Chapter 2
Techniques and Methods of Identification

Abstract In this chapter, techniques and method of chemical analysis are discussed, with the focus on their potential for use in identification procedures. It is demonstrated that analytical techniques providing more information, in particular molecular spectrometry, are preferred for identification. Other techniques are just briefly considered, with for the exception of chromatography, whose combination with spectrometric techniques sharply increases possibilities and trueness of identification. As a whole, mass spectrometry is superior to other spectral techniques in such features as sensitivity, selectivity, generation possibility of molecular mass/formula, and combinability with chromatography. Different types of mass spectrometric instruments are outlined, with many performances tabulated. Experimental conditions for identification of volatile, non-volatile, and high-molecule compounds are discussed. Next, classification of chemical methodologies is given where screening and confirmatory methods are noted. Related procedures, sample treatment, and quantitative determination are also considered as ones affecting qualitative analysis.

2.1 General

Any analytical techniques can be used for the purpose of identification, though their potentialities are not the same.

An analytical process can be considered as a generation of information [1, 2]. In turn, unambiguous true identification, especially that of unknown compound (Sect. 1.5.1), needs a large amount of information. The reason is that the results of the procedure are very often complex chemical compounds. Their molecules differ between each other in elements and the number of their atoms, types of chemical bonds, configurations and conformations. The molecule complexity increases with the number and diversity of atoms, bonds, molecular configurations/conformations. Correspondingly, the amount of information required for the full description of complex molecules and differentiation between them is also increased. This is expressed, for example, in a length of the line notation (see Table 1.2). Thus analytical techniques providing more information (Table 2.1), such as those of

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Table 2.1 Amount of information generated by different techniques [1, 3]

Technique	Potential information, bits
Spot test	1
Titrimetry	100
Emission spectral analysis	≤2,000,000
X-ray spectroscopy	≤50,000
Polarography	800
Gas chromatography	8,000
UV–Vis spectrometry	≤1,000
IR spectrometry	~10,000
Mass spectrometry	~2,000,000

molecular spectrometry, are preferred for identification, other factors being equal. Using proper methods, higher selectivity is achieved, which also expressed in a larger number of identification points (Chap. 5). At the same time, some techniques generating a lot of information such as emission spectral analysis (Table 2.1) are not applicable in molecular analysis with its numerous identification problems.

Statistical data on articles related to identification prove the above conclusion (Fig. 2.1). Spectrometry (-scopy) first, then mass spectrometry and chromatography are the top techniques used for the purpose.

Another three techniques of molecular analysis are also prominent (Fig. 2.1). Electrophoresis is a very important separation technique somewhat analogous to chromatography. Fluorescence techniques are very widespread in biochemical analysis, and often do not provide unambiguous identification; rather, they are techniques for selective detection of certain compounds. The third common technique in this series, X-ray diffraction, is used in structure elucidation of new compounds and qualitative analysis II, e.g., for identification of minerals.

2.2 Elemental Analysis

Qualitative determination of elements/metals/ions is rarely named "identification of elements", though this is what it means. In contrast to molecular qualitative analysis, with a lot of organic compounds having very similar properties, elemental identification is relatively simple in implementation, because elements are not numerous and differ notably in their properties. Elemental analysis is well-described in literature (e.g., see [4, 5]. Here, related techniques are only listed:

- Qualitative reactions: spot/tube tests, other chemical test systems
- Flame test
- Polarography and related methods
- Photometry and spectrophotometry
- Atomic emission/absorption spectroscopy
- X-ray fluorescence analysis

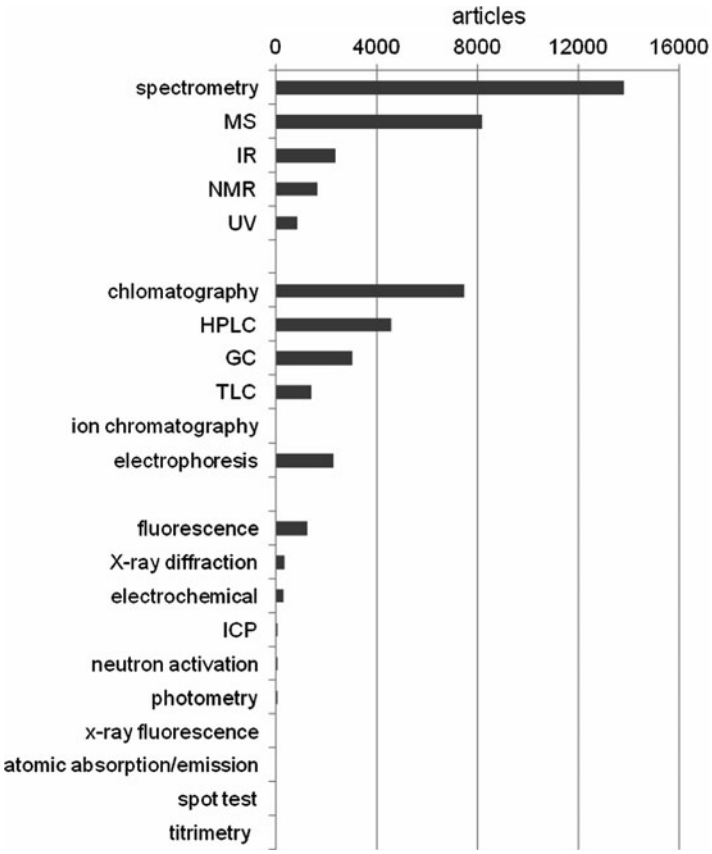

Fig. 2.1 The number of scientific articles on identification performed by different techniques. The Google scholar engine was used for the search between 23 and 25 August 2009. In searches, articles with a combination of (**a**) the "identification" key word and (**b**) the corresponding technique name in the titles were retrieved

- Neutron activation analysis
- Ion chromatography
- Inductively coupled plasma mass spectrometry
- NMR and some others

These techniques mainly refer to inorganic analysis and can be applied to organic one as well. There are also special techniques of organic elemental analysis which use elemental analyzers (e.g., [6]).

Elemental analysis has been advanced in the version of speciation analysis, which may be a combination of the former with a molecular one [7]. Speciation is a determination of the particular chemical form, e.g., a charge/valence of a metal ion or a molecular/complex compound in which an element occurs in a sample. Analytical problems of the second kind are solved using techniques of molecular analysis.

Data obtained from elemental analysis can be required for identification/classification of samples themselves, i.e., in qualitative analysis II (Chap. 8).

2.3 Electrochemistry

Determination of inorganic and organic compounds by electroanalytical techniques includes identification of analytes. For this purpose, polarographic and voltammetric techniques [8] seems to be the most popular ones. The techniques are applicable for identification of electrochemically reducible (e.g., nitro, nitroso, and azo) compounds and oxidizable (aromatic amines, phenols) compounds (e.g., see [9–11]). Voltammetric peak potentials are quantities measured for identification. Two types of electrochemical devices, sensors [11, 12] and selective HPLC detectors [13], are of value for advanced chemical analysis.

2.4 X-ray Diffraction

X-ray diffraction is used for structure determination of inorganic and organic solids and identification of crystalline phases [14, 15]. In these types of analysis, diffraction theory and/or the comparison of the positions and intensities of the diffraction peaks to libraries of known crystalline materials are exploited. Multiple phases in a sample can be recognized. Identification of minerals in geological samples is the best known example of the use of the technique in qualitative analysis II [14].

2.5 Microanalytical Systems

One of the trends in analytical chemistry, miniaturization of techniques [16], is expressed in the appearance of, for example, numerous chemical test systems [17] and sensors [12]. They are very suitable for:

- Purposes of detection and screening
- Field and industrial analysis
- Beginning analysts
- Qualitative analysis II

and applicable in both elemental and molecular analysis, but not sufficiently selective to unambiguously identify most complex molecular species.

2.6 Biological Techniques for Chemical Analysis

Developing biosensor techniques combined with electrochemical devices can be used for screening of some chemicals [12, 18]. Methods based on bioassays, e.g., ones using enzymes, are specific to certain substances and sensitive, i.e., suitable for qualitative confirmation [19, 20]

2.7 Chromatography and Related Techniques

The main chromatography techniques and capillary electrophoresis (CE) are briefly described in Table 2.2. They are not only separation techniques but also complete analytical ones, because the instruments include detectors. The chromatographic signal is at least a two-dimensional one, as are most other analytical signals. One measurand is a retention/migration parameter. The second measurand is an overall intensity of a signal. For detection, just the fact of the presence of a signal itself, i.e., a yes response, is adequate (Chap. 4). In chromatography combined with spectrometry, signals are of complicated structure consisting of individual spectral peaks.

All the quantities are used for identification. The retention/migration parameters are purely chromatographic quantities for qualitative analysis. The range criteria for them are included in both analytical methods (retention times, Chap. 5) and non-target analysis (indices, Chap. 7). Co-chromatography is of prime value in confirmatory analysis (Sects. 5.2 and 5.4). Changes in polarity of stationary or mobile (LC, TLC) phase provides additional evidence for confirmation.

In identification procedures as well as in quantitative analysis, chromatographic resolution is the definitive parameter. As it increases (capillary columns > packed columns in GC, UPLC > HPLC > column chromatography), selectivity of determination also rises, and probability of false and inconclusive results diminishes.

Other identification capabilities depend on the detector type. First, in the case of a specific detector, a chromatographic signal itself may be diagnostic in terms of identification. Examples are nitrogen phosphorous and electron capture detectors in GC (Table 2.2), which indicate the presence of N and P and halogens respectively in an analyte molecule. Second, signals of the basic universal detector, mass spectrometer, and some other spectral tools are multiline spectra unambiguously characterizing many analytes (see below). Chromatographs in such hyphenated instruments can be rather considered as suitable inlet devices.

2.8 Molecular Spectrometry

Main spectrometric techniques usable in identification procedures are outlined in Table 2.3. Mass spectrometry provides more useful information (Table 2.1), and has more analytical applications and less limitations than other methods (see also Sect. 7.8). However, many laboratories use one or more other spectrometric techniques if possible for more reliable qualitative determinations.

Table 2.2 Chromatographic and related techniques

Technique	Principle	Measurand	Detector	Application
GC [21]	In the modern form, separation of a gaseous mixture into individual components (compounds) on passing a gas flow through a thin glass column, the inner walls of which are coated with a special nonvolatile liquid	Time, relative time, and index of retention	Universal (MS, flame ionization, katharometer), specific (nitrogen phosphorus, electron capture), multi-element (atomic emission)	Gas, volatile, and semi-volatile compounds[a]
LC (HPLC, UPLC[b]) [22, 23]	Separation of a liquid mixture into individual components on passing a liquid through a relatively thin steel column packed with particles or a porous layer of stationary phase	Time, relative time, and index of retention	UV–Vis and MS and also electrochemical, refractive index, fluorescence	Non-volatile compounds including almost all biochemicals
TLC [24]	Separation technique for a liquid mixture somewhat resembling LC, where the stationary phase is a layer of solid particles spread on a flat plate	Retention factor	Visual, detection reactions, fluorescence, UV–Vis, densitometers	Non-volatile compounds
Capillary electrophoresis [25, 26]	In the simplest form, separation of dissolved ionizable compounds in silica capillary, due to migration of their ions on application of high electric field	Migration time	UV and also fluorescence, electrochemical, MS	Nucleic acids and nucleotides, pharmaceuticals, proteins, various ionizable compounds

[a]Compounds with boiling points up to approximately 350–400°C at atmospheric pressure. In these cases, vapor pressure of analytes is sufficient to analyze them by GC or GC–MS on sample heating up to not higher than about 300°C

[b]The new version of the HPLC instrument (Waters Corporation, USA), with higher performances for resolution and sensitivity [27]

Table 2.3 Techniques of molecular spectrometry (-scopy) for identification

Technique	Principle	Main applications	Limitations
UV–Vis [28, 29]	Measurements of light absorption at different wavelengths in ultraviolet (wavelengths 190–400 nm) and visible (wavelengths 400–780 nm) part of the spectrum due to electronic excitation	Detection for HPLC	Spectra characterize chromophore types rather than individual compounds
IR[a] [28, 30]	Absorption measurement of IR radiation (wavenumbers from 13,000 to 10 cm^{-1}, wavelengths from 0.78 to 1,000 μm) due to vibration excitation	Structure elucidation (determination of functional groups), qualitative analysis II (polymers, plastics, resins, food, and so on)	Relatively low sensitivity (≥1–10 μg is commonly needed for spectral recording[b]); low compatibility of IR detector with GC and especially LC
NMR [28, 31]	Absorption of radiation in the radiofrequency range of the electromagnetic spectrum (hundreds of MHz) due to changes in the spin states of the atom nucleus	Structure elucidation of pure compounds, metabolomics, qualitative analysis II	Relatively low sensitivity (≥100 μg is commonly needed for ^{1}H spectral recording, with lesser amounts in a few hours acquisition time[c]); slow progress in LC–NMR
MS [28, 32, 33]	Measurement of mass (up to 10^{6} Da) and amount of ions (down to a few counts) generated from atoms/molecules of a substance	All kinds of chemical analysis	Lower applicability in direct analysis of unpolar high-molecular compounds

[a]Raman spectroscopy [34], together with IR called vibrational spectroscopy, provides complementary information for the particular functional groups
[b]It was noted that from 5 to 20 ng was sufficient for recording spectra by GC–FTIR [35]
[c]Measuring limits and analysis times have been sharply reduced with the progress in NMR technique [36]

2.8.1 UV–Vis Spectroscopy

Spectra of this kind rarely lead to unambiguous identification of individual compounds, and rather characterize classes of unsaturated organic compounds [28]. Numerous brand names of UV–Vis spectrometers are manufactured. Such "simple" spectrometers are not easily applicable to identification of substances in mixtures.

However, a liquid chromatograph is easily combined with a photo-diode array detector (DAD), which is the widespread analytical instrument for analyzing complex mixtures [29]. For the purpose of identification

- Reference value tables of λ_{max}, wavelengths at absorption maximums, and ε, molar absorptivities
- Full spectra entered in spectral collections, databases, and e-libraries (Sect. 7.3.2)

are commonly used

2.8.2 IR Spectroscopy

In classical IR spectroscopy, pure organic compounds were elucidated/identified by means of spectral interpretation using reference tables containing (a) specific wavenumber of absorption bands of different functional groups and (b) specific band absorption (strong, medium, or weak) [28, 30, 35]; see also references in Sect. 7.5. The modern state of the technique, typically using FT-IR instruments, is characterized by widespread application of:

- Electronic libraries of IR spectra (Sect. 7.5)
- NIR (13,000–4,000 cm^{-1}), with minimal or no sample preparation, fast determination, and reduced costs, for analysis of foodstuff, pharmaceuticals, chemicals, polymers, and so on, e.g., in qualitative analysis II (Chap. 8)

Substantial limitations of the technique become apparent when mixtures of compounds and their traces are analyzed (see Table 2.3 and also Sect. 7.8).

2.8.3 NMR Spectroscopy

The technique of high-resolution NMR is indispensable for structure elucidation of pure chemical compounds [28, 31]. Depending on the nucleus, the main types are ^{1}H and ^{13}C and also ^{15}N, ^{17}O, ^{19}F, ^{29}Si, and ^{31}P NMR. There may be 1D or 2D versions of the spectra; the role of 2D ^{1}H-^{13}C spectra used for identification has been growing.

In the classical approach to structure elucidation by spectral interpretation, reference tables of corresponding measurand values, chemical shifts and spin–spin coupling constants, accounting for signal multiplicity, are used. The spectral values are very sensitive to changes in molecular configurations and conformations. So NMR techniques are of the first value in the solution of stereochemical problems.

Now, there are two advanced approaches to identification/structure elucidation (Sect. 7.6). First, a computer spectral simulation is applicable. NMR spectra are easily predictable for hypothetical structures, and can be used for comparison with spectra recorded for analytes. Second, such comparisons can be performed if reference databases comprising of experimental NMR spectra are available. Both

approaches are rapidly developed, and well deserve more attention from the analytical chemist. Nevertheless, relatively low (a) sensitivity and (b) identification power in relation to individual components of complicated mixtures (see Table 2.3) still limit NMR applicability in qualitative analysis I. In contrast, NMR applications in qualitative analysis II seem to be in progress (Chap. 8).

2.8.4 Mass Spectrometry and Chromatography Mass Spectrometry

As a whole, this technique is superior to other spectral ones in the combination of features such as sensitivity, selectivity, generation possibility of molecular mass/formula, and combinability with chromatography. Gas or liquid chromatographs as inlet devices to mass spectrometers separate complex mixtures of chemical compounds for their subsequent detection and recognition, with increased selectivity of combined techniques.

Thus, mass spectrometry and chromatography mass spectrometry have the highest potential for qualitative determination of complex organic compounds in complex mixtures/matrices. This advantage, together with the perfect capabilities of quantitative analysis (methods of isotope dilution), results in a rapid development and widespread application of the two techniques. It is clearly proved by the statistical data. Mass spectrometers held a 42% share of the global market for instruments for molecular analysis [37]. The number of mass spectrometers in the world grew from 34,000 in 1999 to more than 200,000 in 2005 [37].

Figure 2.2 shows a typical schematic diagram of mass spectrometers. The type of mass analyzer determines the main features of mass spectrometer and its "generic name." The most popular mass analyzers are specially specified in Table 2.4. The most popular combinations of mass analyzers with different ion sources and chromatographs as commercially manufactured mass spectrometers and chromatograph-mass spectrometers are placed in Table 2.5. The choice of the instrument for identification depends on properties of analytes and also data types necessary for identification. The latter are:

- Masses of the most important ions, i.e., molecular and analogous ones and intensities of the mass peaks
- Masses of individual fragment ions and intensities of their mass peaks
- Accurate ion masses and corresponding molecular formulas
- Full mass spectra, including tandem and high-resolution mass spectra

In mass spectrometry, the most important compound properties are volatility, polarity of molecule, and molecular mass. Based on these properties, all compounds are divided into three groups.

Gases, volatile, and semi-volatile compounds. These compounds are less numerous than non-volatile ones, but until recent years have more often been analyzed by mass spectrometry.

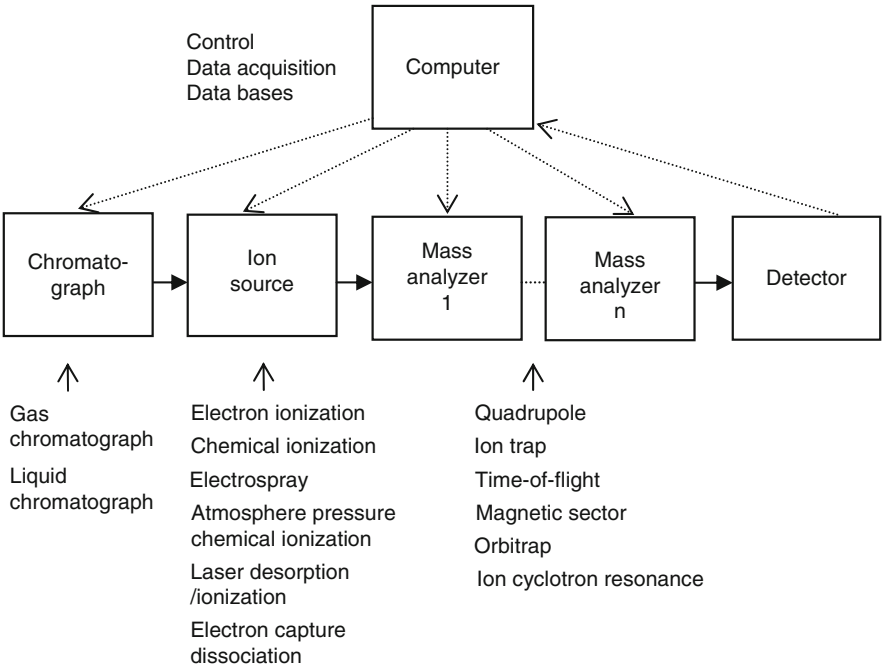

Fig. 2.2 Schematic of mass spectrometer. Main methods of ionization and types of mass analyzer are specified. Tandem mass spectrometers (tandem-in-space instruments) are made of several analyzers. Chromatograph as separation instrument is sometimes substituted by electrophoresis device. Mass spectrometers can be certainly used without chromatographs, e.g., instruments with laser desorption/ionization (MALDI) and also secondary ion emission (SIMS)

Here, the combination GC–MS including EI (CI is less frequently applied) and quadrupole mass analyzer is the standard working instrument. In most cases, identification of volatile compounds is reliable. There are several reasons to form this conclusion. First, the number of the volatile substances is theoretically limited. A difference in properties between them is larger than among a huge number of non-volatile compounds having higher (and indefinitely high) molecular masses. Therefore, gases and volatile compounds are more easily differentiated for forthcoming identification.

A second group of reasons partly related to the first one are:

- The properties of the compounds under discussion are also well-studied, and corresponding values of measurand are well-reproduced
- Databases containing EI mass spectra and GC retention indices are commercially produced (Chap. 7)
- Many efficient analytical methods of quantitative and qualitative determination of this group of compounds have been developed and validated

Table 2.4 Modern mass analyzers and their combinations[a] [38–40]

Mass analyzer	Mass range	Mass accuracy	Fragments for identification	Price[b]	MS^n
Quadrupole	+	+	EI: from ++ to +++ ESI: +	$	no
Triple quadrupole	+	+	ESI: ++	from $$ to $$$	MS^2
Ion trap[c]	+	+	ESI: to ++	from $ to $$	MS^n
Time-of-flight[d]	+++	up to +++	MALDI: +[e]	$$	no
Quadrupole-time-of-flight	from + to +++	up to +++	ESI and MALDI: ++	$$$	MS^2
Orbitrap	+	+++	from + to ++[f]	from $$$ to $$$$	[g] MS^n
Ion cyclotron resonance	+	+++[h]	from + to ++[f]	$$$$	[g] MS^n

[a]General interpretation of symbols unless otherwise stated: +++ high, ++ medium, + low. For mass range and accuracy, + corresponds to a few thousand Da and from a few hundredth through several tenths of Da respectively. In the case of fragmentation: + a few fragments, ++ not very characteristic/reproducible fragmentation, +++ reproducible fragment spectra providing reliable identification

[b]Price grows up from $ (up to about $ 100,000) through $$$$ (not less than about one million dollars)

[c]The common ion trap is a quadrupole one. Now analyzers of the newer type, linear ion traps, with better performances, are also manufactured

[d]Unlimited mass range, high-speed scans, identification based on accurate molecular mass (accuracy about a few ppm)

[e]For recording mass spectrum of fragments in MALDI, the Q-ToF and ToF-ToF instruments have entered into practice

[f]Depends on the ionization technique and the tandem combination with different analyzers providing MS^n capabilities

[g]Combinations with ion traps

[h]The highest mass accuracy

Reference comparisons to full (MS libraries) or partial (a few peak) spectra and GC retention parameters and co-chromatography (co-spectrometry) are general means for identification of these compounds (Chaps. 5 and 7).

Non-volatile low-molecular compounds. In comparison to the above group, they have higher molecular mass and/or are more polar. Some polar compounds can be derivatized into corresponding volatile ones for further identification by GC–MS. If derivatization is impossible, inefficient or not achieved, various mass spectrometers and liquid chromatograph mass spectrometers (Tables 2.4 and 2.5) are used for the purpose.

ESI is the main ionization method. However, corresponding mass spectra are not rich in peaks of fragment ions. Tandem mass spectrometry (MS^n) is required where fragmentation is enhanced, due to collisions (collision activation) of analyte ions with the gas target within the special chamber. Integer-valued molecular masses may be insufficient for differentiating between heavier molecules of many

Table 2.5 The most common mass spectrometers and chromatograph mass spectrometers for organic and bioorganic analysis

Instrument	Application	Comment
Gas chromatograph–mass spectrometers	Volatile and semi-volatile organic compounds	• Rather simple, bench top, and inexpensive instruments • Commonly EI and single quadrupole mass analyzers
Liquid chromatograph low-resolution mass spectrometers	Non-volatile low-molecular organic compounds	• Increasing role of tandem instruments, i.e. ion traps and triple quadrupoles • ESI is the most popular ionization
Liquid chromatograph–high-resolution mass spectrometers	Non-volatile organic compounds including high-molecular bio compounds, proteomics	• Expensive instruments: time-of-flight, Orbitrap, and ion cyclotron resonance ones combined with other analyzers • ESI and some other ionization techniques
Mass spectrometers for non-volatile compounds without chromatography	Bio compounds, proteomics, polymers	• Sample as a thin surface layer of organic compound in matrix • MALDI and also SIMS to a lesser degree

compounds. So HRMS leading to accurate molecular mass is required more than in the case of volatile substances. The combination of $HRMS^n$ is especially advantageous (Chap. 7).

Techniques and methods of identification using HPLC–MC are in progress, and approaches for many analytes have not yet been advanced. General challenges in identification are due to the following factors:

- ESI–MS^n spectra and also HPLC retention parameters are not very reproducible
- Libraries of MS^n spectra are far from being complete

That is why identification means directly based on the use of reference materials, starting with co-chromatography and co-mass spectrometry, are more important than in the case of volatile analytes.

High-molecular compounds. These compounds, both bio substances and synthetic polymers, are non-volatile "by definition." High mass, up to 10^6–10^7 Da, is the challenge because the corresponding mass range is only covered by ToF instruments. Therefore, mass spectrometric analysis of molecular fragments formed in the process of proteolysis (proteins) or pyrolysis (synthetic high molecules) is typical for the field. However, in the case of polar N-containing bio polymers (peptides, proteins and other), ESI produces multicharged ions $[M + nH]^{n+}$ that shorten the m/z range necessary for comprehensive MS analysis of these compounds with the use of non-ToF tools. Therefore, this ionization technique became the leading one in bio mass spectrometry.

Determination of unpolar polymer compounds is a challenge for MS, because they are hardly ionized. Here, some analytical approaches are proposed in which MALDI, for example, is engaged [41].

Another concern is the production of reference materials providing the strongest evidence for identification (Chaps. 1, 8, 9). For example, there are no available standards for most proteins. So identification of many of them (Chap. 7) seems to be tentative.

2.9 Methods

Different techniques form the basis of various analytical methods. In turn, methods are classified according to techniques used for a proper determination of chemical compounds.

There is also another classification of chemical methodologies consisting of a hierarchy of analytical techniques, methods, procedures, and protocols [42]. In its description, the top level placed by *technique* lacks numerous details with regard to chemical operations. As lower hierarchy levels are reached, techniques become more specific. A *method* document consists of descriptions of individual *procedures*. At the bottom level, *protocol*, a complete description of all operations included to perform chemical analyses is represented [42]. Also, *standard operating procedure* occurs in the literature as a sort of synonym for a protocol.

Analytical methods are also divided on the basis of their reliability, i.e., a level of erroneous results. Now, many analysts emphasize that there are *screening* and *confirmatory* methods, with the latter being more reliable than the former [43] (see Table 2.6).

> Screening method means methods that are used to detect the presence of a substance or class of substances at the level of interest. These methods have the capability for a high sample throughput and are used to sift large numbers of samples for potential non-compliant results. They are specifically designed to avoid false compliant results [FN – Author]. Confirmatory method means methods that provide full or complementary information enabling the substance to be unequivocally identified and if necessary quantified at the level of interest [43].

Confirmatory methods are mainly based on mass spectrometry. Earlier, the similar pair of methods was named *routine* and *reference methods* (see [43]). The latter definition often occurs in the modern literature for specifying a method of a

Table 2.6 Identification errors permitted in analytical methods

Method	Probability	
	FN	FP
Screening	$<1:10^4$	$<1:5$
Confirmatory	$<1:10^4$	$<1:10^4$

Proposals for residue analysis [44]

high (the highest) metrological quality. The synonyms for a reference method are a *definitive, absolute, or primary* method. However, these four terms are not often used only as characteristics of qualitative analysis (identification).

Identification using screening and confirmatory methods will be thoroughly treated in Chap. 5.

2.10 Preceding and Related Procedures

If full chemical analysis is performed, operations of qualitative determination are combined with those of quantitative analysis or those preceding/subsequent to the latter. Another preceding procedure or set of procedures is a sample treatment. Identification can be considered as being independent from the two different ones. However, the ways in which sample treatments and quantitative determinations are made have an influence on the result of identification. This will be very briefly outlined.

2.10.1 Sample Treatment

In organic analysis, e.g., carried out by chromatography and mass spectrometry, most samples cannot be directly analyzed because

- The sample phase or chemical form of the analyte is not compatible with the analytical technique
- Non-target sample components and matrices themselves interfere with determination of target compounds and
- Targets present in sample in too low/high amounts

Therefore samples should be treated before analysis. Tens of different procedures for preparing samples for analysis are described [45]. The choice among them depends on whether a sample is gas, liquid, or solid (Fig. 2.3).

Gas samples are often analyzed directly by being injected into chromatographs and spectrometers. Analytes contained in liquids and solids should be isolated, concentrated/diluted, and possibly chemically transformed. Different methods of extraction, separation, clean-up, derivatization, and so on, consisting of many simpler operations, are required (Fig. 2.3). In these procedures, analytes may be lost or not separated from interfering substances; this is one of the sources of false results of detection and identification. Therefore, the presence of the analytes in the samples, rather than only in extracts, should be confirmed (see Sect. 5.3).

Some sample components and the sample itself can be identified, by IR spectroscopy for example, without numerous preparation operations.

In unknown analysis (Chap. 7), many operations of sample treatment, e.g., dissolution in various solvents, different methods of extraction, and even digestion

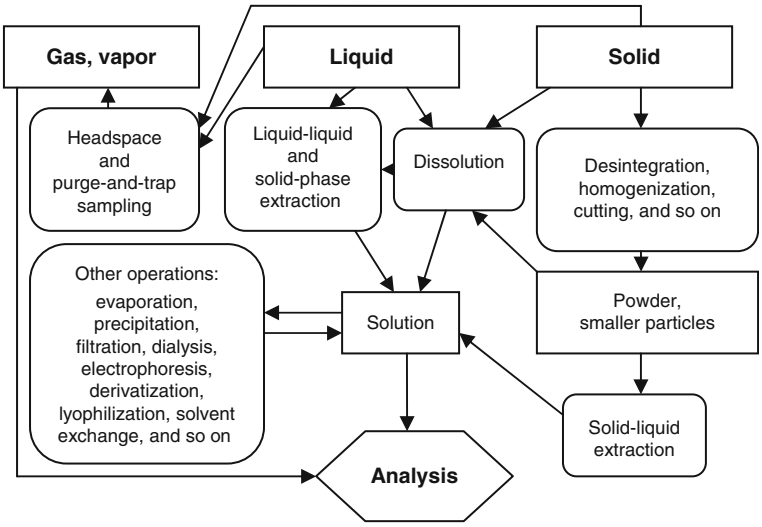

Fig. 2.3 Flow chart for treating gas, liquid, and solid samples in chromatographic and mass spectrometric analysis. Only the most popular procedures and operations without multistep sequences for their implementation are shown

of a matrix itself, are often required to study a qualitative composition in great detail. The information obtained is also useful for qualitative analysis II, although fingerprinting of intact samples may be sufficient to differentiate between them (Chap. 9).

2.10.2 Quantitative Analysis

Measurements for quantitative and qualitative analysis are or may be done simultaneously, but using not the same measurands; e.g., (a) the intensity of the basic spectral peak and (b) the relationship between intensities of several lines in the same spectrum are destined for (a) quantitative determination and (b) identification respectively.

Often, but not always, sensitivity and selectivity/specificity (and also a spectral resolution correlated with selectivity) are inversely dependent. This should be taken into account when it is necessary to choose optimal conditions for implementing identification combined with quantitative analysis within the same experiment. This can be exemplified by MS, where data can be recorded in the mode of SIM/SRM [46] or full scans which are typical for quadrupole instruments. In the first case, a few peaks are recorded, the maximum sensitivity is achieved, and quantitative determination is made. Nevertheless, the amount of information (the number of IP) may be insufficient for unambiguous identification. In the second situation, full spectra are obtained, which make it possible to reliably identify an analyte

(more selective qualitative determination), and this demands a larger amount of substance (analysis at not the best sensitivity).

Quantitative measurements are essential for estimating some limit performances which also are required in qualitative analysis (see Sect. 4.3). Also, quantitative determination may be part of procedures of qualitative analysis II, where a relationship between amounts of sample components is one of the quantities for characterization of a sample itself (Chap. 8).

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