Qualitative Analytical Processes

6

Abstract

This chapter is concerned with Qualitative Analysis, its description and its associated concepts, and also with analytical properties as adapted to the peculiarities of this branch of Analysis. The definition of Qualitative Analysis is followed by a description of screening systems for classifying samples. The YES/NO binary response, which is the output of qualitative analytical processes, is described and exemplified, particularly as regards its special analytical properties and its associated errors (false positives and false negatives). The main types of Qualitative Analysis possible according to the nature of the equipment used in the analytical process (namely, Classical and Instrumental Qualitative Analysis) are briefly discussed, and so is the high information potential of hybrid techniques.

Teaching Objectives

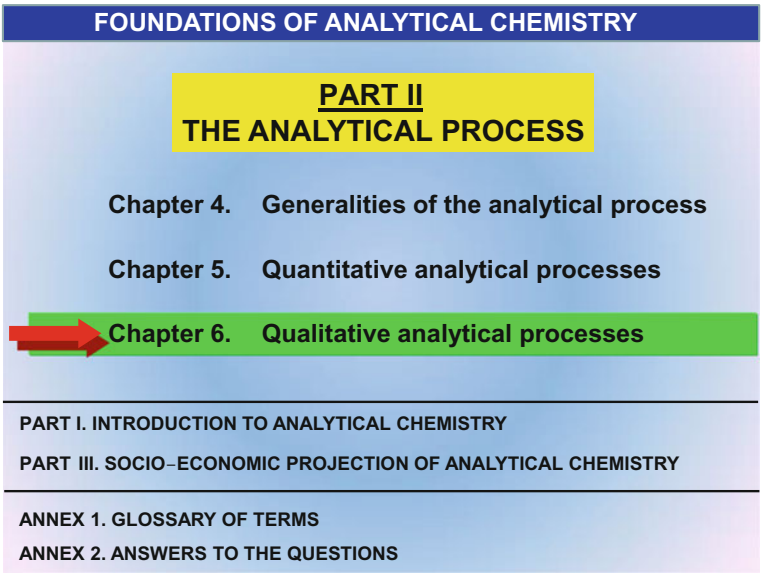
- To introduce students to Qualitative Analysis and underscore its present and future significance.
- To adapt classical analytical properties to the specificities of Qualitative Analysis.
- To define and characterize binary responses, and potential errors in them (false positives and false negatives).
- To describe the most salient classical and instrumental methods of qualitative analysis.

Electronic supplementary material The online version of this chapter (doi:10.1007/978-3-319-62872-1_6) contains supplementary material, which is available to authorized users.

M. Valcárcel Cases et al., *Foundations of Analytical Chemistry*,
DOI 10.1007/978-3-319-62872-1_6

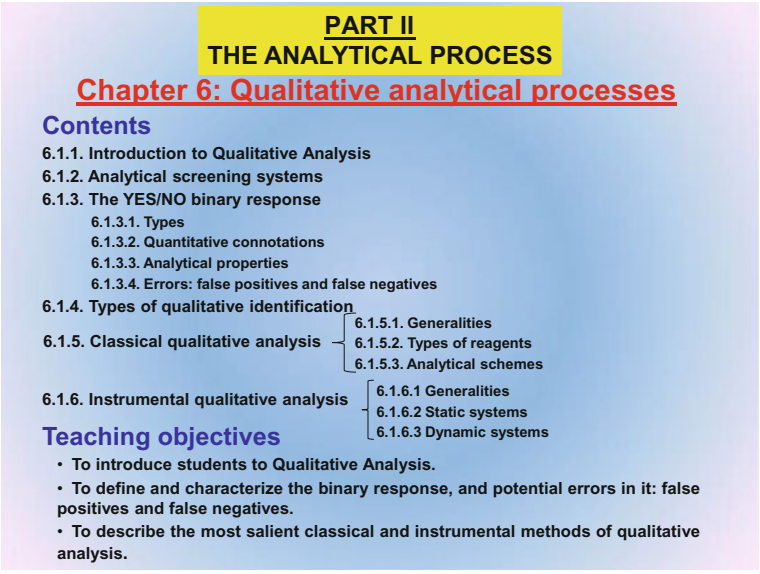
6.1 Explanation of the Slides

Slide 6.1



This slide places in Part II (The Analytical Process) and shows the other two parts. This is the third chapter in Part II and deals with analytical processes that produce qualitative results in the form of YES/NO binary responses.

Slide 6.2

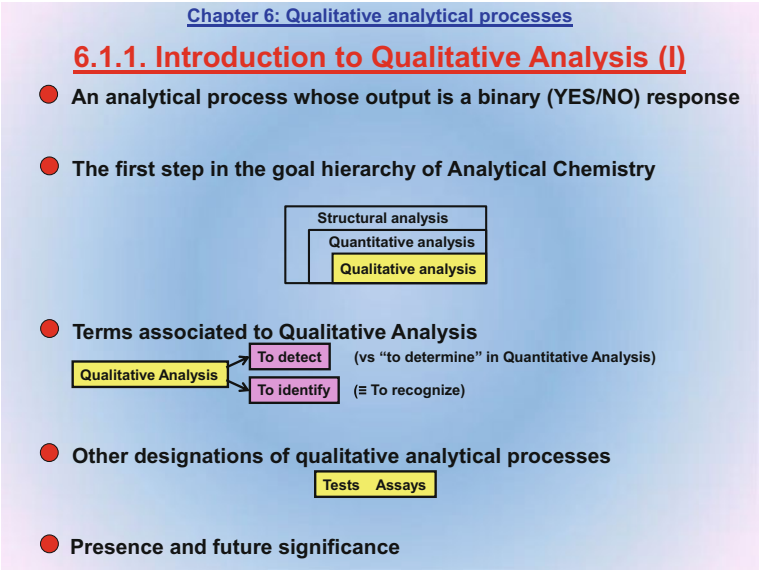


6.2.1. This is an outline of the contents of this chapter. The first section places Qualitative Analysis in context and is followed by a description of screening systems in the second. There follow the most salient features of the binary response in the third and various classifications of Qualitative Analysis in the fourth. The last two sections exemplify Classical and Instrumental Qualitative Analysis.

6.2.2. As can be seen, the primary teaching objectives of this chapter are to introduce students to Qualitative Analysis; and to describe the YES/NO binary response, its features and potential errors (false positives and false negatives) through examples of classical and instrumental qualitative methods.

6.1.1 Introduction to Qualitative Analysis (2 Slides)

Slide 6.3



Qualitative Analysis is the branch of analysis encompassing those analytical processes that yield a YES/NO binary response.

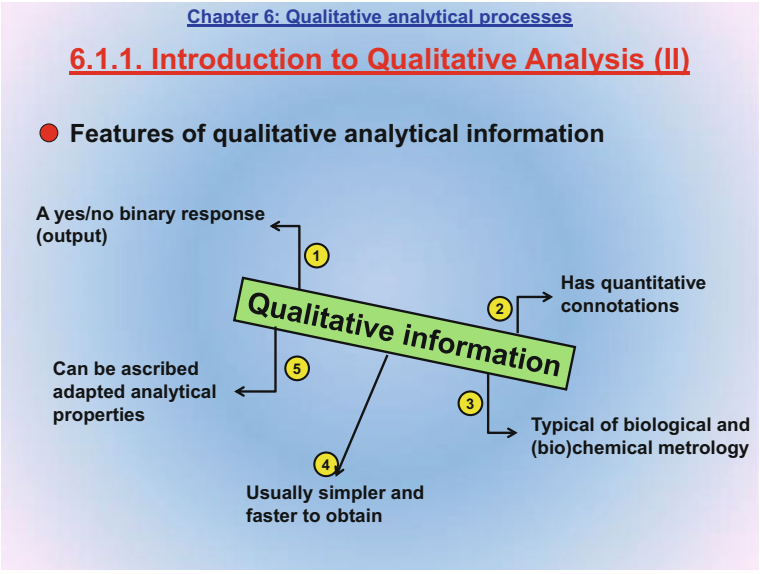
Qualitative Analysis is also the first step in the hierarchy of goals of Analytical Chemistry in Slide 1.9, where it is followed by Quantitative and Structural Analysis. It makes no sense to apply a quantitative analytical process to a sample before the sample is checked to contain the target analyte.

Qualitative Analysis can be described in terms of the verbs *to detect* (vs. "to determine" in Quantitative Analysis) and *to identify* (or, in other words, "to recognize"). Also, it is associated to the verb *to classify* (samples according to qualitative content).

Most qualitative chemical measurement processes (CMPs) are much simpler and faster than quantitative CMPs. As a result, the former are typically designated "tests" or "assays" rather than "methods".

Not long ago, Qualitative Analysis was considered to be the "lesser child" of Analytical Chemistry. As recently as the last quarter of the XX century, Qualitative Analysis was even used to undervalue the significance of this scientific discipline. At present, however, it is gaining increasing recognition as a means of fulfilling clients' information requirements—the ultimate goal as increasingly recognized by many. Very often, such requirements are in the form of binary information (see Slide 6.8) and fulfilled with an expanding array of commercially available qualitative analytical means such as screening systems, portable test kits and biosensors. Some (e.g., planar chromatography on paper) are semi-quantitative and afford estimating the concentration of an analyte in addition to identifying it.

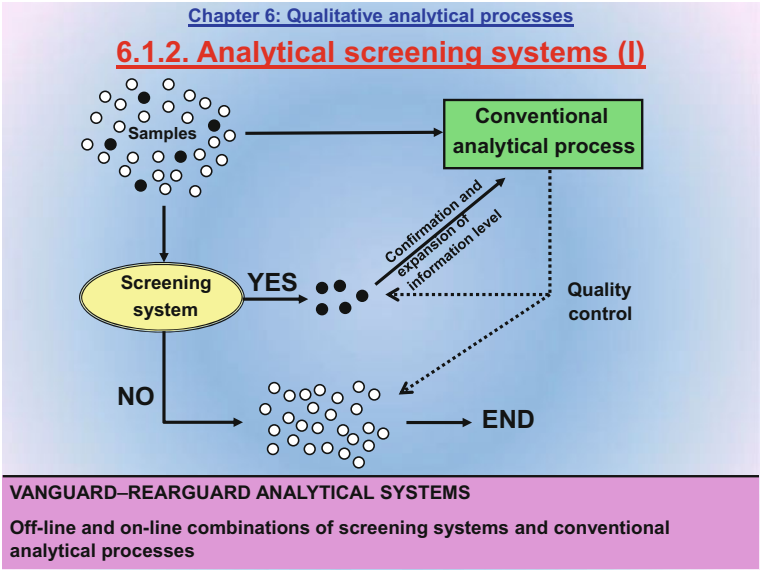
Slide 6.4



Qualitative analytical information therefore takes the form of a YES/NO binary response. As shown in Sect. 6.1.3.2, however, it almost invariably possesses some quantitative connotation. This type of information is rarely needed in Classical (Physical) Metrology; also, it is associated to special analytical properties and usually easier to obtain than other types of analytical information.

6.1.2 Analytical Screening Systems (3 Slides)

Slide 6.5



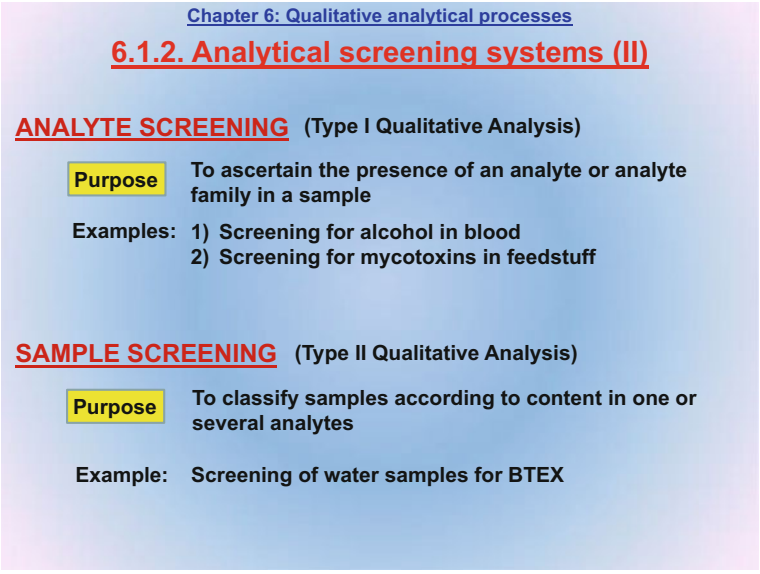
6.5.1. An analytical screening system is usually a simple analytical process used to classify samples into two groups according to whether they give a positive (YES) or negative (NO) response to the binary question posed.

This slide summarizes the operation of a screening system: each sample in a set is independently subjected to the screening process in order to rapidly classify it according to whether it gives a positive (black) or negative result (white).

6.5.2. The process is finished when the response is NO. On the other hand, a YES response requires confirmation with a conventional analytical process (e.g., sample treatment followed by gas or liquid chromatography). This process additionally allows the binary information initially obtained to be expanded. Thus, a sample of imported dried fruits testing positive for mycotoxins in an immunochemical test will be confirmed to contain them and assigned relative concentrations of the different toxins. Also, the results of screening analyses are frequently subjected to confirmation analyses for quality control purposes.

6.5.3. The combination of a screening system and a conventional analytical process constitutes a vanguard–rearguard analytical strategy. The two can be connected off-line (that is, operate independently) or on-line.

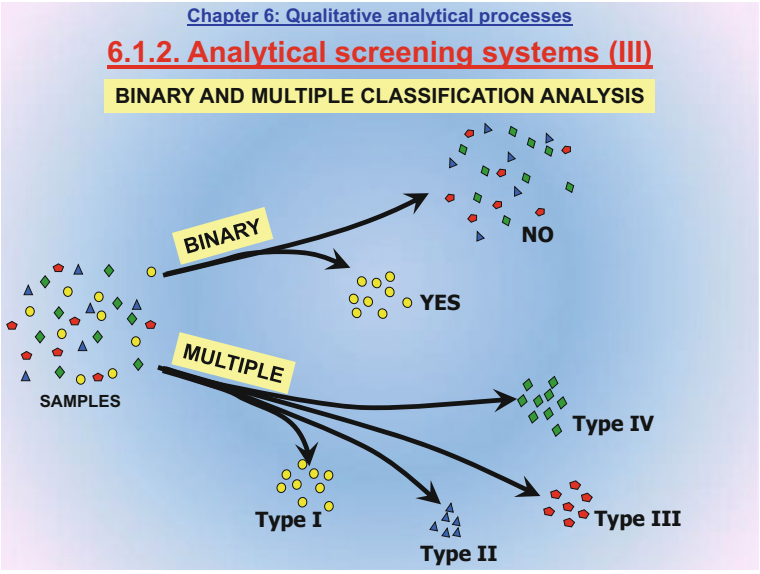
Slide 6.6



There are two types of screening in Qualitative Analysis, namely:

- *Analyte screening,* which is used *to identify* the presence of an analyte or analyte family in a sample and corresponds to Type I Qualitative Analysis in the next slide.
- *Sample screening,* which is used *to classify* samples according to the presence or absence of a particular analyte (e.g., benzene) or analyte family (e.g., BTEX, which comprises benzene, toluene, ethylbenzene and xylene organic molecules). This is the most common type of Qualitative Analysis (Type II in Slide 6.10).

Slide 6.7



It is not uncommon to refer to Qualitative Analysis as Classification Analysis (particularly in chemometric contexts).

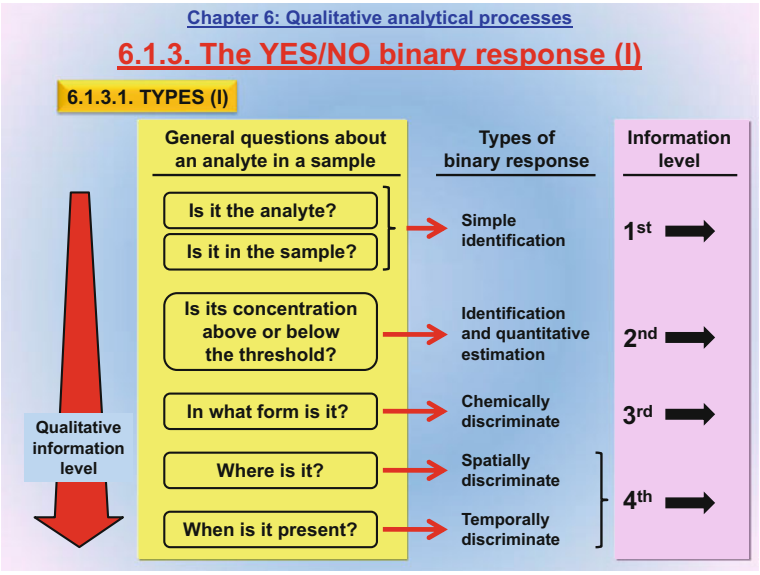
In *Binary Classification Analysis*, samples yielding a positive response (yellow circles) are separated from the rest (see Slide 6.5). One example is the classification of atmospheric samples into two groups depending on whether they contain acid rain gases (SO_x, N_xO_y) at levels exceeding the tolerated limits of the United States Environmental Protection Agency (US-EPA).

Multiple Classifying Analysis is more ambitious: it classifies samples into more than two groups, which usually requires using computers, chemometric software, sophisticated equipment and multiple information (e.g., the presence and concentration of several analytes) for each sample.

6.1.3 The YES/NO Binary Response (18 Slides)

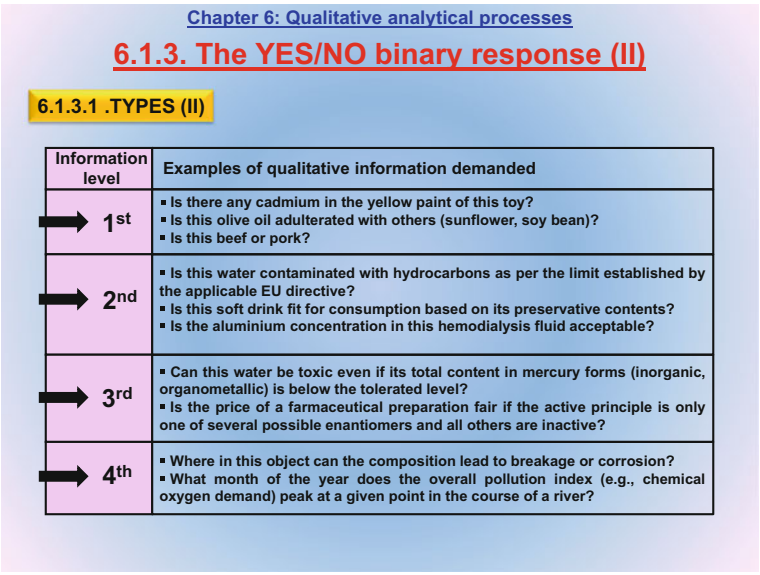
6.1.3.1 Types (4 Slides)

Slide 6.8



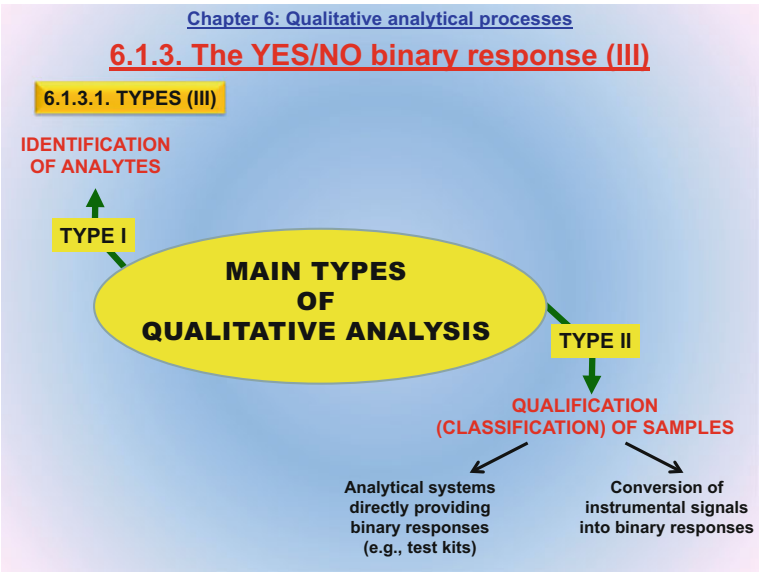
Qualitative Analysis can be classified in various ways. The classification in this slide is based on the type of information sought. The questions to be answered are shown here in increasing order of demand regarding the type of binary information involved. As can be seen, the types of response to be obtained range from simple identification (first level) through identification and quantitative estimation (second level), and chemically discriminate information (third level), to spatially and temporally discriminate information (fourth level).

Slide 6.9



These examples illustrate the four qualitative information levels in the previous slide. As noted earlier, these information requirements are becoming increasingly common, so Analytical Chemistry must gradually aim at their fulfilment.

Slide 6.10



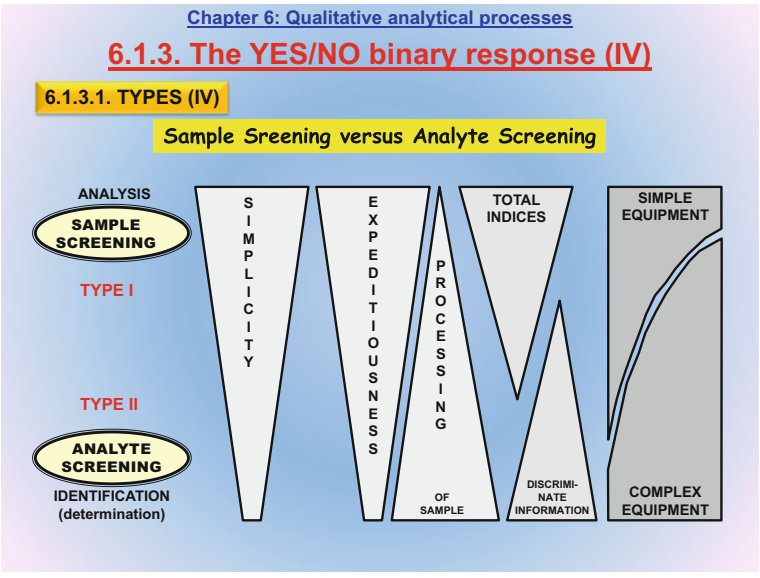
There are two major types of Qualitative Analysis according to primary purpose.

In *Type I* Qualitative Analysis the aim is to identify an analyte (e.g., phenol) or analyte family (several phenols including polyphenols).

In *Type II* Qualitative Analysis the target is the sample and the aim to qualify or classify it (e.g., to find whether it is edible on the basis of its toxin levels). Type II qualitative analyses can be performed in two ways.

- The simpler way involves using a straightforward, fast direct analysis system such as a reagent strip or test kit to obtain binary responses. One example is the pregnancy test kit for urine.
- Alternatively, a conventional analytical system such as a photometer, fluorimeter or chromatograph can be used to obtain instrumental signals for conversion into binary responses in accordance with a preset scheme. These modes of qualitative analysis is illustrated in the next slide.

Slide 6.11

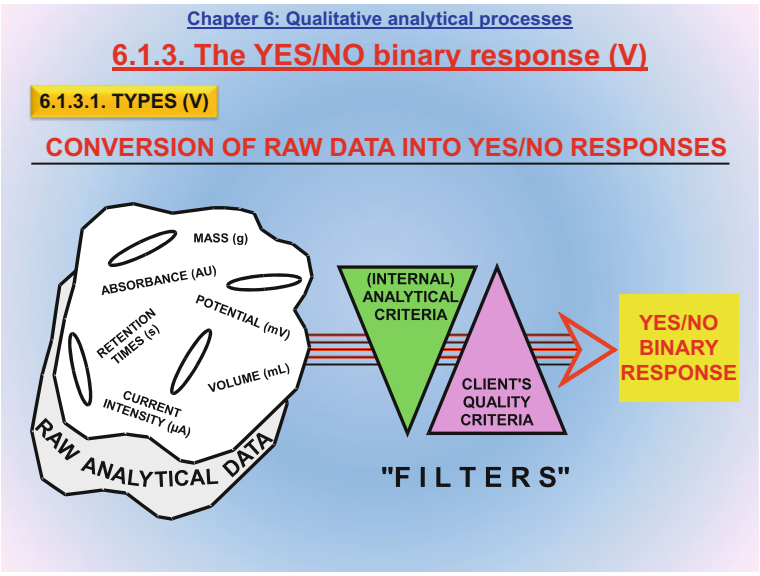


This slide highlights the differences between sample and analyte screening (that is, Type II and Type I Qualitative Analysis).

- Sample screening is more simple and expeditious than analyte screening—and so is the equipment needed by the former.
- Sample treatment is also more simple in sample screening than it is in analyte screening.

- Global information (e.g., total hydrocarbon levels in water) is more frequently managed in sample screening. On the other hand, specific information about individual analytes (that is, discriminate information such as hydrocarbon types in water) is more common in analyte screening.

Slide 6.12

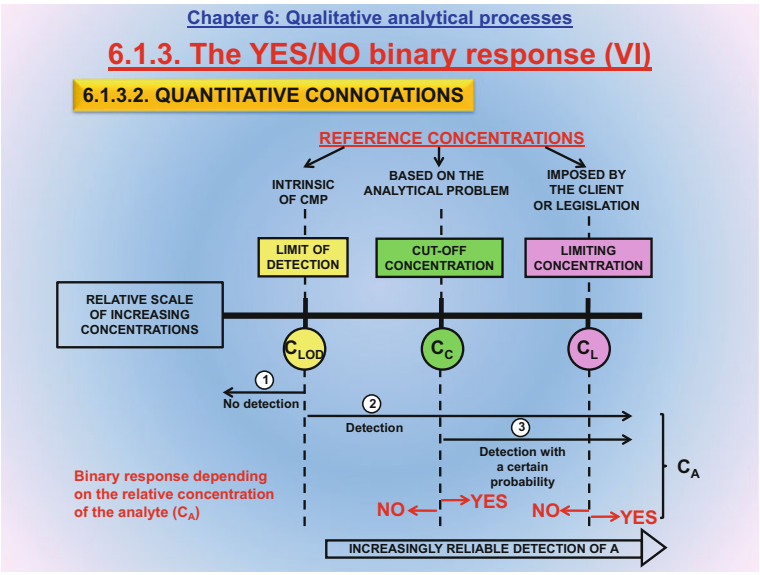


Converting primary instrumental data such as absorbances, fluorescence intensities, current intensities, volumes or potentials into binary responses is no easy task. In fact, it requires considering convergent and divergent criteria that are called "filters" here. The analytical criterion is intrinsic whereas the client's criterion, when applicable, is extrinsic.

In converting an absorbance datum for an analyte in a screened sample, the laboratory criterion materializes, for example, in constructing a calibration curve (see Slide 2.36) to derive a YES/NO binary response from the datum or using the limit of detection (LOD). The client's criterion, however, may differ depending on how strict the conversion is to be. Obviously, laboratory and client's criteria should always be reconciled when needed to solve an analytical problem (see Slides 7.8 and 7.23).

6.1.3.2 *Quantitative Connotations* (1 Slide)

Slide 6.13



6.13.1. As can be inferred from the previous slides, Qualitative Analysis frequently has quantitative connotations, especially when quantitative primary data are to be converted into binary responses (see Slide 6.12).

The conversion involves using a scale of absolute amounts (A) or concentrations (C_A) of analyte. The scale includes the *limit of detection* (C_{LOD}, Slide 2.40), which is typical of the analytical process, and the *limiting* or *threshold concentration* or amount imposed by legislation or the client (C_L). In some cases, the laboratory adopts a *cut-off concentration* C_C as a stricter limit than the threshold concentration for internal quality assurance—to avoid errors. Note that the limit of quantification (C_{LOQ}, Slide 2.41) has been excluded from the scale because it pertains to Quantitative Analysis.

6.13.2. The following two limits in the relative concentration scale define key zones in Qualitative Analysis.

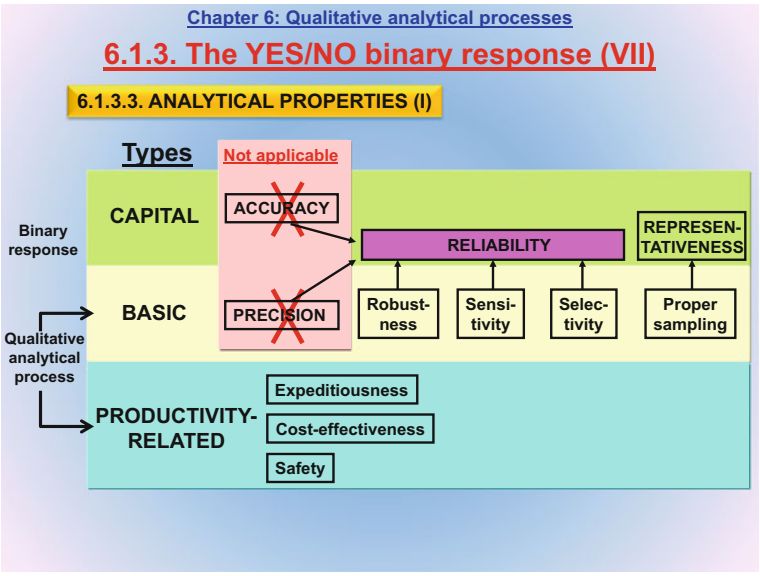
- The limit of detection sets the analyte concentration level above and below which the analyte is detected and not detected, respectively. This limit is inherent in the particular test or qualitative method.
- The cut-off concentration sets the level above which detection with a given probability will occur. This limit is self-imposed by the laboratory.

6.13.3. The cut-off and threshold concentrations also define the zones for the binary responses YES (right) and NO (left).

Interestingly, the proportion of errors in qualitative detection decreases with increasing analyte concentration. A new analytical property called "reliability" is defined in Slide 6.16.

6.1.3.3 Analytical Properties (7 Slides)

Slide 6.14

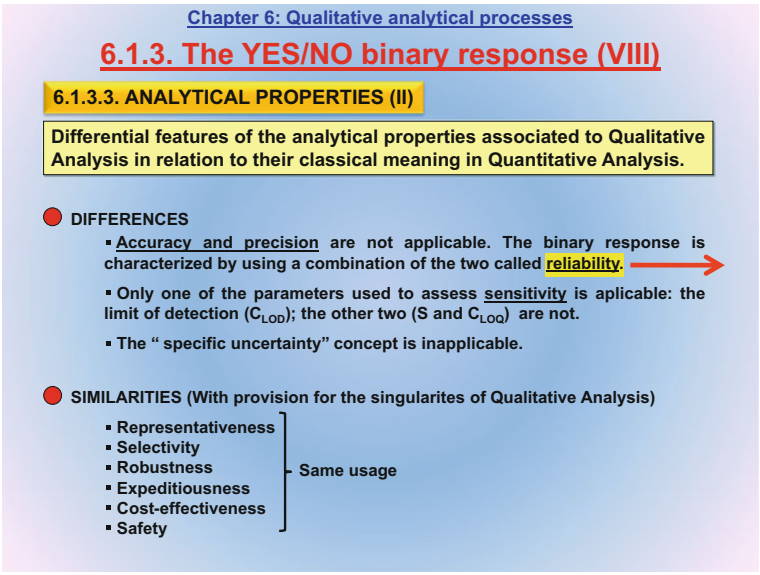


The overview of analytical properties in Slide 2.4 is directly applicable to Quantitative Analysis but requires some adaption for use in Qualitative Analysis. Although the three types of properties (namely, capital properties for the binary response, and basic and productivity-related properties for the analytical process) remain, they differ in two respects from those pertaining to Quantitative Analysis. Thus,

(1) accuracy and precision are not applicable to Qualitative Analysis; and
(2) a new capital property called *reliability*, which rests on the three basic properties (robustness, sensitivity and selectivity), is required.

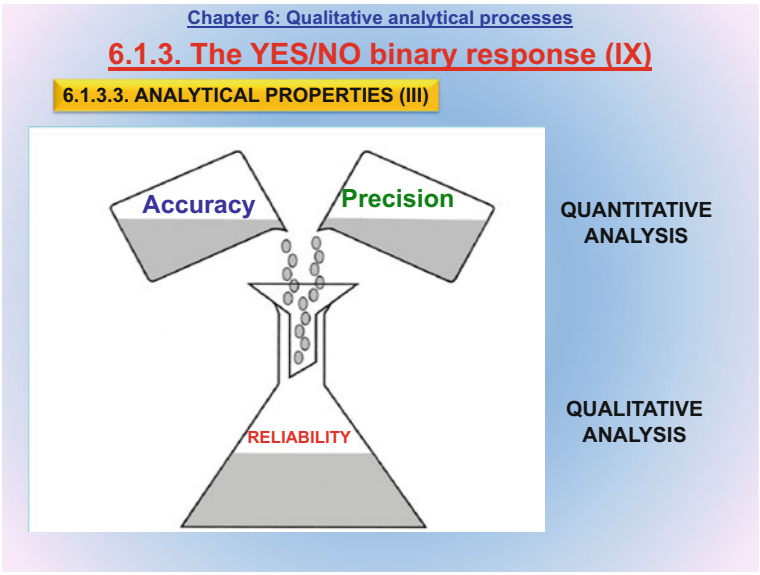
There next slide discusses other, more specific differences.

Slide 6.15



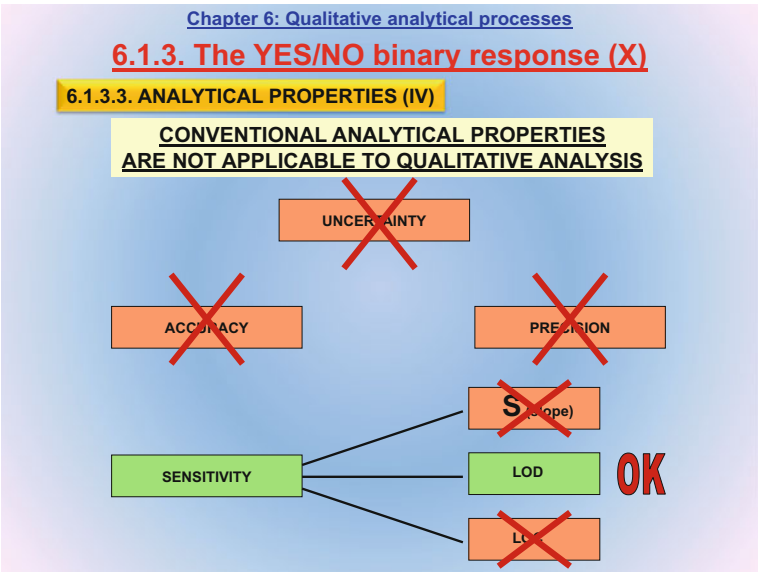
This slide summarizes again the similarities and differences between the analytical properties applicable to Quantitative and Qualitative Analysis. As can be seen, the limit of detection (LOD, Slide 2.40) is the only sensitivity-related parameter applicable to Qualitative Analysis.

Slide 6.16



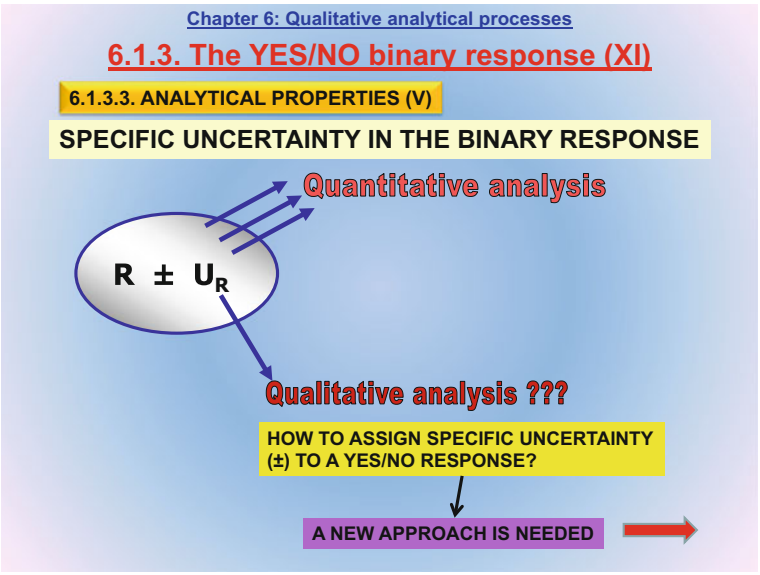
The capital property *reliability* pertains to Qualitative Analysis even though it is a combination of two classical properties pertaining to Quantitative Analysis, namely: accuracy and precision.

Slide 6.17



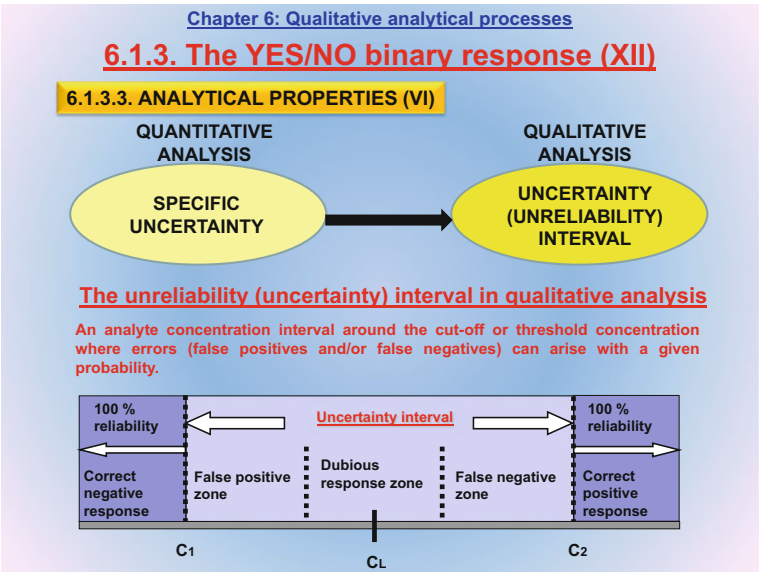
This is a brief summary of the capital (uncertainty and accuracy) and basic properties (precision and two sensitivity measures) not applicable to Qualitative Analysis.

Slide 6.18



The specific uncertainty of a numerical result R, U_R, which is described in detail in connection with Quantitative Analysis in Slides 2.29 and 2.31, cannot be used in Qualitative Analysis. How can a YES/NO binary response thus be assigned an uncertainty interval? By approaching uncertainty in a novel, unorthodox manner that is described in the next slide.

Slide 6.19



Specific uncertainty, which is typical of Quantitative Analysis (see Slide 2.29), should be converted into an *uncertainty* or *unreliability interval* in Qualitative Analysis.

The uncertainty or unreliability interval is defined as the —generally symmetric— range around the cut-off (C_C) or threshold concentration (C_L) where errors—whether false positives or false negatives—can be expected to occur at a given probability level (e.g., 95%). The interval is experimental established as follows:

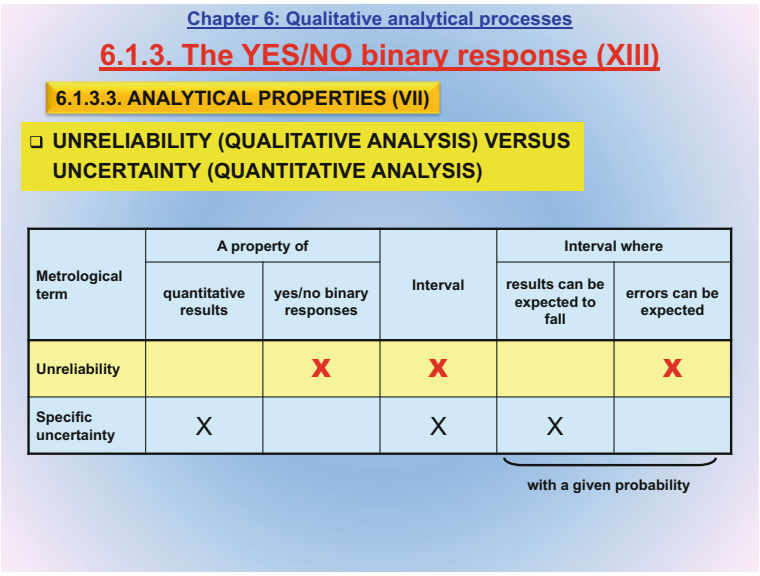
(1) A set of standard samples containing variable concentrations of analyte is prepared. Because the analyte concentration in each sample is known, the result (response) can directly be deemed correct or incorrect and a distinction between false positives and false negatives, which are explained in Slide 6.21, be made. Each sample in the set is subjected to the qualitative analytical process and the results recorded.

(2) The results are plotted on an analyte concentration scale including the threshold or limiting concentration. Around such a concentration is the uncertainty interval (C_1–C_2), which includes a zone of dubious responses, another of false positives at the low-concentration end (near C_1) and a third of false positives at the high-concentration end (near C_2). Outside the uncertainty interval are two zones where

– a negative response at low concentrations ($<C_1$) will be correct; and
– a positive response at high concentrations ($>C_2$) will also be correct.

Therefore, the width of the uncertainty interval determines the reliability or confidence of a qualitative result.

Slide 6.20



This slide compares unreliability in Qualitative Analysis to specific uncertainty in Quantitative Analysis. Both concepts represent a concentration interval which, however, differs between the two types of analysis. Thus,

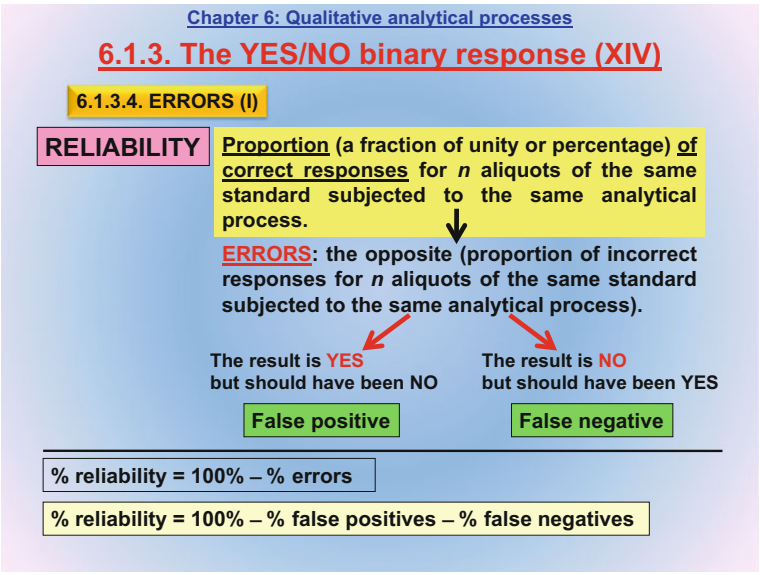
- in Qualitative Analysis, the unreliability interval is the zone where errors (false positives or false negatives) arise;
- in Quantitative Analysis, the specific uncertainty interval is the concentration range where the result for another aliquot of the same sample subjected to the same analytical process can be expected to fall (see Slide 2.7).

Both intervals share a common trait: their width depends on the selected probability level—albeit in a different manner. Thus, the unreliability interval is a range of values where the result is incorrect (that is, a range where trueness in a qualitative result cannot be assured). As a consequence, the higher is the statistical probability in Qualitative Analysis, the narrower will be interval. Conversely, the higher is the probability (confidence) in Quantitative Analysis, the higher will be the specific uncertainty and the wider the interval as a result (see Slides 2.29 and 2.31).

This difference arises from the way the intervals are conceived in Qualitative and Quantitative Analysis. Thus, the unreliability interval is a range of errors—an unwanted outcome—whereas the uncertainty interval is a range where the result can be expected to fall—a desirable outcome.

6.1.3.4 Errors: False Positives and False Negatives (3 Slides)

Slide 6.21



6.21.1. This slide defines "reliability", which appears in the overview of analytical properties applicable to Qualitative Analysis (see Slide 6.14).

Subjecting n aliquots of a standard sample with known binary responses to a qualitative test provides n binary responses the reliability of which will be given by the ratio of accurately identified aliquots to the total number of aliquots.

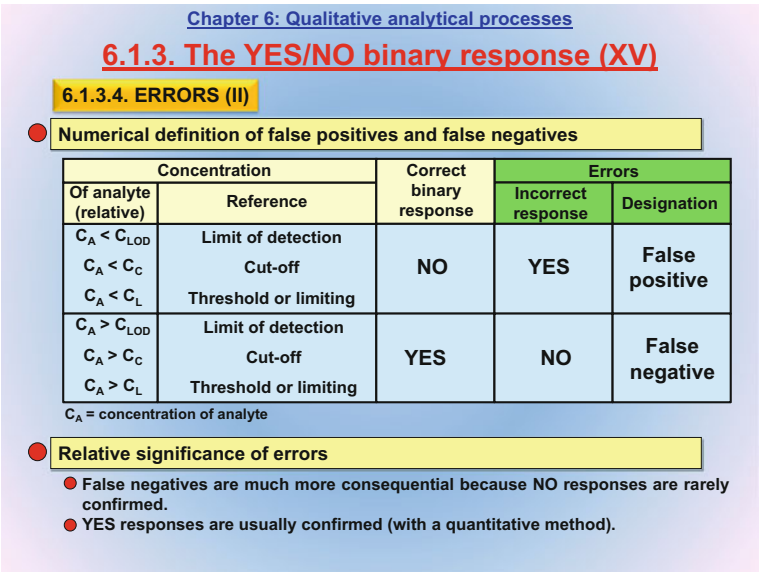
6.21.2. The opposite of "reliability" is "error", which is defined here as the proportion of incorrect responses. In Qualitative Analysis, errors can be of two types, namely:

- *False positives*, which occur when the result is YES but should have been NO and are especially likely at analyte concentrations slightly below the limiting concentration (C_L).
- *False negatives,* which arise when the result is NO but should have been YES and are therefore more likely to occur at concentrations slightly above C_L.

A sample containing an analyte concentration near C_L may give a *dubious result* (see Slide 6.19).

6.21.3. The percent reliability of a qualitative analysis is calculated by subtracting the proportion of errors (that is false positives and false negatives, which can be easily discriminated) from 100.

Slide 6.22



6.22.1. Whether a binary response is correct, a false positive or a false negative can be ascertained by relating the analyte concentration (C_A) to the limit of detection, cut-off concentration or threshold concentration.

- Thus, when C_A is lower than the previous limits, the correct binary response is NO, so YES is incorrect and constitutes a false positive.
- On the other hand, when C_A exceeds the previous limits, the correct response is YES, so NO is incorrect and a false negative.

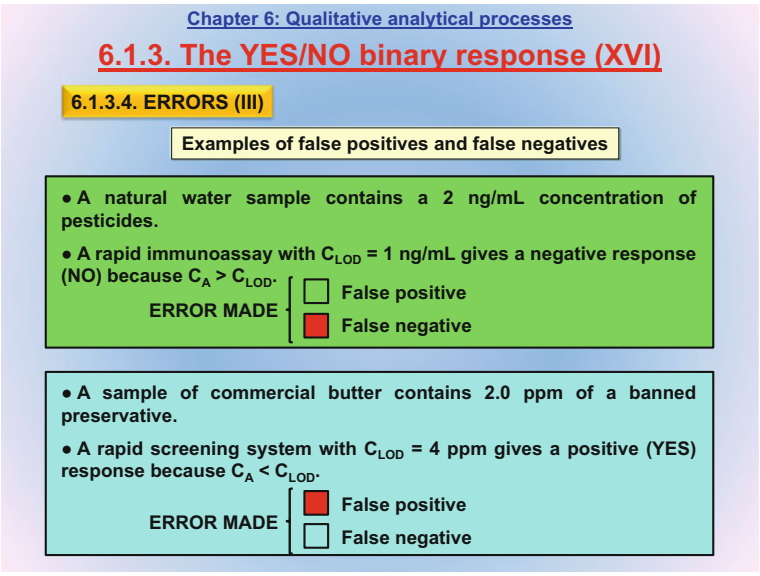
The table is highly illustrative. As can be seen, the concentration level to be used in order to classify a response as YES or NO depends on the particular limit chosen.

6.22.2. It is extremely important to understand that the two types of error in Qualitative Analysis differ strongly in their practical consequences. Thus, as can be seen in Slide 6.5, if the response of a screening system is NO, the analysis is finished; on the other hand, if the response is YES, the samples testing positive are almost invariably subjected to a confirmatory analysis with a conventional analytical process.

Consequently, false negatives should be avoided at any rate because they are not routinely confirmed. For example, a customs laboratory passing an imported batch of peanuts which has incorrectly tested negative for mycotoxins in a screening

analysis can have serious adverse consequences on consumers' health because mycotoxins are carcinogenic. The ensuing risk should be avoided by assuring the complete absence of false negatives in the analysis.

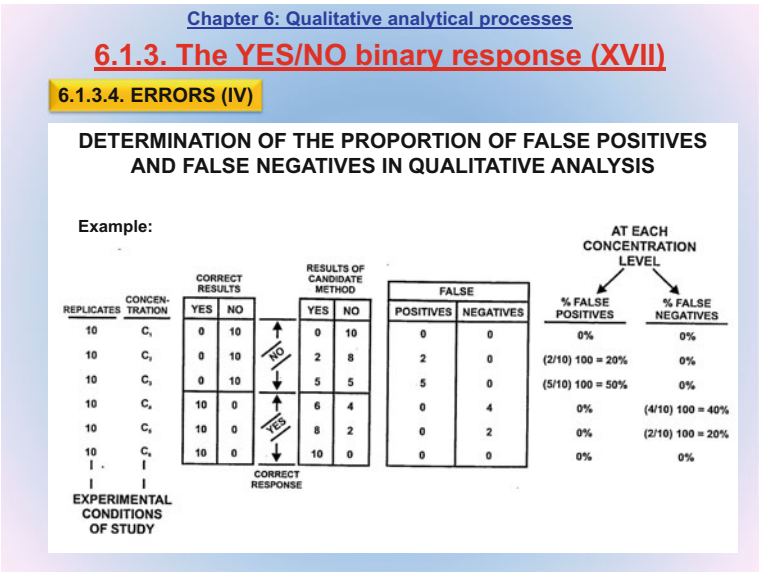
Slide 6.23



6.23.1. These are two examples of errors (false positives and false negatives) in Qualitative Analysis providing for the concepts explained in the previous slide.

6.23.2. The red boxes represent the correct way of labelling the ensuing errors.

Slide 6.24



The experimental procedure for calculating the proportion of false positives and false negatives, and the unreliability interval around the threshold concentration (C_L) for a screening system, is as follows:

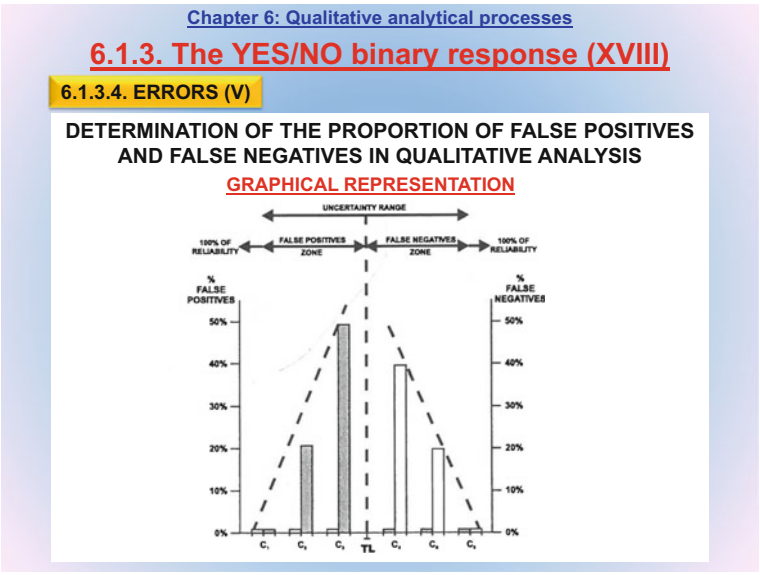
(1) A set of, for example, 60 standard samples containing 6 different analyte concentrations C_1–C_6 (that is, 10 replicates per concentration) is prepared. Three of the chosen concentrations (C_1–C_3) should be higher than the threshold concentration (C_L) and the other three (C_4–C_6) lower.
(2) The samples are subjected to the screening process in a random sequence and a binary response for each is obtained.
(3) Since the correct binary responses for the whose sample set are known, each sample can be classified as "correct", "false positive" or "false negative" by comparison with the actual (experimental) result.

The table shows the results for the 60 samples according to analyte concentration (C_1–C_6).

Although the 30 samples with $C_A < C_L$ should have yielded a NO response, not all did—particularly those containing the analyte at concentrations near the threshold (C_2 and C_3), which gave 2 (20%) and 5 (50%) false positives, respectively. Note that errors increased with increasing nearness of the analyte concentration to C_L.

Likewise, all 30 samples with $C_A > C_L$ should have tested positive (YES) for the analyte; however, 4 samples with C_4 and 2 with C_5 led to false negatives. Again, the number of errors increased with increasing nearness of the analyte concentration to C_L.

Slide 6.25

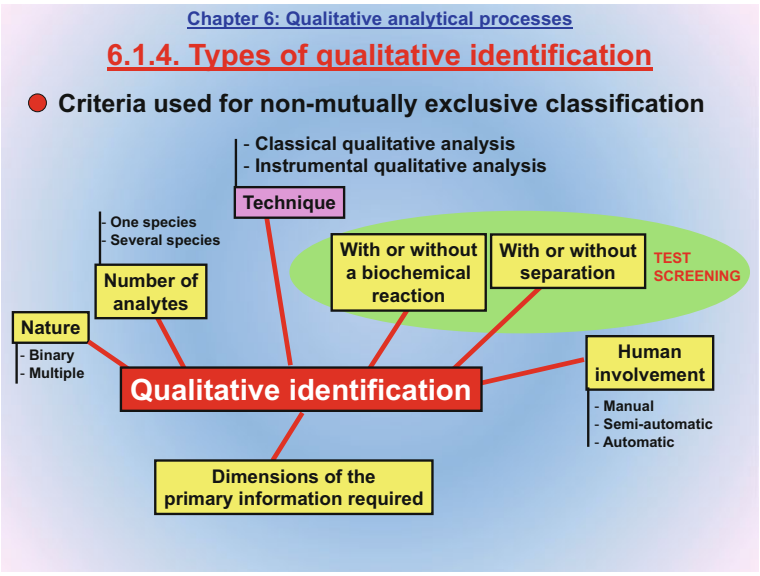


This graph shows the two types of errors in Qualitative Analysis (false positives and false negatives) on two *y*-axes as a function of the analyte concentration on the *x*-axis. The graph focuses on the zone around the threshold concentration, where the three concentrations below C_L and the three above it in the example of the previous slide are placed. As can be seen, the errors exhibit a Gaussian distribution on the analyte concentration scale, which encompasses the zones shown in that slide — that of dubious results excluded.

Both types of errors increase near the threshold concentration. At the two ends of the scale are the zones of total reliability, that is, of error-free classification of samples as YES (left end) or NO (right end).

6.1.4 Types of Qualitative Identification (1 Slide)

Slide 6.26



This is an overview of Qualitative Analysis in the form of non-mutually exclusive classifications according to various, complementary criteria, namely:

(1) *Nature of the response*, which can be binary (YES/NO) or multiple (see Slide 6.7).
(2) *Number of analytes*, which can be one (e.g., phenol) or several (e.g., an analyte family such as phenols).
(3) *Analytical technique*, which can be a classical or instrumental qualitative test or screening system. This criterion is used to describe Qualitative Analysis in Sects. 6.1.5 and 6.1.6.

(4) *Type of qualitative test* or *screening system* used, which can involve

- one or several *(bio)chemical reactions*; and
- With and without a chromatographic or non-chromatographic *analytical separation system.*

(5) Binary and multiple classification of samples entail using a given number of primary data (signals) obtained in a discriminate manner from a single or several instrumental parameters (that is, *specific information dimensions*).

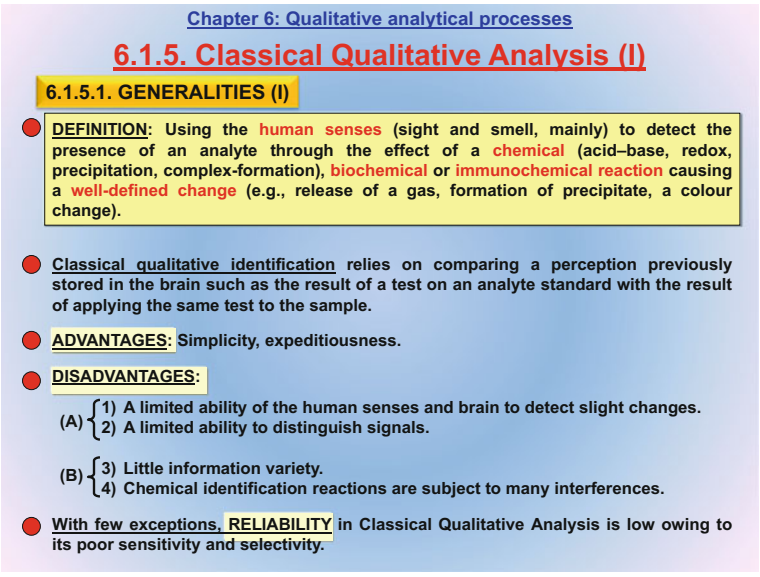
- Classical Instrumental Analysis generally uses a single "instrument" (e.g., human sight) to observe a single signal (e.g., formation of a precipitate).
- Identifying quinine in tonic water by its native fluorescence entails using two instrumental parameters (the excitation and emission wavelengths) to produce a signal (fluorescence intensity).
- Identifying a substance by infrared (IR) absorption spectroscopy requires using a whole IR spectrum, which entails acquiring a large number of absorbance signals at many different wavenumbers. Only in that way can the target substance be identified (see, for example, benzene in Slide 6.37).

In summary, Qualitative Analysis possesses a variety of nuances that substantially enrich it conceptually.

6.1.5 Classical Qualitative Analysis (8 Slides)

6.1.5.1 *Generalities* (2 Slides)

Slide 6.27



This brief description of *Classical Qualitative Analysis* is started with its definition, which comprises the use of

- human senses as "instruments" and the brain as a "computer"; and
- (bio)chemical or immunological reactions between the analyte and a reagent to obtain a product that can be easily seen or smelt.

Qualitative identification additionally involves *comparing* the signal for the analyte—or its absence—with that for a standard. The two are compared by the brain to produce a result: YES or NO. Thus, if an unknown liquid smells of acetic acid (the standard), the liquid can be identified as vinegar.

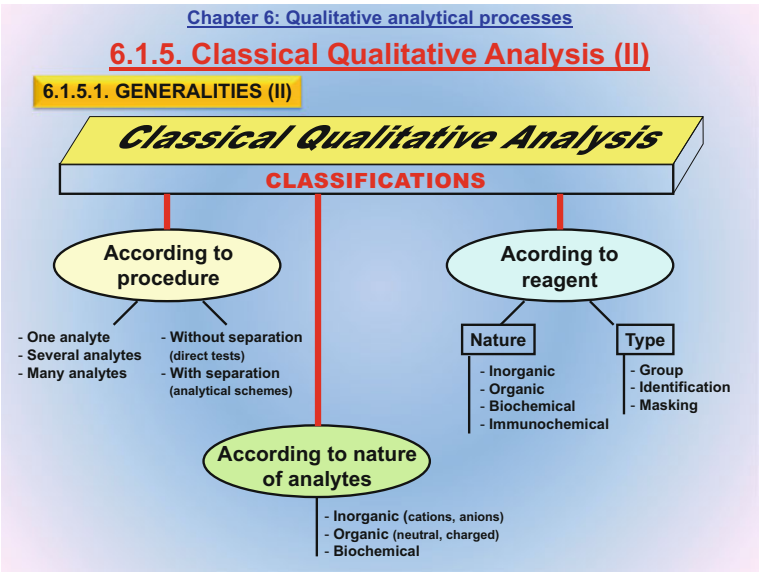
Although Qualitative Analysis possesses doubtless advantages (e.g. expeditiousness and simplicity), it also has major limitations such as the following:

- a low selectivity arising from little variety in the information that can be obtained and the fact that the reactions used—immunoassays excepted—are typically subject to many interferences; and
- an also low sensitivity resulting from the limited ability of human senses to detect small changes.

As a consequence, reliability in Classical Qualitative Analysis is usually modest because it rests on the basic properties sensitivity and selectivity (see Slide 6.14 for a general scheme of analytical properties in Quantitative Analysis).

The previous limitations preclude the use of Classical Qualitative Analysis for multiple classification and restrict it to binary (YES/NO) classification (see Slide 6.7).

Slide 6.28

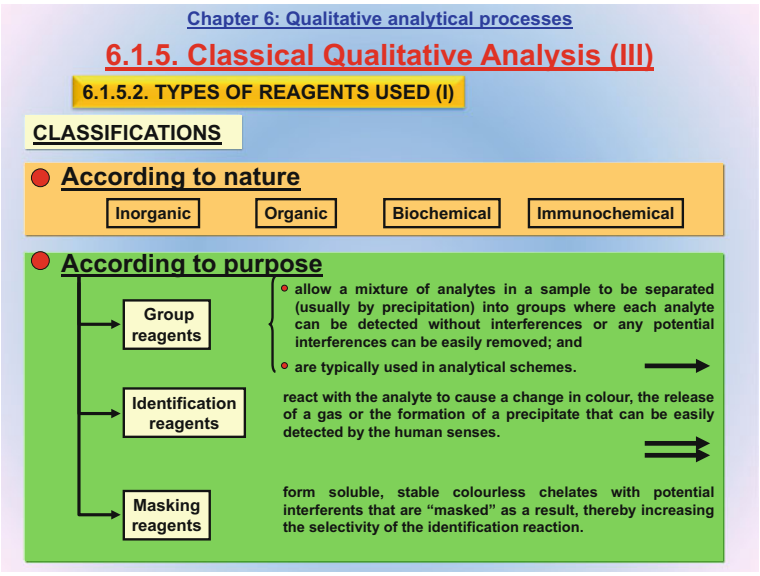


This slide classifies Classical Qualitative Analysis according to the following criteria:

(1) The *experimental procedure* used, which depends on whether one, several or many analytes are to be identified in the same sample. Sensitive, selective reagents, which are very scant, afford *direct analyses* (that is, analyses without separation); most often, however, some *analytical separation* into groups of species (that is, an analytical scheme) is needed to improve the sensitivity and selectivity of the identification.
(2) The *nature of the analytes*, which will require a different type of procedure depending on whether they are inorganic, organic or biochemical.
(3) The *nature and purpose of the reagents*, which is very important (see Slides 6.29 and 6.30).

6.1.5.2 Types of Reagents (3 Slides)

Slide 6.29



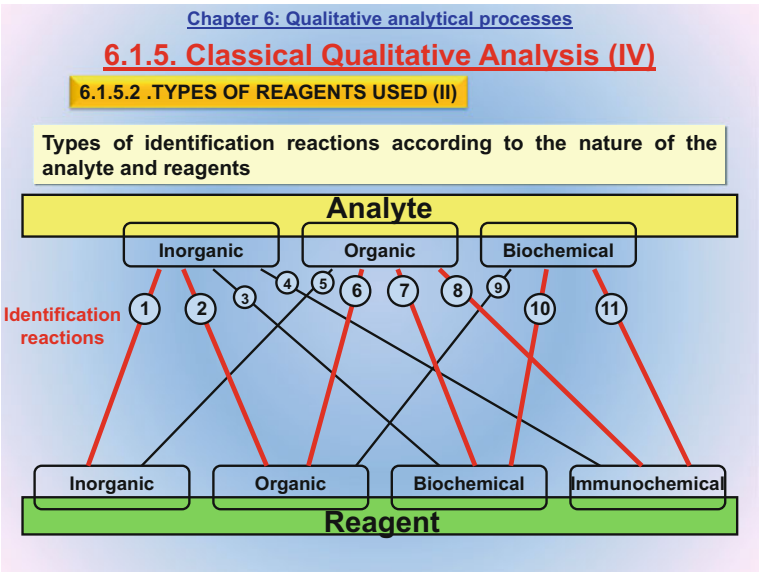
This slide expands on the classifications of Classical Qualitative Analysis according to the nature and purpose of the reagents used for identification.

The first classification, based on the *nature of the reagents*, distinguishes between biochemical and immunochemical reagents—the latter are especially useful by virtue of their high selectivity.

The second classification is based on the *function of the reagents*, namely: separating a group of analytes, identifying an analyte or masking it to facilitate the identification of others.

Both classifications are discussed in greater detail in the next two slides.

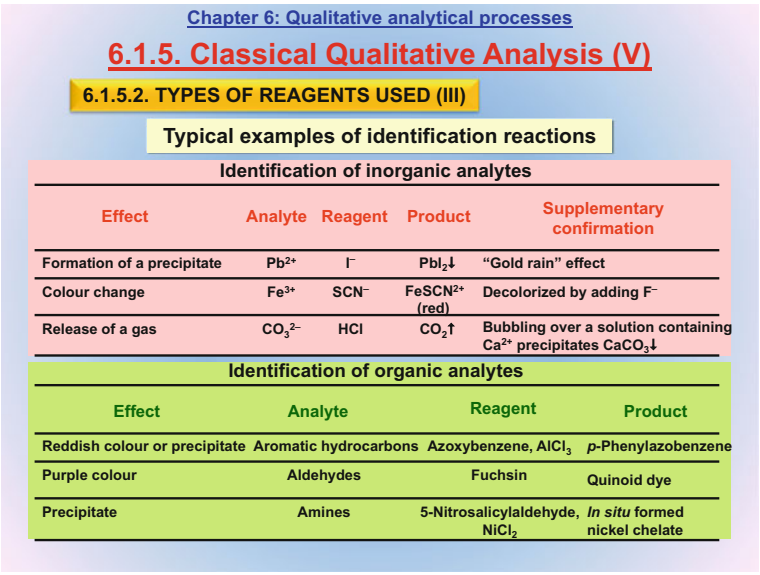
Slide 6.30



Based on their nature, analytes and reagents —largely identification reagents— can be related in eleven different ways for purposes the most common among which are as follows:

- detection of inorganic analytes with inorganic or organic reagents;
- detection of organic analytes with organic, biochemical or immunochemical reagents; and
- detection of biochemical analytes with biochemical or immunochemical reagents.

Slide 6.31

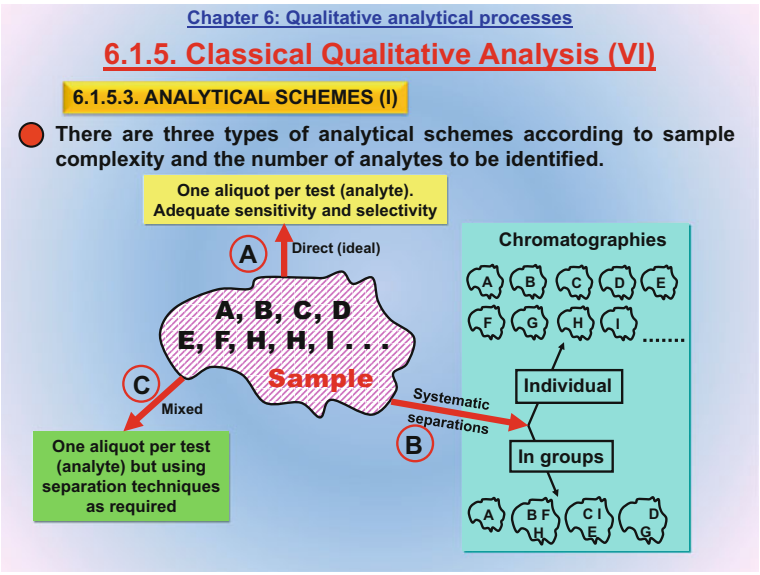


These are several examples of identification reactions.

- The first table exemplifies the detection of inorganic analytes according to the effect to be detected by the human senses (formation of precipitate, a colour change, bubbling of a gas). The analyte, the reagent and the substance producing the effect are stated, and so is the supplementary confirmation reaction to be used.
- The second table shows three examples of identification of organic species (or species families). The effect shown on the first column in each row is due to the product on the last.

6.1.5.3 Analytical Schemes (3 Slides)

Slide 6.32



Analytical schemes are classical qualitative analytical processes that are used to detect many analytes (A, B, C, D, E, F, etc.) in the same sample. There are three different types of systematic detection processes depending on the characteristics of the sample matrix and the number of analytes to be identified, namely:

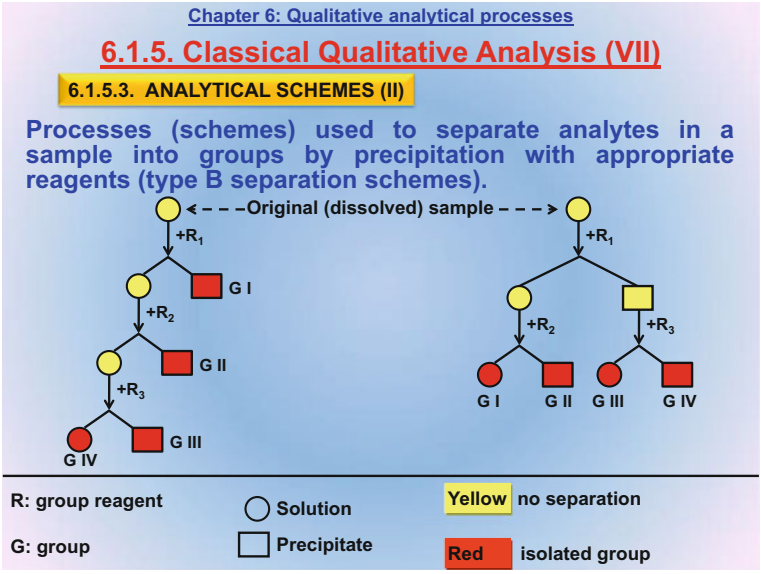
(A) *Direct detection processes*, which can only be used in ideal situations and involve subjecting *n* aliquots of sample to *n* direct, independent tests for each analyte. The low sensitivity and selectivity of Classical Qualitative Analysis make direct detection highly desirable but unfeasible unless only a few analytes contained in a simple sample matrix (e.g., water) are to be identified—which, however, usually requires using highly specific, expensive reagents.

(B) *Processes involving systematic separations* to isolate individual analytes or analyte groups in order to increase the sensitivity and selectivity of the detection tests. Analytes can be separated in two different ways, namely:

- *Chromatographically.* Each analyte is isolated in a given zone of the mobile phase to enable its interference-free detection.
- *By groups*. This requires using so-called "group reagents" (see Slide 6.29), which are usually precipitants, in systematic separations. *Analytical schemes with group separation* are described in Slides 6.33 and 6.34.

(C) *Mixed* processes. These are combinations of the previous two involving the sequential detection of each analyte in a sample aliquot with or without application of a separation system. These processes constitute *analytical schemes without group separation* (see Slide 6.34), a dubious designation because they do involve separations—to identify individual analytes rather that to separate them in groups, however.

Slide 6.33

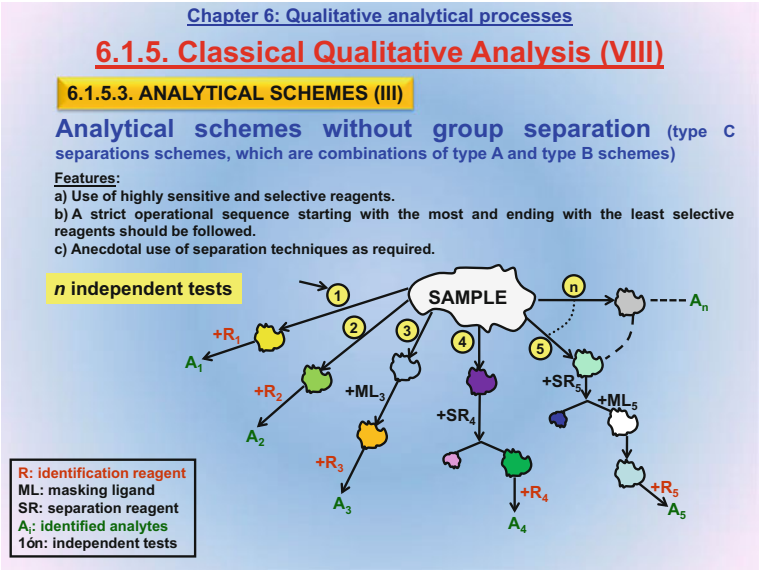


Analytes can be separated into different groups (G) by using precipitating reagents (R) in two different ways, namely:

(1) By sequentially adding the reagents (R_1–R_3 in the slide) to the sample solution in order to successively separate the analytes into groups (I–III), after which the sample will only contain a group of soluble analytes (IV) not reacting with the precipitants.
(2) By using a reagent R_1 to split the analytes in the sample into two large groups: soluble and insoluble analytes. In parallel, the two groups are treated separately with two other reagents R_2 and R_3 in order to eventually obtain two soluble groups (I and III) and another two insoluble groups of analytes (II and IV).

The symbols at the bottom of the slide make it easier to interpret the two types of schemes.

Slide 6.34



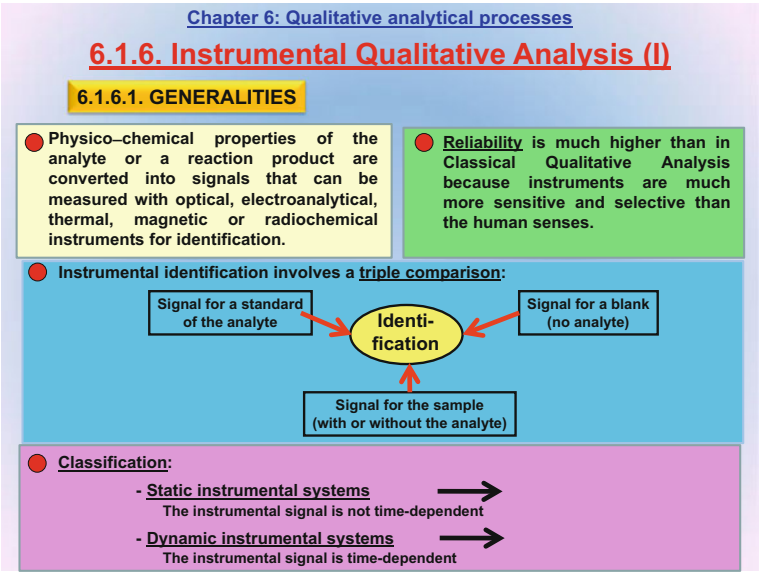
This slide explains analytical schemes without group separation for the identification of a set of analytes A_1–A_n in the same sample, which share several common features, namely:

- they involve performing *n* independents tests (one per analyte to be identified);
- they use highly sensitive and selective—and usually expensive—reagents;
- the identification tests must be conducted in a strictly controlled sequence—from the most sensitive or selective to the least;
- the qualitative information obtained in each test is used to adjust the next—hence the need to use highly sensitive and selective tests first;
- each test requires some separation, usually with a precipitating reagent or, less often, a masking (chelating) reagent (see Slide 6.29); and
- the complexity (number of steps) of the tests increases as the identification process develops.

6.1.6 Instrumental Qualitative Analysis (7 Slides)

6.1.6.1 Generalities (1 Slide)

Slide 6.35



6.35.1. *Instrumental Qualitative Analysis* uses instrumentally measured physico–chemical properties of analytes or their reaction products for identification.

6.35.2. Identification in Instrumental Qualitative Analysis relies on a *triple comparison* involving the measurements (signals) for a blank (the sample matrix containing no analyte), a standard of the analyte and the sample from which analytical information is to be extracted.

For example, the fluorescence intensity I_F at a given excitation and emission wavelength for the blank signal was 0.020; that for a sample standard containing an analyte concentration C_{AP} was 0.210; and that for the target sample 0.280. The signal (fluorescence intensity) corresponding to the analyte concentration is thus 0.260 and a simple proportion allows C_A to be easily calculated.

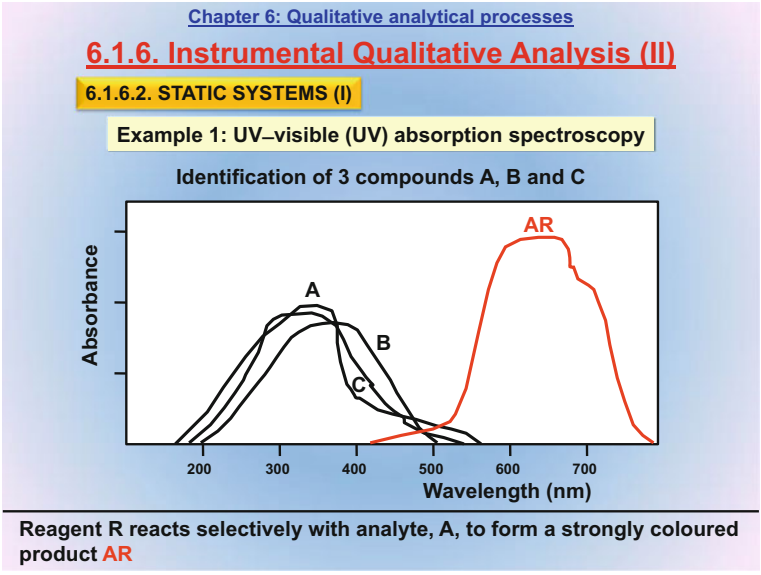
If the analyte concentration is equal to or greater than the limiting concentration ($C_A \geq C_L$), then the binary response will be YES; otherwise ($C_A < C_L$), the response will be NO.

6.35.3. Reliability is much greater in Instrumental Qualitative Analysis than it is in Classical Qualitative Analysis because instruments are much more sensitive and selective than the human senses.

6.35.4. Instrumental Qualitative Analysis can be classified in many different ways. The most immediate way is according to the type of signal (optical, electroanalytical, thermal, mass, radiochemical) used for identification. The following four slides describe an alternative classification based on the time-dependence of the signal, according to which instrumental qualitative analytical systems can be of the *static* or *dynamic* type.

6.1.6.2 Static Systems (2 Slides)

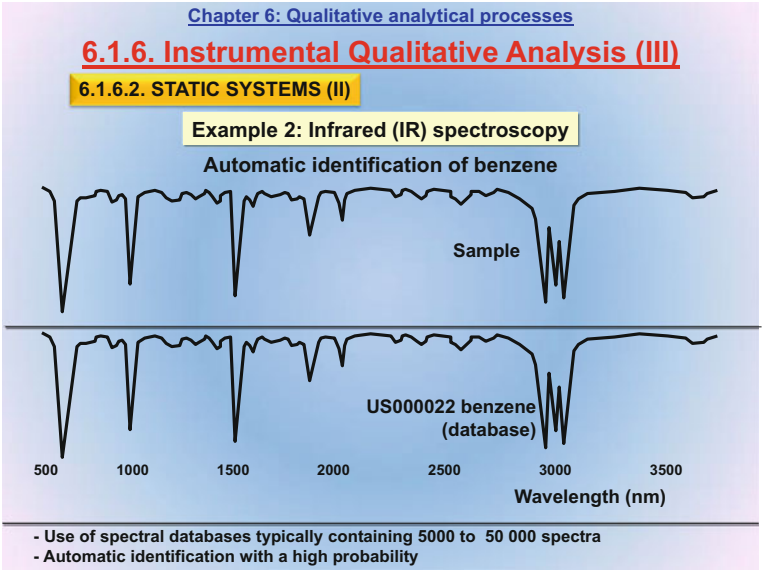
Slide 6.36



In *static systems*, the signal remains constant over time.

Not all measuring instruments are equally capable of discriminating signals for different sample components. Thus, UV–visible absorption spectroscopy has a low discrimination potential. As can be seen in the slide, identifying all three analytes (A, B, and C) in this example is impossible because their absorption spectra (absorbance vs wavelength recordings), in black, are very similar, so no wavelength zone exists where each analyte can be detected in the presence the other two. If no alternative detection equipment is available, the analytes must be discriminated chemically. For example, if a reagent R reacts selectively with only one of them (e.g., A) to form a bluish red chelate AR and the chelate absorbs at 600 nm without interference from the other two analytes, A can be reliably identified.

Slide 6.37



This slide illustrates the automatic identification of benzene by infrared (IR) absorption spectroscopy, which is a widely used technique for qualitative analytical purposes.

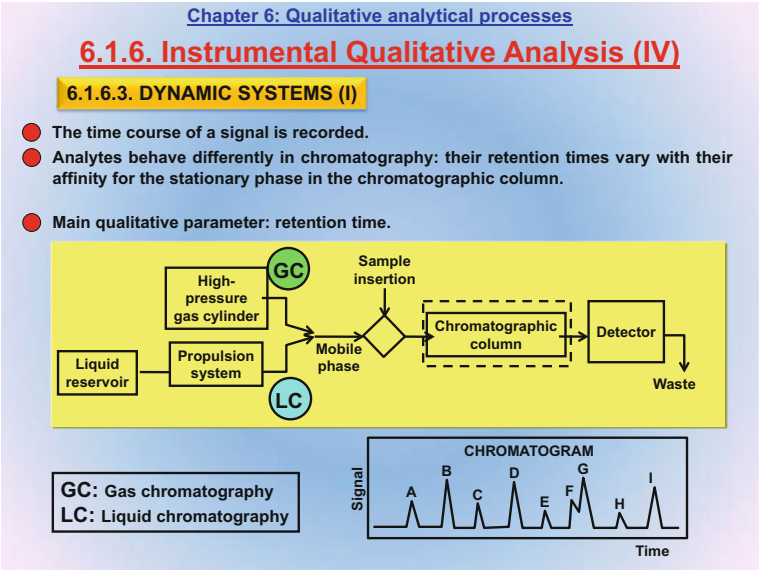
The signals for the sample and the standard (US000022) remain unchanged with time. Comparing a large number of instrumental (absorbance, wavenumber) data ensures a high reliability.

The first IR spectrum corresponds to the sample and the second to an analyte standard as recorded in a spectral database. A computer allows the sample spectrum to be compared to more than 50,000 spectra in a database and, if a coincidence is found, the analyte to be matched to a specific compound with a given level of reliability (98% in our case). Advances in miniaturization have allowed databases to be incorporated into measuring instruments and enormously empowered Instrumental Qualitative Analysis as a result.

A mass spectrometer (MS) is another instrument with a high analyte identification potential.

6.1.6.3 Dynamic Systems (4 Slides)

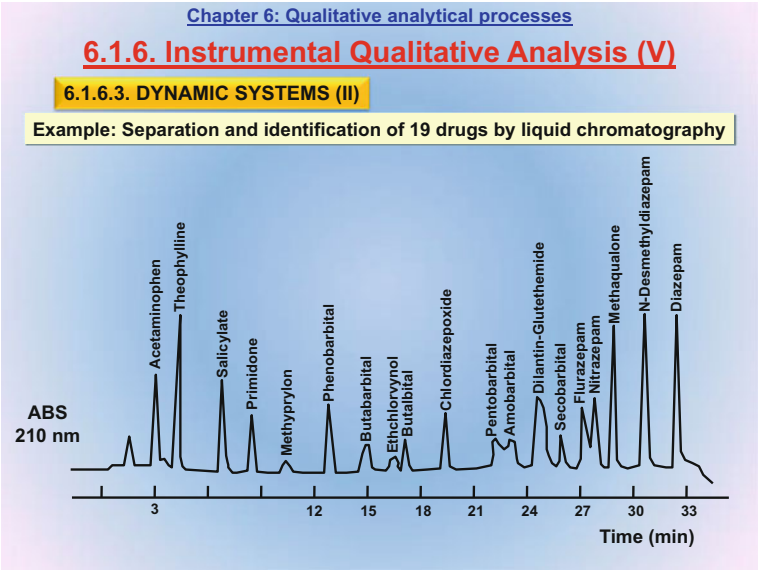
Slide 6.38



In *dynamic systems* for Instrumental Qualitative Analysis, the measured signal changes with time (it is time-dependent). These systems typically use a detector coupled to a GC or LC chromatographic column or electrophoretic capillary for detection after separation. Each analyte is identified in terms of a qualitative parameter called the "retention time". If the chromatogram exhibits a signal at the typical retention time for a given analyte, then the binary response for the presence of the analyte in the sample will be YES.

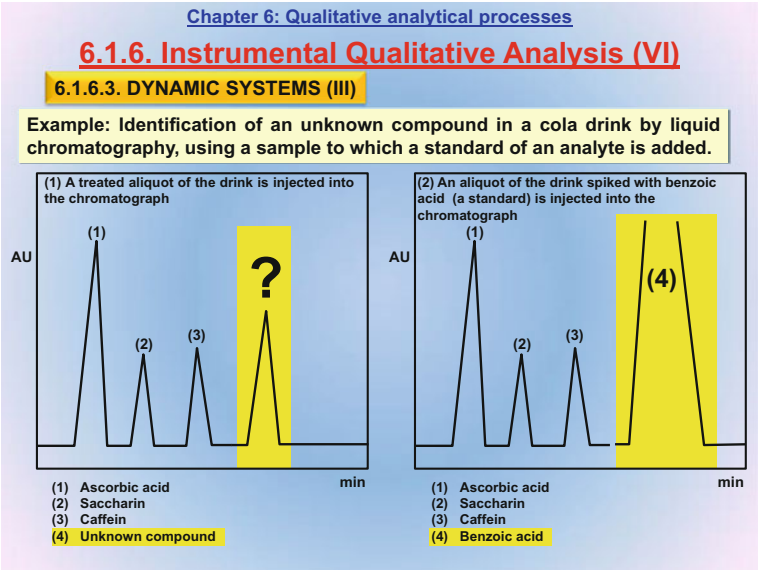
This slide depicts a gas chromatograph (GC) and a liquid chromatograph (LC), which differ in the way the chromatographic fluid is propelled and hence in the nature of the mobile phase. The two use a similar, but not identical, sample insertion (injection) system, column and continuous detector.

Slide 6.39



This slide illustrates the potential of liquid chromatography for multi-identification with the separation and identification of 19 drugs in human serum. Although some peaks in the chromatogram are overlapped, their retention times are different enough for qualitative identification purposes.

Slide 6.40

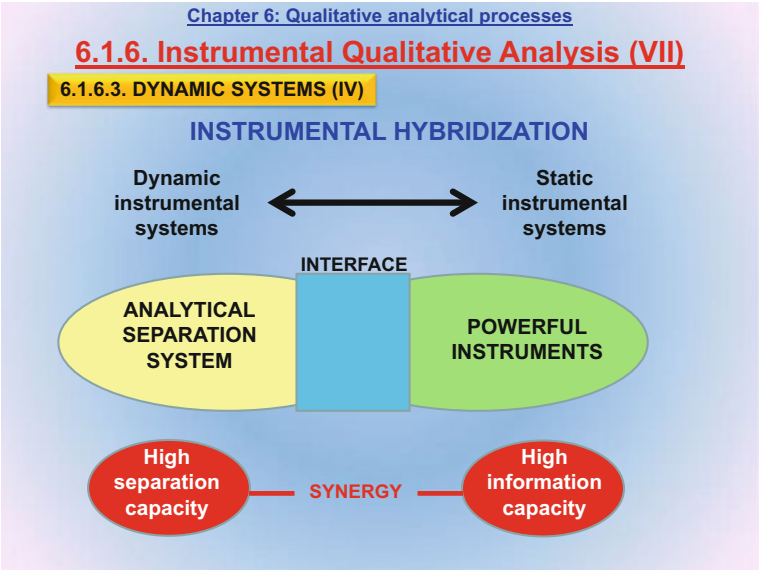


6.40.1. This is a real-life example: the identification by liquid chromatography of an unknown compound in a cola drink.

6.40.2. A sample of the drink gives a chromatograph including three identifiable (expected) peaks and a fourth corresponding to an unknown compound—possibly an acidulant.

6.40.3. Samples of the drink spiked with different preservatives are analysed in the same manner. The sample to which benzoic acid is added gives a peak coinciding with the fourth in the chromatogram for the initial sample, albeit much higher, which confirms that the peak in the original chromatogram corresponded to the acidulant benzoic acid.

Slide 6.41



So-called *instrumental hybridization* is the synergistic combination of two or more instrument systems in order to boost their individual qualitative and quantitative information potentials. The key to an effective connection here is finding an appropriate interface with a view to maximizing analyte separation and information production.

A large number of hybrid instrumental systems are commercially available at present. Especially interesting are those combining a dynamic system and a static system.

Most hybrid systems use a gas or liquid chromatograph, or a capillary electrophoresis system, in combination with a mass spectrometer. The detector coupled to the separation system should never be of the destructive type. Hybrid systems produce vast amounts of information that require computerized processing but afford extremely reliable qualitative identifications.

6.2 Annotated Suggested Readings

BOOKS

Principles of Analytical Chemistry

M. Valcárcel

Springer-Verlag, Berlin, 2000

This was the first book to start the teaching of Analytical Chemistry with its foundations before dealing with methods and techniques in order to provide students with an accurate notion of what Analytical Chemistry is and means.

The contents of this chapter coincide to a great extent with those of Chapter V in the book ("Qualitative Aspects of Analytical Chemistry"). Although this chapter is more synthetic, it contains more examples and elaborates on some topics to better illustrate the state of the art in Qualitative Analysis (particularly as regards screening systems and the adapted description of the "uncertainty" concept). The book provides a source for direct consultation. Few Analytical Chemistry textbooks deal with Modern Qualitative Analysis.

Metrology of Qualitative Chemical Analysis

M. Valcárcel et al.

European Commission, Brussels, 2002.

This 166-page document presents a systematic approach to basic and applied developments in Metrology in Qualitative Analysis. This chapter is based on it.

PAPERS

Qualitative Analysis

M. Valcárcel et al.

Encyclopaedia of Analytical Science, Elsevier (Amsterdam), 2005, 405–411.

This paper expands on the contents of the present chapter. The paper is structured similarly but contains additional figures that complete the message of this chapter.

Analytical Features of Qualitative Analysis

S. Cárdenas & M. Valcárcel

Trends in Analytical Chemistry, 2005, 24, 477–487.

This paper focuses on the singularities of analytical properties as applied to Qualitative Analysis. Thus, it provides firm support for the description of analytical properties and errors here. The paper additionally describes the validation of an analytical process for qualitative purposes.

6.3 Questions on the Topic (Answered in Annex 2)

6.1. Does the qualitative analysis of samples fit in Classification Analysis?

6.2. What name is usually given to qualitative analytical processes?

6.3. Tick the analytical properties that are not applicable to Qualitative Analysis.

[ ] Representativeness
[ ] Accuracy
[ ] Precision
[ ] Sensitivity

6.4. Two methods for the qualitative analysis of milk samples possibly contaminated with pesticides provide wrong information. Thus, method A gives false positives and method B false negatives. Which would you use? Why?

6.5. What are the main differences between Qualitative Analysis and Quantitative Analysis? Tick the correct answers.

[ ] The binary response
[ ] A classical method of analysis
[ ] The use of analytical chemical standards
[ ] The analytical property "reliability"
[ ] Selectivity

6.6. What are the differences between binary and multiple classification in Qualitative Analysis?

6.7. What are the factors dictating the following parameters?
(a) Limit of detection
(b) Cut-off concentration
(c) Threshold concentration

6.8. What is a false positive in Qualitative Analysis? Give an example.

6.9. What is a false negative in Qualitative Analysis? Give an example.

6.10. An immunochemical test (method A) and a chemical spot test (method B) are used to detect the same analyte in the same sample. The results of analysing 100 samples are as follows:

	Reliability (%)	False positives (%)	False negatives (%)
Method A	95	2	3
Method B	94	6	0

Which method provides the better results? Why?

6.11. What analytical properties are applicable to quantitative determinations but not to qualitative tests? Why?

6.12. What are "analytical systems with group separation" in Classical Qualitative Analysis?

6.13. What are the differences between group, identification and masking reagents in Classical Qualitative Analysis?

6.14. Name two identification (Qualitative Analysis) procedures used in dynamic instrumental systems (e.g., chromatography).

6.15. Tick the words directly connected with Qualitative Analysis:

[ ] Detection
[ ] Quantification
[ ] Identification
[ ] Qualification

6.16. How does a "white" sample differ from a "black" sample?

6.17. Is Qualitative Analysis important to modern Analytical Chemistry? Why?

6.18. What are the three quantitative landmarks for the binary response in Qualitative Analysis?

6.19. One brand of canned tuna fish contains 4 ppm tin. A qualitative test with C_{LOD} = 1 ppm for the metal gave a positive (YES) response. What type of error was made?

[ ] None
[ ] A false positive
[ ] A false negative

6.20. What type of error is the more crucial in Qualitative Analysis? Why? Give an example.

6.21. Is "specific uncertainty" applicable to Qualitative Analysis? Why?

6.22. What are the three most important limitations of Classical Qualitative Analysis in relation to Instrumental Qualitative Analysis?

6.23. What are the three types of reagents used in Qualitative Analysis? What is their purpose? Give an example of each.

	Name	Purpose	Example
Type 1			
Type 2			
Type 3			

6.24. What are the three main features of so-called "analytical schemes without group separation"?

6.25. What is the difference between a dynamic and a static instrumental system in Qualitative Analysis?

6.26. What analytical properties are applicable to Qualitative Analysis?

6.27. Are both types of calibration applicable to Qualitative Analysis?

Method calibration [ ] Yes [ ] No
Equipment calibration [ ] Yes [ ] No

6.28. What types of instruments does Classical Qualitative Analysis use?

6.29. What are masking reagents? In what context are they used?

6.30. Define "reliability" in Qualitative Analysis. To which classical analytical properties does it relate?

6.31. Instrumental Qualitative Analysis relies on a triple comparison of signals to be subjected to the analytical process. What do the three signals belong to?

6.4 An Abridged Version of the Chapter

The contents of this chapter can be shortened by about 30% for teaching Analytical Chemistry to students not majoring in Chemistry. The following 12 slides can be omitted for this purpose:

- Section 6.1.2: Slides 6.5, 6.6 and 6.7.
- Section 6.1.3: Slides 6.11, 6.12, 6.17, 6.18, 6.19, 6.20, 6.24 and 6.25.
- Section 6.1.6: Slide 6.41.