# Chapter 11
# Forensic Analytics

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## 11.1 Criminalistic Analytics

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Analysis of various materials for forensic purposes is known as trace analysis. The problem in this field tends not to be the small quantity (concentration) of the analyte in question, but rather the small quantity of material (forming the criminalistic trace at the scene of the crime or incident) that is available for examination. Its mass is often on the order of milligrams or micrograms. This trace amount of disclosed material (e.g. skin fragments, individual fibres, dust, pieces of glass and plastic, soil particles or droplets of blood) constitutes a valuable source of information about the event and persons taking part in it. Disclosure and appropriate securing of material from the scene of the incident is thus crucial for carrying out examinations correctly in the criminalistic laboratory and for explaining the circumstances of the crime.

### *11.1.1 Concept of a Criminalistic Trace*

The concepts of trace and microtrace were introduced into criminalistics to define material that had been found at the scene of a crime and then subjected to examination. The concept of a criminalistic trace was defined by Jan Sehn, who stated that "traces in the criminalistic sense are changes in objective reality (. . .)

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after events that are under investigation", which "may constitute a basis for recreating and establishing the course of these events in accordance with what actually happened" [1]. As understood by this definition, traces are the consequences of some behaviours and phenomena, and thus exhibit a causal link with these behaviours or phenomena. A trace is thus an object or part of an object left at the scene of an incident, an impression of the sole of a shoe or tyre, a dent or scratch on a surface caused by a tool, a fingerprint, a liquid stain (e.g. blood, oil), a hair, a sliver of glass or paint, or a change in the shape of an object caused by heat or force. Traces are perceived via the senses or with the use of technical devices to aid these senses (magnifying glass, microscope, illuminator, etc.). Traces have a material (physical) nature and are thus possible to detect and study.

The importance of traces lies mainly in their reconstructive potential; on the basis of traces it is possible to recreate the course of a particular incident and determine which persons took part in it, as well as their behaviour at the time of the event. Furthermore, traces can be useful aids in enabling direct apprehension of the perpetrator of an incident. Examination of traces can also indicate whether and how defined persons were linked to an event that is of interest to a judicial body. Traces can also play a significant role in the identification of places, persons and things.

Each criminalistic trace can occur at the scene of an incident in various sizes. Advances in measuring equipment, new analytical chemistry techniques and empirical research in this field, as well as the small number of disclosed macrotraces, have led to microtraces acquiring particular importance. In physicochemical terms, microtraces do not differ from macrotraces. They are particles of matter weighing milligrams or less (e.g. soil particles, dust, microfibres, skin fragments and liquid droplets). They can also be gases that are undetectable by the sense of smell or microscopic marks of mechanical action in the form of scratches, dents, or cracks that are invisible or hardly visible to the naked eye. Mirosław Owoc defined them as follows: "Microtraces are those criminalistic traces, which, due to their small dimensions or other particular properties are, without appropriate observational instruments, imperceptible or poorly perceptible by humans, and can only be examined by applying microanalytical methods" [1]. The size of microtraces means that, as a rule, various microscopic and microanalytical techniques must be used to examine them. Impurities and additives are also considered to be microtraces and must be studied using advanced analytical methods.

### *11.1.2 Features of Microtraces*

Microtraces are characterised not only by their microscopic size, but also by their prevalence, the fact that perpetrators are unable to avoid leaving them (irrespective of perpetrators' efforts) and the difficulty in removing them. Microtraces arise as a result of the interactions of the perpetrator (his/her clothes, tools/instruments used) with the surroundings. Most frequently, microtraces are small particles of an originally larger object that have separated from it, such as microfibres, particles

of glass, paint, metal, plastic, soil or explosives. Where macrotraces occur, microtraces also usually occur, constituting a sort of supplement to them. However, from the point of view of criminalistic practice, cases in which there is a lack of macrotraces because they have been destroyed or removed by the perpetrator or other persons, or an experienced criminal has not left any, microtraces are much more valuable. In principal, it is impossible to avoid leaving microtraces. Microtraces occur at the scene of every event, on every tool that the perpetrator has used, as well as on the victim and on objects belonging to him/her. Dust particles or fibres accumulate easily in recesses and cracks of a substrate; hence, it is difficult to remove them. Microtraces are thus "resistant" to destructive factors (e.g. washing and wiping). The action of adhesive forces additionally helps to keep microtraces on the surface of a substrate. In terms of chemical composition, microtraces are usually mixtures of many compounds.

Because of the (tiny) size of microtraces, they are often unconsciously destroyed; for example, they can be accidentally lost or transferred to another substrate, evaporate at elevated temperatures, or destroyed by the action of fire. Their properties might also change. The time elapsed between leaving a trace and its disclosure and securing for examination, as well as atmospheric conditions, are sometimes the cause of chemical and biological breakdown of the trace, or the substrate on which it occurs, making it difficult to carry out analyses.

### *11.1.3 Difficulties in the Study of Criminalistic Traces*

There are many factors that significantly affect the possibility of carrying out analyses of disclosed traces, the choice of analytical methods and interpretation of the obtained results. Among the basic factors are the goal of the examination and the applied methods of disclosure.

#### 11.1.3.1 Aim of the Examination

The aim of chemical analysis of traces is, above all, their identification. Currently, there are many analytical methods and research techniques enabling identification of a wide variety of materials with great accuracy and precision. Qualitative determination of the chemical composition of studied materials and establishing their type does not usually present difficulties, irrespective of the size of the studied sample. In the case of materials such as paints or fibres, the availability of broad databases for reference purposes allows their producer to be identified. However, information obtained about materials in the course of analyses leads only to group identification and not individual identification. This means that, as a result of conducted analyses, the material can be classified into a group of objects (materials) of the same composition and properties, bearing in mind that the size of such a group varies and depends on the amount of information obtained during analysis.

Analytical chemistry methods can help to elucidate whether materials being compared are the same or whether they differ from each other. However, such methods cannot establish whether a studied sample is part of one and the same material system, and thus whether it is of the same material. Therefore, these methods cannot individually identify secured materials. Individual identification is, of course, possible, but achieving it requires going beyond determination of the chemical composition and physicochemical properties of a trace.

Traces are identified by determining their chemical composition and certain physicochemical properties, and comparison with reference material (database) or comparative material collected from the suspect. Criminalistic laboratories create extensive databases on the physical features and production of glass, paints, papers, fabrics and other materials, as well as collecting thousands of samples for comparison purposes.

#### 11.1.3.2 Material

Microtraces are usually very small samples (e.g. milligram or microgram weight) of material such as pieces of paint, a few drops of oil, individual fibres, a hair or fragments of glass or plastic. Once this material has been secured at the scene of the incident, it cannot be collected again for examination. It is therefore quantitatively limited and must suffice for all ordered examinations, and some of it should also be preserved. Furthermore, samples submitted to a laboratory are usually contaminated and often difficult to separate from the substrate on which they occur. The smaller the dimensions of the studied materials and the more subtle their structure, the greater is the influence of the substrate on the results of analyses. Thus, if it is not possible to separate the studied material from the substrate, then the effect of the substrate on the results of analysis should be taken into account. An example might be an examination of initials, signed to confirm receipt of money. Initials are composed of, at most, several letters that have been executed, for example, with regular or India (drawing) ink. They contain only a small amount of the ink in question, which additionally has penetrated into the structure of the paper. Extraction of ink from the substrate damages the document, and thus requires approval of the judicial body requesting the examination. Therefore, it is necessary to examine the ink directly on the document, without separating it from the substrate (paper), using, for example, infrared (IR) spectrometry and Raman spectrometry. In the obtained spectra, absorption bands originating both from ink and paper are then visible. Obtaining information about components of the ink (or India ink) requires correct interpretation of spectral data.

Materials submitted for chemical analysis rarely form a homogenous system. They are usually complex, and particular components are intermixed. In all such cases, it is necessary to separate out one or several significant components from the system and to reject large quantities of ballast material that has no significance for the case.

It often happens that the main components or fractions of the studied material are not of interest to forensic investigators, whereas particles of material attached to cracks on the surface of larger objects and probably linked with the event are significant. An example is the study of material collected from the site of a fire, constituting a mix of products of burning from all sorts of materials. The purpose of their analysis is to find traces of the agent used to start the fire, usually highly flammable liquid, which might, for example, be occluded in the pores of burnt polymer material (flooring, carpets).

Materials that are the subject of criminalistic examinations constitute material evidence in court cases. Their identification is carried out by comparing them with a reference or with so-called comparative material provided for study.

#### 11.1.3.3 Usefulness of Microtraces for Examination

In spite of their durability, the usefulness of microtraces for research can be negatively affected by many factors. One group of factors define the objective state of microtraces at the moment of their formation at the scene of the incident (e.g. heterogeneity) and non-representativeness of particles separated from the larger whole. An example is traces of earth disclosed on clothing in the form of mud. Only a tiny fraction of soil (dust) forms the trace because only such a small sample can stay on the fabric surface.

Other factors act (on the microtrace) in the period from the moment of formation to the moment of securing the microtraces. These include secondary changes, such as ongoing biological decomposition and corrosion, and contamination by dust floating in the atmosphere. Biological material (e.g. fragment of skin containing a gunshot wound) easily degrades, making it impossible to search for traces (e.g. gunshot residue on a gunshot wound). Similarly, the progressive process of corrosion of a metal staple makes it impossible for investigators to see traces of the action of a tool (e.g. a saw blade) on its surface.

Mention should also be made of factors acting in the period between securing a microtrace and examining it, such as environmental influences (humidity), incorrect method of securing and accidental interference. For example, badly stored wet clothes easily become covered with mould, making it difficult or even impossible to look for fibres, particles of paint, and so on on their surface. The action of unfavourable factors is usually compounded by the passage of time.

### *11.1.4 Research Methods*

To identify materials forming a criminalistic trace, it is necessary to study their morphology, determine their chemical composition (mainly qualitative) and study some physico-chemical properties. Because the sample of material forming the trace is small, microanalytical methods are applied for identification. Methods that

do not damage the studied sample or use it to a minimal degree (enabling repeated analysis by the same or a different method) are mainly applied. Instrumental methods currently play the most important role in the examination of traces. They enable results to be obtained quickly and are characterised by high sensitivity and low limits of detection for the analysed component. The results of examinations are then compared against an appropriate database (a collection of results obtained for standard/reference substances) and usually lead to group identification of the studied material. The applied methods are characterised by a high power of discrimination and thus enable differentiation of samples.

The basic principle that applies when conducting examinations is that of cross-checking. A result obtained by one method should be confirmed by other techniques. Trace examinations have an interdisciplinary character. Identification of submitted material requires the cooperation of specialists from various branches of science.

#### 11.1.4.1 Microanalytical Techniques

Until recently, many traces, even those perceptible to the naked eye at the scene of the incident, were not secured because sufficiently accurate methods of analysing them did not exist. It was not until the development of microanalytical methods and their introduction into practice by forensic experts that analysis of traces and their use as a valuable source of information about an incident was possible (i.e. about the course of the event and the persons taking part in it).

The size of a sample forming a criminalistic trace is small, so techniques applied in its analysis should be non-destructive, enabling sample preservation or recovery after performed analyses. All optical microscopy and spectrometric techniques are such methods. Chromatographic techniques, which use the studied sample to a very small extent, are also admitted in the examination of criminalistic traces.

##### Microscopy

The basic method for analysing criminalistic traces is microscopy. A cycle of analyses always starts with microscopy and the obtained results determine the choice of successive examination methods. The fundamental aim of carrying out microscopic analyses is to observe the morphology of the sample, define its structure, thickness and uniformity. In the course of analyses, it is possible to disclose possible inclusions. Sometimes microscopic examination allows identification of a sample (e.g. fibres of natural origin, minerals, gunshot residues) or contamination (e.g. soil). Microscopic examination is usually used in the comparison of samples. Obtaining a similar microscopic image for two samples constitutes a premise for making inferences about their similarity and suggests a common origin. Microscopic examinations are non-destructive; even when it is necessary to

prepare microscopic preparations, a sample can always be recovered for further examination. There are two types, optical and electron microscopy.

Optical microscopy enables observation of a sample at magnifications ranging from a few times to 1000 times. The source of illumination of the sample in optical microscopy is white light, which passes through the studied sample (transmitted light microscopy) or is reflected from its surface (reflected light microscopy). Polarised light can be applied (polarised light microscopy) or fluorescence can be induced in a sample by illuminating it with short wavelength light (fluorescence microscopy).

In electron microscopes, a completely different source of energy is used for illumination – accelerated electrons. Images of surfaces obtained using electron microscopy are characterised by very good resolution and depth of field, which cannot be achieved with optical microscopes. In the electron microscope, the beam of "radiation" does not form an image in a direct way, but serves only to excite the sample. The image is formed on the basis of analysis of results of the collision of the stream of electrons with the surface of the studied object. The diameter of the beam striking the specimen is always small, of the order of hundredths of a micrometre. Electrons do not penetrate through the sample, which is why the obtained images constitute a reflection of the topography or composition of the surface layer of the studied object.

### Microspectrometry

Microspectrometry is an indispensable technique in criminalistic analyses, being a combination of optical microscopy and spectrometry. Microscopy creates, records and interprets magnified images, whereas spectrometry uses emission, absorption and reflection of radiant energy by matter to determine its structure, properties and composition. On the basis of the type of energy applied, microspectrometry can be divided into IR, visual and ultraviolet (UV-vis), and Raman microspectrometry. This group also includes X-ray microspectrometry, in which an electron microscope takes the place of an optical microscope. Infrared and Raman microspectrometry enable determination and comparison of the chemical composition of studied samples; UV-vis microspectrometry serves to compare the colour of samples in an objective way that is independent of the observer; and X-ray microspectrometry allows determination of the elemental composition.

Fundamental advantages of microspectrometry are that extremely small quantities of a sample can be analysed, often without the necessity of separating it from the substrate; there is no burdensome process of preparing a sample for analysis; and multiple repetitions of measurements can be performed without destroying the sample. A further, specific, advantage of microspectrometry is the possibility of photographing and archiving the measured areas of the sample. However, a fundamental disadvantage is the fact that, in principle, microspectrometry only allows point analysis, and that is why non-uniformity of a sample and its contamination can significantly influence the results of spectrometric measurements.

The advantages of microspectrometry mean that it is now used in most criminalistic laboratories for examination of traces disclosed at the scene of an event.

*Infrared Microspectrometry*

The apparatus used for IR microscopy is a Fourier-transform infrared (FTIR) spectrometer coupled on-line with an optical microscope. The microscope serves to observe the sample in white light at significant magnification for the purpose of determining its morphology, as well as to select the area for analysis. The spectrometer, on the other hand, enables study of the sample by transmission or reflection measurement for the purpose of determining the chemical composition. It also provides information about the microstructure and optical properties (orientation) of the sample. It is possible to apply polarised light both in the observation of the sample and in spectrometric measurements.

In order to obtain a good quality spectrum, it is essential that a large amount of IR radiation energy should reach the detector, and also that the area of the sample analysed spectrometrically should be accurately defined. Failure to meet these conditions leads to reduced signal-to-noise ratio. This also happens in the case of non-uniform illumination of the field of vision and measurement. It is easy to demarcate the area of the sample that is of interest to the analyst using a beam of white light. The area to be analysed spectrometrically is larger as a result of diffraction of IR rays. Therefore, in the case of small samples, exact demarcation of the area for spectrometric analysis is crucial in obtaining a good IR spectrum.

This method is particularly useful for analysis of the qualitative composition of trace amounts of various substances secured as material evidence in court cases, analysis of the homogeneity of a sample, identification of inclusions and contaminations on a surface, and detection of defects in a structure. Its main drawback is the fact that the physical nature of the microsample can affect the photometric accuracy of measurement and cause distortion of the obtained spectra.

Infrared microspectrometry (FTIR) is most frequently applied for identification of microtraces such as particles of paint, plastic, fibre, rubber and glue, as well as for analysis of the chemical composition of, for example, ink or toner.

*Microspectrometry in the Visible and Ultraviolet Range*

This method allows comparison of the colour of very small samples of various materials (e.g. individual fibres, particles of paint, traces of ink or ballpoint pen ink on a forged document) in an objective way that is independent of the observer (i.e. the acuity and quality of his/her vision). The apparatus consists of an optical microscope with a spectrometer for analysis in the UV-vis range via an analogue digital converter with a computer. The method enables information to be obtained about the spectral differences existing between two samples of similar colour, which are indistinguishable using a comparative optical microscope. Obtaining fully consistent spectra for the compared samples attests to consistent colour and, thus, consistent pigment/dye composition of samples. Additionally, applying

appropriate software for analysis of microspectrometric results allows accurate definition of the colour by assigning a numerical value to it, the chromaticity coordinates. These coordinates ascribe to each colour a point in colour space, determined by three components describing colour (i.e. hue, brightness and saturation). Overlapping of points in colour space attests to the identical colour of samples. Measurement and description of colour were carefully standardised by the International Commission on Illumination (Comission Internationale de L'Eclairage, CIE) for the first time in 1931 [2]. Digital colour description and calculation of chromaticity coordinates were proposed about 40 years later.

*Raman Microspectrometry*

Raman microspectrometry is a complementary method to IR microspectrometry. An optical microscope coupled to a spectrometer enables measurement of radiation scattered by a sample with a diameter of several micrometres. Depending on the type of laser used to excite the sample, information about different components is obtained. Advantages of the method include good sensitivity and spectral resolution, very short duration of measurement, high power of discrimination and the possibility of spatial imaging of selected components within the sample. These advantages have aroused the interest of forensic chemists, among others. Most frequently, the method is used for analysis of the pigment composition of traces in the form of paint particles, individual fibres and inks on a document. As a method that is non-invasive and non-destructive (of the studied sample), it is the basic technique for examination of the authenticity of documents, enabling differentiation of inks and ballpoint pen inks directly on the questioned document. However, sometimes samples exhibit fluorescence and then the application of several excitation lasers to obtain a readable Raman spectrum (or the use of surface-enhanced resonance Raman scattering, SERRS) is required.

*X-Ray Microspectrometry*

X-ray microanalysis is performed using a scanning electron microscope coupled with an energy dispersive X-ray detection system (SEM-EDX) or using an X-ray microfluorescence spectrometer (μ-XRF). Characteristic X-ray radiation is emitted from the studied material as a result of bombarding the surface of the sample with a beam of accelerated electrons emitted by a cathode (in the electron microscope), or with X-ray radiation arising in the X-ray tube of the XRF spectrometer during excitation of the atoms of the anode target with a stream of electrons. Detection of this radiation, and determination of its intensity, provide information about the elemental composition of the analysed sample.

The greater the energy of the electrons of the beam and the smaller the mean atomic number of the elements making up the studied sample, the greater the depth of penetration of electrons into the sample. In the electron microscope, the penetration varies from a few tenths to several micrometres, and in μ-XRF it is significantly greater, even of the order of millimetres.

Thanks to the linear relationship between the intensity of the characteristic X-ray radiation generated in the sample by electrons and the concentration of the given element, quantitative elemental analysis is also possible. X-ray microanalysis performed using SEM-EDX is, in principle, point analysis and is suitable for studying very small samples of solid materials that are stable in an electron beam. The X-ray fluorescence method, on the other hand, can be applied to the study of both solids and liquids. The signal reaching the detector always originates from a certain sample volume, and thus it is not point analysis. It is more sensitive than the SEM-EDX method.

## Pyrolysis Gas Chromatography

Among other microanalysis techniques, it is worth mentioning pyrolysis gas chromatography. The apparatus set consists of a pyrolyser, where the breakdown of the studied sample to simple volatile substances occurs, and a gas chromatograph coupled with a mass spectrometer for separation and identification of the volatile substances. In contrast to microspectrometry, this technique is considered destructive (of the studied sample) to a small extent. It is an indispensable technique for studying the chemical composition of macromolecular materials (polymers, plastics, rubber). The breaking of chemical bonds that occurs during pyrolysis of the studied sample under the influence of temperature or electromagnetic radiation in an inert gas atmosphere leads to degradation of the sample and to creation of stable fragments that are characteristic for it. Their separation on a chromatographic column and identification of particular compounds by MS gives information about the composition of the starting sample. Selection of pyrolysis conditions allows control of sample fragmentation and the formation of defined particles, which enable samples of a similar chemical composition to be distinguished (e.g. samples belonging to the same chemical class). By maintaining the same pyrolysis conditions and stable measurement conditions, one can obtain, in a repeatable way, the same type of fragment from the same starting sample. For improvement of detection of some compounds, it is beneficial to carry out preliminary derivatisation of the sample in an on-line system with the help of an appropriately selected reagent.

An advantage of this method is the fact that the amount of sample needed for analysis is of the order of 3–5 μg, depending on the type of polymer in the sample and the type of applied instrument. Its accuracy varies in the range 10–20 %. It is used in the study of traces of polymer materials such as paints, plastics, rubbers, glues and adhesive tapes.

### 11.1.5 Interpretation of Results

The most difficult stage of the whole process of identification of materials making up the criminalistic trace is interpretation of the obtained results of analyses, taking into account the fact that each measurement result is burdened with error. The most important thing is for the obtained results to be repeatable. Therefore, the precision of measurements must be high and the results should not be burdened with systematic errors, but close to the true values. The applied method should be accurate and reliable. Each measurement of a quantity is repeated several times, the scatter of the results observed and the measurement error determined. Practically each applied measurement method must be validated.

When processing results, simple statistical methods as well as more complex chemometric methods are used. Significance tests are applied to assess measurement results obtained for two compared samples and to establish whether small observed differences between them are the result of real differences in measured values, or whether they are the result of accidental errors.

Chemometric methods such as analysis of correlation coefficients, cluster analysis or neural network analysis are used, for example, in the classification of fragments of glass on the basis of their elemental composition or refractive index. Such methods allow the test material to be classified into the appropriate group of products on the basis of the measured parameter.

Criminalistic interpretation of the results of examinations is an important issue. Results of chemical examinations of materials constituting material evidence in a given case are helpful in identification of the perpetrator on the basis of traces. A forensic chemist thus seeks to use reliable methods and research procedures to obtain accurate data on studied samples. All the methods applied in the identification of traces allow determination of their most characteristic features (i.e. composition and properties). However, ascertaining the consistency of chemical composition and properties of studied materials is insufficient to state that they are identical. Knowledge is necessary about differentiation of the studied type of materials, variability within type resulting from non-compliance with technological norms, application and prevalence in the world around us. Knowledge of the circumstances of the course of the incident itself is also useful. That is why databases of defined types of materials that form criminalistic traces secured for examination at the scene of an incident (databases of paints, glass, plastics and fibres) are being created in individual criminalistic laboratories. Such databases contain both technological information about the products and the results of their laboratory examination.

Processing of research results leads to determination of the following conclusion: if as a result of conducted comparative physicochemical analyses, the properties and chemical composition of material forming the evidence trace are found to be consistent with those of the reference material, then, on this basis, the materials could have a common origin. This means, in the case of analysis of samples of paint, glass, plastic and fibres, that they could have constituted a single entity before

the incident. In the case of traces of soil, it means that they could have originated from one place in a given area. Categorical determination is not possible, because there is a finite but small probability that the studied materials originate from two different products, belonging albeit to the same type but, for example, from two production batches. Thus, their chemical compositions only differ insignificantly. On the other hand, if a difference is demonstrated in properties or composition of the compared materials, then one can assume that the studied materials are significantly different.

### *11.1.6 Examination of Chosen Microtraces*

The most common microtraces examined in criminalistic laboratories are so-called contact traces (i.e. small fragments of paint coating, glass, single fibres, soil, writing materials). Moreover, traces of flammable liquids originating from fire debris or traces pointing to the use of firearms are revealed and identified.

#### 11.1.6.1 Paint

Paint traces are revealed most often in connection with events such as car accidents, robberies or burglaries. They occur in the form of microfragments of paint, frequently with an area of several square millimetres or less, or visible smears of paint in the form of coloured streaks found on the clothing of persons involved in these events or on other substrates. The aim of paint examination is to establish the degree of similarity between the sample forming the paint trace and the sample originating from the suspect (from his vehicle, tools used in the act, etc.). Identification analysis is also carried out to determine the type of paint product, its use, the producer and the year of production. Routine examination of the paint encompasses establishing the colour and shade of the sample, the structure of the paint fragment and analysis of the chemical composition [3].

Most often, fragments of paint have a multilayer structure. Each layer (about 10–50 μm thick) is made up of paint material and is a mixture of many chemical compounds. Paint smears, on the other hand, are usually made up of one or two layers of paint material mixed with and sunk into the base (e.g. among fibres of the fabric). The morphology of the paint trace can be observed under an optical microscope. The number of layers visible on a cross-section of paint chip, their colour and thickness are characteristic for the coat of paint from which it originates (Fig. 11.1). The layers are often better visible if the sample is illuminated with polarised light or if fluorescence of sample is excited by illumination with UV light.

Colour is one of the most characteristic features of paint samples. It can be precisely described by comparison with the colour of standard paint samples from a catalogue prepared by paint producers. In the case of automobile paints, a good fit between the colour of the examined sample and the colour of a sample from a

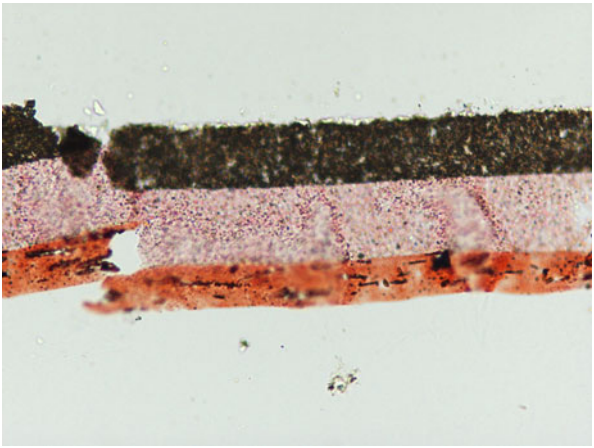

**Fig. 11.1** Cross-section of a car paint chip: microscopic image in transmitted light

catalogue enables definition of the model of the car and its maker. However, visual colour comparison is subjective and depends on proper sample illumination and the sharpness and quality of the vision of the observer. Microspectrometry in the visible range (MSP-vis technique) allows comparison of the colour of very small samples of various materials in an objective way, independently of the observer, without delving into the pigment composition of the analysed samples. Each colour can be described using three variables (hue, brightness and saturation), therefore each colour is represented by a single point lying in the colour space marked out by these variables. Overlapping of points in the colour space attests to the identical colour of samples. A mathematical way of establishing variables was elaborated about 40 years ago. Parameters of colour (chromaticity coordinates) defined on the basis of measured spectra serve in assessment of the similarity of the colour of studied samples. Modelling studies have determined threshold values, which are helpful in assessment of the differences and similarities in colour [4, 5].

Every paint contains binder, which is composed of synthetic resins and a combination of organic and inorganic pigments, extenders and decorative (effect) pigments. Pigments provide the coat of paint with its colour, whereas extenders are responsible for the decorative effects of a paint coat (e.g. covering and polish) and its resistance to the activity of atmospheric factors. Paints of the same colour can contain the same polymeric base but a different set of pigments and extenders, which depend on the use of the paint and the producer of the article. The composition of the polymer binder is routinely established using IR spectroscopy. Application of GC-MS for analysis of gaseous products of paint samples enables differentiation between resins (polymeric binders) belonging to the same chemical group. An example is shown in Figs. 11.2 and 11.3. Three paint samples contain the same type of polymer binder (styrene-acrylic-urethane binder) and the same main inorganic pigment (titanium dioxide). Their IR spectra are very similar, whereas their pyrograms are clearly different. This indicates that the polymer contents of the

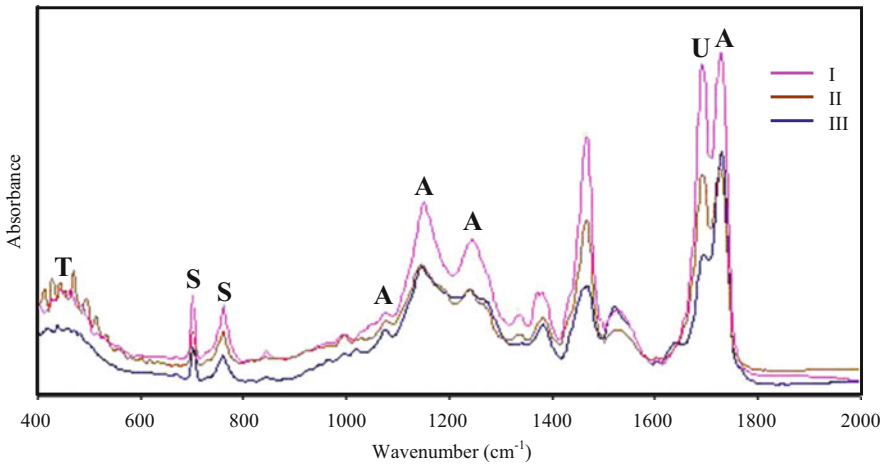


**Fig. 11.2** Infrared spectra of three styrene acrylic urethane paints (*I*, *II*, *III*). *S* styrene, *A* acrylic resin, *U* urethane resin, *T* titanium white

paint samples differ significantly and that there are, in fact, three different paints [6–10].

Inorganic pigments and extenders are usually identified on the basis of the elemental composition of the paint sample, established using SEM-EDX or XRF methods. The identification of pigment content is possible based on the elemental composition of a paint sample and data on possible pigment sets used in the paint industry. Organic pigments added to paint in a very small amount can be found only with the use of Raman spectroscopy. Comparison of the obtained Raman spectra with the spectra of standard pigments from a library provides identification.

The obtained analytical data allow a conclusion to be drawn about whether the compared paint samples could have originated from the same coat of paint or not. If the reference material (i.e. from the suspect) is not available, only establishment of the kind of paint and the type of paint coat is possible. In the case of automobile paints, it is also possible to find the model of car involved in an accident. For this purpose, analytical data are compared with a database containing information about the type of paint coatings (layering, chemical composition of each layer) used in various types and models of vehicles in Europe. Such a collection has existed in Europe since 1995 and is updated every year with information on new products. Experts from many criminalistic laboratories have participated in its creation. Comparison with such a database provides information about the make and model of the vehicle involved in the incident and its year of production. It is thus helpful in identifying the perpetrator's vehicle. It should be emphasised that identifying the make and year of production of a vehicle on the basis of a paint database only applies to vehicles with a factory-new coat of paint.

There is usually only a small amount of paint visible on clothing or other substrate. So, the amount of information about the coat of paint from which it

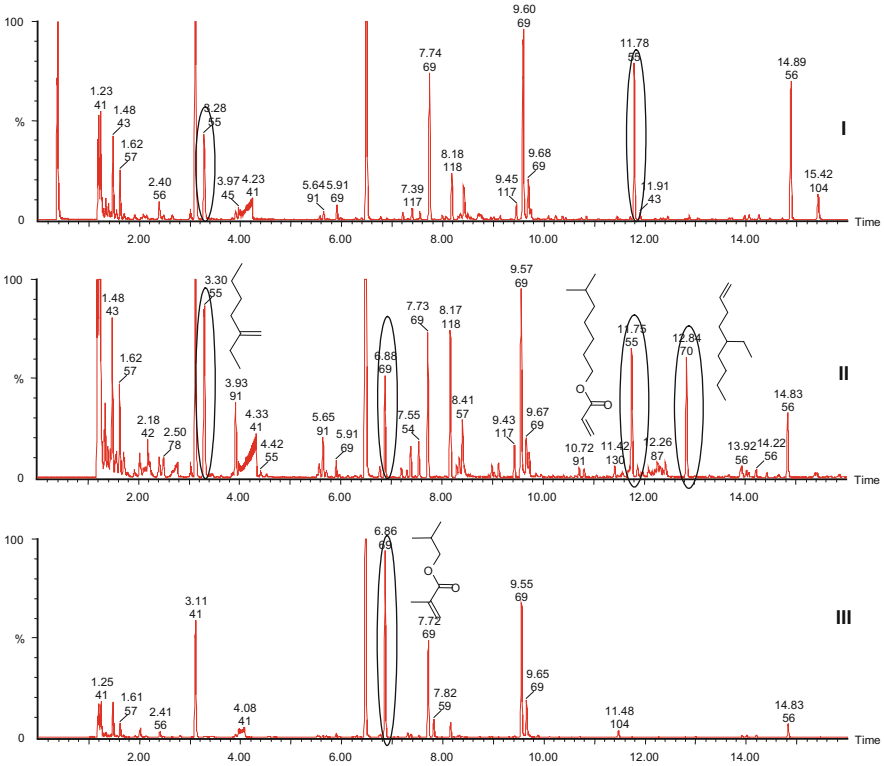


**Fig. 11.3** Pyrograms of three styrene acrylic urethane paints (*I*, *II*, *III*). The differences are marked with *ellipses*

originates is smaller than in the case of a paint chip. The evidential value of it is, therefore, smaller than that of a paint chip.

### 11.1.6.2 Glass Microtraces

Glass fragments are known to transfer to the clothing of a person breaking a window (e.g. in a house, shop or car). Traces in the form of glass fragments are also revealed in cases of traffic accidents, fights, robberies and shots. Small fragments of glass can dislocate several meters from the broken glass object and be kept (hidden) between fibres of clothing of the breaker for a long time. Fragments of glass have various sizes. Those found at the scene of the event are large fragments, whereas those revealed on the clothing, hair or body of persons (the glass-breaker) are small, with linear dimension less than 1 mm. Routine examination of glass fragments encompasses establishing their elemental composition and determining some physical properties, such as the refractive index [11, 12]. These data can be used by a forensic scientist for comparison of samples of glass, for

ascertaining the kind of object they could have broken off from (window, bottle, headlight) and, hence, for establishing their origin.

It is worth noting that the chemical composition and properties of glass are very similar, irrespective of the type and application of the glass. Technological advances in glass manufacture have led to less variability in physical and optical properties between products manufactured by different companies, and also to less variability between different types made by the same manufacturer. Consequently, the ability to distinguish between glass fragments (the discrimination potential) has been diminished.

The major raw materials employed for the manufacture of soda–lime–silica glasses are soda ash ($Na_2CO_3$), limestone (CaO) and sand ($SiO_2$). The other components of glass are different for different types of glass. The main elements (Na, Ca, Si and Al) are present in all glass categories at nearly the same level. The differences concern other elements originating from various additives used to improve the properties of the glass or in connection with its later application, or originating from impurities in raw materials used in the production process. Their concentration is significantly lower (at trace level).

The chemical composition of glass can be determined by many methods [13, 14]. Forensic scientists prefer non-destructive methods, allowing the sample to be examined using two or more analytical methods. Another desirable feature is the possibility of simultaneous determination of several elements (analytes), using the smallest possible amount of studied material. In the case of analysis of glass microfragments, these requirements are fulfilled by SEM-EDX and XRF methods. Other instrumental techniques such as inductively coupled plasma–mass spectrometry (ICP-MS) and its modification, laser ablation (LA) ICP-MS) [8, 9], are especially valuable. They enable evaporation of a glass sample using laser and give quantitative data on elemental content. These techniques enable elucidation of more than 30 traces of elements in glass samples.

The measurement of major (by SEM-EDS), minor and trace (by ICP-MS) elements is very important for discrimination and classification of samples into glass types. It is usually helpful to be able to classify the glass into a category such as sheet, container, vehicle window, vehicle headlamp or tableware. However, it is also necessary to apply statistical methods in the characterisation of glass evidence according to its elemental composition.

A thermo-immersion method is used for the measurement of the refractive index of glass fragments. It makes use of the change in the refractive index of immersion oil with temperature. Oil containing an immersed glass fragment is heated to a temperature at which the (observed) edges of the glass fragment disappear (i.e. up to the moment when the refractive indexes of glass and liquid are the same). The glass fragment in the immersion liquid on the microscopic slide is generally put directly on the heating stage of the microscope. Measurement of the refractive index is determined by a refractometer.

Means of refractive indexes of various glass samples differ only slightly. Therefore, various statistical methods are used for comparison of glass samples when differences in their refractive indexes are being evaluated. Such methods enable

evaluation of whether the observed differences are a result of instability of equipment, non-homogeneity of glass or different origin of samples. The comparative analysis of glass microtraces on the basis of their refractive indexes and elemental composition requires the application of statistical methods to evaluate the significance of observed differences and decide whether examined samples could originate from the same glass object.

#### 11.1.6.3 Fibres

Fibres are valuable criminalistic evidence. Fibres are a few millimetres long and loosely connected with the surface of clothing, curtains, rugs or furniture coverings. Every mutual contact between two people is accompanied by transfer of microfibres from clothing of one person to clothing of the other. Fibres are revealed mostly on clothing of people taking part in such events as murder, robbery or a fight. They are also found on underwear or under the nails of a rape victim. Fibres are revealed on the edges of an obstacle that was forced by the perpetrator (i.e. window, door or fence) as well as on tools used in the act (e.g. knife). During examination of a car accident, they could be found on a safety belt or seat covers and are useful in determination of the car driver. Moreover, recovery on the car body or chassis of fibres that are consistent with fibres of the victim's clothing confirms contact between the victim and a car.

Fibres are collected during optical examination of evidence [15, 16]. The aim of their examination is classification of fibre type and establishment of the type of textile from which they originate. During examination, chemical composition and some physical properties are established. The methods applied are optical microscopy, spectrometry in the UV-vis-IR range and Raman spectroscopy. Optical microscopy in transmission and reflection mode provides information on morphology as well as the shape of cross-section and thickness (Fig. 11.4). If polarised light is applied, the crystallinity can also be observed. The information obtained enables ascription of the fibre to one of the main types. Some synthetic fibres such as polyamides (PAs) and polyethylenes (PEs) have a very characteristic appearance in polarised light; therefore, it is possible to differentiate between fibres of similar colour and morphology observed in white light. The presence of fluorescence, originating from pigments, additives and detergents, is also helpful in identification


**Fig. 11.4** Microscopic images of common fibres: (**a**) cotton, (**b**) wool and (**c**) acrylic

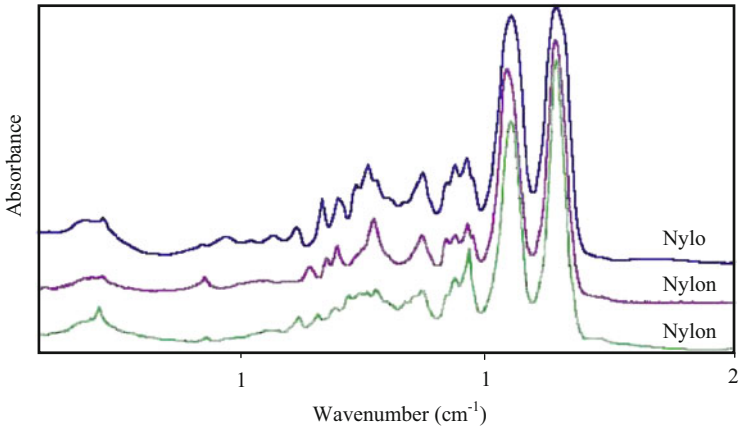


**Fig. 11.5** Infrared spectra of different polyamide fibres

of fibres. Generally, it is possible to differentiate between natural and synthetic fibres, to identify some kinds of samples and to determine the distribution of pigments and additives in fibres. The main components of fibres are natural (e.g. cellulose, casein, keratin) or synthetic (e.g. PA, PE, polyacrylonitrile, polyolefins) polymers. They can be identified using IR spectroscopy (Fig. 11.5). The readable spectrum of single short microfibres can be obtained using microscopy or the diamond cell technique. It is possible to differentiate between polymers within the same chemical group; for example, different polyamides have different spectra in the range 1000–15,000 $cm^{-1}$. Normally, pigments and dyes are not visible in the IR spectrum of a fibre. For their identification, Raman spectroscopy is applied [9] and the spectra clearly show peaks originating from pigments, dyes and extenders.

For comparison of the colour and shade of fibres, UV-vis microspectrometry is routinely applied. Consistent spectra obtained for compared fibres confirm consistent pigment content; however, the pigment and dye set are not identified. Black and colourless fibres are exceptions because they have no colour (i.e. the most characteristic feature of a fibre) and so cannot be compared in this way.

Information about the type of fibre and its components helps in comparative analyses that aim to state similarity between transferred fibres and fibres of the suspect's clothing. The identity of analysed features provides the conclusion that the fibres originate from the same fabric, which means that fibres from the clothing of one person were transferred to the clothing of another. The evidential value of revealed fibres is different and depends on the kind of fibres. The most prevalent fibres (i.e. white cotton) have the smallest evidential value. They originate from underwear, bed linen or dust and so their recovery does not mean that they were transferred from the clothing of the suspect. Similarly, the value of jeans fibres is small because of the popularity of jeans clothing. The significance of fibres that are seldom met in the environment is greater.

#### 11.1.6.4 Gunshot Residue

Examination of gunshot residue (GSR) plays an important role in establishing some circumstances of a crime involving use of a firearm. This kind of examination is complementary to ballistic examination of weapons and ammunition.

Powder gases leaving the barrel of a firearm contain products of explosive reactions of both the primer and the propellant, as well as products of interactions of these materials with other parts of the cartridge and weapon. The chemical composition and properties of GSR depend directly on the kinds of materials used in production of the ammunition. The most characteristic GSRs are metallic particles arising from components of the primer, demonstrating characteristic morphology (size of the order of micrometers, approximately spherical shape) and specific chemical composition (lead, antimony and barium in the case of lead ammunition) (Fig. 11.6). Found around the gunshot hole and on the clothing and body of the shooter, GSR provides information on which to base, among other things, inferences about the shooting distance and the kind of ammunition used (and thus the weapon). Most importantly, GSR serves to link the suspect to the shooting [17–21].

Micron-sized particles, having a morphology consistent with rapid cooling, form a liquid state. Such GSR particles contain elemental combinations of either lead/barium/antimony or barium/antimony and are unique to detonation of the primer of a round of ammunition.

For many years there was no sufficiently specific method for the identification of characteristic GSRs. One could not see metallic particles because of their small size (5–50 μm) and their presence was ascertained indirectly by means of colouring chemical reactions or such instrumental methods as atomic absorption spectroscopy (AAS), neutron activation analysis (NAA) or XRF. These methods, however, are

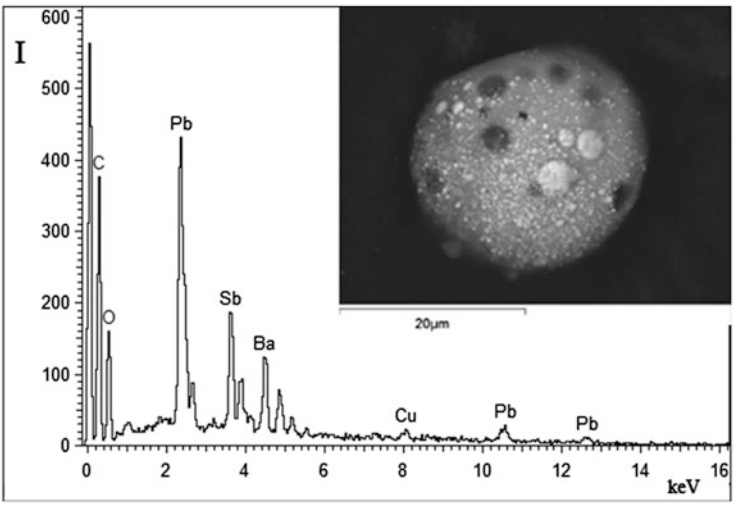


**Fig. 11.6** Gunshot residue particle (*inset*) and its elemental composition

not specific and record all impurities, independent of their source of origin. The most successful technique to date for the analysis of GSR particles is, without doubt, SEM-EDX [17].

Only a little sample preparation is needed. Mostly, half-inch diameter aluminium stubs with an adhesive layer of double-sided tape are used for sampling. When the sample has been secured on the stub, it is then examined for spherical metallic particles of defined diameter and chemical composition. The method has many advantages, but its basic drawback is that it is time-consuming if carried out manually. Suitable software for automatically searching through the secured material on the stub (in order to detect particles with specific features) shortens the time of investigation significantly [17].

According to the formal approach to evaluation of analytical results, metallic particle classification can be carried out in the following way: the most characteristic particles (Pb, Sb, Ba) are singled out, followed by indicative one- and two-component particles (e.g. antimony, lead, lead/antimony), which always accompany the former and occur in considerably greater quantities. Finding GSR on material received from a suspect confirms his or her participation in the event, meaning that the person was shooting, present in the near vicinity of a firing gun or came into contact with an object highly contaminated with GSR.

Because the technology of ammunition production has changed towards leadless solutions, the evidential value of GSR should be individually established for every case in a case-by-case approach. For this, knowledge of the persistence and prevalence of GSR in certain environments is crucial [17, 18].

Solid particles of material other than metal, such as powder grains and the products of their conversion, found on targets not far from the gun can be identified from their IR spectra, taking into account the main composition of smokeless propellants (i.e. nitrocellulose or nitroglycerine). Components occurring in smaller amounts (e.g. diphenylamine or centralites) can be identified by means of Raman spectrometry. This information can be used to determine the shooting distance, to recognize the entrance and exit gunshot holes, as well as for differentiation between residues from various types of ammunition [18].

Volatile and gaseous products of propellant combustion remain inside the gun barrel and cartridge case for some time and their detection and quantitative determination by means of gas and liquid chromatographic methods (thermal desorption GC-MS, GC-thermal energy analysis, LC-MS/MS) can help in establishing, whether the gun was fired up to 3 days, 2–3 weeks or more than 3 weeks before examination [19, 20].

#### 11.1.6.5 Fire Debris

The identification of various flammable liquids that can be used to start a fire is one of the tasks of the forensic laboratory. These products (usually petroleum products) include gasoline, fuel oil, lubricant oils and diluents. They are often used by perpetrators because of their physico-chemical properties, such as volatility and flammability.

The spilled flammable liquids sink into the base (e.g. soil, textile, wood). The burning process only takes place on the surface of the base. Deep down, liquids hardly evaporate and burn very slowly; some are occluded in porous materials created from synthetic materials during the fire. That is why it is possible to find traces of flammable liquids in fire debris in spite of them being burnt out of almost everything [23, 22].

The fire scene is carefully examined after a fire has been extinguished. If arson is suspected, samples for laboratory analysis are collected from the place that is considered to be the probable source of the fire. Usually, such examinations are limited to a search for traces of flammable liquids, which are commonly used as accelerants. Although the problem of analysis of fire debris is not a new issue, detection and identification of flammable liquids still remains a challenge. Difficulties arise because there are many different commercially available agents that can be used to light fires, most of which are multicomponent mixtures. They contain hydrocarbons (petroleum products) mixed with other solvents and flammable liquids (e.g. ether, alcohol, turpentine) and condensed by adding substances such as resins or plastics. Mostly, liquids such as benzene, kerosene, motor oil, alcohols and solvents are present. In the course of a fire, compounds contained in these mixtures evaporate to different extents, and some of them undergo thermal decomposition [22]. Burnt materials could also undergo pyrolysis, which results in the presence of numerous interferents in the sample, making analysis more difficult. Therefore, accelerants present in a sample of fire debris are available only in trace amounts and, moreover, their chemical composition significantly differs from the composition of the unburnt liquids An additional factor that can influence the effectiveness of detection of accelerants is the use of various fire-fighting agents, which introduce additional substances to the analysed material.

The main method applied in analysis of fire debris is GC. The analysis of fire debris has three stages. The first stage is isolation of accelerants from the matrix and their concentration, followed by separation of particular components and their chromatographic analysis and, last, identification of potential accelerants. The efficiency of the first stage strongly determines the possibility of identification of the isolated and adsorbed organic compounds. An improperly performed first stage could make it impossible to identify the questioned substances.

The isolation and concentration of petroleum products can be performed in several ways. The most efficient method is passive adsorption. In this method, the sample along with a tube filled with Tenax TA adsorbent is placed in a thermostated (60–70 °C) tightly closed container, such as a glass jar, for over 10 h. Under these conditions, a balance between compounds present in the headspace of the sample and the sample adsorbed on the polymer adsorbent is established. Adsorbed compounds are subjected to thermodesorbtion; then, the desorbed compounds together with the carrier gas are injected onto a GC column, where they are separated and then identified. This approach has enabled easy detection and identification of trace amounts of petroleum products. Headspace analysis with passive adsorption on Tenax TA is normally used for separation and concentration of analytes. Gas chromatography coupled with an autothermal desorber and a mass spectrometer (ATD-GC-MS) is the best technique for separation of multicomponent mixtures

and identification of components in the analysed product. GC-MS enables qualitative and quantitative analysis of traces of flammable liquids.

Interpretation of the obtained chromatograms must take into account the changes that flammable liquids undergo during a fire. One change is evaporation of light volatile compounds from the examined material, causing a decrease in the content of volatile components and an increase in content of components of lower volatility. The second change is thermal breakdown of components of liquids and burnt materials. The effect of this is appearance of volatile substances that were absent in the flammable mixture at the beginning, which makes it harder to identify the liquid used to start the fire. It is also possible that these compounds were present in burnt material at the beginning and are the components of burnt material.

During experiments carried out to study whether various fire-fighting substances influence the detection of traces of accelerants in samples taken from a fire scene, it was suggested that the application of extinguishing foam is linked to introduction of substances into the samples. Although these substances might be detected in the process of analysis, their presence does not hinder identification of the accelerant, provided the person conducting the analysis knows how to interpret the obtained results correctly.

Currently applied methods enable easy recovery of trace amounts of flammable liquids, provided that samples are properly collected from the place of fire, saved in hermetic containers and sent directly to a criminalistic laboratory. Figure 11.7

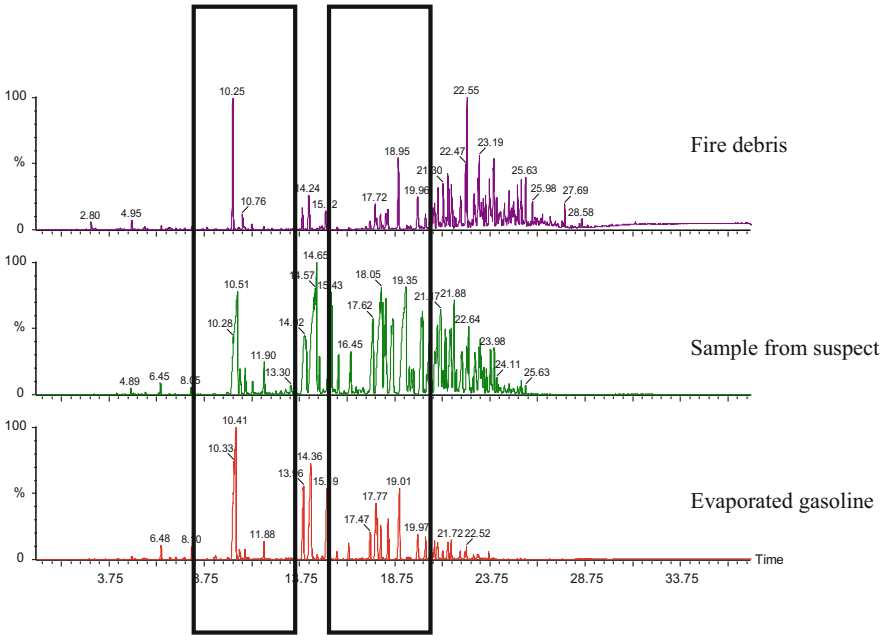


**Fig. 11.7** Chromatograms of samples of fire debris, from the can found in the subject's possession and gasoline evaporated to 50 %. The characteristic patterns are marked with *rectangles*

shows the results of analysis of fire debris collected from an office. Comparative material was found at the suspect's house in an empty plastic container. Chromatograms were obtained for debris and for vapour from the recovered gasoline container. On both chromatograms, characteristic profiles consistent with the profile of gasoline evaporated by 50 % were visible. It was concluded that the flammable liquid used to start the fire was gasoline.

#### 11.1.6.6 Writing Materials

Chemical analysis of writing materials such as inks and toners is a very important part of forensic examination of questioned documents. The investigations are most often aimed at authenticating a document or determining its age or origin. Non-destructive analytical methods, such as microscopic and optical techniques, are applied first in routine examinations of inks. Selected parameters of the ink, such as its colour, luminescence and absorption of radiation can be determined using these methods [24]. Use of transmission, reflection and fluorescence spectra in the range 220–900 nm makes it possible to differentiate samples obtained for inks, ballpoint pen inks and printing paints placed on the surface of paper in a more objective way than by comparing their colours using a tintometer.

However, such optical and spectrometric methods do not provide information on all components of ink and do not allow identification of ink on the basis of its chemical composition. They take into account only those components that strongly interact with a given region of electromagnetic radiation and, therefore, they merely show differences between compared samples. Ballpoint pen ink contains a dye or several dyes in a viscous liquid, which is a mixture of natural or synthetic polymers and an oil or olein. The ink also contains acidic compounds, which decrease its coefficient of friction during writing, and substances that inhibit drying of the paste and ensure its suitable viscosity. The detailed recipes are patented. In the literature, there is no information on the composition of gel inks. It is only known that, apart from other components, they contain insoluble pigments.

As a rule, chemical methods used in the examination of writing materials require initial preparation of a sample for study. Paper chromatography, thin-layer chromatography and capillary electrophoresis are experimental techniques often applied. These methods lead primarily to separation of the dyes contained in the ink under examination and to the discrimination of ink samples. The techniques are simple to use, require a small amount of sample for examination, are selective and give reproducible results. Their basic disadvantage, however, is the necessity to isolate the ink from the substrate (e.g. paper) on which the examined document has been prepared. Solvent extraction of the ink often leads to partial damage of the document.

Spectrometric methods such as IR spectroscopy give information on the main components of the examined samples (dyes, resins and oily liquids). The main pigments are easily detectable in the IR spectra of inks. Because of its non-destructive nature, Raman spectroscopy is applied in forensic investigations for the identification of inks directly on a document, and for determination of the

sequence of handwritten lines. Attempts have been made to utilise this method to examine the process of ageing of inks. Additionally, HPLC and GC-MS, as well as quantitative elemental analysis, are used for identification of some ink components. Each method applied for ink examination has its advantages and limitations. In practice, several analytical methods are required for characterisation and identification of inks [24].

Differentiation of inks through their IR spectra is based on the position of peaks and their relative intensity. In Raman spectroscopy, the course and shape of the background curve, which depicts the fluorescence intensity of the examined material, is also relevant and should be taken into account.

The elemental composition is useful for characterisation of inorganic pigments and organic dyes. However, a database is needed for their identification. In all ballpoint pen inks, sulfur, copper, silicon and phosphorus are present in the elemental composition. Some samples also contain zinc, chlorine, bromine and calcium. In black inks, chromium and lead are additionally found. Samples differ with respect to the elemental composition quantitatively rather than the qualitatively. A greater variability in elemental content is observed for gel inks.

Toners are another type of writing material used in printers and copiers. They are black or coloured and contain grains of synthetic polymers with characteristic plastic and electrostatic features. During printing, the grains of toner are connected with the paper, but it is easy to remove them without damaging the surface of the paper. Their size, shape and composition depend on the kind of toner. Using IR spectroscopy it is possible to identify these polymers (e.g. styrene, polyethylene, acrylates and methacrylates). Elemental composition using SEM-EDX has identified iron as the main element in ferromagnetic toners (Figs. 11.8 and 11.9). In non-ferromagnetic toners, the main elements are Zn or Cr and the content of iron is smaller. The determination of polymers and elements makes it possible to classify toners into several groups and sometimes to identify the kind of printer [25–27].

### *11.1.7 Conclusions*

In forensic examination of different materials, various analytical methods are applied. These methods should enable analysis of small amounts of samples (milligrams or micrograms) and be non-destructive, making it possible to repeat analysis using the same or different methods. The methods are quick and simple and do not require time-consuming sample preparation before analysis. They provide information about chemical composition and about some physico-chemical features of analysed samples, enabling group identification of the examined sample. Forensic chemists look for microcomponents of the examined sample or features that are distinctive and enable differentiation of the sample from other samples belonging to the same chemical group. Therefore, in forensic examination it is necessary to examine small amounts of sample and to identify trace components, because the main components do not usually characterize the examined criminalistic trace.

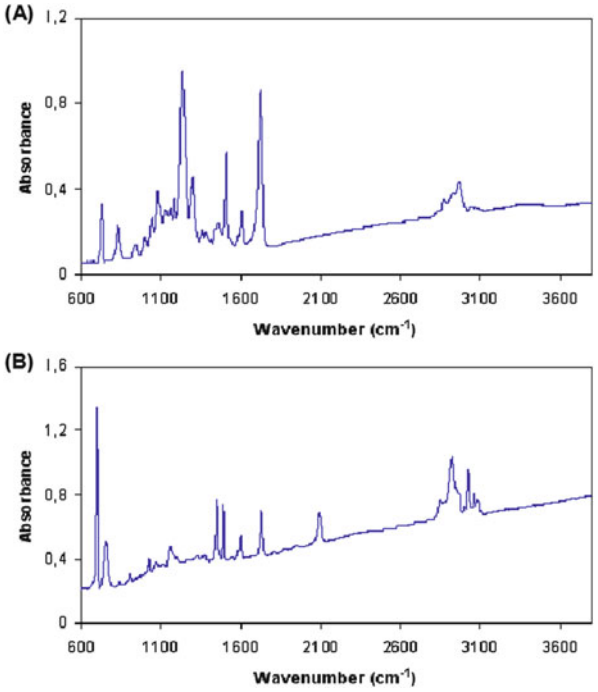


**Fig. 11.8** Infrared spectra of two different toners (**a**, **b**)

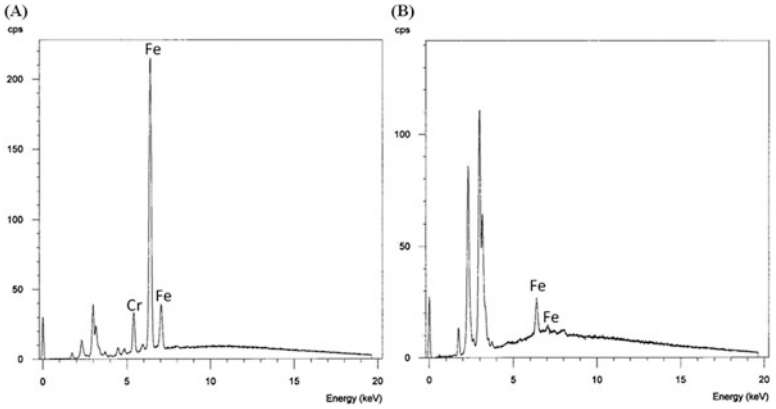


**Fig. 11.9** XRF spectra of toners: (**a**) ferromagnetic toner and (**b**) non-ferromagnetic toner

Forensic chemistry applies new and sensitive analytical methods in identification of traces and collects technological information about various materials to create a database. For the evaluation of results obtained, statistical methods enable assessment of significance of differences observed between the examined samples, as well as the statistical error.

## 11.2 Toxicological Analysis of Microtraces

Maria Kała

### *11.2.1 Introduction*

Toxicology is the science of poisons. It was established as a separate academic discipline, separating from forensic medicine, at the beginning of the nineteenth century. At that time it dealt only with detecting poisons in autopsy material; in other words, it assisted forensic medical doctors in issuing opinions on the cause of death in cases of poisoning. Since then, toxicology has developed in many directions and is currently an interdisciplinary science. It builds on the achievements of the basic sciences and cooperates with various applied disciplines. This has led to specific research areas being separated into areas such as the toxicology of drugs, pesticides and food; environmental, industrial, clinical and forensic toxicology; and various sub-disciplines (e.g. toxicological analytics). Modern toxicology deals with the analysis of qualitative and quantitative effects of the harmful action of chemical compounds on living organisms.

This paper presents contemporary toxicological analysis for the presence of poisons, which are increasingly frequently detected in the human organism at very low concentrations. It starts with a discussion of those elements of toxicology that are of greatest relevance to (toxicological) analyses performed at the behest of the administration of justice. Attention is paid to types and sources of poisons, the influence of the route of introduction of the chemical compound into the organism on the course of poisoning, the type of compound whose presence must be demonstrated in order to confirm or rule out exposure, the scope and directions of toxicological analysis, and research methodology.

Currently, at least 1200 scientists, members of The International Association of Forensic Toxicologists (TIAFT), work in the field of toxicological analysis for the needs of the administration of justice. Two reference books hold a key position in the work of forensic toxicologists (the issuing of expert opinions). One is the extensive monograph *Clarke's analysis of drugs and poisons* [28], bearing the name of an eminent English toxicologist and currently available in its fourth edition. The second work, *Disposition of toxic drugs and chemicals in man*, is updated every couple of years (the tenth edition is currently available) [29].

### *11.2.2 Types of Poisoning*

The harmful action of chemical compounds, affecting humans and other forms of life, can occur everywhere (i.e. in the wider human environment, including the workplace). Poisoning can occur accidentally or as a result of deliberate human action (suicide, murder). Depending on the speed of development of the harmful

action of a toxic compound on the organism, the poisoning can be defined as acute (quick development of harmful changes after taking a single dose), sub-acute (changes occur less rapidly) or chronic (action of poison in small doses over a long period of time). From these definitions, it is clear that we are dealing with varying amounts of toxic factor. A large single dose of a toxic compound usually results in a high concentration in the organism, whereas small multiple doses lead to low concentrations of the compound and/or its metabolites (i.e. products formed in the organism as a result of biotransformation processes). Analytical methods applied to confirm or rule out the presence of a chemical substance in the organism of a human must be characterised by high accuracy, sensitivity and specificity.

### *11.2.3 The First Poisons*

Harmful chemical compounds called poisons have accompanied humanity since ancient times. The first poisons were substances of natural origin, that is, toxins (produced by plants), venoms (produced by animals) and mineral substances (As, Sb, Sn and Cu). Dynamic advances in the synthesis of chemical compounds, initiated at the turn of the nineteenth and twentieth century, as well as the rapid progress of civilisation, led to introduction into the human environment of a huge number of synthetic chemical substances. Currently, therefore, we are faced with an increasing number of toxic substances of natural and synthetic origin.

**Toxicity** Since ancient times, people have been aware of the dual nature of chemical substances. Many substances are applied in low doses in the treatment of diseases, and these same substances are used as poisons in high doses. Not all chemical substances introduced into the living organism or produced in it are equally harmful. Very toxic substances cause dysfunction or death of an organism in amounts (doses) of a few drops or tens of milligrams. The lethal dose for relatively harmless or practically non-toxic substances is defined at a level equal to or greater than a kilogram. The relationship between dose and toxicity was described by the father of toxicology, Theophrastus Bombastus von Hohenheim Paracelsus, in the sixteenth century in the first definition of poison: "All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing poison." This definition remains true to the present day, taking into account not only the dose (administered or absorbed), but also the route of administration (oral, inhalation, intravenous or dermal) and frequency of administration (once or many times).

### *11.2.4 Modern Spectrum of Poisons*

**Factors Affecting the Course of Poisoning and Interpretation of Results of Toxicological Analysis** These days it is rare to come across poisoning as a result of a single substance or high dose of low-toxicity chemical substance. Most frequently, poisonings are caused by introduction into the organism of a mixture of many substances. This leads to "interaction" effects of these substances. As a result of interaction, the action of many substances gives results that are qualitatively or quantitatively different from the predicted actions, arising from the sum of the effects brought about by the individual components of the mixture. Thus, small doses of various compounds lead to low concentrations of each separately, and can result in severe intoxication. Revealing all the components of an administered mixture in biosamples (body fluids and/or sections of internal organs) is important in assessment of the severity of poisoning and requires application of sophisticated, very sensitive, specific and selective analytical methods.

Many substances (agents) that are a threat to the health or even life of the consumer can be the subject of toxicological analysis. In view of our ability to detect smaller and smaller amounts of xenobiotics (chemical substances that are not natural components of a live organism) in the analytical process, interpretation of the obtained results assumes particular significance. Interpretation must take into account both the concentration of the detected xenobiotic and the individual sensitivity of the victim. Individual sensitivity varies according to the properties of the xenobiotic and intra-individual factors (i.e. race and sex). Furthermore, sensitivity changes with age and is linked with congenital and acquired disease states (allergies, diseases of civilisation) and genetic defects, which are more and more prevalent. Drug addiction, addiction to medication, polytoxicomania and polypragmasia (abuse of drugs that harm health), which frequently lead to dependence, tolerance (long-term drug use necessitating an increase in dose in order to induce the desired effects) and abstinence syndromes, are currently widespread. The broad use of polytherapy (treatment with several medications) has resulted in the availability of many different types of pharmaceutical drugs.

**Medications** In cases of poisoning by a single harmful agent or a mixture of medications and/or toxic substances, compounds of various chemical nature and numerous metabolites are present in biological material. The possibility of occurrence of various types of interaction (synergistic, additive, hyperadditive and antagonistic) at each stage of the course of poisoning (absorption of the xenobiotic, distribution in the organism, biotransformation, action and excretion) after administration of several drugs makes it much more difficult to interpret the result in terms of the severity of the poisoning and adapt remedial treatment. Independent administration of medications, without consulting a medical doctor, is noted increasingly frequently. The type and dose of substances introduced into the body are rarely known. Knowledge of the type and toxicity of the compound facilitates the choice of an appropriate method and suitable material for analysis.

Currently, substances that are characterised by many times the toxicity of compounds regarded as the greatest poisons (cyanide, As, Tl) are used in medical treatment and are thus potentially (widely) available. A striking example of the dual nature of a substance is the botulinum toxin, which is an exceptionally strong poison but finds application in medicine (for treatment of spasticity of muscles, involuntary muscle contractions and excessive sweating) and even in the cosmetic field for aesthetic purposes (i.e. temporary reduction of wrinkles). Forensic toxicologists increasingly frequently encounter an expanding assortment of drugs used for in-patient healthcare, each dose of which when introduced into the human organism without medical supervision can be fatal. Use of such drugs during an unsuccessful operation necessitates asking whether the therapeutic dose of the medicine had been exceeded.

**Environmental Poisons** Environmental and industrial poisons constitute another group of very toxic compounds. Among the environmental poisons are dioxins, which humans themselves produce by burning various types of waste in their homes. As a result of increasingly stringent requirements concerning safety at work, industrial poisoning most frequently occurs accidentally or as a result of negligence. In spite of the fact that acute poisoning by inorganic compounds (metals, semi-metals and anions) is becoming increasingly rare, its significance in contemporary toxicology cannot be overlooked. People living in heavily industrialised regions are more exposed to low doses of industrial and environmental poisons as a result of environmental contamination. Food, atmospheric air and water from these areas are constant sources of exposure (of inhabitants) to small doses of metals, which can accumulate in the body. Ruling out chronic poisoning, which may have occurred in conditions of environmental or industrial contamination, requires knowledge of normal levels of metals (i.e. their concentrations in the organs of non-poisoned persons). Metals can be divided into the following groups: very toxic (As, Be, Cd, Cr, Pb, Hg and Ni); essential for the correct functioning of the human organism, but characterised by a high potential for toxic action (Co, Cu, Mn, Se, and Zn); and low toxicity (Sb, Ba, In, Mg, Ag, Te, Tl, Sn, Ti, U and V). Such categorisation is useful in the assessment of exposure of humans to metals. A fourth group is made up of metals whose toxicity results from their application in therapy (Al, Bi, Ga, Au, Li and Pt). Detection of toxic anions such as nitrates(III) and nitrates(V), fluorides, oxalates, chlorates, phosphides and sulfides is also within the area of interest of toxicologists. Many of these are used as artificial fertilizers, in the household, and even in the food industry.

**The Drugs (Narcotics) Market** An unlimited source of substances that are the subject of contemporary toxicological analyses is the drugs (narcotics) market. It has long provided a broad range of synthetic substances (e.g. amphetamine and numerous derivatives) and substances of plant origin (e.g. marijuana, hashish, hallucinogenic mushrooms) that are under international control. Here, they are classed together under the term “classic psychoactive drugs”, enabling discussion of these agents to be kept brief. Changes in drugs available on the Polish drugs (narcotics) market over the years, from substitutes for drugs of abuse to the current

drug scene, have already been described many times [30, 31]. However, it is impossible not to mention the large group of new compounds, the so-called designer drugs, which are structural analogues of controlled substances. Among them, the following are most prominent: derivatives of phenylethylamine, cathinone, piperazine, tryptamine and the so-called synthetic cannabinoids. Many of these substances are smuggled, but they can also be produced in large illegal clandestine laboratories. Another group consists of home-made substances, for example, from medicines available without prescription [32, 33]. Many substances that are newly introduced onto the drugs market are characterised by increasingly strong action. Unpredictable mixtures of known substances have become the cause of lethal poisonings of many uninformed users [34]. In 2006, genetically modified cannabis appeared on the drugs market, containing small amounts (below 0.20 %) of $\delta^9$-tetrahydrocannabinol (9THC) and a high content of $\delta^9$-tetrahydrocannabinol-2-carboxylic acid (9THCA-A), which is converted to 9THC during smoking. In order to determine whether cannabis secured by the police is subject to the provisions of the Act on the Prevention of Drug Addiction (i.e. whether it is of the fibrous or narcotic variety), methods allowing determination of each compound separately (high-performance liquid chromatograph, HPLC) or both together (gas chromatography, GC) should be applied to their analysis [35].

**Online Shopping** Online purchases from websites remain beyond any sort of control. It is possible to put anything from anabolic steroids, through doping agents to various slimming products, especially natural products of Chinese origin, into your "basket". The latter are advertised most frequently as safe preparations of plant origin that aid slimming, but often contain large doses of synthetic compounds with the structure and activity of amphetamine derivatives. Furthermore, some Chinese herbal preparations have such a high lead content that if they are administered long term they engender symptoms of poisoning by this metal. The same applies to pesticide residues contained in these products.

**New Psychoactive Substances** The term "new psychoactive substances" (NPS) (legal highs, boosters) is used to describe various types of preparations or substances contained in them, which are sold as collectors' items, bath salts, agents for rinsing river stones, plant care agents, incense and the like, not intended for human consumption but in fact used for intoxication purposes. These products are often advertised as "legal alternatives to drugs". The attractive names of these substances, for example, Energy pills, Euphoric pills, Psychedelic pills, *Salvia divinorum* (Diviner's sage), Magic garden, *Amanita muscaria* (Fly agaric) and Indian warrior, under which they are available in brick-and-mortar and internet shops, and even by telephone order with home delivery (as well as assurances about their low harmfulness), have encouraged many young people to experiment with them, often ending in poisoning. Since November 2010, the psychoactive components of many of these types of products have been placed on the list of substances controlled by the Act on the Prevention of Drug Addiction. Various types of preparations were and are on sale, from powders and tablets to capsules with herbal mixtures. Laboratory studies have shown that, in most preparations, the plant

material only constituted the carrier onto which synthetic cannabinoids were deposited (JWH-type, followed by a digital symbol such as 081, 007, 018, or similar) [36]. Since 2008, the presence of more than 30 synthetic cannabinoids has been ascertained in legal-high products around the world. In the last 10 years in Europe, about 100 new substances with psychoactive activity have been discovered. According to the reports of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the largest group is phenethylamines (32 compounds), then tryptamines (22), cathinones (15) and piperazines (12) [37, 38]. The mass spectra of these new compounds, together with the spectra of various additives present in commercial preparations, have been published in a combined study [39].

**Date-Rape Drugs** The colloquial term "date-rape drug" encompasses about 70 different drugs or psychoactive substances that are applied to facilitate commission of a crime (rape or looting). A typical scenario of such an event consists in adding such a substance unnoticed to a drink, which the potential victim of the future crime consumes. The victim quite quickly loses awareness of what is happening, and all memory of what has happened is impaired. After regaining consciousness, amnesic symptoms are accompanied by physiological disorders such as lack of spatial orientation, dizziness, drowsiness, difficulty moving, nausea and sometimes hallucinations. Sometimes the victim sees the attacker but cannot defend him- or herself, because the administered substance has caused difficulty in moving or temporary paralysis. Generally, the victim's recall of the event is so unclear that s/he is not a credible witness for courts and other judicial bodies. In such situations, only chemico-toxicological analysis of body fluids can confirm the use of a pharmacological substance. Usually material for testing is collected after several days, and thus the applied methods must be very sensitive and encompass many compounds and their metabolites [40, 41].

**Agents Acting Similarly to Alcohol** Use of agents acting on the central nervous system modulates the behaviour of people and affects driving ability. That is why provisions of the Road Traffic Act forbid driving of a vehicle by a person "in a state after use of alcohol" and "in a state after use of substances acting similarly to alcohol". Drivers can be subjected to tests for the presence of these substances at the place where they are stopped by police, using methods that do not require laboratory facilities. Breathalysers are used to demonstrate the presence of alcohol in a driver's body. These devices enable determination of alcohol in exhaled air. This result has evidential value. Drivers are controlled for the presence of substances acting similarly to alcohol on the basis of observation of symptoms of action of these substances on the body of the driver, as well as by using on-site oral fluid drug screening devices. Such tests enable determination of illegal compounds from five groups (opiates, tetrahydrocannabinols, cocaine and its metabolites, amphetamines and benzodiazepines) in drivers' saliva. Each positive result of analysis using an on-site oral fluid drug screening device must be confirmed by a blood test carried out on the driver using laboratory methods such as GC or HPLC) with various types of detection, especially mass spectrometry (MS). Only then does the result of toxicological analysis constitute a basis for prosecuting a driver. The applied

laboratory methods must be characterised by limits of quantification (LOQ) imposed by the Order of the Minister of Health of 16 July 2014. All LOQ values are defined at the level of nanograms per millilitre of blood and are 1 ng/mL for 9THC, 10 ng/mL for morphine and cocaine, 25 ng/mL for amphetamine and its derivatives and 100 ng/mL for benzoylecgonine. The LOQ has not been established for drugs of the benzodiazepine group, because of the numerous derivatives applied in very diverse doses (varying from 2 to 25 mg), which result in the occurrence of a very broad range of concentrations in blood [42].

**Toxins and Venoms** Because human interest does not always develop in the right direction, the number of venoms and toxins that the forensic toxicologist can encounter in his or her everyday work is growing. Several hundred toxins in various species of plants, and venoms in many species of marine organisms, poisonous fish, venomous snakes, tics, scorpions and spiders are known. Furthermore, there are species of birds whose feathers can be triggered to act toxically almost by touch alone. On occasion, such exotic specimens, for example, acquired during travel abroad or bought over the internet and then bred in unsuitable conditions (and sometimes abandoned) may become the cause of laborious toxicological tests of body fluids taken from a bitten victim.

### *11.2.5 Routes of Introduction of Poison into the Body*

A toxic agent can enter the body by various routes. The development of poisoning depends primarily on the toxicity of the substance, which is linked to the dose but also the route of administration. Intravenous xenobiotic administration results in the entire dose being quickly introduced into the organism. The toxic dose is smallest for a poison given intravenously. A dose of xenobiotic given orally is not always completely absorbed into the organism and, therefore , the administered dose is not always the acting dose. Part of the administered dose is removed as a result of natural defensive mechanisms of the organism against poisoning, for example, vomiting. A large part of the administered dose of many xenobiotics first passes from the stomach via venous blood through the portal vein to the liver, where it is metabolised, and then goes into the systemic circulation in the form of metabolites. This process is known as the first-pass effect. If the metabolites are less toxic than the administered (parent) substance, then the toxic effect is reduced. In the opposite case, when the metabolites are more toxic than the administered substance, the course of intoxication is more severe. Volatile xenobiotics (toxic gases and solvents) enter the body through the respiratory system (by inhalation) and, with air, enter the alveoli, which are richly supplied with blood and have a large surface area. A large momentary dose of xenobiotic can result in the occurrence of very rapid changes, which does not, however, translate into a high concentration in the bloodstream (and by the same token in the organism). Many poisons, such as organophosphate pesticides, are absorbed through the skin and not only have

negative health effects, but can also be a threat to life itself. Other routes of administration, such as topical, rectal and intramuscular, have great significance in medicine, but are also encountered in cases of suicidal and deliberate poisoning, for example, dowsing a victim with a corrosive or burning substance. Depending on the route of introduction of the xenobiotic into the organism, the analyst has to deal with a lower or higher concentration of xenobiotic and/or its metabolites.

### *11.2.6 Symptoms of the Action of Poisons*

The effect of the action of a toxic compound on an organism is the occurrence of symptoms of its action. There is practically no symptom that is specific for a single specific compound. Recognised symptoms of the action of a toxic compound (i.e. a syndrome of clinical features, toxidrome) can usually be related to poisoning by a given group of compounds. The most frequently mentioned toxidromes are anticholinergic syndrome (antihistamines, scopolamine, atropine, Jimson weed, tricyclic antidepressants), cholinergic syndrome (organophosphate compounds, derivatives of carbamic acid, mushrooms), hallucination syndrome (ring derivatives of amphetamine, LSD, hallucinogenic mushrooms, Diviner's sage, cocaine, cannabinols), opiate syndrome (opiates, opioids, fentanyl), sedative-hypnotic syndrome (barbiturates, benzodiazepines, ethanol) and stimulant syndrome (amphetamine and its chain derivatives, cocaine). Smellable (odoriferous) and perceptible changes are also useful for directing toxicological analyses. In particular, chemical odour (almonds, garlic, rotten fish or rotten eggs) suggests toxic gases such as cyanide, arsine, phosphine, or hydrogen sulfide, respectively. Discolouration of the skin (redness, hyperaemia) suggests nitrates(III) and nitrates(V); discolouration of the blood and lips (chocolate) is indicative of methemoglobinogenic compounds; and discolouration of urine (red-orange, blue-green, pink-red, dark yellow, dark brown and even black) suggests various medicines or iron compounds. Furthermore, burns (of the skin, oral mucosa, eyes) are indicative of acids and bases, whereas diarrhoea suggests fungi or metals. The size of pupils and their reaction to light are also indicators: dilated pupils indicate amphetamines, atropine and cannabinols, but also opiate withdrawal syndrome; constricted pupils indicate opiates, barbiturates, organophosphates, carbamates and phenothiazines. Bedsores can suggest barbiturate use. Results of analysis of the acid–base balance (acidosis) can indicate methanol and ethylene glycol.

### *11.2.7 Interpretation of the Results of Toxicological Analysis*

**Interpretation of the Concentration of Xenobiotic** The final stage of the work of the toxicologist-analyst is the interpretation of results. This interpretation must be consistent with the circumstances of the event, the symptoms resulting from the

action of the poison or the autopsy results in cases of fatal poisoning. Most frequently, the analyst interprets the determined concentration of the compound or compounds with reference to a therapeutic concentration or low range of concentrations (if the toxic factor does not have application in medicine) as well as normal (metals) and toxic concentrations and those encountered in cases of fatal poisoning.

**Scope of Interpretation** Interpretation of results of toxicological analyses for the purposes of judicial decisions encompasses a very broad range of issues. Besides confirming or ruling out the administration of a xenobiotic or exposure to a toxic compound, toxicologists are frequently asked to solve problems such as determining the method (active administration, passive exposure) of introduction of the psychoactive substance (9THC, amphetamine, cocaine) into the organism, determining the source of the compounds (medical treatment, diet, deliberate induction of a state of intoxication), determining the time of the last administration (in cases of frequent administration of psychoactive substances), conducting a retrospective account (e.g. in order to establish the concentration of ethyl alcohol in the body a few hours prior to sampling blood) and commenting on the advisability of carrying out an exhumation for the purpose of confirming or ruling out a possible cause of death from poisoning (e.g. amphetamine derivatives, LSD).

The range of activities of the toxicologist-analyst also encompasses estimating the production capacity of an illegal clandestine laboratory in which controlled substances are produced, as well as determining the yield from cultivation of crops for the production of substances of abuse, such as cannabis.

**Profiling** The task of the toxicologist-analyst is not only to confirm or rule out poisoning, and identify and quantitatively analyse the main components of non-biological materials secured by the police, but also to characterise samples of drugs by a detailed definition of their physical features and chemical composition (enabling their classification and comparison), and determine the relationship between them. Such a detailed identification of components occurring in trace amounts in samples is known as profiling. Profiling allow explanation of many facts that are significant for judicial bodies, such as the link between two or many samples, the link between a dealer and a drug addict, the distribution network of samples on the local, national or international level (by showing a similarity between samples at the microtrace level), the origin of samples and the method of synthesis. Furthermore, this type of identification is helpful in establishing the geography of the drug trade, tracking the emergence of new sources of substances that are under international control, estimating the size and activity of clandestine laboratories, and distinguishing between substances originating from illegal and legal sources [43].

**Thanatochemical Processes** When interpreting the results of toxicological analysis of autopsy material, knowledge of putrefactive decomposition processes occurring in corpses (thanatochemical processes) is very important. The direction and intensity of progressive post-mortem changes occurring over time depends on

many factors, among which significant roles are played by the physical condition of the dead person, illness prior to death, injuries, the course of the agony and environmental factors. Moreover, degradative transformations can occur in different ways in different organs of the same corpse. Processes occurring on the surface of organs (aerobic) are faster than those in internal parts (anaerobic). As a result of putrefactive decomposition processes, substances (alcohols such as ethyl, methyl and higher; cyanides) can be produced in corpses that are significant from a toxicological point of view. These substances are of endogenous origin, and thus were not introduced into the organism before death. To date, no specific explanation has been found for the production of cyanide ions at high concentrations in some post-mortem blood samples, comparable with values occurring in cases of poisonings. On the other hand, as a result of degradation of biological material, many toxic compounds that had been taken while the person was still alive (i.e. compounds of exogenous origin) undergo decomposition. These are esters of higher alcohols, numerous volatile organic compounds and many drugs, especially with a quaternary ammonium base structure. As a result of degradation of amino acids, which are natural components of biological material, alkalisation of the environment occurs, which at the stage of far-reaching putrefactive decomposition of biological material makes it impossible to draw conclusions about poisoning by acids, especially hydrochloric acid [44].

**Endogenous Compounds** Interpretation of the results of toxicological analysis of compounds that are natural components of the organism, but also poisons, can be a big challenge for forensic toxicologists.

The physiological level of ethyl alcohol is of the order of 0.01 ‰, whereas in persons suffering from diabetes or in hunger states, it can be somewhat higher, but never exceeds the statutory threshold of a state indicating consumption of alcohol (i.e. 0.2 ‰). However, as a result of production of endogenous alcohol in corpses, interpretation of its origin is sometimes very difficult. In order to establish whether we are dealing with alcohol produced post mortem (in vitro) or consumption of alcoholic drinks before death (in vivo), two types of post-mortem material should be subjected to analysis: blood (in this material, production of endogenous ethyl alcohol occurs very quickly after death) and vitreous humour (whose decomposition is slower), or blood and urine.

Methanol, a poison acting through its metabolites, is a component of all alcoholic drinks produced by the descendant companies of the Polish Spirits Monopoly, and occurs naturally in living organisms. The origin of endogenous methanol has not yet been unambiguously explained. Its concentrations range from 0.1 to 3.4 mg/kg (i.e. several orders of magnitude lower than concentrations of methanol occurring in cases of poisonings).

The carbon monoxide derivative of haemoglobin (carboxyhaemoglobin, HbCO) also occurs in small amounts (2–7 %) as a natural component of the living organism, being a product of the decomposition of haemoglobin. HbCO can also be produced as a result of inhaling carbon monoxide from the environment (in the case of smoking cigarettes, HbCO reaches a concentration of the order of 11–13 %),

or through post-mortem decomposition processes (leading to values of the order of 5–7 %). Long-term exposure to carbon monoxide causes severe poisoning by carbon monoxide, but the concentration of HbCO may be very low (not exceeding 2 %).

Gamma-hydroxybutyric acid (GHB) is also a natural component of living organisms. In the past it was used in medicine, whereas currently this compound is increasingly abused for recreational purposes, and as a date-rape drug. It occurs naturally in the organism in very diverse concentrations, which change during storage of biosamples collected when the subject was alive and from post-mortem examinations. Furthermore, it undergoes rapid elimination, even when administered at very high doses (detectable up to 8 h in blood and up to 12 h in urine). For these reasons, GHB causes very great interpretation difficulties in relation to its in vitro or in vivo origin [45].

**Competency of the Analyst** The forensic toxicologist-analyst interprets the results of analysis within his or her competency, in relation to general toxicological knowledge and the general population. A medical doctor specialising in clinical toxicology pronounces on the defined behaviour of a specific person under the influence of a given drug. Solving problems relating to criminal responsibility (type of punishment, offence, indictment, prosecution, ban) lies clearly within the remit of the adjudicating body (the court), in other words, issues such as substantive assessment of the validity of conclusions in the case of conflicting opinions of two experts.

## 11.3 Contemporary Toxicological Analysis

**Fundamentals of Toxicological Analysis** Toxicological analysis is the cornerstone of clinical and forensic toxicology. Currently, a forensic medical doctor will not issue an opinion on the cause of death as a result of poisoning without the results of toxicological analysis of post-mortem material. Without analysis of body fluids taken from a living person suspected of having been poisoned, it is impossible to issue a categorical opinion. In clinical toxicology, the results of analysis most often serve to diagnose poisoning and monitor the success of treatment, but in cases of hospitalisation of victims and perpetrators of various criminal activities, the results of analysis are used for the needs of the administration of justice. The evidential value of the results of toxicological analysis depends on the method applied for determinations and on the type of material collected for study (as a result of legal, scientific and analytical requirements, etc.).

The basis of modern toxicological analysis is a two-stage examination of biological and non-biological material. In the first stage, screening methods are applied, and in the second stage, confirmatory methods. The analyst uses screening methods to obtain preliminary results (i.e. non-categorical results; negative and positive), after which the positive results must be verified by confirmatory methods.

Screening methods are aimed at analysing as broad a spectrum of various chemical compounds as possible. Confirmatory methods are more specific and are characterised by a lower limit of detection (LOD) and limit of quantification (LOQ) than screening methods.

**Validation of Methods** For any analytical process to be applied for forensic toxicological purposes, it must be subject to control from the moment of collecting the sample to obtaining the result and then documenting this result. Standardisation of the method (i.e. validation) ensures such control. Thus, validation parameters must be determined for each new method (screening, confirmatory, qualitative and quantitative) in accordance with international requirements. According to the Scientific Working Group for Forensic Toxicology (SWGTOX), parameters such as the following must be determined for all methods used in the analysis of biosamples: precision, dilution integrity, interference studies, LOD, carryover and stability. For quantitative methods, the following are required: bias, calibration model and LOQ [46]. The following are considered to be additional parameters that should also be determined: recovery, reproducibility and sensitivity of the method to small changes (ruggedness or robustness). For methods using LC-MS, the matrix effect should always be studied, in other words, ionisation suppression or enhancement, especially in an electrospray ionisation (ESI) chamber [47]. Applying deuterated derivatives of analytes as internal standards, checking the correctness of methods by analysis of reference materials and participation in interlaboratory comparisons facilitates continuous control of result uncertainty .

Validation of a method is not research work, but an integral part of the process of quality assurance of results of examinations in every modern analytical laboratory applying principles of good laboratory practice, and constitutes a condition that is necessary both for gaining accreditation and for recognition (in the international arena) of results of examinations conducted in the laboratory. Determination of validation parameters undoubtedly prolongs the period of development of a method, but is essential to confirm its usefulness for achieving the intended analytical goal. This process does not conflict with Albert Einstein's statement that "everything should be made as simple as possible, but not simpler". On this basis, simplification of the method by omitting the process of validation is not permissible.

**Material** Selection of appropriate material for examination is mainly determined by the time that has elapsed from administration of the toxic substance to collection of the material, as well as the site of conducting examinations (a clinical or forensic laboratory, or the site of the incident, for example, during a roadside check of a driver). Various compounds occur in various materials – parent, and active and inactive metabolites. Active metabolites influence life processes, and the presence of inactive metabolites in the organism could attest to consumption of a substance a long time ago. Currently available analytical techniques are applied to detect, identify and determine chemical substances in classical biological material (i.e. blood, urine and sections of internal organs) as well as in so-called alternative materials (i.e. hair, saliva and sweat). In recent years, numerous studies aimed at

showing the correlation between concentrations of various compounds in saliva and blood have been conducted [48]. Assuming that a given poison is excreted via sweat, a pillowcase can also be a useful, indirect material for toxicological tests. Many analysts undertake analysis of fresh or old blood stains revealed on various materials. It is worth mentioning that modern analytical methods allow conclusions to be drawn about the cause of death as a result of poisoning on the basis of analysis of fly larvae developing on human remains [49, 50]. This material also serves for estimation of the time of death, both in hot [51] and cold climates [52].

Sometimes the amount of material delivered for examination is very limited for completely unjustified reasons. When developing methods, analysts take this fact into account. Increasingly frequently, blood samples of only 1 mL are collected for screening analyses, whereas as little as 0.1 mL blood is sampled for targeted or confirmatory analyses.

**Directions of Analysis** Analytical procedures depends on the type of problem set. Unknown circumstances of an event or an unknown toxic factor require the application of systematic toxicological analysis (STA), so that the analytical procedure encompasses as many toxic substances as possible. In cases where the administered toxic compound is known, first of all a course of analysis targeted at this compound is conducted, and a positive result must be confirmed by another independent method. When working on a case in which only the symptoms of the action of an unknown toxic factor are given, the ability to use complementary techniques as well as knowledge of the fields of medicine, pharmacology and pharmacokinetics are of particular importance [53].

### *11.3.1 Screening Methods*

**Immunochemical Methods** Commercial tests that make use of immunochemical reaction (ICh), enzyme immunoassay (EIA), radioimmunoassay (RIA), fluorescence polarisation immunoassay (FPIA) and the kinetic interaction of microparticles in solution (KIMS) are most frequently applied as screening methods. They are designed to detect compounds from defined, but not numerous, groups (e.g. opiates, cannabinols, derivatives of amphetamine, tricyclic antidepressants and benzodiazepines), and, more rarely, single compounds (e.g. digoxin). These tests enable very quick analysis of a specific body fluid (urine, serum or saliva). The obtained result relates to the whole group of compounds, and is defined as group-positive or group-negative. Depending on the method of detection (visual or electronic reading using an analyser), the obtained result can be quantitative, semi-quantitative or only qualitative.

ICh tests have many advantages: they are very sensitive, rapid, do not require pre-treatment of the biological material (which they are designed to analyse), and use small quantities (0.01–0.1 mL) of body fluids. Their principal drawback is low specificity.

**Principles of Immunochemical Tests** All ICh tests use an antibody or other binding protein, an antigen and a label. The basic principle underlying these tests is competition between the unlabelled antigen (the drug from the biosample) and the labelled antigen for a binding site in antibodies. The type of label (radioactive, chemical and fluorescent labels are used) with which the antibody or antigen is labelled determines the detection technique. In RIA tests, labels are nuclides ($^{3}H$,$^{14}C$,$^{125}I$,$^{131}I$) introduced into the antigen, antibody or enzyme. Tests using measurement of radioactivity are very sensitive. When using a fluorescent label or luminophore we measure, respectively, a change in fluorescence in polarised light (FPIA) or chemiluminescence. Various types of enzymes can also be labels. The principal components are the components of the sample, antigen labelled with a specific enzyme, a specific antibody for the antigen and a substrate that causes a measurable change in optical signal when it takes part in a reaction catalysed by the enzyme. In tests involving measurement of absorbance, the co-presence in the sample of compounds characterised by a high molar absorbance coefficient influences the result, causing a false positive result to be obtained. Chemical compounds or dyes that effect a change in colour of a solution or indicator zone can also be used as labels. The simplest tests make use of test strips (e.g. the American Frontline test) that are immersed in a urine test sample. On the paper strip, the components of the sample are subject to chromatographic processes and reach the zone where antibodies are deposited. The drug from the urine binds with antibodies. The surplus of free antibodies reaches the second zone, in which antigens are immobilised. The antigens capture all unbound antibodies. Only particles of antibody bound to the drug from the sample undergo further chromatographic processes. These conjugates reach the label zone, which often contains colloidal gold as a label. A change in colour of this zone occurs, whereby a red band indicates a positive result. In other strip tests (e.g. Hydrex), in spite of the same label, the appearance of a coloured band indicates a negative result.

**Chromatographic Methods** Chromatographic methods such as thin layer chromatography (TLC), GC and HPLC, with various types of detection, are more universal and specific, especially when they are coupled with MS. These methods, with the inclusion of MS, are defined as open (i.e. they allow inclusion of successive new compounds into a previously developed analytical procedure). Screening methods using both GC and HPLC for separation and, for detection, tandem mass spectrometry (MS/MS) with selected ion monitoring (SIM), selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) encompass a limited number of compounds. This is often a result of the limitations of the equipment, which restrict the number of analytical signals (ions or reactions) that can be simultaneous recorded at an appropriately high sensitivity in one process. That is why in recent years screening methods targeted towards a strictly defined group of compounds have been developed in place of so-called general screening methods. Examples include a method for detecting substances affecting the psychomotor performance of drivers, and a method for detecting substances use to facilitate rape or robbery. This approach ensures that the sensitivity of the screening method is

satisfactory, although all screening methods are less sensitive than confirmatory methods or methods targeted towards a specific compound.

Note that with the need to analyse an ever-increasing number of analytes at increasingly small concentrations (trace analysis), various methodological pitfalls arise [53].

**Spectrometric Methods** Screening methods are not just restricted to organic compounds. In addition to flame (F-AAS) and flameless atomic absorption spectrometry (including cold mercury vapour atomic absorption spectrometry, CV-AAS), which are established methods for studying metal content in biological material, other techniques such as inductively coupled plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS) are used increasingly frequently. The latter two techniques enable analysis of about 70 elements in one analytical process, the specific number being dependent on the number of standard substances that the analyst has at their disposal. The method of choice for determination of Hg, As and Se is AAS with hydride generation (HG-AAS), whereas AAS with electrothermal atomisation (ET-AAS) enables determination of trace amounts of heavy metals not only within the normal range, but also at levels encountered in chronic poisoning and, in some cases (e.g. Tl, Pb or Se) in acute poisoning. In order to rule out or confirm a poisoning, especially a chronic poisoning, by toxic metals, semi-metals and non-metals, knowledge of normal levels of elements occurring in trace amounts in particular types of biological material is essential.

**Systematic Toxicological Analysis** The number of toxic compounds that must be taken into account in cases where the circumstances of an incident are completely unknown (e.g. a corpse found in a forest, an unconscious person found in a park) is continuously growing. It is not possible to encompass all the various chemical compounds that are significant from a toxicological point of view in one analytical process.

Toxic substances can be classified in various ways (e.g. alphabetically) or in terms of pharmacological activity (tricyclic antidepressants, anticonvulsants, antihypertensives) or chemical structure (derivatives of benzodiazepine, barbituric acid, phenothiazine). For the purposes of STA, the best way seems to be to divide compounds into groups depending on the type of technique used to extract them from various biological materials. This gives six basic groups [53]. These groups are listed below in the (chronological) order that should be maintained when performing toxicological analysis:

- Gases and volatile compounds, which can be isolated by diffusion into the headspace
- Toxic anions such as nitrates(III), nitrates(V), phosphides and oxalates, isolated by dialysis
- Sparingly volatile organic compounds, for which the most suitable method is pH-dependent extraction with organic solvents (liquid–liquid extraction, LLE) or the solid phase extraction (SPE) screening technique

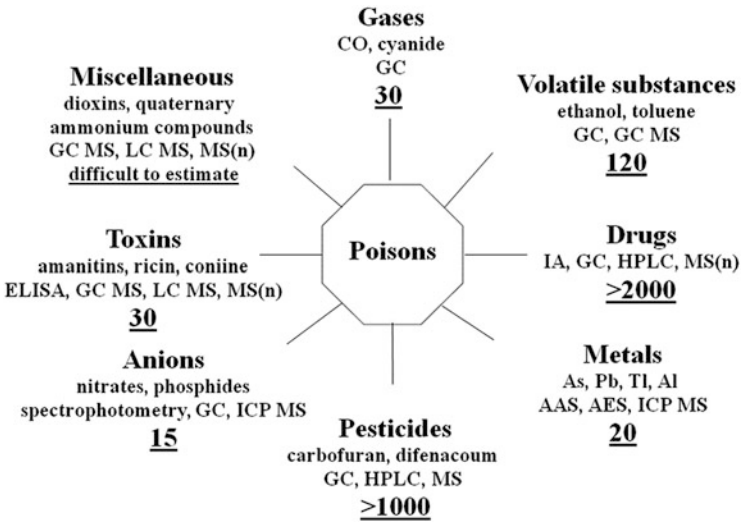


**Fig. 11.10** Classification of poisons for analytical purposes. The most commonly used methods for their determination and estimated numbers of toxicologically relevant compounds (*underlined*) are indicated

- Pesticides, although many require a defined extraction procedure because a general procedure is not effective
- Metals and non-metals, which require application of various mineralisation techniques (wet, ashing, microwave-assisted)
- Toxins and a large group of compounds (e.g. quaternary ammonium bases and dioxins) that require special isolation techniques, using ion pairs or ion exchange resins, formation of derivatives, continuous extraction, precipitation and concentration

Detecting or ruling out the presence of a broad spectrum of compounds from each of these groups in biological material (Fig. 11.10) requires the application of increasingly sensitive instrumental techniques.

### *11.3.2 Confirmatory Methods*

Modern instrumental techniques, particularly the coupling of GC-MS, LC-MS or LC-MS/MS with various types of ionisation (electron, EI; chemical, CI; electrospray, ESI; photoionisation, APPI) leading to the formation of positive and negative ions, as well as various modes of monitoring these ions (total ion current, TIC; selected ion monitoring, SIM; parent ion scanning, PIS; daughter ion scanning, DIS; selected reaction monitoring, SRM), enable – in one analytical process – separation of a mixture of compounds, identification of individual components and

their determination at concentrations of the order of picograms in tens of microlitres (of blood, serum, urine) or milligrams of material (hair). Methods using these techniques require preliminary, multistage (hydrolysis, extraction, derivatisation) preparation of biosamples, application of appropriate standard and reference materials and determination of validation parameters, which ensure control of the whole analytical process. Such methods are thus very time-consuming and costly. Constantly improved design solutions for mass analysers (quadrupole, Q; ion trap, IT; triple quadrupole, $Q_3$; time of flight, TOF; Fourier-transform ion cyclotron resonance, FT-ICR) enable lowering of the LOD to very low values.

### *11.3.3 Identification Systems*

Each laboratory should have its own analytical procedures. These procedures can be based on commercial or published systems, but their reproducibility and sensitivity (robustness) in new laboratory conditions must be checked. To date, most systems have been developed for screening analysis of organic compounds. Numerous systems of identification have been developed for one of the oldest chromatographic methods (TLC), each encompassing over 1000 different compounds, including medicines and their metabolites [54]. For the GC method with classic detection, two systems are best known. One method is used in screening analysis for the presence of sparingly volatile organic compounds and is based on two types of detectors (flame-ionisation detector, FID, and nitrogen phosphorus detector, NPD) and retention indices of 4500 compounds [55]. The second method is used for the identification of volatile organic compounds of the solvent type [56]. Computerised systems of identification of drugs can be purchased with liquid chromatographs equipped with diode array detectors (HPLC-DAD). Using these systems, compounds divided into acidic and basic (encompassing neutral compounds in both groups) are identified on the basis of the relative retention time and spectrophotometric spectrum. Another significant HPLC-DAD system, compatible with high performance chromatography systems from various companies, encompasses about 2682 compounds, taking into account, in addition to the above mentioned identification elements, the molecular structure by comparing 1600 chromophores or combinations thereof [57, 58].

Chromatographic techniques coupled with mass spectrometry (GC-MS, GC-MS/MS, LC-MS and LC-MS/MS) have also been applied in many combinations. A library of reference mass spectra is an integral part of each type of GC-MS apparatus, enabling typing of spectra that are potentially similar to the identified compound. For the GC-MS-EI technique, a combination of two previously separate libraries, Wiley and NIST 2008 (W8/N08), should be mentioned because of its extensiveness. Library W8/N08 contains 562,000 EI spectra, 5308 spectra of parent ions (precursors) subjected to fragmentation by the MS/MS technique, over 2 million names of chemical compounds and their synonyms, 35,000 structural formulae and 43,000 GC retention indices. The Automated Mass Spectrometry

Deconvolution and Identification System (AMDIS) is also integrated into this library. The Pfleger/Maurer/Weber library is most useful for the needs of toxicological analysis, containing 7800 spectra of drugs, pesticides, their metabolites, derivatisation products (especially methyl and silyl derivatives) and artefacts (compounds that might be produced during analysis, for example, under the influence of high temperature in the injection chamber of the chromatograph). This library also contains other data on compounds, such as Kovats retention indices, structural or empirical formulae, molar mass, the Chemical Abstract Service Registry Number, the name of the pharmacological group into which the compound is classified, the type of biosample and a description of the method of preparation of the sample [47]. In spite of this, not all parent compounds and their metabolites from this library can be encompassed by one analytical procedure.

When developing an analytical procedure using the LC-MS technique, it should be remembered that mass spectra after ionisation of a given compound in the ESI chamber or in APCI conditions obtained after single fragmentation contain, in contrast to EI-type spectra in GC, very few fragments. The pseudomolecular ion obtained in APCI mode is also characterised by low identification value. The identification value of spectra increases after the application of multiple fragmentation and collection of spectra of daughter ions. An important problem for ESI is the possibility of reduced ionisation of the analyte by co-present compounds in the sample, which is called suppression of ions and can lead to overlooking a strongly toxic compound whose concentration in the sample is low. Bearing the above in mind, a library of daughter mass spectra (MS/MS) of the ESI type with fragmentation within the source by the method of collision-induced dissociation is very useful [59]. The library contains spectra of over 800 (pharmaceutical) drugs at three (low, medium and high) collision energies. Furthermore, the same authors have created a library of mass spectra for the LC-MS-Q method, whereas Schreiber [60] has established a library of ESI and APCI-type spectra for identification of pesticides and explosive compounds. These libraries have been commercialised. For LC-MS or LC-MS/MS methods, most libraries have so far been created in a "homemade way" in individual laboratories. These libraries work well for particular instruments or the same types of instruments.

### *11.3.4 Analytical Strategies*

Often in identification systems, one pre-treatment procedure is applied to samples, but various end determination techniques (GC, HPLC and, increasingly rarely, TLC). Because of the high sensitivity and broad range of linearity (often encompassing three orders of magnitude) of coupled techniques (GC-MS or LC-MS), some analysts apply one method of sample preparation (extraction and derivatisation) to all analytes, in spite of the low efficiency of the process of isolation of acid compounds subjected to extraction from an alkaline medium. The most extensive method to date, enabling detection and identification of over

2000 compounds (medicines and their metabolites from 20 pharmacological groups) in one extract (pH 8–9) from urine, was developed by Maurer et al. using the GC-MS-EI technique [61]. With the help of modern liquid chromatographs, it is possible to record mass spectra under planned changing measurement conditions (e.g. at two fragmentor voltages) in the course of one process. The basis of other analytical procedures is the application of complementary methods using different types of detection (MS/MS, electron capture and FID) and ionisation (EI, APCI and ESI). Numerous analysts are advocates of pH-dependent extraction and combining extracts before instrumental analysis, or their separate analysis. In many laboratories, methods designed for studying a defined type of biological material (blood, urine, saliva or hair) are being developed with maximum a posteriori probability (MAP) [62], as well as for studying specific pharmacological groups (e.g. benzodiazepines [63, 64], antidepressants [65], beta-blockers [66]). More and more developed methods relate to a specific problem and biological material. For example, the LC-MS-APCI method serves to detect and determine substances facilitating commission of crime (rape, looting, robbery) in blood [41] and urine [67]. The LC-MS-ESI method determines substances acting similarly to alcohol in drivers' blood [68], such as phenylalkylamines of plant origin in plasma [69]; phenylalkylamines considered designer drugs classified into group 2C and, more precisely, containing two dimethoxy groups attached to the benzene ring in positions 2 and 5, in plasma [70]; and α- and β-amanitines (Death Cap toxins) in urine [71]. Coupled techniques enable the development of universal methods that allow screening analysis, identification and quantitative analysis to be conducted in one process. In the case of the LC-MS, the successive stages of the procedure for one extract from a biosample are screening analysis, in which suspected compounds are typed, and then identification of these compounds using the total ion current mode (TIC, SCAN). For quantitative analysis of a compound identified in this way, it is sufficient to monitor one ion per compound. Among the most extensive screening methods developed to date using the LC-MS technique are the LC-MS/MS-QTrap method, encompassing 301 compounds in blood and urine [59], and the LC-MS/MS-ESI method [72], encompassing 238 drugs in blood. Alder's team [73] developed and compared two methods, LC-MS/MS and GC-MS-EI, for the identification of 500 of the most frequently applied pesticides. Pang et al. [74], applying gel chromatography to preliminary separation, conducted validation for 660 pesticides and then, using quantitative analysis by GC-MS and LC-MS/MS, encompassed 437 active components of plant protection agents, dividing them into four groups.

### *11.3.5 Summary*

As a result of the increasing number of samples that are subjected to toxicological analysis, efforts are being made to automate the analytical process. Software is being developed to aid the measurement process by automatic tuning, collection, editing and archiving of data; creation of reports; searching through libraries; and

quantitative analysis. Extensive literature and internet publications on the subject are available. Continuously developed analytical procedures are verified by internal and external systems of quality control for the results of analyses. All this enables forensic analyst-toxicologists to screen about 3000 compounds in the everyday work of a typical well-equipped laboratory, in cases where there is a lack of information about a particular event. The remaining compounds of significant toxicological importance, whose number is estimated at about 100,000, require application of a specific analytical procedure targeted towards a given compound.

Although analysts know the analytical canons and methods, they are conscious of methodological traps and apply generally accepted analytical procedures for seeking and identifying poisons. Moreover, they detect poisons with the help of biomarkers of exposure, effects and sensitivity in biological material, enabling confirmation of poisoning in the period of occurrence of symptoms (in blood, saliva and urine) or in the period after symptoms of poisoning have subsided (in urine, hair and sweat). General knowledge, incisiveness, an ability to discern logical associations between facts and, increasingly rarely, chance play important roles in determining whether or not analysts are successful in their investigations.

Currently, toxicological analysis of biological material increasingly frequently boils down to the need to detect, identify and determine trace amounts of toxic compounds. Criteria of identification of compounds vary, depending on current knowledge and new requirements, which in turn depend on the field and the problem to be solved. It is obvious that the most stringent requirements are set in those fields in which results lead to legal sanctions. In these fields in particular, the analyst-toxicologist should make use of the best knowledge and fulfil all formal conditions. After their fulfilment, the result of identification should be described in terms such as "nothing stands in the way of stating that it is this compound" and, in the case of a quantitative result, "the compound at the determined concentration could have caused a health disorder or threat to life, but it is not an unequivocal condition". The analyst, acting within his/her strictly limited remit, works in partnership with a forensic medical doctor in issuing opinions for judicial bodies. It is currently difficult to imagine a ruling on the cause of death as a result of poisoning without, at the very least, a toxicological analysis of post-mortem material. In such cases, the consistency of factual findings, the picture of the autopsy and the results of toxicological analysis authorise the forensic medical doctor to issue an opinion about the cause of death. The toxicologist-analyst can assess the number of active single doses of the substance of abuse or psychoactive substance, estimate the yield of crops cultivated for the purpose of producing substances of abuse (cannabis) or the production capacity of an illegal laboratory producing a controlled substance (amphetamine), but a substantive assessment of the results of these investigations in relation to criminal liability clearly lies within the remit of the adjudicating body.

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