdose in eight patients: insulin pharmacokinetics and review of the literature. *Medicine*. 1985;64(5):323–32.
6. Mork TA, Killeen CT, Patel NK, Dohnal JM, Karydes HC, Leikin JB. Massive insulin overdose managed by monitoring daily insulin levels. *Am J Ther*. 2011;18(5):e162–6. <https://doi.org/10.1097/MJT.0b013e3181f4eadb>
7. Ashawesh K, Padijakara RNK, Murthy NP, Nizar H, Anwar A. Intentional overdose with insulin glargine. *Am J Health Syst Pharm*. 2009;66(6):534. <https://doi.org/10.2146/ajhp080532>
8. Lu M, Inoriboon PC. Lantus insulin overdose: a case report. *J Emerg Med*. 2011;41(4):374–7. <https://doi.org/10.1016/j.jemermed.2010.04.007>
9. Groth CM, Banzon ER. Octreotide for the treatment of hypoglycemia after insulin glargine overdose. *J Emerg Med*. 2013;45(2):194–8. <https://doi.org/10.1016/j.jemermed.2012.11.099>
10. Kim CCL, Rosano TG, Chambers EE, Pai MP, Desemone J. Insulin glargine and insulin aspart overdose with pharmacokinetic analysis. *AACE Clin Case Rep*. 2016;2:E122–8. <https://doi.org/10.4158/EP15689.CR>
11. Li YX, Ke Y, Li R, Chen X, Mollah S, Wang X. Quantitation of insulin analogue glargine and its two metabolites M1 and M2 on triple quad 6500 and triple TOF 5600 LC-MS/MS systems in a dog toxicokinetics study. *J Anal Bioanal Tech*. 2013;5:004. <https://doi.org/10.4172/2155-9872.55.004>
12. Arbouche N, Godard E, Gheddar L, Raul JS, Kintz P. Attempted murder of a young child followed by an attempted suicide of the mother by injection of insulin aspart: identification of quantification of insulin by LC-HRMS and UPLC-MS/MS in blood of the two cases. *J Anal Toxicol*. 2021;bkab097 <https://doi.org/10.1093/jat/bkab097> Epub 2021 Sep 16.
13. Watson WA, Litovitz TL, Rodgers GC Jr, Klein-Schwartz W, Reid N, Youniss J, et al. 2004 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am J Emerg Med*. 2005;23(5):589–666.
14. Gummin DD, Mowry JB, Beuhler MC, Spyker DA, Brooks DE, Dibert KW, et al. 2019 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 37th annual report. *Clin Toxicol*. 2020;58(12):1360–541.
15. Fromont I, Benhaim D, Ottomani A, Valéro R, Molines L, Vialettes B. Prolonged glucose requirements after intentional glargine and aspart overdose. *Diabetes Metab*. 2007;33(5):390–2. <https://doi.org/10.1016/j.diabet.2007.05.002>
16. Takahiko N. Severe hypoglycemia associated with tiapride in an elderly patient with diabetes and psychosis. *Innov Clin Neurosci*. 2017;14(1–2):12–3.
17. Karatas F, Sahin S, Karatas HG, Karsli PB, Emre C, Kivrakoglu F. The highest (3600 IU) reported overdose of insulin glargine ever and management. *Indian J Crit Care Med*. 2015;19(12):750–1. <https://doi.org/10.4103/0972-5229.171418>
18. Kumar AR. Management of intentional overdose of insulin glargine. *Endocrinol Nutr*. 2012;59(9):570–2. <https://doi.org/10.1016/j.endonu.2011.12.007>
19. Fuller ET, Miller MA, Kaylor DW, Janke C. Lantus overdose: case presentation and management options. *J Emerg Med*. 2009;36(1):26–9. <https://doi.org/10.1016/j.jemermed.2007.02.038>
20. Sandooja R, Moorman JM, Kumar MP, Detoya K. Critical low ca-tastrophe: a case report of treatment-refractory hypoglycemia following overdose of long-acting insulin. *Case Rep Endocrinol*. 2020. <https://doi.org/10.1155/2020/8856022>
21. Danne T, Becker RHA, Ping L, Philotheou A. Insulin glargine metabolite 21(a) -Gly-human insulin (M1) is the principal component circulating in the plasma of young children with type 1 diabetes: results from the PRESCHOOL study. *Pediatr Diabetes*. 2015;16(4):299–304.
22. Agin A, Jeandidier N, Gasser F, Grucker D, Sapin R. Glargine blood biotransformation: in vitro appraisal with human insulin immunoassay. *Diabetes Metab*. 2007;33(3):205–12. <https://doi.org/10.1016/j.diabet.2006.12.002>
23. Werner U, Tennagels N, Fanelli CG, Bolli GB. Equipotency of insulin glargine 300 and 100 U/mL with intravenous dosing but differential bioavailability with subcutaneous dosing in dogs. *Diabetes Obes Metab*. 2021;23(1):166–74. <https://doi.org/10.1111/dom.14212>
24. Thornton S, Gutovitz S. Intravenous overdose of insulin glargine without prolonged hypoglycemic effects. *J Emerg Med*. 2012;43(3):435–7. <https://doi.org/10.1016/j.jemermed.2011.06.038>
25. Lucidi P, Porcellati F, Candeloro P, Cioli P, Marinelli Andreoli A, Marzotti S, et al. Glargine metabolism over 24 h following its subcutaneous injection in patients with type 2 diabetes mellitus: a dose-response study. *Nutr Metab Cardiovasc Dis*. 2014;24(7):709–16. <https://doi.org/10.1016/j.numecd.2014.02.008>
26. Warriner D, Debono M, Gandhi RA, Chong E, Creagh F. Acute hepatic injury following treatment of a long-acting insulin analogue overdose necessitating urgent insulin depot excision. *Diabet Med*. 2012;29(2):232–5. <https://doi.org/10.1111/j.1464-5491.2011.03385.x>
27. Shibutani Y, Ogawa C. Suicidal insulin overdose in type I diabetic patient: relation of serum insulin concentrations to the duration of hypoglycemia. *J Diabetes Complications*. 2000;14(1):60–2. [https://doi.org/10.1016/s1056-8727\(00\)00057-x](https://doi.org/10.1016/s1056-8727(00)00057-x)
28. Brvar M, Mozina M, Bunc M. Poisoning with insulin glargine. *Clin Toxicol*. 2005;43(3):219–20. <https://doi.org/10.1081/CLT-56408>
29. Eldred AE, Mustafa OG, Hunt KF, Whitelaw BC. Problem based review: the patient who has taken an overdose of long-acting insulin analogue. *Acute Med*. 2013;12(3):167–72. <https://doi.org/10.52964/AMJA.0312>
30. Tofade T, Liles EA. Intentional overdose with insulin glargine and insulin aspart. *Pharmacotherapy*. 2004;24(10):1412–8. <https://doi.org/10.1592/phco.24.14.1412.43147>
31. Barmanray R, Chiang CY, Yeoh K, Yates CJ. A case of surreptitious glargine overdose confirmed by insulin pharmacokinetic time curves. *J Anal Toxicol*. 2019;43(6):4–6. <https://doi.org/10.1093/jat/bkz025>
32. Kuhn B, Cantrell L. Unintentional overdose of insulin glargine. *Am J Health-Syst Pharm*. 2008;65(6):508. <https://doi.org/10.2146/ajhp070602>
33. Dogan FS, Onur OE, Altinok AD, Goneysel O. Insulin glargine overdose. *J Pharmacol Pharmacother*. 2012;3(4):333–5. <https://doi.org/10.4103/0976-500X.103694>

How to cite this article: Arbouche N, Walch A, Raul J-S, Kintz P. Intentional overdose of glargine insulin: Determination of the parent compound in postmortem blood by LC-HRMS. *J Forensic Sci*. 2023;68:1077–1083. <https://doi.org/10.1111/1556-4029.15247>