Review

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Murder by poisoning: successful analytical investigations of spectacular cases in Austria

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To prove murder by poisoning requires the application of analytical toxicology to detect the fatal substance and clear up the cause of death. Improvements in the development of mass spectrometry in combination with high-resolution chromatographic methods are steadily enhancing detection and identification power but making use of these advances relies on proper sample preparation as well as on knowledge about the chemical nature of the substances and their bio-transformation products. This review gives examples of case reports with successful analytical investigations of murder by poisoning in spectacular Austrian cases involving low molecular weight.

Keywords: murder; poison; strychnine; physostigmine; glibenclamid

Introduction

Murder by poisoning is a violent crime and charging a culprit for it requires evidence of the use of toxic substances. Relevant motives of the perpetrator are the unnoticed adduction of the substance to the victim, and a good chance of successfully accomplishing the fatal effect without detection of the toxic agent itself. Offenders will try to avoid anything that could give rise to suspicion of intoxication. For example, their strategy will ensure that the substances are acquired discreetly and that the fatal effect cannot easily be distinguished from a competing natural cause of death.

The police and courts have a difficult task in proving criminal activity as there are countless toxic substances. Moreover it is not the presence of the substance itself but rather its quantity and the duration of exposure that relates to the fatal effect.

To detect such criminal actions the authorities rely on follow-up investigations, including statements of witnesses, which might be biased due to individual perceptions, and various traces, which might have degraded. The character of the perpetrator might also arouse suspicion due to his social activities (chatting) or evidence that he overestimates his power over life. However, substantive arguments need objective data, such as evidence concerning location, time and – in the case of toxic compounds – their identity and concentration. These facts remain even after the retraction of confessions or comments made by the suspect.

One of the first and more important steps in the perception of findings is the autopsy – including detailed reporting about the crime site and the victim's clothing.[1] Clues regarding the administration route of the toxic substance are essential and can only be obtained during the autopsy; failures brought to mind much later remain irrevocable and limit the range of interpretation of the results later on. Photo-documentation of macroscopic and microscopic substrate, as well as the acquisition of various samples from the body, are therefore mandatory.

Death due to intoxication does not usually produce typical variations in morphological appearance compared to death from natural causes, but the number of competing causes of death is restricted only by exclusion of intoxication, requiring proof by chemical analysis.

Three constraints distinguish the strategy of the forensic chemist from certain procedures of chemical analysis, such as using codified methods to control the quality of a product:

- Several natural causes of death compete with fatal effects of intoxication and might prevent any initiative to authorize chemical analysis.
- The countless number of toxic substances demands a broad range of investigations.
- The interpretation of quantitative results has to take individual conditions into consideration.

Of course, the practical considerations involved in successfully detecting an otherwise concealed murder by poison are also not simple – mostly for the same reasons. First, the number of substances involved is typically in the thousands; second, detection requires modern high-performance instrumentation. The latter requires money and is therefore not always available.

The objective of this short review is not to highlight the differences between several practically used chemical methods but to hint at a successful strategy in coping with the set challenge to detect murder by poisoning. No claim is laid on completeness in every detail and the examples presented are individual cases.

Analytical Strategies

Historical development

Paracelsus (1493–1541) proclaimed the relation between dose and toxicity. This rule has been proven correct and, consequently, mere identification of a substance in suspicious material does not facilitate the solution of a difficult case. The only way

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to start chemical investigations into the nature of undetermined potentially toxic compounds is to analyse the complex mixture of countless different compounds with which one is presented according to their gaseous, liquid or solid components.[2,3]

While direct sampling of gaseous compounds is a standard procedure, the forensic chemist also looks for dissolved gases in body fluids by using head space gas chromatography (volatile organic compounds including HCN) and proper detectors such as mass spectrometers; whereas appropriate stationary phases applied in gas chromatography are carbon molecular sieves, silica gel and polyaromatic resins. If the dissolution of the gaseous compound is followed by an irreversible reaction, such as the formation of carboxy-haemoglobin (COHb), a spectrophotometric analysis of a diluted blood sample will easily identify this odourless fatal intoxicant.

The number of gaseous substances or compounds that are volatile at room temperature is rather small, and therefore direct identification by their chromatographic or mass spectrometric properties is promising. A more sophisticated approach is necessary to separate the more-or-less countless non-volatile components in biological samples. Following the historical development of forensic chemistry, Mathieu Joseph Bonaventur Orfila (1787–1853) thought that only inorganic compounds could be detected successfully. Using the chemical methods available in his time the organic compounds were separated by burning and only chemical elements were monitored. The same strategy is applied today, using the methods based on atomic-absorption-spectrometry (AAS) or inductively coupled plasma-mass-spectrometry (ICP-MS) to trace the elements down to the ppb range.

Jean Servais Stas (1813–1891) was the first to realize that the solvent ethanol could be used to extract organic substances from tissue material;[4] conspicuous components could be 'identified' for instance by odour or bitter taste. The use of solvent extraction together with chemical clean-up and chromatographic separation ahead of spectrometric identification proved successful in the search for organic compounds of molecular weights up to approximately 1000 Da. Improvements were achieved not only with high-performance separation instruments coupled to mass spectrometers but also by introducing polystyrene resins for the separation of the analyte from the biological matrix, avoiding protein precipitation. Solid-phase extraction by organic sorbents minimizes any irreversible loss of analyte due to adsorption and occlusion onto proteins in the course of precipitation; thus the procedure improves the yield of non-volatile organic compounds with lipophilic properties – properties that are typical of many drugs and metabolites.[5]

Human sample material, body fluids, tissue, hair and contents of the gastro-intestinal tract can be subjected to chemical analysis using chromatographic separation combined with mass spectrometric detection, and the chances are good of identifying not only substances that were suggested or expected in the course of preliminary inquiries but also intoxication of unknown origin.[6,7,8]

The strategy used when searching for toxins has to consider limits in terms of time and financial resources and therefore a combination of exclusion and positive proof is indispensable. Remarks from witnesses and rumour about an incident induce the medical post mortem examination, which, in most fatal intoxications, is based on the exclusion of other natural causes of death. Preliminary chemical tests, like immunoassays, can be used to narrow down important groups of pharmaceuticals and illicit drugs that could be neglected or that should be followed up further.

Applying liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods (multi-target-analysis) routinely, several hundred different organic compounds can be detected in biological samples within the ppb range in the course of a single chromatographic run, combined with a mass spectrometric screening programme. Subsequent computer-assisted selective – but broadly applied – detection identifies and quantifies the conspicuous substance beyond reasonable doubt.

Analytical Strategy to Detect Fatal Poisoning

The strategy described briefly above allows screening for most of the relevant poisons with low molecular weight and includes fast multi-target screening using highly sophisticated methods like LC-MS/MS as well as general-unknown detection by scanning gas chromatography-mass spectrometry (GC-MS) before and after chemical modification. Gaseous poisons are regularly identified by Headspace-gas chromatography (GC) and flame ionization detector (FID), Nitrogen Phoshorous Detector (NPD) and/or mass spectrometry (MS). The detection of inorganic compounds relies on ICP-MS or AAS after oxidative digestion of the acidified specimens.

Unambiguous identification and a quantitative estimate of the range of concentrations can be achieved, although interpretation of any quantitative result is limited due to inter- and intra-individual variability of biological effects. Consequently, fixation to the 'exact value' instead of evaluating the range of concentration might cause misleading interpretation of data.

The following should be considered as general checklist for the detection of fatal poisoning in biological material:

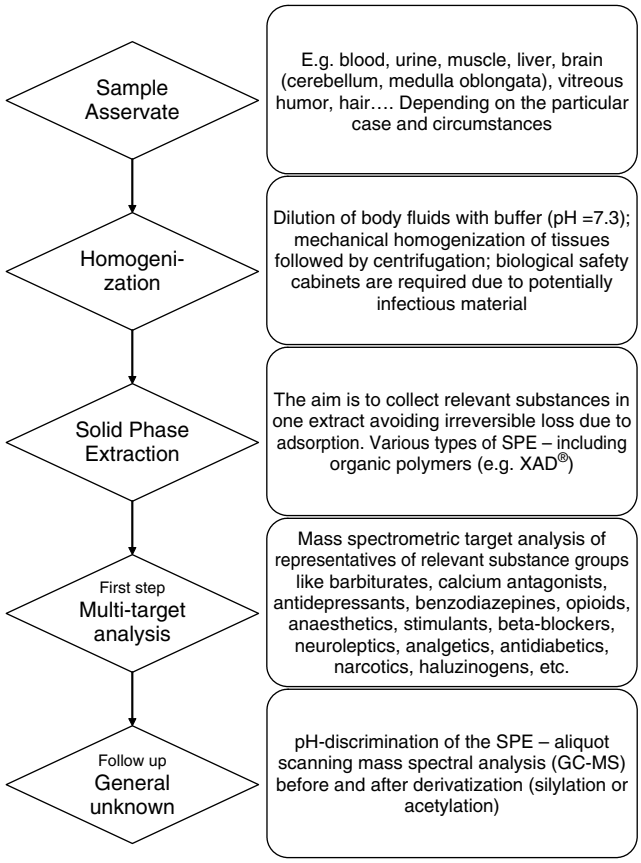
- Background. Detection of fatal poisoning starts on the site of the event. The analyst is to be informed about the case history and emerging assumptions about the nature of the poison.
- Sample collection. Specially trained pathologists should confer with the analyst about which specimen is to be collected according to the circumstances of the particular case, taking into account the possible path of the poison. Promising specimens could be solids (such as tissues from liver or brain, hair), liquids (for example, body fluids) or gases (such as gaseous lung content).
- Transportation. Cooled specimens are transported to the analytical lab as soon as possible.
- Pre-analytical screening. Pre-analytics tests on a sample aliquot (for example, weight, pH-determination, immunological screening methods, spot tests) are to be included.
- Multi-target analysis. Multi-target analysis is performed after appropriate clean-up procedure – for example, LC-MS/MS screening for the most relevant poisons and impairing substances after using SPE via organic polymers (such as XAD®) from a neutralized aqueous extract of the biological sample.
- General unknown analysis. This is performed according to the rules of organic analysis in cases where no relevant substances can be found. pH discriminated organic extracts are examined via GC-MS before and after derivatization

Reports of Fatal Cases

Identification of strychnine in chocolate candy

Case history[9]

After having breakfast with his wife, an approximately 50-year-old mayor of a small village swallowed a 'Mon Cherie' chocolate that



Scheme 1. Analytical approach for the detection of unknown poisons including target analysis representatives of selected substance groups as well as general unknown analysis.

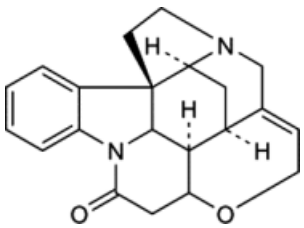


Figure 1. Chemical structure of strychnine.

he had found in an anonymous letter on his car the evening before and departed by car for his workplace in the neighbourhood. Approximately 10 to 15 minutes later he had to stop because of very severe convulsions. Just before losing consciousness he managed to inform a citizen nearby about his consumption of the 'Mon Cherie' chocolate and articulated his suspicion of having been poisoned.

About 11 minutes later first aid arrived and resuscitation was started. Due to assumed poisoning with potassium cyanide the antidote hydroxycobalamine was administered together with midazolam and fentanyl on the way to the hospital, where he arrived approximately one hour after the first symptoms of poisoning. The emergency physician collected samples of urine, blood and gastric content for subsequent toxicological analysis.

Assuming possible cyanide intoxication, a colour test for cyanide in the stomach content was performed using ferrous sulphate[10] but no traces of cyanide in relevant concentrations above 200 µg/ml sample could be detected.

Immunological screening of the urine sample performed simultaneously resulted in evidence of benzodiazepine-like substance intake, which was confirmed as midazolam by a later LC-MS/MS analysis.

Systematic toxicological analysis of the basic extracts of gastric content and urine were started subsequently via GC-NPD/MS analysis of non-derivatized analytes and via multi–target screening by LC-MS/MS.

Approximately two hours after having received the body fluids from the poisoned person the lab reported the identification of strychnine in the gastric content and in the urine, the result relying on the application of two independent analytical methods.

Figure 2 shows the reduction in the amount of strychnine in serum and urine concentrations during the medical treatment of the intoxicated person in the hospital over a period of five days following intoxication.

The strychnine concentrations were determined using LC-MS/MS simply by diluting the gastric content and urine, as well as serum samples, with methanol; after centrifugation an aliquot of the supernatant solution was injected into the analytical system using diazepam-d3 as internal standard. The gastric content revealed a remaining strychnine amount of about 30 mg in 5 ml, confirming oral administration.

The suspected dose of strychnine was estimated from the amount of strychnine parent compound excreted in urine; according to the medical expert the excretion rate of unchanged

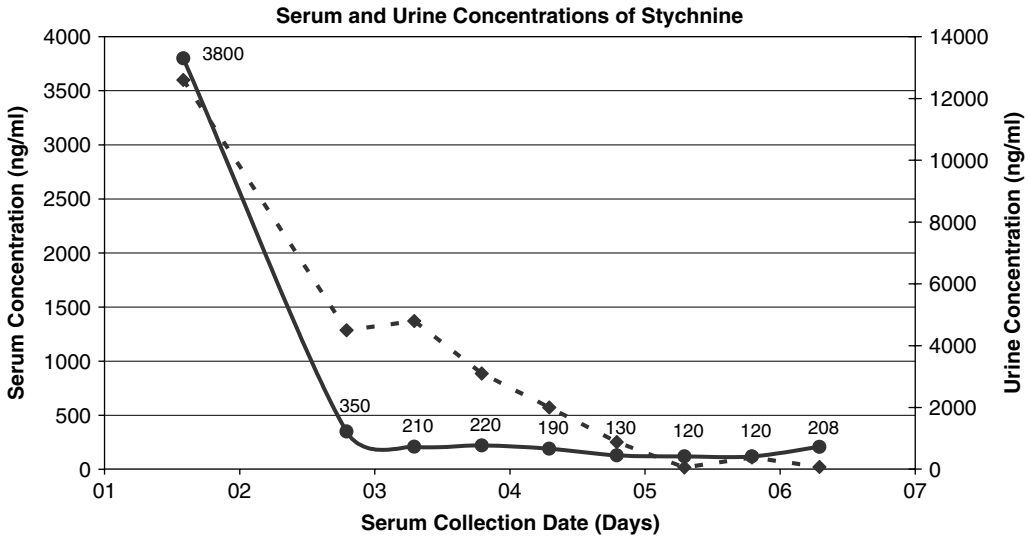


Figure 2. Serum (full line) and urine (dotted line) concentrations in ng/ml; the time interval on the *x*-axis is marked in days, collection started on day 01, the day of the poisoning.

strychnine averages approximately 6%, which would correspond to a total amount of approximately 700 mg ingested strychnine.[11]

The victim was delivered to hospital unconscious, suffering from circulatory arrest and paralysis of the limbs, several weeks later an irreversible brain damage due to oxygen deficiency was diagnosed.

The presumed murderer was arrested because traces of his DNA had been identified on the anonymous greeting card that accompanied the chocolate candy. He was initially given 20 years in prison but after an appeal the Supreme Court increased the penalty to life imprisonment.

Origin of physostigmine in body fluids

Case history

An approximately 50-year-old male was transferred to hospital suffering from diarrhoea and vomiting and was released after one week of successful medical treatment. One month later he experienced the same symptoms and was hospitalized again. Toxicological analysis of the stomach content detected physostigmine, a parasymphatomimetic antidote. The respective amount calculated from the concentration in the total amount of gastric lavage (450 ml) came to about 100 mg physostigmine. The minimum dose associated with fatal physostigmine ingestion has been reported as 6 mg.[12]

A urine sample collected simultaneously with the gastric lavage contained 17 µg physostigmine per millilitre. After approximately two months in hospital the patient's health declined dramatically; cardiogenic shock was given in the emergency protocol as the possible cause of death. Using LC-MS/MS technology after XAD®–4 extraction of the homogenized post-mortem asservates (cerebellum, medulla oblongata, blood, liver and urine) physostigmine was not observed above a limit of detection of 10 ng/ml.

Given the long time delay between the intoxication with physostigmine and the time of death (approximately two months) the impairment and weakening of the body functions caused by the overdose of physostigmine seemed to be the main cause of death.

One of the questions asked by the criminal court concerned clues to the origin of the consumed physostigmine. This substance, synonymous to eserine, is naturally present in calabar beans[13] (see Figure 3), as well as in a pharmaceutical preparation (Anticholium® ampoules, 2 mg physostigmine salicylate in 5 ml) and can be purchased as fine chemical in quantities of 1 g and more.

The analytical strategy to investigate the possible reason for the physostigmine intoxication included the detection of associated alkaloids from the calabar bean. According to literature this plant material does not only contain (−)-physostigmine, but structurally familiar substances like (−)-norphysostigmine and (−)-geneserine[14] (see Figure 4), which are supposed to be absent

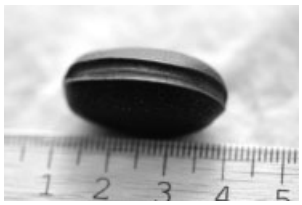


Figure 3. A calabar bean.

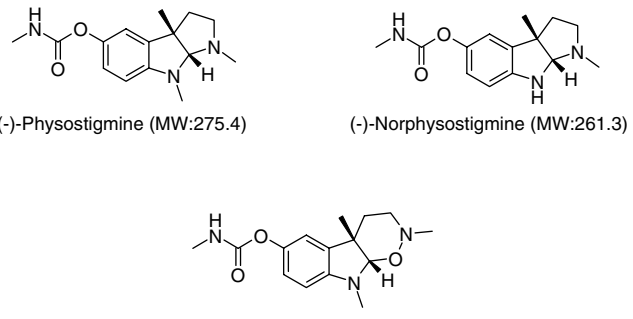


Figure 4. Chemical structures and molecular weights of three alkaloids present in calabar beans.

in the pharmaceutical preparation as well as in the fine chemical preparation.

Reanalysis of the gastric content showed that none of the two associated alkaloids were present; only a single signal of physostigmine was detected. The methanolic extract of the calabar bean showed all three alkaloids (approximately 35% of geneserine and approximately 12% for norphysostigmine, in relation to physostigmine), whereas the pharmaceutical preparation 'Anticolium®' showed a strong signal for physostigmine and a weak signal (approximately 2% compared to physostigmine) for geneserine (see Figure 5). Due to this analytical finding the pure chemical was concluded to be the possible source of this physostigmine intoxication.

After the analytical results had been transmitted the court suspended the trial.

The 'black widow' – general LC-MS/MS analysis of unknown substances

In November 1995 the body of a 76-year-old man was brought to autopsy because neighbours had noticed a dramatic change in the condition of a previously healthy man shortly after an elderly lady (E.B.) had started caring for him. Until then he had carried his age well. Lingering illness had started only a few weeks earlier, when E.B. started her 'nursing' activities. Autopsy did not reveal any significant natural cause of death, so blood and urine from the corpse were sent for chemical analysis and the forensic chemist was asked to screen for generally harmful substances. The clean-up procedure included solid-phase extraction with polystyrene. Separation and detection were performed using GC-MS; the number of substances covered exceeded 10 000, represented in the MS-library files by Pfleger/Maurer/Weber.[15] Surprisingly, the tricyclic antidepressant clomipramine was detected in blood at a concentration high above the therapeutic range.

Faced with the results of autopsy and chemical analysis the suspect informed the officer in the course of inquiry that she had administered glibenclamid several times before to those whom she had offered to take care of. The authorities consequently charged her with the murder of several people between 1981 and 1996, accusing her of having used the pharmaceuticals Euglucon® and Anafranil® intentionally to obtain the victims' money.

The cases reported exemplify instances where no special substances were suggested or where the analytical strategy followed the principles of the general unknown analysis and finally led to legal proceedings. (The verdicts in two of the cases – 'strychnine' and E.B. 1[16] were affirmed – proceedings in the 'physostigmine' case was stopped by the public prosecutor before the prosecution was opened.) But, of course, courts have not only demanded investigations for unknown substances. A great number of cases have been carried out in which a limited number of relevant compounds were subject to analysis.

A combination of mass spectrometry with chromatographic separation offers detection limits down to the femtomol range, even in complex matrices. But in forensic chemistry even target analysis must be performed in accordance with nature. In the E.B.

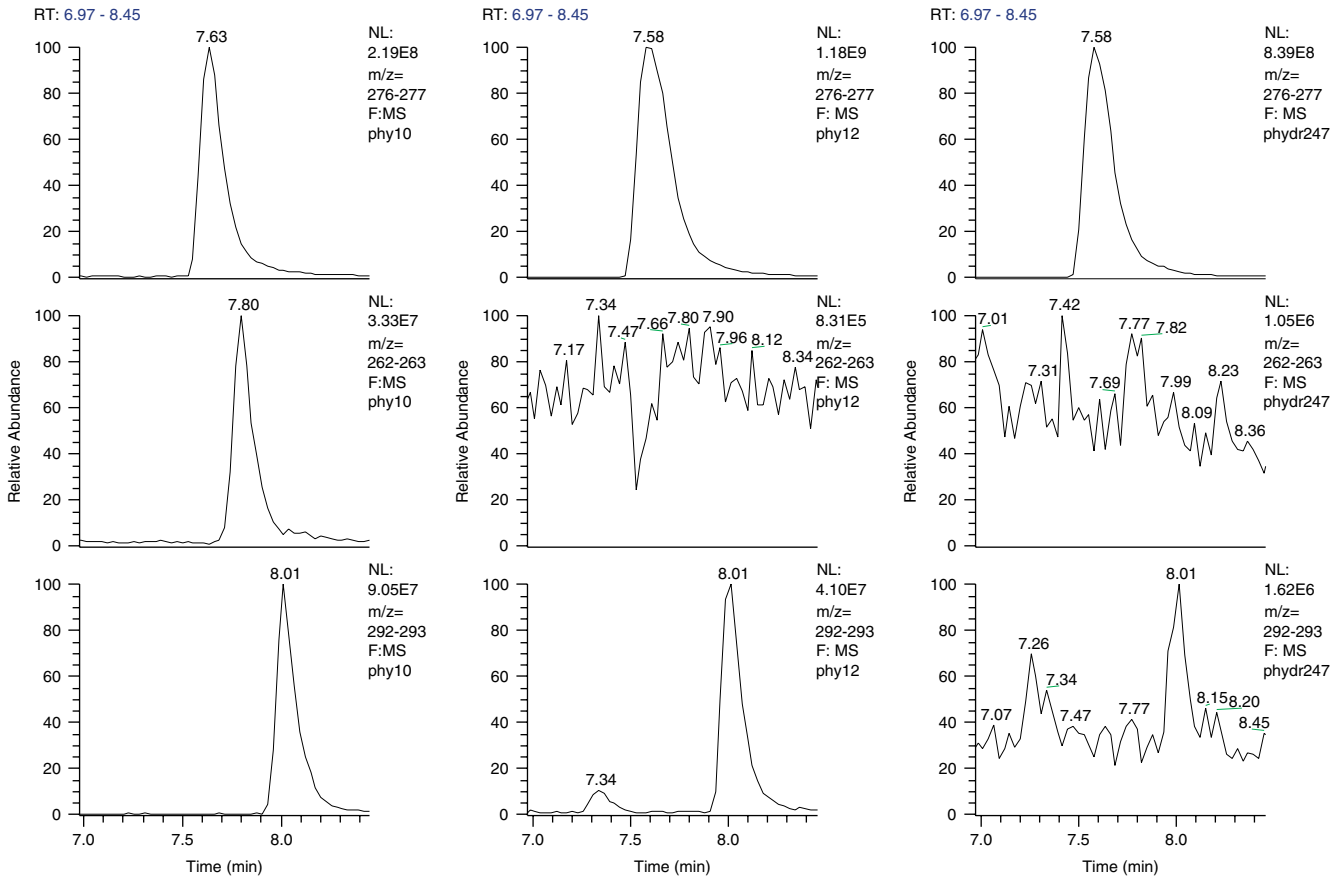


Figure 5. Chromatograms of a methanolic extract of the calabar bean (A), the pharmaceutical preparation 'Anticholium®' (B) as well as gastric content (C). Mass traces of the molecular ions are numbered with 1 (physostigmine), 2 (norphysostigmine) and 3 (geneserine).

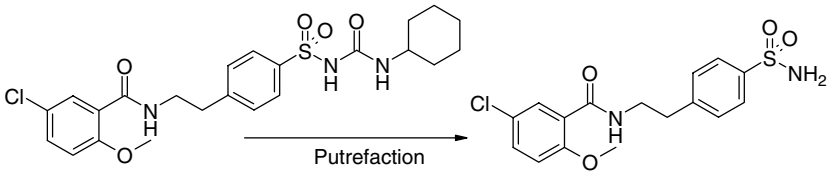


Figure 6. Transformation of glibenclamid to its putrefaction artefact.

case mentioned above, the use of Euglucon® had to be proved. The victim's body had been buried in a sealed zinc coffin and after four years' burial an aliquot of the developed putrefaction fluid was analysed by LC-MS/MS for glibenclamid, the active ingredient of Euglucon®, with no positive evidence for the consumption of the anti-diabetic pharmaceutical. The use of glibenclamid was detected after glibenclamidsulfonamid had also been considered as a reasonable candidate of relevant substances formed from glibenclamid in the course of putrefaction (see Figure 6). Although the analytical instrumentation was possibly not as powerful as it is today, the relevant artefact was doubtlessly detected in the course of the chosen chemical procedure and the judgment affirmed later.[17,18]

Without LC-MS/MS instrumentation the identification of the relevant substance in this case could not possibly have been achieved, but we also had cases that were solved by slight but effective changes in the clean-up procedure.

Murdering nurses – effective changes in sample-preparation processes

In spring 1989 four nurses from a hospital in Vienna confessed to the police that they had shortened the lives of terminally ill patients by first using tranquilizers and then introducing water into the upper respiratory system. A year before rumours had spread that patients had been immobilized using flunitrazepam. Internal investigations discovered that the consumption of flunitrazepam was about ten times higher in this department than in all the others and that even patients in a relatively stable condition had died when those four nurses were on duty; in the previous two years these four nurses attended a number of death cases six times higher than their colleagues. The public prosecutor accused the four nurses of murdering 40 people, requesting expert evidence in regard to the proof of flunitrazepam, diazepam and prothipendyl in 14 exhumed dead bodies. Owing to the expected relatively low concentration of the target analytes and the advanced decay of the biological material – further impeding the analytical detection due to high background signals – the analytical strategy was based on extensive separation including solid-phase extraction with organic resin and mass spectrometric identification. The detection of flunitrazepam was a particular challenge as no substance-specific and high-sensitive GC-MS or LC-MS/MS method was available at this time; the answer was found by reducing the chemical noise in the relevant fraction by methylation. By esterifying the huge amount of free fatty acids in the crude acidic fractions of blood, brain and fat tissue and a supplementary clean-up by C-18 chromatography portions were obtained that could be analysed by high-resolution mass spectrometry, measuring the exact formula weight of flunitrazepam, i.e. 313.2881. Afterwards, mass spectrometric detection of flunitrazepam could be achieved using negative chemical ionisation. Flunitrazepam or prothipendyl could be detected in four of the 14 exhumed bodies. The separately examined recovery of flunitrazepam over the total clean-up procedure was in the range of 20% to 30% and the total loss of substance from putrefying tissue was in the range of 90%. As the administration of flunitrazepam to the patients was not authorized at any time, mere discovery of the tranquilizer could be taken as proof of the wilful murder of the patients. The Supreme Court judged the sentence accordingly.

Although the murder of seriously ill patients was not evident at the beginning, the subsequent rumour caused administrative authorities to keep a careful eye on the department; moreover any authorized prescription of flunitrazepam was precisely controlled. This later permitted the detection of the tranquilizer that could be used as proof in court.

A similar case of suspected murder was published by Daldrup in 2001,[19] reporting that even though quantitative determination of a relevant pharmaceutical compound was carried out by three different and independent agencies, the court of appeal could not find a relationship between its concentration in blood and tissue on the one hand and the cause of death on the other, as the patient was seriously ill.

An alternative effort to shed light on sinister practices in the care of elderly people involved analytical chemistry at a much earlier stage. Due to a vague rumour, the administrative authority of a retirement home appointed an expert in forensic chemistry to conduct a segmental hair analysis to test for the presence of sedatives; in close cooperation with the medical service all from the patient consumed pharmaceuticals could be detected and correlated with significant symptoms.

Conclusion

Frequently it is not the sensitivity of the detection instrument that solves the crime. Chemical analysis, although essential, is only one part of the process and high-performance instrumentation is necessary, but not sufficient, to reveal murder by poisoning. One faulty step in the process can lead to failure. The clean-up procedure used with the sample matrix is an important link in the chain, being related directly to the responsibility of the executing analyst – instrumentation is much more a question of money and infrastructure.

Agents and metabolites of interest may be present in tiny quantities. Several thousand different compounds might be involved. The careful and innovative analytical strategy of the analyst determines whether or not joint efforts lead to success.

When considering murder by poison as the cause of death, chemical analysis cannot always be restricted to the search for a handful of substances in a few standard samples of biological residues. Even substances that were not suggested or expected in the course of preliminary inquiries should be identified to detect poisonings of unknown origin. Discovery of murder by poison is not to be taken for granted – it is the result of a wide range of knotted episodes.

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